Insulin Resistance and Polycystic Ovary Syndrome Through Life

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Abstract: The heterogeneity of Polycystic Ovary Syndrome (PCOS) emphasizes the need for a consensual review of the data concerning its diagnosis and treatment and for determination of the relationship between the development of PCOS and the ethnic origin, the social status and the lifespan. Insulin resistance is an important characteristic in women with PCOS that aggravates features of PCOS. This review is focused in the diagnosis and treatment of insulin resistance and the risk factors for PCOS during childhood, adolescence and postmenopause. The role of endocrine disruptors and/or their interaction with PCOS have also been analyzed.

Keywords: Polycystic ovary syndrome, insulin resistance, child, adolescence, menopause, treatments.

1. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the commonest endocrine and metabolic pathologies that affect women of reproductive age. The current consensus states that two of the following disorders are necessary for the diagnosis of PCOS, related features having being excluded: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, or 3) polycystic ovaries [1-5]. Hyperinsulinemia and its consequence, insulin resistance, aggravate the features of PCOS [6]. Synthetic chemicals such as pesticides, plasticizers and antimicrobials as well as components extensively involved in industrial and agricultural production are leading to widespread contamination of the environment [7]. These environment insults have been demonstrated as having a direct link with the development of PCOS [7]. Pursuance of a modern-day lifestyle characterized by obesity, stress, sedentary life as well as unhealthy diets (typically featuring high consumption of advanced glycation end products: AGEs) and certain modes of cooking are also linked with PCOS [8]. The main points discussed in the present revision work were the reproductive age for definition of the diagnosis of PCOS and the abnormalities present in the development of the syndrome such as insulin resistance and contamination of the environment.

2. TISSUES AND INSULIN RESISTANCE IN PCOS

Insulin resistance is an important feature in women with PCOS (Fig. **1**). With regards to the mechanisms involved in the development of insulin resistance, it has been reported that type 2 diabetes and obesity are not necessarily components of insulin sensitivity [9-12] by the contrary, a tissue-specific response was established [13] (Fig. **2**). It appears that each tissue contributes differently to the systemic state of insulin resistance by Akt phosphorylation or by other mechanisms not established yet [13]. It has been proposed that the low insulin sensitivity index in women with PCOS is a consequence of the higher trunk/peripheral fat ratio [14].

Insulin Resistance and Ovarian Tissue

Hyperandrogenism indirectly contributes to insulin resistance by regulating adipose cell functions [15]. The *"paradox of women with PCOS"* explains the fact that in spite of the systemic insulin resistance, the ovaries of women with PCOS remain sensitive to insulin action. Therefore, the blockade of the insulin receptor abolishes the stimulatory action of insulin, indicating that insulin stimulates human ovarian testosterone production by activating its own receptor [21, 22]. These data and the fact that the number of the insulin receptor is not altered suggest post-receptor defects in the insulin pathway [16- 22].

Insulin Resistance and Cardiovascular Diseases

Cardiovascular diseases, including cerebrum-vascular, coronary heart and vascular diseases, are today leading causes of death, though they appear later in women than in men [23]. The Third Report of the National Cholesterol Education Program in the Adult Treatment Panel III, 2002 [24] cited the ages of 55 years and 45 years as the risk-factor ages in women and men respectively. Mainly androgen levels, but also hormonal status account for the latter gender difference [25]. In agreement with these data, it has been revealed that women with PCOS show a higher cardiovascular risk than healthy women at the same age [26]. Other features of women with PCOS, such as dyslipidemia, low levels of high density lipoprotein (HDL)-cholesterol and increased levels of low density lipoprotein (LDL)-cholesterol and triglycerides (TG) have also been implicated in the increased cardiovascular risk of PCOS patients [27, 28]. Therefore, other non-classical markers of cardiovascular risk, such as C-reactive protein and homocysteine, and subclinical atherosclerosis (as carotid intima-media thickness, coronary artery calcium) are altered in PCOS patients [29]. Moreover, insulin resistance, hyperinsulinemia and abdominal obesity increase the cardiovascular risk [30]. Rizzo *et al*. [31] failed to detect a relationship between hyperandrogenism and increased cardiovascular risk; however, they found that women with the classical phenotype of PCOS display dyslipidemia, increased levels of C-reactive protein and values of carotid intima-media thickness as compared with women with dyslipidemia but without PCOS [31]. These findings led the authors to conclude that in PCOS patients, androgens have a limited role in cardiovascular risk whereas other features such as abdominal obesity and insulin resistance seem to play a central role in cardiovascular risk in these individuals. Recently, Bayram *et al* [32] reported a positive correlation between malondialdehyde production and free testosterone levels; and a negative correlation between paraoxonase 1 activity and free testosterone in serum sample of PCOS patients. These data led the authors to conclude that insu-

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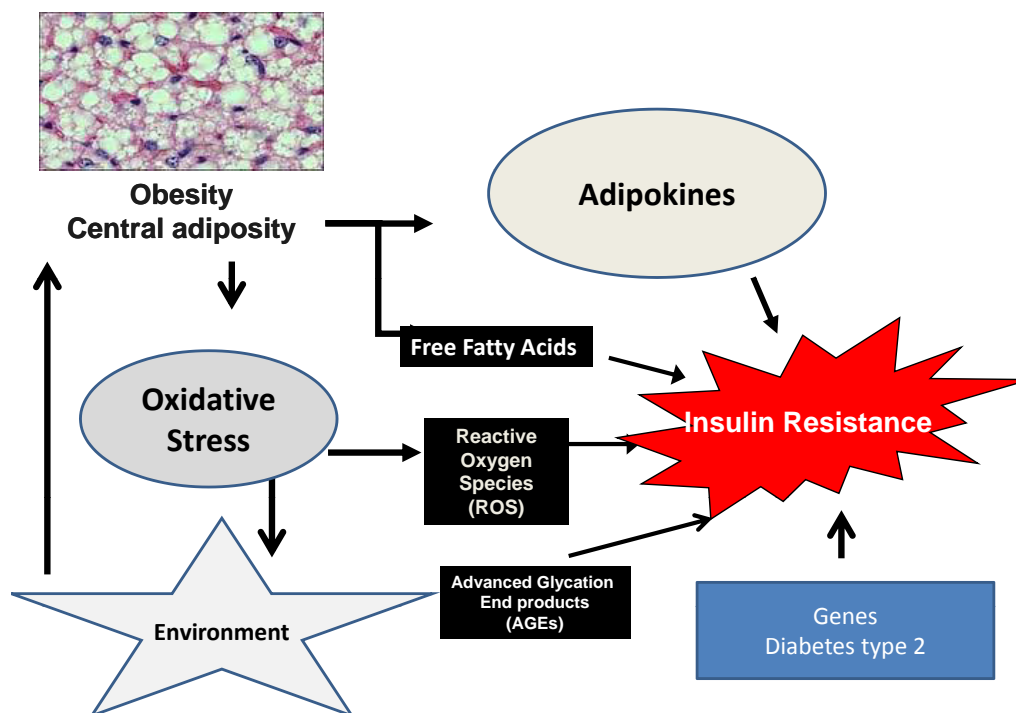E-mail: aliciabmotta@yahoo.com.ar

Fig. (1). Endogen and environmental factors involved in Insulin Resistance in PCOS. Pursuance of a modern-day lifestyle characterized by obesity, stress, sedentary life as well as unhealthy diets (typically featuring high consumption of advanced glycation end products: AGEs), certain modes of cooking and genes are linked with Insulin Resistance.
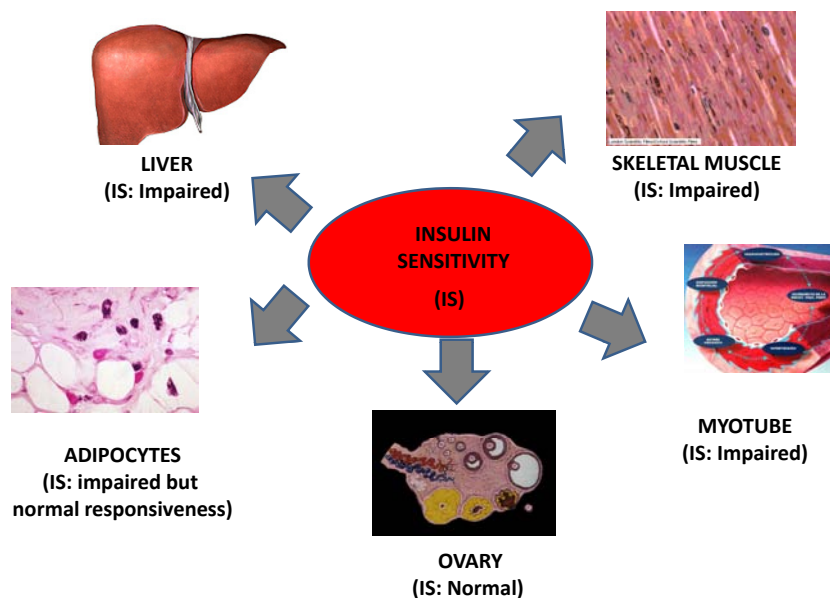

Fig. (2). Tissue-specific response of insulin resistance. Insulin sensitivity shows a tissue-specific response. The *paradox of ovary* is the fact that impaired insulin sensitivity in liver, adipocytes, muscle and myotube could be accompanied with normal insulin sensitivity in the ovary.

lin resistance, dyslipidemia, endothelial dysfunction, and oxidative stress might contribute to the high risk of cardiovascular disease show in PCOS patients.

3. PCOS THROUGH LIFE

Risk Factors for PCOS in Childhood and Adolescence

Precocious pubarche is defined as the appearance of pubic hair before the age of 8 years old in girls and before the age of 9 years old in boys [33]. Although the relationship between low weight at birth and precocious pubarche remains controversial, both features are associated with hyperinsulinemia, dyslipidemia of prepubertal onset, ovarian hyperandrogenism and ovulatory dysfunction during adolescence [34-39]. For these reasons precocious pubarche is considered an early marker of future pathologies, including PCOS, during adult life [40- 47]. These findings led to the hypothesis that by identifying children at risk of PCOS it is possible to prevent some of the long-term complications associated with the syndrome [48].

Sir- Petermann *et al.* [49] reported that serum levels of the anti-Mullerian hormone are increased in prepubertal daughters of women with PCOS, this pointing to an altered follicular development of these girls early during infancy and childhood. These findings were subsequently confirmed by the same group in peripubertal daughters of women with PCOS [50]. Prepubertal daughter of women with PCOS display increased ovarian volume, insulin, luteinizing hormone (LH), testosterone and 17-hydroxyprogesterone levels, thus regarding as good markers for endocrine and metabolic features [51].

It have to consider that, during the first two years post-menarche, it is common to observe physiological changes such as menstrual disorders, hyperandrogenism and ovarian cysts which could be wrongly associated with PCOS. Hickey *et al.* [52] conducted a prospective cohort study with 244 girls aged 15.2 years and found that clinical hyperandrogenism was uncommon (3.5% of girls) whereas menstrual irregularity (51% of the girls) and mean ovarian volumes were greater than those reported in adult women. Moreover, the 35% of these girls had polycystic ovary morphology by transabdominal ultrasound. Interestingly, the authors found that taking the upper 5% of free testosterone as hyperandrogenism, 18.5% fit the Rotterdam criteria for PCOS, 5% the Androgen Excess Society criteria and 3.1% the National Institutes of Health criteria. These findings led to conclude that menstrual irregularities, common during adolescence, are not related to clinical or biochemical hyperandrogenism and that the diagnostic criteria for PCOS, as well as ovarian volume and morphology, may be of limited use in adolescence. In agreement with these findings, polycystic ovarian morphology during adolescence has been found to be related to increased levels of anti-Mullerian hormone [53]. In order to define the age for diagnosis of PCOS in girls, Bronstein *et al.* [54], conducted a retrospective study and systematic review including 28 studies. The authors demonstrated that, based on the Rotterdam criteria, 26% preadolescents (aged <13 years) and 74% adolescents (aged between 13 to 18 years), were diagnosed with PCOS. No significant differences were found between the two groups on risk factors for PCOS, such as ethnicity, body mass index (BMI), family history of maternal PCOS, birth weight, hyperandrogenism or insulin resistance. The preadolescents with PCOS had an earlier onset of pubarche and were taller for their age than adolescents. Menstrual irregularities, ovarian morphology and clinical hyperandrogenism should not be accurate markers for diagnosing PCOS during adolescence. Even more important, however, is prevention of obesity, given that it aggravates insulin resistance and induces development of PCOS.

PCOS in Post-menopausal Women

Although cardiovascular diseases appear later in women than in men, they are a leading cause of death among postmenopausal women [55]. A cross-sectional study of 1423 healthy women aged 18-75 years demonstrated that the physiology of androgen production decreases with age [56]. This decrease is greater in the earlier than in the later decades of reproductive ages [56]. These findings and the fact that the levels of androgen are not modified by natural menopause support the hypothesis that the postmenopausal ovary is a site of ongoing site of testosterone production [56]. Considering that ovarian volume of peri-menopausal women is smaller than those of younger women [57], it appears that in postmenopausal women other features than androgens are responsible for the increased cardiovascular risk. It has been reported that the risk of central obesity may increase almost five times for postmenopausal women [58]. In this context, Maturana and Spritzer [59] found that postmenopausal women with androgen levels in the high median values show greater BMI, hyperinsulinemia, waist circumference, TG and 2-hour post-glucose insulin levels than women with lower testosterone values. Moreover, a positive association was found between androgens and insulin to glucose ratio independently of BMI, age or time since menopause.

The relationship between cardiovascular risk and PCOS during menopause was studied by Krentz *et al.* [60] in a cross-sectional study of 713 postmenopausal white women. They found that 9.3 % of these women showed different phenotypes of PCOS and the cardiovascular risk concomitantly increases with features of PCOS. In fact, endogenous testosterone levels are part of a proatherogenic profile in early postmenopausal women (61) and, independently of BMI, women with PCOS have a two-fold higher risk of arterial disease as compared to women without PCOS [62]. In agreement with these data, Maturana *et al.* [63] reported that in postmenopausal women, the lipid accumulation index, androgen and sexual hormone binding globulin (SHBG) are positively related to cardiovascular risk. They also showed that in postmenopause the polymorphism rs9939609 in the fat mass and obesity-associated (FTO) gene is related to abnormal glucose levels and to the lipid accumulation index and cardiovascular risk [63].

4. ENDOCRINE DISRUPTORS AND PCOS

Advanced glycation end products (AGEs), emerging as factors triggering PCOS are reactive derivatives of non-enzymatic glucose-protein reactions. The interaction between environmental factors and the development of PCOS led researchers to study the role of such environmental insults as certain dietary products and endocrine disruptors. AGEs are endogenously produced by aging, hyperglycemia and oxidative stress and exogenously created by thermally processed foods rich in proteins. These data suggest that it is necessary to be careful with the use of recently promoted high-protein diets [64]. High-protein diets are nephrotoxic because of their excessive dietary AGE content and their enhanced amino acid load that also increases the production of AGEs [64]. Moreover, it has been demonstrated that AGEs contribute to the elevated risk for diabetes and cardiovascular disease in women with PCOS [64]. Therefore, insulin-resistant women with PCOS have increased both levels of serum AGEs and their respective receptor (RAGE) localized in theca and granulosa cells [64]. In addition, women with classic PCOS have higher serum AEGs levels than ovulatory women with PCOS, thus clearing indicating that AEGs levels increase with the phenotypical aggravation of PCOS [65]. AEGs and the anti-Mullerian hormone interact in the anovulatory mechanism of women with PCOS [66]. In addition, AGEs are related to the number of oocytes and pregnancy rates [67]. With regard to the treatment, Diamanti-Kandarakis *et al.* [8] demonstrated that the lipase inhibitor, Orlistat has a beneficial effect in the absorption of food glycotoxins.

Endocrine disruptors are synthetic chemicals (among others, pesticides, plasticizers, antimicrobials and flame retardants) that disrupt the hormonal balance and result in developmental and reproductive abnormalities [7]. Neonatal exposure of rats to reusable plastic containers made with bisphenol A (BPA) induces an endocrine and metabolic disorder which features resembling those of PCOS [68]. BPA decreases fertility rate by altering serum testosterone, estradiol and progesterone levels [68]. Therefore, BPA in combination with insulin accelerates the *in vitro* conversion of fibroblasts to adipocytes [69]. Recently, Diamanti-Kandarakis *et al.* [70] reported higher levels of BPA and a positive association between androgens and BPA in women with PCOS as compared to controls. These data support the hypothesis that BPA has a potential role in the development of PCOS with a direct effect on ovarian theca cells that alters enzymes involved in steroidogenesis [71].

5. TREATMENTS OF PCOS

Diet and Physical Activity

It has been accepted that obesity - one of the risk factors for reproductive failure- needs to be treated prior to initiation of the treatment for PCOS. Obesity is associated with anovulation [4, 72], pregnancy loss [73] and late pregnancy complications [74]. In PCOS patients, obesity is linked to the failure or delay to respond to

treatments [75- 79]. Weight loss is associated with improved spontaneous ovulation rates and spontaneous pregnancy [72, 80, 81] and increases live birth rate in obese women with and without PCOS [80]. For these reasons, the first line for PCOS treatments targets central obesity and insulin resistance [4, 82] and markers of central adiposity and insulin resistance may be potential surrogate markers of specific intervention in PCOS. One of these predictors of central obesity is the waist circumference, which is a better measure than the BMI [83]. Wiltgen *et al.* [84] proposed the lipid accumulation product index (LAP=[waist (cm) - 58] x triglyceride (mmol/L) as a reliable marker of cardiovascular risk in PCOS patients. In addition, a direct correlation between serum protein rations and insulin resistance has been recently reported in PCOS patients [85].

Moreover, it has been reported that waist circumference, TG and homeostasis model assessment values (HOMA) are higher in classic PCOS than in the other PCOS phenotypes even after adjustment for BMI [3, 86].

It seems that obesity, and not diet is related to higher cardiovascular risk. Carmina *et al.* [87] compared the cardiovascular risk between women with PCOS who live in Pennsylvania (USA) with women with PCOS living in Sicilia (Italy). Although the total daily caloric intake of both groups was similar, the women from Pennsylvania had a higher BMI and a worse metabolic profile (insulin resistance, higher TG and lower HDL-cholesterol) than the women from Sicily. These findings led the authors to conclude that di*et al*one does not account for the differences in BMI, and that it appears that genetic and lifestyle factors appear likely contribute. They also found that increased saturated fat intake (as that consumed by patients from Pennsylvania) may worsen the cardiovascular risk profile.

Insulin resistance is not strictly associated with energy intake or diet in PCOS patients. Toscani *et al.* [88] found that although a group of PCOS patients and healthy ovulatory controls had similar intake of energy and micronutrients, the total body fat, the waist circumference, androgens, HOMA and lipids were higher in PCOS patients than in controls. The authors also demonstrated that calorie reduction rather than protein content seems to affect body composition and hormonal profile [88]. In agreement with these findings, Moran *et al.* [89] reported that lifestyle intervention improves body composition, hyperandrogenism and insulin resistance in women with PCOS.

Hypocaloric diet (500 to 1000 kcal/day) minimizes cardiovascular and metabolic risks and reduces body weight by 7 to 10 % in 6 to 12 months [90]. The dietary pattern should be < 30 % of calories from fat, < 10% calories from saturated fat and increased consumption of fiber, whole-grain breads and cereals and fruits and vegetables [90]. Physical activity plus diet offers additional benefits to dietary weight loss for the improvement of reproductive and metabolic features of PCOS. It has been widely reported that exercise enhances the quality of life, and its role in anxiety and depression is increasingly recognized. Hutchison *et al.* [90] described an etiologic role for visceral fat on insulin resistance in PCOS and reported that changes in exercise intervention do not support a causal relationship. They also found that TG are strongly modulated by exercise training in PCOS than in non-PCOS women [91]. These findings suggest that weight loss should not be the sole focus of exercise [91]. After reviewing five database and three cohort studies, Harrison *et al.* [91] reported that 12 to 24 weeks of moderate exercise improves ovulation and reduces insulin resistance and weight, independently of the type, frequency or length of the exercise sessions [92].

Lifestyle changes also improve the results of pharmacological treatments. Palomba *et al.* [93] reported that six weeks of structured exercise training and hypocaloric diet increases the probability of ovulation after clomiphene citrate treatment in overweight and obese women with PCOS.

Clomiphene Citrate-treatment

The first choice to induce ovulation is the clomiphene citrate (CC) treatment, with a dose of 50-100 mg CC per day for 5 days starting from day 2-5 of spontaneous or induced bleeding. If this treatment does not result effective, the dose has to be increased 50 mg per day each cycle until an ovulatory cycle is achieved. It has been reported successful ovulation in the 70-85% of treated women, resulting in a pregnancy rate of 40-50% [94, 95]; however, some patients who were able to ovulate after CC treatment suffer early miscarriage as a consequence of a failure in the implantation process [96]. If the PCOS patients do not response after three consecutive treatment cycles, they are considered as "clomiphene-resistant". In this case, the treatment with insulin-sensitizing drugs is recommended.

Insulin-sensitizing Agents

The fact that insulin resistance aggravates the pathophysiology of PCOS led to the use of insulin-sensitizing drugs in the treatment of PCOS. In this context, it has been reported that the N,N´dimethylbiguanide: metformin restores the sexual cycles and protects early pregnancy [96- 99]. In addition, it is well known the property of metformin as scavenger of reactive oxygen species [100-106]; however, this drug is clinically used without a complete understanding of its mechanism of action.

By activating the AMP-protein kinase pathway (AMPK) [107, 108], metformin increases oxidation of fatty acids in hepatocytes [107], bovine aortic cells [109], skeletal muscle cells [109] and ovarian tissue [104] and increases the uptake of glucose by tissues [110]. The relationship between metformin and AMP causes the drug to indirectly to be involved in changes in the cellular energy charge.

Recently, Crisosto *et al.* [111] evaluated ovarian function during early infancy in daughters of women with PCOS treated with metformin during pregnancy as a means to reduce androgen and insulin levels. The authors found that the improvement of the altered endocrine-metabolic environment of mothers with PCOS resulted in reduced levels of anti-Mullerian levels in their daughters, which reflects a decrease in their follicular mass. In summary, metformin is able to restore ovulation but also to modulate the cellular energy charge, to ameliorate cardiovascular diseases, to act as an anti-oxidant metabolite, to modulate gestational diabetes and, when administrated during pregnancy, to improve metabolic and endocrine parameters in daughters of women with PCOS.

Gonadotropin Treatment

Gonadotropin therapy is based on the physiological concept that initiation and maintenance of follicle growth is achieved by a transient increase in FSH above a threshold dose to generate a limited number of developing follicles, then, the risk to use gonadotropins to induce ovulation in women with PCOS is the multiple follicle development [111, 112]. The recommended dose is 37.5-50.0 IU gonadotropin/day and the treatment should not exceed six ovulatory cycles and intense ovarian response monitoring is required to reduce complications [113-118].

Statins

Statins modulate plasma cholesterol levels. In a prospective, crossover trial of 48 women with PCOS, it has been recently reported that simvastatin improves endocrine/clinical aspects of PCOS by regulating the lipid profile [119]. Simvastatin modulates testosterone levels by inhibiting the mevalonate pathway and by improving the hypothalamo-pituitary function [119]. Statins had a lower impact on atherogenic lipoprotein phenotype showing a moderate beneficial effect [120] and reduce the concentration of total testosterone, total cholesterol (TC), TG and LDL [121, 122]. Considering that simvastatin modulates endometrial proliferation [123],

the treatment with statins would be the additional benefit in the uterine tissue of PCOS patients (Table **1**).

Table 1.   Summarizing Treatment Options for PCOS

Treatments of PCOS	References
Diet and physical activity	[72, 80-93]
Clomiphene citrate	[94-96]
Insulin-sensitizing agents	[96-111]
Gonadotropin treatment	[112-118]
Statins	[119-123]

6. CONCLUSIONS

Data presented here indicate that insulin resistance is a frequent and important characteristic of women with PCOS since it aggravates the features of the syndrome. Hyperandrogenism indirectly contributes to insulin resistance by regulating adipose cell functions. In lean women with PCOS insulin resistance is due to the higher trunk/peripheral fat ratio. The "*paradox of women with PCOS*" is the fact that ovaries of women with PCOS are sensitive to insulin action. Obesity should be prevented because, at all ages, it aggravates insulin resistance.

There is agreement to the need of early diagnosis of PCOS and the importance to define premature markers. Precocious pubarche and low weight at birth (both associated with adverse in-uterus environment) are linked to hyperinsulinemia, dyslipidemia of pre-pubertal onset, ovarian hyperandrogenism and ovulatory dysfunction during adolescence. Menstrual irregularities, ovarian morphology and clinical hyperandrogenism, when considered as isolated signals, are not good markers to diagnose PCOS during adolescence. These parameters could be altered because of the immaturity of the ovarian function.

In postmenopausal women with PCOS, central obesity, rather than hyperandrogenism, is highly associated with cardiovascular risk.

High-protein diets are nephrotoxic because of their excessive content of Advanced glycation end products (AGEs). AGEs contribute to the increased risk for diabetes and cardiovascular diseases in PCOS patients and insulin-resistant women with PCOS have increased AGEs as compared with ovulatory women with PCOS. The neonatal exposure of bisphenol A (BPA), a component of some plastic containers, induces endocrine and metabolic disorders resembling PCOS features.

With regard to PCOS treatments, it depends on age and desire of patients; however, weight loss is associated with improved spontaneous ovulation rates in women with PCOS. Lifestyle modifications, including dietary and exercise interventions, improve metabolic features of women with PCOS with or without any other treatment. Clomiphene citrate (CC) seems to be the first line of treatment to induce ovulation. Metformin indirectly induces ovulation by reducing the concentration of circulating insulin. The treatment with metformin of pregnant women with PCOS seems to improve the ovarian function of their daughters. The significantly higher hyperstimulation rate, the associated risk of multiple pregnancies and the cost do not currently justify the routine use of gonadotropins during ovulation induction in women with PCOS. Beneficial effects produced by statins over and above the reduction in plasma cholesterol levels, led to propose the use of statins in the treatment with PCOS.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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