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REVIEW ARTICLE

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Myo-inositol in polycystic ovary syndrome: a systematic review and positioning of its use

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Abstract

Introduction: Polycystic Ovary Syndrome (PCOS) is the most common endocrine disease among women of reproductive age 6 to 15% its presentation is complex and heterogeneous and is characterized by clinical and laboratory findings of hyperandrogenism, oligo-anovulation, metabolic abnormalities such as insulin resistance (IR), overweight, obesity, type 2 diabetes mellitus, dyslipidemia and increased risk of cardiovascular disease. **Objective:** To evaluate the effects of myo-inositol in pregnant and non-pregnant women with polycystic ovary syndrome in relation to fertility improvement, gestational outcomes, and metabolic and hormonal parameters. **Methods:** Data from randomized clinical trials on the efficacy of myo-inositol in women with PCOS were used in this review. The PubMed database was used for literature search. **Results:** Insulin resistance is one of the mechanisms that explain the emergence of metabolic and reproductive changes in women with PCOS. When compared to placebo, myo-inositol was effective in improving fertility, increasing ovulation and fertilization rates, in addition to improving metabolic parameters as indicators of insulin resistance (HOMA index). Even when compared to more traditional insulin sensitizers like metformin, myoinositol showed similar efficacy in restoring fertility. Myo-inositol was also effective when associated with clomiphene acetate and in women undergoing in vitro fertilization processes. There was also an improvement in pregnancy outcomes and a reduction in the risk of developing gestational diabetes with the use of myo-inositol. **Conclusions:** Myo-inositol improves clinical and laboratory parameters in both pregnant and non-pregnant PCOS patients, increasing the fertility rate and improving pregnancy outcomes.

Keywords: Inositol. Myo-inositol. Gestational diabetes. Fertility. Polycystic ovary syndrome. Insulin resistance.

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disease among women of reproductive age 6 to 15% **[1]** its presentation is complex and heterogeneous and is characterized by clinical and laboratory findings of hyperandrogenism, oligo-anovulation, metabolic abnormalities such as insulin resistance (IR), overweight, obesity, type 2 diabetes mellitus, dyslipidemia and increased risk of cardiovascular disease. Hyperandrogenism is the most striking clinical manifestation. Hyperandrogenism profoundly affects the functioning of the granulosa layer cells of the granulosa ovarian follicle and follicular development through a complex mechanism that ultimately leads to the development of obesity and IR.

This hyperandrogenism alters the hormonal secretion pattern leading to defective folliculogenesis, in addition to causing obesity, hirsutism, androgenic alopecia, and acne **[2]**. Drugs such as birth control and spironolactone help lower androgen levels, and substances that promote insulin sensitization (metformin, pioglitazone, and myoinositol) improve pregnancy rates in addition to helping to lower androgen levels. As we said, PCOS has a heterogeneous character and both hyperandrogenisms lead to obesity and insulin resistance, and IR and obesity worsen hyperandrogenism, forming a vicious cycle that leads to the emergence and worsening of PCOS. Although numerous studies have already been conducted, the

exact pathogenic mechanism of PCOS remains unclear **[3]**.

Its diagnosis is made using the Rotterdam criteria. Two criteria are needed to close the diagnosis (polycystic ovaries on imaging, oligo-anovulation, and hyperandrogenism). It is also necessary to rule out endocrine diseases that mimic PCOS (congenital adrenal hyperplasia, Cushing's syndrome, androgen-producing adrenal and ovarian tumors, hyperprolactinemia, and hypothyroidism). The "two cell theory" says that the synthesis of ovarian androgens requires the participation of LH, FSH, theca, and granulosa cells. The first step is the formation of androgens in the theca stimulated by LH. The frequency and speed of the LH secretion pulse determine the intensity of ovarian production of androgens, which in turn are transported to the granular layer where they undergo the action of the aromatase enzyme, transforming into estradiol. Women with PCOS have attenuated estradiol production due to decreased expression of the aromatase enzyme caused by androgens **[4]**.

Also, hyperandrogenism causes some pathophysiological changes in women with PCOS including IR, dyslipidemia, hyperinsulinemia, and an imbalance in the LF/FSH ratio. Insulin promotes cell growth, proliferation, and differentiation through the action of intracellular mediators such as phosphatidyl-inositol-3-kinase (PI3-kinase) and mitogen-activated protein kinase (MAPK). Insulin can directly stimulate androgen production in the theca. IR also promotes a decrease in sex hormone-binding globulin (SHBG) levels, which leads to an increase in the concentration of free androgens. Obesity has a strong link with PCOS. Patients with PCOS, both obese and non-obese, have more visceral fat compared to healthy patients. And the greater the amount of visceral fat, the greater the androgen levels. Likewise, obese patients with PCOS have higher levels of free androgens than patients with PCOS, but with a normal BMI. Androgens stimulate IR and abnormal accumulation of visceral fat in women, and increased adiposity leads to increased blood levels of unesterified free fatty acids, cholesterol, triglycerides, and other apolipoprotein abnormalities. The increase in free fatty acids further aggravates the IR picture. A vicious cycle occurs where the increase in androgens promotes obesity and IR and obesity + IR aggravates hyperandrogenism. Obesity also affects other adipokines. It increases leptin levels which in turn inhibits aromatase, worsening hyperandrogenism. Obesity also decreases levels of adiponectin, an adipokine that reduces insulin resistance **[4]**.

In this sense, there is a strong genetic and epigenetic component associated with PCOS, an example of this is that some genetic polymorphisms associated with increased BMI (body mass index) are related to PCOS **[2]**. The detail is that IR in PCOS tends to be selective, affecting different organs differently. While muscles, adipose tissue, and liver are most affected, the ovaries remain responsive to insulin action. The main neuroendocrine characteristic of PCOS is the increase in the pulsatility of GnRH and, consequently, of LH/FSH. Changes in the LH/FSH ratio (with a predominance of LH) are a typical finding in PCOS.

Also, insulin hypersecretion acts synergistically with the increase in LH levels to generate hyperandrogenism in PCOS. Inhibition of hepatic SHBG synthesis contributes to the increase in free testosterone levels. The polycystic appearance of the ovaries (several antral follicles with a size between 2-8 mm) is due to an interruption in their maturation as a result of the aforementioned pathophysiological changes (hyperandrogenism, hyperinsulinemia, and increased LH levels) **[1]**.

Due to this complex interaction with the endocrine-metabolic system, PCOS can trigger a series of complications such as **[1,2]** psychological (depression and anxiety), metabolic (insulin resistance, pre-diabetes, metabolic syndrome, obesity), cardiovascular diseases (hypertension and dyslipidemia), endometrial cancer and complications infertility and pregnancy.

Therefore, the present systematic review study aimed to evaluate the effects of Myo-inositol in pregnant and non-pregnant women with polycystic ovary syndrome to improve fertility, gestational outcomes, and metabolic and hormonal parameters.

Methods

Study Design

The present study followed a systematic review model, following the rules of systematic review – PRISMA (Transparent reporting of systematic reviews and meta-analyses-HTTP: //www.prisma-statement.org/).

Research Strategy And Information Sources

The PubMed, Embase, Ovid, Web Of Science, ScienceDirect Journals (Elsevier) and Scielo databases were used to search for randomized clinical trials that evaluated the efficacy and safety of myo-inositol. The search was done using the terms: *Inositol. Myo-inositol. Gestational diabetes. Fertility. Polycystic ovary syndrome. Insulin resistance*.

Articles on the improvement of metabolic parameters and fertility with the use of myo-inositol when compared with placebo or other insulin sensitizers (such as metformin) were included. Articles on

outcomes in pregnant women using myo-inositol and women with PCOS undergoing *in vitro* fertilization (IVF) procedures or ovulation induction with clomiphene acetate and rFSH were also included. Articles published before 2009, in other languages, in animals, and in vitro were excluded.

GRADE Ratings And Risk of Bias

According to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria. The quality of the evidence was classified as high, moderate, low, or very low, according to the risk of bias, clarity of comparisons, precision, and consistency of analyses. The Cochrane instrument was adopted to assess the risk of bias of the included studies.

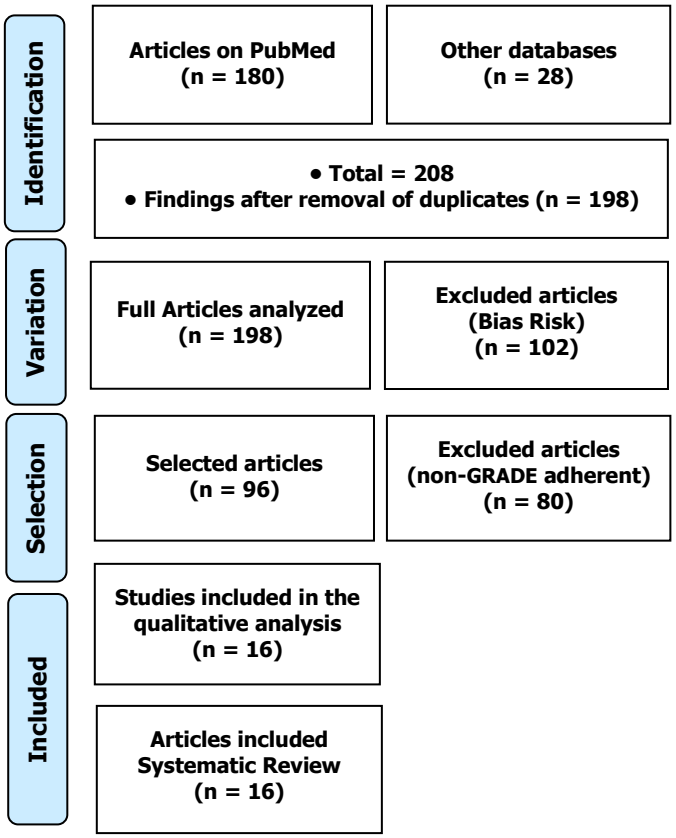
Results

A total of 208 articles were found with the keywords described, but only 16 met the inclusion and exclusion criteria necessary for this review (**Figure 1**). The many beneficial effects of inositols on follicular development, hormone regulation, and glucose homeostasis support their use as therapeutic agents in patients with PCOS. Many studies confirm its positive effects on metabolic, hormonal, and reproductive disorders in PCOS, both alone and in combination with other substances. In addition, IM treatment is safe, with few side effects compared to other substances used in the treatment of insulin resistance and ovulation induction **[4]**.

In 2015, the International Consensus Conference on MI and D-chiro inositol (DCI) in Obstetrics and Gynecology recognized that both MI and DCI are present in several biological pathways involved in the pathogenesis of PCOS and many clinical data are demonstrating that inositol supplementation can be beneficial in improving the metabolic and reproductive aspects of this disorder. Several clinical studies prove the effectiveness of treatment with IM for both improving fertility and improving symptoms of PCOS, in addition to reducing pregnancy complications in these patients **[5]**.

Costantino D. et al.2009 (grade of recommendation A, level of evidence 1B) evaluated the replacement of IM (4g + folic acid 400 mcg) x placebo (folic acid only 400 mcg) in relation to clinical and laboratory aspects in women with PCOS in a randomized, double-blind, placebo-controlled clinical trial of 42 women aged between 18 and 40 years. The MI group had a 65% drop in total testosterone (99.5±7 to 34.8±4.3 ng/dL x placebo 116.8±15 to 109±7.5 ng/dL; p=0.003) and free testosterone (0.85 ±0.1 for 0.24±0.33 ng/dL x placebo 0.89±0.12 for 0.85±0.13 ng/dL; P=0.01). Plasma triglyceride levels were also reduced: 195±20 to 95±17 mg/dL x placebo: 166±21 to 148±19 mg/dL; P=0.001). There was an improvement in systolic blood pressure: 131±2 to 127±2 mmHg x placebo: 128±1 to 130±1 mmHg; P=0.002) and diastolic blood pressure: 88±1 for 82±3 mmHg x placebo 86±1 for 90±1 mmHg; p=0.001) Area under the curve (AUC) of plasma insulin after oral glucose administration: 8,540±1,149 for 5,535±1,792 μU/mL/min x placebo: 8,903±1,276 for 9,100±1,162 μU/mL/min; P=0.03). 16 out of 23 women in the MI group ovulated (69.5%) vs 4 out of 19 in the placebo group (21%) (p=0.001). MI treatment provided a reduction in testosterone and insulin levels, as well as an improvement in several metabolic factors and ovulation rate **[6]**.

Figure 1. Article Selection Process.



Regidor et al. 2016 (grade of recommendation B, level of evidence 2B) evaluated improved fertility and reduced hyperandrogenism in an observational study of 3602 women with PCOS and infertility according to the Rotterdam criteria. The intervention group used MI 2000mg + folic acid 200 mcg twice daily for a period of 2 to 3 months. A subgroup of 32 patients performed an analysis of total testosterone, free testosterone, and progesterone levels after 12 weeks of therapy. Seventy

percent of patients had ovulation restored and 545 pregnancies occurred, which represents a pregnancy rate of 15.1% in patients undergoing treatment. The fertility levels achieved were similar to those found with metformin. MI proved to be much safer when compared to metformin, which is often associated with side effects such as abdominal pain, nausea, diarrhea, and lactic acidosis (rare). Total testosterone levels dropped from 96.6 ng/dL to 43.3 ng/dL and progesterone levels rose from 2.1 ng/mL to 12.3 ng/mL (p=0.05) after 12 weeks of treatment **[7]**.

Raffone et al. 2010 (grade of recommendation B, level of evidence 2B) compared the efficacy of MI and metformin alone or in combination with r-FSH for the treatment of menstrual irregularities, chronic anovulation, and infertility in women with PCOS. One hundred and twenty patients with PCOS (by Rotterdam criteria) followed in an outpatient infertility clinic for a period of 14 to 16 months were treated with oral metformin 1500 mg/day (n=60) or oral myo-inositol 4 g/day + acid folic acid (n=60) both in continuous use. If pregnancy did not occur, r-FSH (37.5 U/day) was added for a maximum of three attempts. Fifty percent of patients who used metformin had spontaneous ovulation restored and 18.3% were able to become pregnant. The other 42 patients were treated with metformin + r-FSH. There was a 26.1% pregnancy rate. The total pregnancy rate in this group was 36.6%. 63% of patients treated with myo-inositol + folic acid had spontaneous ovulation restored and 30% of these were able to become pregnant. The 38 patients who did not become pregnant were treated with MI + folic acid + r-FSH. Pregnancy occurred in 11 of these patients (28.9%). The total pregnancy rate in this group was 48.4% **[8]**.

Zeng et al.2017 (grade of recommendation A, level of evidence 1A) performed a systematic review with a meta-analysis showing the effectiveness of inositol in women with PCOS. There were 10 randomized clinical trials with a total of 573 patients. Myo-inositol decreased insulin resistance levels measured by the HOMA index (-0.65; 95% CI -1.02, -0.28; p=0.0005), increased estradiol levels (16.16; 95% CI 2.01, 30.31; p=0.03); There was no statistical significance regarding total testosterone levels. (-16.11; 95% CI -46.08, +13.86; p=0.29). There was an increase in SHBG levels in the myo-inositol group compared with placebo (24.01; 95% CI 3.96, 44.06; p=0.02) **[9]**.

A meta-analysis by Zheng et al 2017 (Evidence Grade A, Recommendation Level 1A) evaluated the effectiveness of myo-inositol in women with infertility in inducing ovulation before intracytoplasmic sperm injection (ICSI) or in vitro fertilization with embryo transfer (IVF-ET). 7 studies were included, 6 of which were randomized and a cohort study with a total of 935 patients. Four hundred and fifty-three women used myo-inositol and 482 used a placebo. The intervention group used myo-inositol 4g + folic acid 400mcg and the placebo group used folic acid alone. Patients used the medication once or twice daily for 3 months before and during ovulation induction. The age of the patients ranged between 31.5 and 36.2 years. The mean duration of infertility ranged from 21.6 to 46.1 months and the BMI ranged from 22.7 to 26.7kg/m2. The pregnancy rate was significantly higher in the myo-inositol group compared to placebo (33.33% vs. 27.62%) (OR, 1.45; 95% CI, 1.08–1.95; p=.01). The miscarriage rate was significantly lower in the myo-inositol vs placebo group (5.92% vs 17.61%). (OR, 0.26; 95% CI, 0.12–0.59; p=.001). The proportion of grade 1 embryos was significantly higher in the myo-inositol group compared to placebo. (45.17% x 41.93%). (OR, 1.73; 95% CI, 1.10–2.74; p=.02). The total amount of drugs used to induce ovulation was significantly lower in the myo-inositol group compared to placebo (-327.40; 95% CI, -567.38 to -87.41; p=.007). The meta-analysis showed that the myo-inositol group needed less r-FSH and less gonadotropin compared to the placebo. There were no significant differences between the groups regarding the total number of oocytes removed (-0.11;95% CI, -0.79 to +0.58; p=.75), proportion of stage MII oocytes (58.65% X 57.10%) (OR, 1.06; 95% CI, 0.97–1.15; p=.21) or stimulation days (-0.55; 95% CI, -1.17 to -0.07; p=.08) **[10]**.

Kamenov et al. 2014 (grade of recommendation B, evidence level 2B) did an open-label prospective study comparing the efficacy of myo-inositol with the combination of MI + clomiphene in women with PCOS associated with proven IR, in inducing ovulation and pregnancy. Fifty patients between 20 and 35 years old with PCOS + proven insulin resistance and infertility for more than 1 year received MI (2 g MI + 200 mcg folic acid 2X daily) for three consecutive spontaneous cycles starting between the 3rd and the 5th day after menstrual bleeding. Patients who remained anovulatory after the third cycle with MI had their menstruation induced by the use of dydrogesterone (Duphaston 10 mg twice a day for 10 days) and clomiphene 50 mg was associated with IM (on the fifth day after the onset of menstruation) and maintained for 5 days for another three cycles. If pregnancy did not occur, the clomiphene dose was increased by 50 mg to a maximum of 150 mg on the third attempt. The primary endpoints were

ovulation rate (via the serial US) and the onset of pregnancy. Secondary endpoints were changes in BMI and HOMA score. Three patients were lost to follow-up throughout the study. After using IM, 29 patients ovulated (61.7%) and 18 (38.3%) were resistant. Among the patients who ovulated, 11 managed to get pregnant (23.4% of the total). Among the 18 patients who ovulated but were resistant to MI, 6 (12.8% of the total) became pregnant after the association of clomiphene with MI. Among the 18 patients who did not ovulate with IM alone, 13 (27.7%) managed to ovulate with the combination of clomiphene, and 6 (12.8%) managed to get pregnant. The total pregnancy rate after 6 months of study was 48 .9% (23 patients). Most patients who became pregnant with MI had a normal BMI, while most patients resistant to MI were obese. Obese patients were 50% less likely to ovulate and 33% less likely to become pregnant when compared to normal BMI patients with this protocol. There was a small but statistically significant reduction in BMI after 6 months of study ($p<0.001$) in all groups, but the group that ovulated had a lower initial BMI. There was a reduction in the HOMA index in all groups, but the patients who ovulated had a lower HOMA at baseline. The authors concluded that MI therapy is effective for reducing IR and provides an improvement in ovulation and pregnancy rate, which is corroborated by other studies, which also demonstrate an improvement in the quality of oocytes and a reduction in the number of gonadotropins used to induce ovulation **[11]**.

Zheng et al, 2015 (grade of recommendation A, level of evidence 1A) in a meta-analysis of studies with pregnant women with diabetes, evaluated the improvement of insulin resistance with the use of myo-inositol. Five studies with 513 participants were included. The global incidence of hyperglycemia during pregnancy is 16.9% and more than 90% of these patients live in poor countries or with developing economies. The meta-analysis showed a significant reduction in the incidence of gestational diabetes (RR, 0.29; 95% CI, 0.19–0.44) and birth weight (-116.98; 95% CI, -208.87 to -25.09). There was also an improvement in oral glucose tolerance test (OGTT) results (-0.36; 95% CI, -0.51 to -0.21); 1h-OGTT (-0.63; 95% CI, -1.01 to -0.26); 2-h OGTT (-0.45; 95% CI, -0.75 to -0.16) and complication rate (respiratory distress syndrome, shoulder dystocia, neonatal hypoglycemia, macrosomia, polyhydramnios, and preterm delivery) (odds ratio [OR], 0.28; 95% CI 0.14 to 0.58). Current evidence shows that myo-inositol supplementation reduces the development of gestational diabetes, although additional large-scale, multicenter, double-blind, randomized, placebo-controlled studies are needed **[12]**.

Guo et al, 2017 (grade of recommendation A, level of evidence 1A) in a meta-analysis of randomized trials, evaluated the benefits of MI in pregnant women at high risk of developing gestational diabetes mellitus (GDM). The primary endpoint was the development of GDM + birth weight and the secondary endpoints were the 1 h and 2 h oral glucose tolerance test and pregnancy complications. 4 studies were included, with a total of 586 patients. Compared with placebo, patients using MI had a significantly reduced chance of developing GDM (RR = 0.44, 95% CI [0.32, 0.62], $p<0.0001$), with the number needed to treat (NNT) from 6.2 which was rounded to 7 (one out of 7 patients treated benefited from the therapy). There was no statistical significance in the reduction in birth weight in patients treated with MI (60.60g, 95%CI [-177.21, 56.02], $p=0.31$). The MI group had a significant reduction in the incidence of preterm birth (0.30, 95% CI [0.11, 0.79], $p = 0.01$) and a significant improvement in OGTT: 1h (-0.55, 95% CI [-0.81, -0.28], $p<0.00001$) and 2h (-0.58, 95% CI [-0.94, -0.23], $p=0.001$). There was no statistical significance for other types of complications. A sequential analysis of the studies (sequential trial analyses-TSA) was performed for the primary outcomes, which demonstrated the robustness of the results, but as the level of evidence is small, the authors suggest further research to reinforce these findings **[13]**.

A systematic review with the meta-analysis by Zhang et al, 2019 (grade of recommendation A, level of evidence 1A) evaluated the influence of myo-inositol supplementation on the incidence of gestational diabetes. Five randomized clinical trials were included. Again myo-inositol supplementation demonstrated a significant reduction in the incidence of gestational diabetes compared to placebo (RR=0.43; 95% CI=0.21 to 0.89; $p=0.02$). There was also a reduction in the incidence of preterm delivery (RR=0.36; 95% CI=0.17 to 0.73; $p=0.005$), but there was no improvement in other indicators such as OGTT, macrosomia, and birth weight **[14]**.

Laganà et al. 2018 (grade of recommendation A, evidence level 1A) in a meta-analysis of 8 randomized clinical trials with 812 women, the impact of myo-inositol supplementation on reducing the duration of controlled ovarian hyperstimulation and reducing the amount of gonadotropin used to perform of IVF therapies in both women with PCOS and women without PCOS. There was a significant reduction in the amount of gonadotropins used (-493.66, [95% CI - 582.76, -

404.56], p<0.00001), and in the duration of controlled ovarian hyperstimulation (- 0.71, [95% CI-1.12, - 0.30], p=0.0007). The total number of patients studied with PCOS was 515 (n=244 with MI and n=271 in the control group). The total number of patients studied without PCOS was 112 (n=58 with MI and n=56 in the control group). Regarding gonadotropins, the greatest reduction in use was in patients with PCOS (-507.20, [95% CI - 600.54, - 413.86], p< 0.00001), but patients without PCOS also experienced a reduction in the total amount of gonadotropins, but to a lesser extent than PCOS patients (-354.54, [95% CI - 653.74, -55.33], p=0.02). Regarding the duration of time of controlled ovarian hyperstimulation, there was only statistical significance in the reduction in duration in the group of patients with PCOS (MD = - 0.95, [95% CI - 1.27, - 0.63], p<0.00001) x (- 0.05, [95% CI - 0.56, -0.66], p=0.87 in the group without PCOS) **[15]**.

Regarding safety, a review of the literature showed that myo-inositol is completely safe at the standard dose (4g/day) with an incidence of side effects equal to zero at this dose. However, with daily doses equal to or greater than 12g/day, patients showed an increased incidence of gastrointestinal symptoms such as nausea, flatulence, and diarrhea **[6]**. Similar safety results were described in all studies cited in this review **[16]**.

Discussion

Insulin Resistance

Insulin signaling begins after the binding of this hormone with the alpha subunit of the cell receptor, with a structural modification of the beta subunit of the same receptor, located inside the cell (autophosphorylation). Once activated, the beta subunit of the insulin receptor phosphorylates IRS 1,2,3 and 4 (insulin-responsive substrates), which in turn activate both the MAP kinase pathway and the PI3-kinase pathway (phosphatidyl-INOSITOL). 3-kinase). Once activated, the enzyme phosphatidyl-INOSITOL 3-kinase (PI3K) will increase the production of two molecules contained in the cell membrane, phosphatidylinositol-4,5-bisphosphate (PI [4,5] P2) and phosphatidylinositol-3,4, 5-trisphosphate (PIP3). (PI[4,5]P2) and (PIP3) continue the cascade of reactions (when they are released from the cell membrane in the form of myo-inositol-inositolphosphoglycans (MI-IPGs) and D-Chiro-inositol-inositolphosphoglycans (DCI-IPGs) as we will see below), activating protein kinase b (PKB). Activated PKB translocates glucose transporters (GLUT4) to the cell membrane, thus promoting the entry of glucose into the cell **[3,4]**.

Simplified sketch of the insulin signaling mechanism. A minimal diagram of the mitogenic and metabolic pathways of insulin signaling is shown. GLUT 4 (type 4 glucose transporter); GRB2, (type 2 growth factor receptor-binding protein); GS (glycogen synthase), (P indicates the inactive phosphorylated form); GSK 3 (glycogen synthase kinase 3); IRS, (insulin receptor substrate (4 different proteins); MAP kinase (activated mitogenic protein kinase); mTOR (mammalian target of rapamycin); PDK (phospholipid-dependent kinase); Pl 3 kinase (phosphatidylinositol 3 kinase) PKB/ Akt (protein kinase B); AKR8 (murine tumor protein kinase 8); PP-1 (glycogen-associated protein phosphatase 1); Ras (rat sarcoma protein); SHC (Src and collagen homolog protein); SOS (son-of-sevenless protein); TK (tyrosine kinase) **[3]**.

The importance of Inositol in the intracellular signaling of insulin and FSH

Inositol plays a crucial role in the transmission of intracellular signals from both insulin and FSH, Inositol is formed within cells from glucose-6-phosphate (G6P), G6P is converted to Inositol-3-phosphate (Ins3P) which in turn is transformed into myo-inositol. MI, through the action of insulin, can be converted into D-Chiro-Inositol. Both (MI and DCI) participate in the insulin signaling process. The normal MI: DCI ratio in plasma is 40:1, but because of its importance in insulin and FSH signaling, the ratio of MI: DCI in the ovary is 100:1. Inositol can be found free inside the cell, or present in the cell membrane in the form of Inositol-phosphoglycans **[2,3]**.

Cleavage of phosphatidylinositol-4,5-bisphosphate (PI[4,5]P2) gives rise to phosphatidylinositol-3,4,5-trisphosphate (PIP3) an intracellular second messenger of the insulin signal. (PI[4,5]P2) and PIP3 are present in the cell membrane and when stimulated by insulin they release myo-inositol-inositolphosphoglycans (MI-IPGs) and D-Chiro-inositol-inositolphosphoglycans (DCI-IPGs) that also participate in intracellular signaling of insulin and FSH. MI-IPG promotes the release of GLUT 4 to the cell membrane. MI-IPG stimulates the release of calcium (another intracellular mediator of the insulin signal) from the endoplasmic reticulum. DCI-IPG promotes intracellular glycogen storage and inhibition of the aromatase enzyme (which converts testosterone to estradiol). MI also participates in FSH signaling, stimulating the proliferation and maturation of the cells of the granular layer of eggs and the synthesis of anti-Müllerian hormone, which improves the sensitivity of follicles to FSH, accelerating their maturation. Still, concerning FSH signaling, MI also stimulates the

MAPK/PKA pathway, promoting estrogen synthesis through the action of aromatase **[2-4]**.

Inositol and Its Role in PCOS

Inositols are chemically identified as cyclohexanes and include a family of nine stereoisomers. Myo-inositol (MI) is the most widely distributed in nature. The main dietary sources of MI are fruits, beans, grains and nuts. Daily MI intake does not exceed 500–700 mg/day for a standard Western diet. MI can also be actively synthesized (up to 4 g/day) in the human body (especially in the liver and brain) **[1]**. The cellular precursor of MI is glucose-6-phosphate, which is isomerized to inositol-3-phosphate (IP3) by D-3-myo-inositol-phosphate synthase. IP3 is then dephosphorylated to free MI by inositol 1-monophosphatase. Free inositol can also be obtained by recycling inositol-1,4,5-triphosphate and inositol 1,4-bisphosphate. MI biosynthesis varies between tissues, depending on specific functional requirements for each organ. There is a complex relationship between glucose and MI metabolism. On the one hand, MI inhibits duodenal glucose absorption and reduces the rise in blood glucose, suggesting the existence of a competition for the same transport system. On the other hand, glucose significantly neutralizes cellular uptake of inositol and can induce MI depletion by activating the glucose-sorbitol pathway **[4]**. Inhibition of aldose reductase in cultured cells restores MI levels by neutralizing the depleting effect of sorbitol. Sodium-glucose transporter (SGLT) 1/2 inhibitors prevent the uptake of both glucose and inositol, which suggests that the two molecules share the same transport systems.

Hyperglycemia and insulin resistance modify the relative proportions between the different isomers of inositol present in the tissues. Myo-inositol and DCI are abundant in the ovaries and follicular fluid and have specific roles in insulin signaling and follicular development. MI stimulates FSH signaling as a second messenger, while DCI is responsible for insulin-mediated androgen synthesis in addition to acting as an aromatase inhibitor. In the normal ovary, the balance between these two isomers maintains normal hormone secretion and ovarian function. Under physiological conditions the MI/DCI ratio is between 100:1 in follicular fluid and 40:1 in plasma. In patients with PCOS and insulin resistance, hyperinsulinemia induces a higher DCI-MI ratio because of the activity of stimulating the activity of the epimerase enzyme, which transforms MI into DCI. Despite the chemical similarities between MI and ICD and their synergistic effects on insulin sensitivity, they exert different functions in the ovary. MI can affect aromatase activity in an opposite way compared to DCI **[2,3]**.

Thus, higher MI/DCI ratios (100:1) promote aromatase activity in granulosa, increasing estrogen, while lower MI/DCI ratios (2:1) stimulate androgen production in theca cells. This may explain why DCI supplementation produces an increase in testosterone levels and a concomitant reduction in estrogens. This leads to hyperandrogenism and suppression of FSH signaling. This mechanism is involved in the so-called "ovarian paradox", defined in 2011 by Carlomagno et al. who hypothesizes that in the ovaries of patients with polycystic ovary syndrome, increased epimerase activity leads to a local deficiency of MI which, in turn, is responsible for the poor quality of the oocyte. Under these conditions, glucose uptake and metabolism in oocytes and follicular cells are negatively affected, thus compromising oocyte quality, which depends on the availability of adequate amounts of IM. This paradox is further supported by later studies in animals and humans that show the differential effect of supplementation with MI alone and MI+DCI in different proportions, proving that restoration of physiological levels of the two inositol isomers may be crucial for proper functioning of the ovary **[2]**. Myo-inositol and D-chiroinositol intracellular pathways as second messengers.

Abbreviations: AC (Adenyl cyclase); AMH (anti-Müllerian hormone); DCI (D-chiroinositol); E2 (estradiol); ER (endoplasmic reticulum); FSH (follicle stimulating hormone); FSHR (FSH receptor); G (glucose); Gas (heterodimeric protein G); GLUT-4 (type 4 glucose transporter); GS (glycogen synthase); insP3 (insositol 3 phosphate); IPG (inositol phosphoglycan); IP3-R (insP3 receptor); IR (insulin receptor); MI (myo-inositol); P (phosphate); PDH (phosphate dehydrogenase); PKA(protein kinase A) **[4]**.

Conclusion

Insulin sensitizing agents are considered the therapy of choice for PCOS, as insulin resistance and associated hyperinsulinemia are recognized as important pathogenic factors of the syndrome. Nearly all obese women with PCOS, and more than half of normal-weight women with PCOS, are insulin resistant and therefore have some degree of hyperinsulinemia. For this reason, the use of insulin sensitizers has been suggested in most patients with PCOS, as a useful treatment in reducing serum androgen levels and in the use of gonadotropins in assisted reproduction therapies,

in addition to providing an improvement in serum lipids. These therapies have also been associated with a decrease in acne and hirsutism, an improvement in menstruation regulation, and an improvement in ovulation and fertility. Changes in inositol metabolism are related to the pathogenesis of insulin resistance. Administration of myo-inositol improves physiological insulin receptor activity, restoring spontaneous ovulatory function in most women with PCOS. The clinical studies evaluated clearly showed an improvement in insulin sensitivity and glucose tolerance in both pregnant and non-pregnant women. MI supplementation also improved fertility by increasing the rate of spontaneous pregnancy. In patients undergoing assisted reproductive therapies, IM supplementation provided a reduction in the number of gonadotropins used and a decrease in the time of controlled ovarian hyperstimulation. The studies also showed a reduction in birth complications in patients with gestational diabetes who used MI. Compared with metformin (the most commonly used insulin-sensitizing agent), MI achieved similar or slightly superior results in restoring fertility in women with PCOS, either alone or in combination with clomiphene or r-FSH. with the advantage of having fewer side effects than metformin. It is noteworthy that more double-blind randomized trials with MI are needed, as most have a low number of participants and short durations. Furthermore, the long-term benefits (more than 6 months) of using MI remain undefined.

Recommendations of the Brazilian Association of Nutrology (ABRAN - Associação Brasileira de Nutrologia)

Considering that:

1. PCOS can be associated with several metabolic pathologies such as obesity, metabolic syndrome, and diabetes;

2. PCOS is due to multifactorial causes, such as lifestyle inappropriate, genetic tendencies and epigenetic factors;

3. PCOS is a clinical condition with specific diagnostic criteria.

4. Myo-inositol is a food supplement registered and approved by Anvisa and meets specific rules for proof of safety and efficacy;

5. The insulin resistance mechanism plays a crucial role in the pathophysiology of PCOS.

6. Myo-inositol acts as an intracellular second messenger for insulin and its levels are reduced in PCOS.

It is recommended:

1. That the diagnosis of PCOS is based on the Rotterdam criteria and the treatment follows two lines of action (the central route and the peripheral route), focusing on three objectives (restoration of fertility, controlling signs and symptoms, and improving the metabolic profile of patients).

2. That the prescription of myo-inositol be supported by clinical studies and evidence found in the literature;

3. That the prescription of myo-inositol be made in the clinical conditions presented (improvement in ovulation and pregnancy rates, improvement in pregnancy outcomes, control of gestational diabetes, improvement in laboratory and metabolic parameters, and improvement in indices related to in vitro fertilization processes), at a dose of 4g/day alone or associated with conventional therapies already established;

4. That the cost-benefit ratio for the choice of myo-inositol be evaluated, considering its excellent safety profile, low incidence of side effects, and low cost when compared to current therapies.

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Conflict of interest
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