

Novel strategies in the management of polycystic ovary syndrome

P. M. SPRITZER¹, A. B. MOTTA², T. SIR-PETERMANN³, E. DIAMANTI-KANDARAKIS⁴

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting reproductive-aged women. PCOS has been recognized as a syndrome combining reproductive and metabolic abnormalities with lifelong health implications. Cardiometabolic alterations require regular screening and effective and targeted lifestyle advice to lose weight as well as to prevent weight gain. Pharmacological therapy includes insulin-sensitizer drugs and agents that act directly on metabolic comorbidities, such as statins and antiobesity drugs. Bariatric surgery may be an option for severely obese women with PCOS. Regarding reproductive aspects, ovulation induction with antiestrogens such as clomiphene citrate or letrozole is the first-line medical treatment. Exogenous gonadotropins and *in vitro* fertilization (IVF) are recommended as second-line treatment for anovulatory infertility. Laparoscopic ovarian diathermy may be used in special cases and metformin is no longer recommended for ovulation induction. Combined oral contraceptives (OCs) are the first-line treatment for the management of menstrual irregularities in women not seeking pregnancy, also providing endometrial protection and contraception. Progestin-only pills or cyclical progestins are recommended for those with contraindications to OCs. Metformin is also considered a second-line choice for improving menstrual cycles in women presenting insulin-resistance and dysglycemia. Hirsutism requires cosmetic

¹Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre and Department of Physiology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

²Laboratory of Ovarian Physiopathology, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina

³Endocrinology and Metabolism Laboratory, West Division, School of Medicine, University of Chile, Santiago, Chile

⁴Third Department of Medicine, Endocrine Unit, Medical School, University of Athens, Sotiria General Hospital, Athens, Greece

procedures and medical treatment with OCs. More severe cases may need anti-androgen drugs added to the OCs. In conclusion, strategies regarding the management of reproductive issues in PCOS encompass a tailored approach to individual needs of each patient.

KEY WORDS: Insulin resistance - Menstrual cycle - Hyperandrogenism - Ovulation induction - Contraceptives, oral, combined.

The polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 6.8% of reproductive-aged women.¹ PCOS has been recognized as a syndrome combining reproductive and metabolic abnormalities with lifelong health implications. Anovulation and androgen excess are the hallmark clinical features of the syndrome, while insulin resistance is a significant con-

Corresponding author: E. Diamanti-Kandarakis, Third Department of Medicine, Endocrine Unit, Medical School, University of Athens, Sotiria General Hospital, 1A Zefyrou Ekali, Athens 145 78, Greece.
E-mail: e.diamanti.kandarakis@gmail.com

tributor to the pathogenesis of PCOS. Insulin resistance participates in the reproductive as well as metabolic abnormalities associated with PCOS.² Increasingly appreciated are the metabolic and cardiovascular sequelae of the syndrome. The expanding knowledge in the pathophysiology and clinical spectrum of PCOS has modified therapeutic management of affected women. The research focus has been placed in the discovery of novel strategies for the global, multilevel therapeutic management of this syndrome. A pathophysiologically rationalized therapeutic approach should take into account the fact that reproductive and cardiometabolic abnormalities coexist and interact with each other in the context of PCOS.

Pathophysiology of PCOS

The pathophysiology of PCOS is complex involving multiple components of the reproductive and metabolic functions. The major pathophysiologic mechanisms of PCOS include androgen overproduction due to intrinsic theca cell defects, impaired function of the GnRH axis and insulin resistance.

The ovary is located at the pathophysiologic core of PCOS. The ovarian disarray involves various processes inside and outside the ovary. Theca cells propagated from women with PCOS display an intrinsic steroidogenic defect which leads to a constitutive increase of androgen production.

In turn, ovarian androgen excess can exert local/ paracrine as well as neuroendocrine effects on the hypothalamic-pituitary-ovarian (HPO) axis. In the ovary, androgens modulate gonadotropin-induced steroidogenesis in granulosa cells and folliculogenesis.³ Intraovarian androgen excess could impair folliculogenesis in a dual fashion, by stimulating the growth of small follicles and by hindering follicular maturation towards the dominant stage.³

Available literature suggests that androgens contribute to the control of follicular fate, acting in a developmental stage-spe-

cific manner and in co-operation with the intrafollicular hormonal microenvironment.

Additionally, androgens may disturb the normal feedback control of the hypothalamic GnRH pulse generator.⁴ In humans sustained androgen exposure during critical windows of human neuroendocrine maturation may dysregulate the HPO feedback loop. However, in a proportion of individuals, progesterone feedback is preserved despite the presence of androgen excess and the mechanisms of this varied susceptibility may partly reflect genetic differences.

In particular, PCOS is associated with an increased frequency of pulsatile GnRH release, which results in a selectively increased frequency of pulsatile LH secretion while simultaneously suppressing FSH release.⁴ LH stimulates increased androgen production by the theca cells of the ovarian follicle.⁵ Because of the relative deficiency of FSH, follicular growth is arrested resulting in a lack of granulosa cell maturation.³

Overall, it seems that the central neuroendocrine alterations associated with PCOS are not primary but rather secondary to the events that take place within the ovary.⁵ Androgen excess contributes to reproductive aberrations including impaired function of the ovary and the HPO axis.^{3, 5} In fact recent studies in experimental androgenized animals have provided evidence that androgens interfere with the intraovarian antioxidative system by decreasing the activity of glyoxalase -1, leading to accumulation of detrimental oxidative molecules like AGEs.⁶

Another major pathophysiologic component of PCOS is insulin resistance accompanied with hyperinsulinemia. These interdependent factors contribute to the ovarian disorder as well as the metabolic derangement in PCOS. The resultant metabolic phenotype displays a variable clustering of features typical of the metabolic syndrome.⁷ Obesity, a common characteristic of PCOS women, aggravates insulin resistance and its reproductive and metabolic sequelae.⁸

Insulin resistance in PCOS is tissue specific and pathway selective involving various molecules of insulin signaling. The PCOS ovary remains sensitive to the ster-

oidogenic and mitogenic actions of insulin, despite metabolic insulin resistance.⁹ Insulin plays a central part in ovarian function, since insulin receptors are present in both ovarian theca and granulosa cells. By binding to its own receptor, insulin retains its classic metabolic effects but also modulates specific pathways of steroidogenesis and folliculogenesis in ovarian cells. Thereby, both theca and granulosa cells are capable of responding to prevailing hyperinsulinemia, which overdrives steroidogenic and mitogenic pathways.⁹ Hyperinsulinemia acts as a co-gonadotropin to increase LH-induced androgen synthesis in theca cells. Additionally, insulin excess in PCOS may trigger premature LH receptor expression leading prematurely to terminal differentiation of granulosa cells in small follicles. The latter phenomenon called premature luteinization and leads to the arrest of follicular growth and linked to failure of ovulation.^{10, 11} The signaling events that mediate intraovarian insulin action, as well as the pathophysiologic importance of insulin in ovarian function and dysfunction are currently actively investigated. Moreover, inflammation and oxidative stress may play an intriguing in the pathophysiology of PCOS,¹². Dietary triggers such as glucose and glycotoxins in PCOS rat models are capable of modifying the expression of scavenger receptors and are reinducing oxidative stress and inflammatory responses independent of obesity.¹³ The inflammatory

response may aggravate insulin resistance and pro-inflammatory stimuli may upregulate androgen production in ovarian theca¹². Furthermore, accumulating data suggest that a class of oxidative molecules, known as advanced glycated end products (AGEs), may be increased in PCOS women, independently of insulin resistance and obesity.^{14, 15} AGEs are produced endogenously as well as consumed by diet and may affect various processes of reproduction, metabolism and cardiovascular function.^{6, 16, 17}

Overall, insulin resistance and androgen excess appear to be the major pathophysiologic mechanisms in PCOS. Oxidative stress, in the form of AGEs, may act independently and participate in the pathophysiologic pathways of PCOS. It remains unclear which one of the above mechanisms precedes the other and how they interact with each other in the developmental course of PCOS.

Cardiometabolic aspects

It is well known that PCOS is a general health pathology involved not only in infertility and menstrual alterations but also in metabolic disturbances. Among these metabolic disturbances, cardiometabolic aspects, including higher risk of insulin resistance, hyperinsulinemia, impaired glucose tolerance, dyslipidemia, hypertension, subclinical atherosclerosis, endothelial dysfunctions and other risk factors for cardiovascular dis-

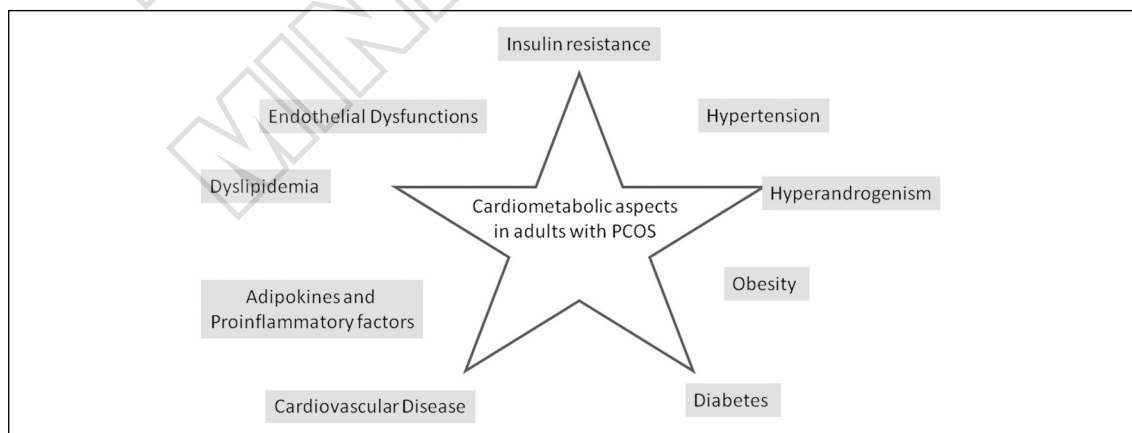


Figure 1.—Strategies to be considered in adult patients with PCOS

ease (CVD), are highly prevalent in women with PCOS.¹⁸ In addition, PCOS patients show increased prevalence of sleep apnea, altered secretion of adipokines and adipose tissue-derived proinflammatory factors, which influence the metabolism, insulin sensitivity and energy homeostasis.^{19, 20} Thus, the management of cardiometabolic alterations is an important issue in women with PCOS and comprises metabolic and cardiovascular risk aspects (Figure 1).

Lifestyle changes

Although the recommended first line of treatment of PCOS is lifestyle management (physical exercise and an appropriate diet), the optimal dietary composition remains unknown. Regarding anthropometric, reproductive, metabolic and psychological outcomes in women with PCOS, it has been found that a monounsaturated fat-enriched diet is more effective in weight loss and that low-glycemic diet reverses menstrual irregularities, improves insulin sensitivity and quality of life, and decreases total fibrinogen and HDL-cholesterol.²¹ In addition, it has been reported that a high-carbohydrate diet increases the free androgen index, that a high-protein diet improves depression and self-esteem, and that a low-carbohydrate diet decreases insulin resistance, total fibrinogen levels and HDL-cholesterol.²¹

In the treatment of PCOS patients, it is important to consider the role of vitamins as supplements. In fact, in PCOS patients vitamin D deficiency is related to insulin resistance and obesity²² and the regulation of vitamin D receptor is associated with glucose and lipid metabolism and blood pressure regulation.²³ In addition, it has been established that administration of vitamin B12 is associated with increased insulin sensitivity, and decreased obesity and homocystein levels.

Finally, both the diet and the cooking methods (precooked fast-food meals heated at high temperatures) dramatically increase AGEs, thus, increasing the atherosclerosis risk in women with PCOS and/or metabolic syndrome.¹⁴ These findings

together with the fact that serum AGEs levels and testosterone and anti-mullerian hormone levels are positively associated in PCOS women^{15, 24} allow to suggest that low AGEs dietary content changes during lifestyle changes in women with PCOS.²⁵

Endothelial dysfunction markers are surrogate markers of CVD risk, which is high in PCOS patients. Physical exercise improves endothelial function in PCOS patients thus reducing CVD risk factors.²⁶ It has been reported that after a 16-week exercise program, brachial artery endothelial function is improved, independently of changes in body weight or composition,²⁷ and that dietary management and exercise, either alone or in combination, modulate serum anti-mullerian hormone and improve insulin sensitivity in overweight/obese women with PCOS.²⁸ Lifestyle intervention restores menstrual cycles, prevents androgen excess and CVD and improves intima media thickness.²⁹

Pharmacological treatments

COMBINED ORAL CONTRACEPTIVES

Combined oral contraceptives (OCs) have been the main therapy for PCOS patients. However, the effects of OCs on cardiometabolic aspects are controversial. When comparing with other therapies, such as insulin sensitizers and insulin-lowering agents, OCs are more effective in improving menstrual cycles and reducing serum androgen levels.^{19, 30} However, OCs containing antiandrogenic progestins increase the risk of cardiovascular and thromboembolic events. In addition, OCs have limited effect on carbohydrate metabolism and the dyslipidemic effect has been linked to the progestogen component of OCs. The effects of OCs on lipid alterations are also controversial. Some reports have indicated that OCs increase LDL-cholesterol and total cholesterol and decrease HDL-cholesterol,³¹ whereas others have shown that OCs increase³² or have no effect on triglycerides. As a way to neutralize some of the negative effects of OCs, some authors have proposed the combina-

tion with lifestyle modifications.³³ In summary, as OCs may have a negative effect on metabolic aspects of women with PCOS, their administration should be based on the PCOS phenotype, and the metabolic state and clinical history of the patient.

INSULIN SENSITIZERS

There is a general consensus that insulin resistance, in particular hyperinsulinemia, leads to hyperandrogenemia and is associated with risk for CVD. Then, insulin sensitizers, mainly metformin and thiazolidinediones (TZDs) ameliorate the metabolic aberrations of PCOS patients.

Although the way in which metformin prevents these aberrations is not yet established, it is known that the molecular mechanism is complex and tissue-specific.³⁴⁻³⁶ Metformin suppresses gluconeogenesis and hepatic glucose output in the liver, enhances peripheral insulin action in skeletal muscle and reduces glucose absorption from the digestive tract, but has a controversial effect on adipose tissue lipolysis.³⁷ Metformin has additional benefits on weight loss in the combined treatment with lifestyle changes (*via* diet or exercise). This combined treatment also results in a significantly greater decrease in hyperandrogenism and insulin resistance. Metformin also ameliorates cardiometabolic parameters by enhancing insulin sensitivity and lowering blood glucose and androgen levels³⁸ and contributes to fewer atherothrombotic events by reducing the circulating levels of plasminogen activator inhibitor-1 (PAI-1).³⁸ Several studies support that metformin has a beneficial effect on lipid profile but no effect on normolipidemic patients.³⁹ In fact, it has been reported that metformin is least effective in women who have a Body Mass Index >35 kg/m².

Regarding insulin-sensitizing drugs versus combined OCs in PCOS patients, it has been reported that metformin is more effective than OCs in reducing fasting insulin and triglycerides⁴⁰ and AGE products,³⁰ but there is insufficient evidence on their effects on fasting glucose and cholesterol levels.⁴⁰

Metformin also interacts with the peroxisome proliferator-activated receptor gamma (PPAR γ) pathway, which binds TZDs.³⁴ In summary, metformin has multiple favorable actions on cardiometabolic aberrations of PCOS patients and together with lifestyle changes, it appears to be a good option of treatment.

TZDs (pioglitazone and rosiglitazone) are synthetic ligands of PPAR γ . The activation of PPAR γ by TZDs increases insulin sensitivity and stimulates differentiation of adipose cells, increases HDL-cholesterol levels, reduces triglycerides, improves hepatic insulin signaling and reduces overproduction of hepatic lipoprotein. However, rosiglitazone treatment has been suspended due to its association with increased cardiovascular mortality. On the other hand, as ligands of PPAR α , fibrates have a major impact on triglyceride metabolism and on atherogenic dyslipidemia by increasing HDL-cholesterol and decreasing LDL-cholesterol.

ANTI-OBESITY AGENTS

Orlistat, a pancreatic lipase inhibitor, reduces the absorption of dietary fat, body weight, insulin resistance and total testosterone levels in PCOS patients and a long-term orlistat treatment reduces increased AGE levels independently of the BMI⁴¹. It has been recently reported that orlistat is as effective as metformin in improving the lipid profile and pregnancy rates in obese PCOS patients. However, orlistat has minimal side-effects and is better tolerated than metformin. On the other hand, a recent study has shown that metformin and sibutramine, but not orlistat, reduce PAI-1 levels, which are increased in insulin-resistant and obese women with PCOS.⁴² In combination with lifestyle changes, orlistat induces substantial weight loss in women with PCOS, resulting in improvements in insulin sensitivity, hyperandrogenemia and cardiovascular risk factors.⁴³

Sibutramine, a selective serotonin and adrenergic reuptake inhibitor, also has a positive effect on metabolic abnormalities in obese PCOS patients.⁴⁴ In obese women

with PCOS, sibutramine reduces the waist-hip ratio, serum triglyceride levels⁸ and PAI-1 levels.⁴² However, as sibutramine can cause a rise in blood pressure and can increase cardiovascular risk, it has been withdrawn from the market in both the United States and Europe.

STATINS

Atorvastatin and simvastatin improve the cardiometabolic aspects, including lipid metabolism of PCOS patients. However, the effect of simvastatin remains controversial. Some authors have reported that simvastatin has a lower impact on the atherogenic lipoprotein phenotype with a moderate beneficial effect⁴⁵ whereas others have reported a reduction in total cholesterol, triglycerides and LDL-cholesterol. In a multicenter, open-labeled, randomized trial, Park *et al.* (2010) found that rosuvastatin was more effective than atorvastatin on lipid and glycemic profile in patients with metabolic syndrome.

BAIATRIC SURGERY

In the recent consensus statement of the Androgen Excess and Polycystic Ovary Society,⁴⁶ it has been recommended that bariatric surgery should be performed only after standard weight loss strategies have failed in PCOS patients with a BMI greater than 40 kg/m² or greater than 35 kg/m² with a high-risk obesity-related condition according to the 1992 NIH Consensus Development Conference Statement (1992).

In conclusion, based on all the above-described data and considering the long-term health, cardiometabolic alterations present in PCOS patients require more regular screening for such risks as well as effective and targeted lifestyle advice to prevent weight gain (Table I). The diagnosis and subsequent management of women with PCOS are continuously in discussion. Although most PCOS patients are overweight, there is a significant number of lean women with PCOS who have cardiometabolic abnormalities. The most common pharmaceutical products deal with obesity, hyperten-

sion, dyslipidemia, diabetes, inflammation and atherogenic risks. However, further research should focus on these issues in patients with PCOS.

Reproductive aspects

The management of reproductive aspects in women with PCOS should focus on the treatment of infertility in women seeking pregnancy or the improvement of menstrual abnormalities and clinical hyperandrogenism as well as endometrial protection in women not seeking pregnancy.

Management of infertility

Infertility in women with PCOS is associated not only to dysovulation but also to low oocyte quality and disturbed endometrial receptivity. In addition, these reproductive disturbances are worsened in the presence of obesity and/or insulin resistance.¹⁸ Therefore, the first non-pharmacological approach for overweight or obese women with PCOS is lifestyle changes and weight loss. Modest decreases in body weight have been associated with improvement in ovulation and pregnancy rates as well as on requirements for ovulation induction drugs.¹⁸

CLOMIPHENE CITRATE

The first-line pharmacological treatment for infertility secondary to anovulation in PCOS is ovulation induction. Classically, clomiphene citrate (CC) is the most frequent choice because of their known effects on hypothalamus, competing with estradiol by the estrogen receptor and blocking its negative feedback on the gonadotropin secretion. In consequence to higher gonadotropin levels, ovaries are stimulated leading to follicular development (Figure 2). Effectiveness of CC is around 23% of live birth rate.^{47, 48} In turn, the risk for ovarian hyperstimulation syndrome (OHSS) is rare and the risk for multiple gestations is around 6%, being almost limited to twin pregnancies.⁴⁷

TABLE I.—*Management of cardiometabolic aspects of adult patients with PCOS.*

Treatment	Positive and negative effects on cardiometabolic aspects
Lifestyle changes (Physical exercise and an appropriate diet)	<ul style="list-style-type: none"> – Induce weight loss – Improve insulin sensitivity – Decrease total fibrinogen – Regularize blood pressure – Improve endothelial functions – Restore menstrual cycle
Combined oral contraceptives (OCs)	<ul style="list-style-type: none"> -Controversial evidence – OCs are more effective in reducing serum androgen levels than affecting cardiometabolic aspects – Some OCs may increase thromboembolic events – Flutamide improves lipid profiles and adipokine levels, decreases visceral fat content and improves insulin sensitivity. Presents a dose-dependent hepatic toxicity
Insulin sensitizers	<p>Metformin</p> <ul style="list-style-type: none"> – Enhances peripheral insulin action and reduces glucose absorption from the digestive tract – In combination with lifestyle changes results in a significantly greater decrease in insulin resistance and hyperandrogenism – Decreases plasminogen activator inhibitor-1, thus reducing atherothrombotic events – Improves lipid profile in dyslipidemic patients <p>Thiazolidinediones</p> <ul style="list-style-type: none"> – Increase insulin sensitivity and stimulate differentiation of adipose cells, increase HDL-cholesterol, reduce triglycerides, improve hepatic insulin signaling and reduce overproduction of hepatic lipoprotein. May promote weight gain – Rosiglitazone treatment has been suspended due to its association with increased cardiovascular mortality. <p>Fibrates</p> <ul style="list-style-type: none"> – Improves triglyceride metabolism (decreases triglycerides, LDL- and total -cholesterol and increases HDL-cholesterol)
Antiobesity agents	<p>Orlistat</p> <ul style="list-style-type: none"> – Reduces the absorption of fat, body weight, insulin resistance and total testosterone – A long-term orlistat treatment reduces advanced glycated end products – Improves lipid profile – In combination with lifestyle changes, orlistat induces substantial weight loss reducing cardiovascular risk <p>Sibutramine</p> <ul style="list-style-type: none"> – Reduces plasminogen activator inhibitor-1 – Reduces the waist-hip ratio, triglyceride levels and plasminogen activator inhibitor-1 – As adverse effects: sibutramine raises blood pressure and cardiovascular risk thus, sibutramine has been withdrawn from market in United States and Europe
Statins	<p>Atorvastatin improves cardiometabolic aspects including lipid metabolism</p> <p>The effect of simvastatin remains controversial (low impact on the atherogenic lipoprotein phenotype and high effect in reducing total cholesterol, triglycerides and LDL-cholesterol)</p> <p>Rosuvastatin is more effective than atorvastatin on lipid and glycemic parameters in patients with metabolic syndrome</p>
Bariatric surgery	Recommended only after standard weight loss strategies have failed in PCOS patients with body mass index greater than 40 kg/m ²

METFORMIN

Metformin was first proposed for inducing ovulation in women with PCOS, based on the high prevalence of obesity, insulin resist-

ance and compensatory hyperinsulinemia in these patients. Insulin is known to act as a co-gonadotropin in the ovary, activating the cytochrome P-450 enzyme complex, leading to increased androgen production. Therefore,

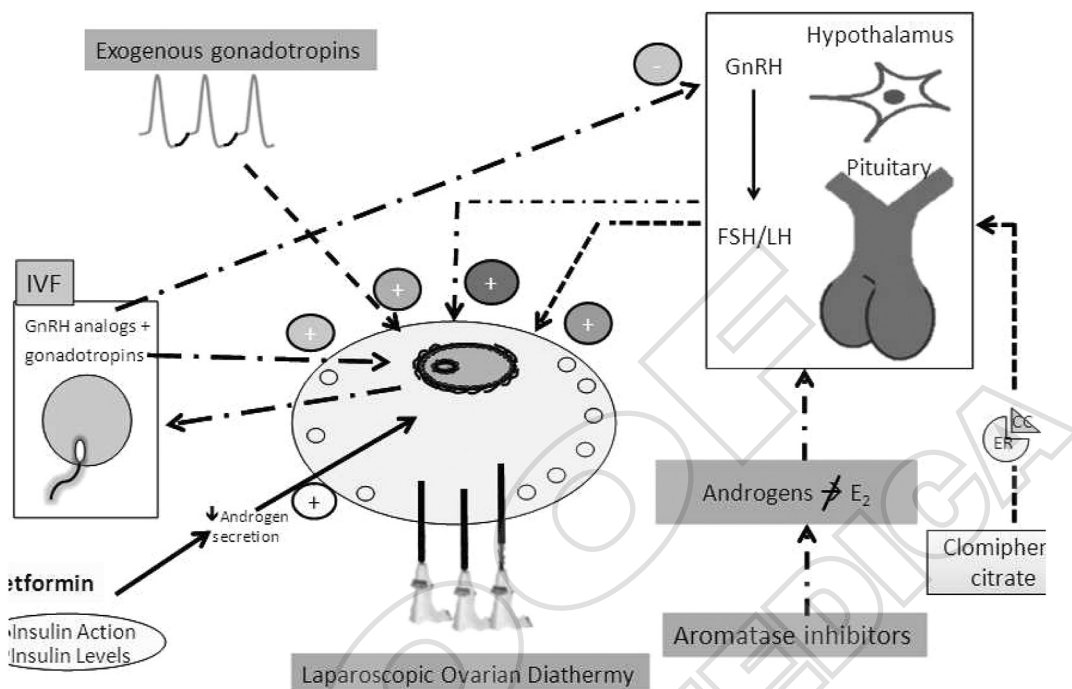


Figure 2.—Mechanisms of action of induction of ovulation and IVF treatments. Clomiphene citrate acts on the hypothalamus-pituitary axis, competing with estradiol for estrogen receptor binding and blocking its negative feedback on gonadotropin secretion. In consequence to higher gonadotropin levels, ovaries are stimulated leading to follicular development (---); aromatase inhibitors (letrozole) act by inhibiting androgen conversion to estrogen, leading to a weaker feedback effect of endogenous estrogen on the hypothalamus and pituitary, thereby enhancing follicle recruitment (-.-.-); exogenous gonadotropin (low-dose FSH) directly stimulates follicular development (= =>); in IVF GnRH analogs promote suppression of the hypothalamus-pituitary axis and gonadotropins stimulate multiple follicular development (=>=>); Laparoscopic ovarian diathermy promotes a thermal injury by the insertion of laparoscopic needle into the ovarian stroma (==>); metformin improves insulin action and decreases ovarian androgen secretion and may exert a direct ovarian effect improving the follicular microenvironment; however it does not improve live births rates and it is no longer considered as first-line drug to induce ovulation in PCOS (___).

as metformin improves insulin action and, in consequence, decreases insulin levels the final effect might be a decrease on ovarian androgen secretion (Figure 2). Metformin may also exert a direct ovarian effect improving the follicular microenvironment. However, evidence indicates that metformin alone or associated to CC does not improve live births rates in PCOS^{47, 49}. Therefore, currently, metformin is no longer considered as first-line drug to induce ovulation. In contrast, specific cases such as those presenting impaired glucose tolerance or BMI>35 kg/m² associated to CC resistance might benefit with this drug associated to lifestyle changes or other ovulation inducing agents, as described later. In addition, metformin may also be used as an

adjuvant therapy in women with PCOS undergoing IVF in order to prevent ovarian hyperstimulation syndrome (OHSS)⁵⁰.

AROMATASE INHIBITORS

More recently, accumulating evidence suggests aromatase inhibitors, such as letrozole, may be effective for ovulation induction in women with PCOS. Letrozole acts by inhibiting androgen conversion to estrogen, leading to a lower feedback effect of endogenous estrogen on the hypothalamus and pituitary (Figure 2).⁵¹ Aromatase inhibitors seem to exert an adequate endometrial stimulus and a more physiological follicle recruitment.⁴⁸ In fact, in a recent randomized clinical trial com-

paring letrozole with CC in 750 PCOS women, Legro *et al.*⁴⁸ found a higher cumulative live birth in the letrozole group (27.5%) than in the CC group (19.1%, $P=0.007$). In addition, the study showed no differences on the rates of miscarriage, multiple pregnancies, or congenital malformations between the groups, suggesting letrozole might be superior to CC for treating infertility in PCOS. Moreover, a recent meta-analysis, including other nine studies and totalizing 1783 women also showed that letrozole is superior to CC in the treatment of infertile women with PCOS (OR 1.64, CI 1.32-2.04), although the quality of the evidence was low.⁵¹ However, concerns with letrozole are related to its potential fetal teratogenicity, as reported previously, although not confirmed in the study of Legro *et al.*⁴⁸

EXOGENOUS GONADOTROPINS

A second-line therapy for ovulation induction in women with PCOS is the use of exogenous gonadotropins, mainly low-dose FSH (Figure 2). Both the step-up protocol, in which the dose of gonadotropin is gradually increased according to follicular development at ultrasound and the step-down protocol, that begins with higher doses and advances with lesser doses may be used. Using low-dose FSH the risk for multifetal pregnancies and cancellations rate due to multifollicular recruitment has been reported to be less pronounced than in the past with conventional doses.^{52, 53} In addition, one meta-analysis reported a possible beneficial effect of metformin, added to low-dose FSH treatment on live birth and pregnancy rates in patients with PCOS, although the quality of the evidence was low⁵⁴ indicating that further randomized clinical trials are needed to confirm these data.

IVF

IVF is also recommended to women with PCOS who are not responsive to ovulation induction. While classically regarded as a third choice for treating infertility in women with PCOS, after low dose FSH, IVF may be a better approach than FSH for older women

with PCOS. IVF presents a specific benefit of limiting multiple pregnancies but not the risk of OHSS, by employing single embryo transfer (Figure 2).⁵⁵ In addition, there is inconclusive evidence regarding any influence of metformin before or during IVF or intracytoplasmic sperm injection (ICSI) on live birth rates but metformin added to women with PCOS undergoing IVF was associated with a decrease in the risk of OHSS.⁵⁵ Obesity exerts a negative impact on live birth rate with IVF treatment,⁵⁶ supporting the common sense that obese women with PCOS should lose weight before IVF procedures. *In vitro* maturation (IVM) of oocytes obtained from women with ovaries minimally stimulated has been proposed as an alternative to IVF because of the potential lower risk of OHSS. However, up to now there is no convincing evidence, which could allow deciding the more effective and safe procedure, IVM *versus* IVF or ICSI.⁵⁷

LAPAROSCOPIC OVARIAN DIATHERMY

Laparoscopic ovarian diathermy is an option to CC-resistant women with PCOS, particularly for those who cannot follow the intensive monitoring required for low-dose FSH. The procedure, using either electrocautery or laser, includes the insertion of a laparoscopic needle into the ovarian stroma promoting a thermal injury (Figure 2). Around 50% of women undergoing laparoscopic ovarian diathermy have an improvement on menstrual cycles and ovulation. Live births rates are similar to low-dose FSH.⁵⁷ Concerns related to this procedure are the possibility of adhesion formation and the potential reduction of ovarian reserve.⁵⁸

Management of menstrual abnormalities, endometrial protection and hirsutism in women not seeking pregnancy

Menstrual disturbances

Menstrual disturbances are a very frequent clinical feature in women with PCOS,

varying from menstrual irregularity to oligo/amenorrhea and signalize the occurrence of anovulatory cycles. While weight loss in overweight and obese patients may improve menstrual cyclicity and restore ovulation in some cases, most of patients will need a medical treatment for this complaint. Effective treatment will additionally protect endometrium against unopposed estrogen stimulation and the recognized higher risk for endometrial hyperplasia and cancer.

COMBINED ORAL CONTRACEPTIVES

Combined oral contraceptives (OCs) are the first-line pharmacological treatment for the management of menstrual irregularities in PCOS, providing endometrial protection and contraception. The estrogen component increases SHBG, reducing bioavailable androgen levels and the progestin component suppresses circulating luteinizing hormone, thereby decreasing ovarian androgen secretion. Currently, OCs contain low doses of estrogens and synthetic progestins, equivalent to ≤ 35 μg ethinyl estradiol and between 0.1 and 3 mg of different progestins. There is a consensual idea that no evidence exists until now showing any difference in the effectiveness of distinct OCs in the treatment of PCOS.^{50, 59}

While the benefits of OCs in the long-term treatment of PCOS outweigh the risks, concerns emerge regarding the relative risk of venous thrombosis as well as the potential metabolic adverse effects of these drugs.^{18, 60} A meta-analysis including 35 studies found OCs in PCOS were not associated with alterations in fasting glucose or laboratorial markers of insulin resistance.⁶¹ Overall, screening for contraindications to OCs is recommended before prescription in women with PCOS.^{18, 50, 59}

PROGESTIN-ONLY HORMONAL CONTRACEPTIVE PILLS AND CYCLICAL PROGESTINS

Medical alternatives for treating menstrual irregularities, providing endometrial protection and guaranteeing contraception

to women with contraindications to OCs are progestin-only hormonal contraceptive pills⁶² or intrauterine devices, although these methods may be associated with spotting or intermenstrual bleeding. Metformin is also considered a second-line choice for improving menstrual cycles in women presenting insulin-resistance and dysglycemia. In turn, cyclical progestins, such as micronized progesterone, dydrogesterone or medroxyprogesterone acetate for 10-14 days/month are plausible options for those women who do not need contraception.¹⁸

Clinical hyperandrogenism

NON-PHARMACOLOGICAL AND TOPICAL TREATMENT

Clinical manifestations of hyperandrogenism, such as hirsutism, acne and androgenetic alopecia are also common in women with PCOS and are a source of anxiety and stress to these patients. The most frequent feature of clinical hyperandrogenism is hirsutism and non-pharmacological management includes cosmetic procedures such as shaving, waxing and bleaching. In addition, more efficient for long-term hair removal is laser therapy (photoepilation).⁵⁰ Another choice for mild or moderate facial hirsutism, is the topical application of eflornithine, an irreversible inhibitor of the enzyme ornithine decarboxylase, related to cellular growth and proliferation.

COMBINED ORAL CONTRACEPTIVES

In women with moderate or severe hirsutism pharmacological therapy is recommended in addition to cosmetic procedures. The aims of the treatment are to suppress ovarian androgen excess and inhibit androgen action on hair follicle. OCs are the first-line choice to treat hirsutism, acting on suppressing LH secretion, leading to a decrease on ovarian androgen production. OCs also increase SHBG hepatic secretion, thus reducing circulating free testosterone levels.^{18, 50, 63} At long-term OCs may decrease the binding of dihydrotestosterone to the androgen receptor.⁶⁴

ANTIANDROGEN DRUGS

Antiandrogen therapy is recommended in addition to OCs for more severe hirsutism⁶⁵ or if the response is not complete after 6 months of treatment with OC pills.^{18, 50} Both cyproterone acetate (CPA) and spironolactone are effective for treating hirsutism. CPA is a progestin with antiandrogenic properties that reduces the androgen action by competing with endogenous androgens for binding to androgen receptor. In addition, due to its progestogenic activity, CPA also suppresses gonadotropin secretion and reduces androgen production,⁶⁶ and may be administered in a cyclical way. Spironolactone, an antagonist of the mineralocorticoid receptor, presents antiandrogenic properties, having a structure that is very close to the testosterone and also binds competitively to the androgen receptor. The reduction of hirsutism starts to be evident after around 6 months of treatment with CPA or spironolactone.^{66, 67} It is important to point out the need to guarantee contraception during anti-androgen therapy, because of the teratogenic effects of these drugs and their effects on fetal sexual differentiation.

Among other antiandrogens that have been proposed for the treatment of hirsutism, flutamide, a nonsteroidal molecule, is effective in reducing androgen action, although it presents a dose-dependent hepatic toxicity.^{68, 69} For this reason, it is not recommended as first-line option to treat hirsutism. Another antiandrogen drug is finasteride that acts by inhibiting the activity of the enzyme of 5 α -reductase, and in consequence reduces the conversion of testosterone to its more potent metabolite DHT in the hair follicle. Although finasteride is not extensively used it may present the same effects as spironolactone or flutamide in the treatment of hirsutism.⁷⁰ However, no definitive evidence emerges from literature regarding the effectiveness and safety of finasteride in comparison with other antiandrogens.^{71, 72}

In conclusion, management strategies for women with PCOS desiring pregnancy should focus on weight loss for overweight

and obese women. Ovulation induction with antiestrogens such as clomiphene citrate or letrozole is the first-line medical treatment. Exogenous gonadotropins and in vitro fertilization are recommended as second line treatment for anovulatory infertility. Laparoscopic ovarian diathermy may be used in special cases and metformin is no longer recommended for ovulation induction because evidence has shown that it does not increase live birth rates. However, metformin might be administered during IVF cycles to prevent ovarian hyperstimulation syndrome. Combined OCs are the first-line treatment for the management of menstrual irregularities in women not seeking pregnancy, restoring menstrual cycles and providing endometrial protection and contraception. Progestin-only pills or cyclical progestins are recommended for those with contraindications to OCs. Metformin is also considered a second-line choice for improving menstrual cycles in women presenting insulin-resistance and dysglycemia. In the presence of mild hirsutism cosmetic procedures are recommended, while moderate hirsutism requires medical treatment with OCs. Management of more severe cases of hirsutism or cases with incomplete response to OCs involves anti-androgen drugs, mainly cyproterone acetate or spironolactone. In conclusion, strategies regarding the management of reproductive issues in PCOS encompass a tailored approach to individual needs of each patient.

Novel strategies in adolescents

PCOS is a common disorder among reproductive-age women. This syndrome is increasingly recognized in adolescent girls and is one of the most frequent causes of androgen excess in this age group.⁷³ Although the clinical and metabolic features are similar to those found in young adult women with PCOS, the diagnosis may be overlooked during adolescence, as irregular menses with anovulatory cycles, polycystic ovarian morphology, obesity, and acne are frequent in teenagers without this syn-

drome.^{74,75} Therefore, strict diagnostic criteria have been proposed to limit premature and overdiagnosis of PCOS in adolescents.

Management of the adolescent with PCOS is complex and requires a multidisciplinary team approach, including endocrinologists, gynecologists, dermatologists, psychologists, and nutritionists, who should have a deep knowledge of the disease for optimal results. Due to the fact that PCOS is a lifelong condition, patients should be carefully monitored during adolescence and thereafter in adulthood. Early treatment of menstrual irregularity and hirsutism may improve an adolescent's self-image and quality of life. Moreover early intervention for PCOS-associated morbidities such as insulin resistance, obesity, and dyslipidemia may modify the progression of these chronic conditions later in life.⁷⁶

In general treatment of PCOS in adolescents is symptomatic and preventive. Lifestyle changes are the first-line intervention in women with PCOS, who are overweight. Management of menstrual abnormalities, endometrial protection and cutaneous manifestations of hyperandrogenism is similar to those employed in young PCOS women who do not desire pregnancy. Insulin resistance can be managed by diet and exercise, and with appropriate weight control. Metformin improves insulin sensitivity and glucose metabolism,⁷⁷ and ameliorates hyperandrogenism and irregular menses in adolescents.^{78,79} Metformin is also beneficial in normalizing the lipid profile.⁷⁸ However, questions about how long treatment should be continued and regarding the long-term safety are controversial. Actually the major debate in the management of PCOS is the long-term treatment with insulin sensitizers starting in adolescence.

Lifestyle changes

Early lifestyle changes may prove to be the most effective approach as younger adolescents with PCOS and obesity are more prone to adopt healthy lifestyle changes than older adolescents.²⁹ It has been demonstrated that a reduction of 5-10% in body

weight in obese women with PCOS can, for example improve hirsutism in 40-55% within 6 months of weight reduction⁸⁰ and restore regular menstrual cycles. Similar findings have been observed in adolescents with PCOS. Nevertheless, there is limited data regarding the most effective dietary intervention, exercise regimen, or behavior modification program for obese adolescents with PCOS.⁸¹ Caloric reduction will lead to weight loss, however it is not clear if low carbohydrate or low fat diets are preferred for achieving and maintaining long-term weight loss.⁸² In a multidisciplinary clinic approach for adolescents with PCOS, nearly 70% of patients succeeded in short-term weight stabilization, with 57% demonstrating weight loss. Interactions with the health psychologist and dietitian appeared to play a key role in successful weight control, supporting the importance of psychology and nutrition expertise in the management of this disorder.⁸³

Reproductive abnormalities

Menstrual irregularities such as persistent oligomenorrhea, primary or secondary amenorrhea and menorrhagia are the most frequent cause of consultation of PCOS adolescent. As previously mentioned, lifestyle changes are the first line of treatment in these girls.

The use of cyclic progestins or OCs constitutes the second line of treatment. Progestin regulates menstrual cycles and may protect against endometrial hyperplasia, which is secondary to chronic anovulation. Treatment with cyclic progestin is useful in girls with PCOS who are not candidate for OCs or have not initiated their sexual activity. Nevertheless they must be advised that cyclic progestin will not treat androgenic symptoms and is not a contraceptive. In adolescents requiring contraception OCs or a progestin intrauterine device could be the option.

OCs and anti-androgens are also useful tools for the treatment of the clinical manifestations of hyperandrogenism. Nevertheless, concerns exist about the risk of

venous thromboembolism (VTE), insulin resistance and dyslipidemia caused by OCs in adolescents with PCOS. PCOS may represent an independent risk factor for VTE in young women with PCOS and OCs may further increase the relative risk of VTE in these women. Concerns regarding the risk of VTE with OCP containing drospirinone have emerged. Nevertheless, observational trials have not considered other factors such as prolonged immobility, obesity, smoking history, and family history of VTE.⁸⁴ Increased risk for IR caused by OCs in adolescents with PCOS has been suggested by a number of clinical trials.^{85, 86} A 6-month study with drospirinone/ethinilestradiol (EE) showed no effect on insulin sensitivity or in insulin secretion measured by hyperinsulinemic-euglycemic clamp.⁸⁷ On the contrary the combination of desogestrel/EE and cyproterone acetate/EE showed an increase in insulin resistance estimated by HOMA-IR.⁸⁸ In addition, OCs increase LDL and HDL cholesterol, leading to unchanged total cholesterol-to-HDL ratio.^{87, 88} However, the long-term impact of the use of OCs in adolescents on cardiovascular and diabetes risk is not known; further studies remain to be conducted.

For cosmetic improvement of acne and hirsutism, low dose antiandrogen drugs used alone or in combination with OCs had beneficial effects on hyperandrogenism or hirsutism in adolescents.^{89, 90} Nevertheless, the use of antiandrogens in late adolescence requires concurrent use of an effective contraception to prevent the risk of feminization of a male fetus if they get pregnant. Spironolactone at a dose 100 mg/day has been shown to be more effective than placebo in reducing hirsutism in women with PCOS.⁷⁰ The addition of spironolactone (100 mg/day) to an OCs containing drospirinone did not lead to elevated potassium levels in small trials but the safety of this combination has not been evaluated in larger trials. More recently, it has been shown that the combination of low-dose spironolactone (50 mg/d) and metformin improved insulin sensitivity to a magnitude superior to either drug alone.⁹¹ Regarding

flutamide, an androgen receptor antagonist, the group of Ibanez *et al.*, published a series of clinical trials using combination therapy with low dose flutamide and insulin-sensitizers in adolescents showing improvement of clinical manifestations as well as cardiovascular risk factors.^{92, 93}

Metabolic derangement

In the last years there is a growing concern about the increase in dyslipidemia, impaired glucose tolerance, and hypertension in adolescents with PCOS, especially in obese girls. Therefore, management of PCOS with insulin sensitizers like metformin³⁷ and thiazolidinediones,⁹⁴ has generated significant interest.

Metformin, an oral biguanide, is well established for the treatment of diabetes and it is now considered the first-line drug in the management of women with PCOS and insulin resistance. In addition to its insulin sensitizing properties, metformin may also have a direct action on human theca cells, reducing androgen synthesis, through an insulin-independent mechanism.⁹⁵ A recent survey indicated that 30% of pediatric endocrinologists consider metformin appropriate treatment for adolescents with PCOS, and 68% for obese adolescents with PCOS.

Many studies has demonstrated the efficacy of metformin in improving menstrual cyclicity, anovulation, inflammation, endothelial dysfunction, and various metabolic parameters. Low dose metformin alone or in combination with flutamide has been used in lean adolescents with PCOS and insulin resistance by Ibañez *et al.*⁹⁶ showing an improvement in the lipid profile. Few studies that compare OCs and metformin in adolescents have been published, making difficult to draw conclusions. Studies that have evaluated the effectiveness of metformin therapy alone or in conjunction with lifestyle modifications suggest that metformin decreases serum androgens and clinical hyperandrogenism and improve lipid profile and chronic anovulation.⁷⁶

In summary, studies indicate that the degree to which metformin therapy is ef-

fective in improving the androgenic and metabolic profile in adolescents with PCOS may be related to 1) the dose used, particularly when metformin is used as monotherapy, necessitating higher doses;⁷⁷ 2) the agent with which metformin is combined, estrogen-progestin combination pills or antiandrogenic agents such as flutamide, spironolactone, and cyproterone acetate;⁹⁷ and 3) the characteristics of the PCOS patient being treated, whether obese or lean, hyperinsulinemic, or normoinsulinemic.^{77, 79} All these aspects have to be considered before initiating metformin therapy in these girls.

Thiazolidinediones (TZDs) are a family of insulin-sensitizer drugs that act through the nuclear receptor PPAR- γ that when stimulated, are able to mediate the genetic transcription and regulate the glucose metabolism, inflammatory response and the differentiation of adipocytes. These drugs have also shown very similar effects to the ones obtained with metformin, improving hyperandrogenism, anovulation, and insulin resistance in adult women with PCOS. In addition they show a favorable effect on visceral adipose tissue which was also observed in young PCOS women.⁹⁴ Nevertheless, despite its proven efficacy in adult women with PCOS these medications have not been rigorously studied in adolescents, in either traditional states of IR or in late adolescent girls with PCOS. Therefore, it is not recommended in this age group. As previously mentioned, metformin is recommended as first line insulin sensitizer given the larger evidence in the beneficial use of this drug in the clinical practice.

Other treatment alternatives

Recently the employment of electroacupuncture and the use of berberine have emerged as new therapeutic options. Electroacupuncture and Chinese kidney-nourishing medicine in obese Chinese women with PCOS improve obesity-related indexes, insulin sensitivity and adiponectin levels. This favorable effect was not demonstrated in a previous study using electroacupuncture

and exercise in Caucasian PCOS women.⁹⁸

Berberine, the major active component of rhizomacoptidis, exists in a number of medicinal plants and displays a broad array of pharmacological effects. In Chinese medicine, Berberine has long been used for its anti-diabetic effects. Recently it has been shown to have positive effects on insulin resistance, lipid metabolism, nitric oxide production, and metabolic syndrome.

The mechanisms of berberine in treating PCOS are partially known. Berberine acts through the activation of AMP-activated protein kinase (AMPK), improving insulin resistance in theca cells and granulosa cells in a way similar to metformin.⁹⁹

Risk factors and prevention

Risk factors such as premature pubarche, ethnicity,¹⁰⁰ and a positive family history¹⁰¹⁻¹⁰³ should alert the clinician about the possibility of PCOS in adolescent girls. Moreover, in recent years a relationship between early puberty,¹⁰⁴ low and high birth weight have been described¹⁰⁵ and the events that may occur during pregnancy are becoming very relevant in the ontogeny of this syndrome. Understanding how perturbations of the maternal-fetal environment influence the developmental origins of PCOS will allow the set up of preventive strategies.

References

1. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG *et al.* A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *J Clin Endocrinol Metab* 1999;84:4006-11.
2. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev* 2012;33:981-1030. PubMed PMID: 23065822.
3. Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, be the main culprit for the follicular arrest. *Hum Reprod Update* 2004-Apr;10:107-17. PubMed PMID: 15073141.
4. Blank SK, McCartney CR, shall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. *Hum Reprod Update* 2006-Aug;12:351-61. PubMed PMID: 16670102.
5. Diamanti-Kandarakis E. Polycystic ovarian syn-

- drome: pathophysiology, molecular aspects and clinical implications. *Expert Rev Mol Med* 2008;10:e3.
6. Chatzigeorgiou A, Kandaraki E, Piperi C, Livadas S, Papavassiliou AG, Koutsilieris M *et al.* Dietary glycotoxins affect scavenger receptor expression and the hormonal profile of female rats. *J Endocrinol* 2013;218:331-7.
 7. Kandaraki E, Christakou C, Diamanti-Kandarakis E. Metabolic syndrome and polycystic ovary syndrome... and vice versa. *Arq Bras Endocrinol Metabol* 2009;53:227-37.
 8. Diamanti-Kandarakis E. Role of obesity and adiposity in polycystic ovary syndrome. *Int J Obes (Lond)* 2007;31 Suppl 2:S8-13; discussion S31-2.
 9. Diamanti-Kandarakis E, Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. *Trends Mol Med* 2006;12:324-32.
 10. Willis D, Mason H, Gilling-Smith C, Franks S. Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. *J Clin Endocrinol Metab* 1996;81:302-9.
 11. Willis DS, Watson H, Mason HD, Galea R, Brincat M, Franks S. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. *J Clin Endocrinol Metab* 1998;83:3984-91.
 12. Gonzalez F. Inflammation in Polycystic Ovary Syndrome: underpinning of insulin resistance and ovarian dysfunction. *Steroids* 2011 10;77:300-5.
 13. Kandaraki E, Chatzigeorgiou A, Piperi C, Palioura E, Palimeri S, Korkolopoulou P *et al.* Reduced ovarian glyoxalase-I activity by dietary glycotoxins and androgen excess: a causative link to polycystic ovarian syndrome. *Mol Med* 2012;18:1183-9.
 14. Diamanti-Kandarakis E, Katsikis I, Piperi C, Kandaraki E, Piouka A, Papavassiliou AG *et al.* Increased serum advanced glycation end-products is a distinct finding in lean women with polycystic ovary syndrome (PCOS). *Clin Endocrinol (Oxf)* 2008;69:634-41.
 15. Diamanti-Kandarakis E, Piperi C, Kalofoutis A, Creatsas G. Increased levels of serum advanced glycation end-products in women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2005;62:37-43.
 16. Daffu G, del Pozo CH, O'Shea KM, Ananthakrishnan R, Ramasamy R, Schmidt AM. Radical roles for RAGE in the pathogenesis of oxidative stress in cardiovascular diseases and beyond. *Int J Mol Sci* 2013;14:19891-910.
 17. Merhi Z. Advanced glycation end products and their relevance in female reproduction. *Hum Reprod* 2013;29:135-45.
 18. Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A *et al.* European survey of diagnosis and management of the polycystic ovary syndrome: results of the ESE PCOS Special Interest Group's Questionnaire. *Eur J Endocrinol* 2014;171:489-98. Cardiometabolic aspects of the polycystic ovary syndrome. *Endocr Rev* 2012;33:812-41.
 20. Spritzer PM, Lecke SB, Satler F, Morsch DM. Adipose tissue dysfunction, adipokines and low-grade chronic inflammation in PCOS. *Reproduction* 2015;149:R219-R227.
 21. Moran LJ, Ko H, Misso M, Sh K, Noakes M, Talbot M *et al.* Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. *Hum Reprod Update* 2013;19:432.
 22. Hahn S, Haselhorst U, Tan S, Quadbeck B, Schmidt M, Roesler S *et al.* Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes* 2006;114:577-83.
 23. Freundlich M, Quiroz Y, Zhang Z, Zhang Y, Bravo Y, Weisinger JR *et al.* Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. *Kidney Int* 2008;74:1394-402.
 24. Diamanti-Kandarakis E, Piouka A, Livadas S, Piperi C, Katsikis I, Papavassiliou AG *et al.* Anti-mullerian hormone is associated with advanced glycosylated end products in lean women with polycystic ovary syndrome. *Eur J Endocrinol* 2009;160:847-53.
 25. Tantalaki E, Piperi C, Livadas S, Kollias A, Adamopoulos C, Koulouri A *et al.* Impact of dietary modification of advanced glycation end products (AGEs) on the hormonal and metabolic profile of women with polycystic ovary syndrome (PCOS). *Hormones (Athens)* 2014;13:65-73.
 26. Tanaka LY, Bechara LR, Dos Santos AM, Jordao CP, de Sousa LG, Bartholomeu T *et al.* Exercise improves endothelial function: A local analysis of production of nitric oxide and reactive oxygen species. *Nitric Oxide* 2015 22;45C:7-14.
 27. Sprung VS, Cuthbertson DJ, Pugh CJ, Aziz N, Kemp GJ, Daousi C *et al.* Exercise training in polycystic ovarian syndrome enhances flow-mediated dilation in the absence of changes in fatness. *Med Sci Sports Exerc* 2013;45:2234-42.
 28. Nybacka A, Carlstrom K, Fabri F, Hellstrom PM, Hirschberg AL. Serum antimullerian hormone in response to dietary management and/or physical exercise in overweight/obese women with polycystic ovary syndrome: secondary analysis of a randomized controlled trial. *Fertil Steril* 2013;100:1096-102.
 29. Lass N, Kleber M, Winkel K, Wunsch R, Reinhr T. Effect of lifestyle intervention on features of polycystic ovarian syndrome, metabolic syndrome, and intima-media thickness in obese adolescent girls. *J Clin Endocrinol Metab* 2011;96:3533-40.
 30. Christakou C, Kollias A, Piperi C, Katsikis I, Panidis D, Diamanti-Kandarakis E. The benefit-to-risk ratio of common treatments in PCOS: effect of oral contraceptives versus metformin on atherogenickers. *Hormones (Athens)* 2014;13:488-97.
 31. Lemay A, Dodin S, Turcot L, Hene F, Forest JC. Rosiglitazone and ethinyl estradiol/cyproterone acetate as single and combined treatment of overweight women with polycystic ovary syndrome and insulin resistance. *Hum Reprod* 2006;21:121-8.
 32. Nader S, Diamanti-Kandarakis E. Polycystic ovary syndrome, oral contraceptives and metabolic issues: new perspectives and a unifying hypothesis. *Hum Reprod* 2007;22:317-22.
 33. Bargiota A, Diamanti-Kandarakis E. The effects of old, new and emerging medicines on metabolic aberrations in PCOS. *Ther Adv Endocrinol Metab* 2014;3:27-47.
 34. Elia EM, Pustovrh C, Amalfi S, Devoto L, Motta AB. Link between metformin and the peroxisome proliferator-activated receptor gamma pathway in the uterine tissue of hyperandrogenized prepubertal mice. *Fertil Steril* 2011;95:2534-7 e1.
 35. Motta AB. Mechanisms involved in metformin action in the treatment of polycystic ovary syndrome. *Curr Pharm Des* 2009;15:3074-7.
 36. Motta DA. Metformin in the treatment of polycystic ovary syndrome. *Curr Pharm Des* 2008;14(21):2121-5. PubMed PMID: 18691121. Epub 2008/08/12. eng.

37. Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN. Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. *Eur J Endocrinol* 2010;162:193-212.
38. Diamanti-Kandarakis E, Mitrakou A, Raptis S, Tolis G, Duleba AJ. The effect of a pure antiandrogen receptor blocker, flutamide, on the lipid profile in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1998;83:2699-705.
39. Sahin Y, Unluhizarci K, Yilmazsoy A, Yikilmaz A, Aygen E, Kelestimur F. The effects of metformin on metabolic and cardiovascular risk factors in non-obese women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2007;67:904-8.
40. Costello M, Shrestha B, Eden J, Sjoblom P, Johnson N. Insulin-sensitising drugs versus the combined oral contraceptive pill for hirsutism, acne and risk of diabetes, cardiovascular disease, and endometrial cancer in polycystic ovary syndrome. *Cochrane Database Syst Rev* 2007:CD005552.
41. Diamanti-Kandarakis E, Katsikis I, Piperi C, Alexandraki K, Panidis D. Effect of long-term orlistat treatment on serum levels of advanced glycation end-products in women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2007;66:103-9.
42. Koiou E, Tziomalos K, Katsikis I, Delkos D, Tsourdi EA, Panidis D. Disparate effects of pharmacotherapy on plasma plasminogen activator inhibitor-1 levels in women with the polycystic ovary syndrome. *Hormones (Athens)* 2014;12:559-66.
43. Panidis D, Tziomalos K, Papadakis E, Chatzis P, Kandaraki EA, Tsourdi EA *et al.* The role of orlistat combined with lifestyle changes in the management of overweight and obese patients with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2013;80:432-8.
44. Sabuncu T, Harma M, Harma M, Nazligil Y, Kilic F. Sibutramine has a positive effect on clinical and metabolic parameters in obese patients with polycystic ovary syndrome. *Fertil Steril* 2003;80:1199-204.
45. Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba AJ. Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: prospective, randomized, crossover trial. *J Clin Endocrinol Metab* 2007;92:456-61.
46. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W *et al.* Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab* 2010;95:2038-49.
47. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA *et al.* Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med* 2007;356:551-66.
48. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P *et al.* Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 2014;371:119-29.
49. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* 2012;5:CD003053.
50. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R *et al.* Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2013;98:4565-92.
51. Franik S, Kremer JA, Nelen WL, Farquhar C. Aromatase inhibitors for subfertile women with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2014;2:CD010287.
52. White DM, Polson DW, Kiddy D, Sagle P, Watson H, Gilling-Smith C *et al.* Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. *J Clin Endocrinol Metab* 1996;81:3821-4.
53. Hugues JN, Cedrin-Durnerin I, Avril C, Bulwa S, Herve F, Uzan M. Sequential step-up and step-down dose regimen: an alternative method for ovulation induction with follicle-stimulating hormone in polycystic ovarian syndrome. *Hum Reprod* 1996;11:2581-4.
54. Palomba S, Falbo A, La Sala GB. Metformin and gonadotropins for ovulation induction in patients with polycystic ovary syndrome: a systematic review with meta-analysis of randomized controlled trials. *Reprod Biol Endocrinol* 2014;12:3.
55. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2014;11:CD006105.
56. Bailey AP, Hawkins LK, Missmer SA, Correia KF, Yanushpolsky EH. Effect of body mass index on in vitro fertilization outcomes in women with polycystic ovary syndrome. *Am J Obstet Gynecol* 2014;211:163 e1-6.
57. Siristatidis CS, Vrachnis N, Creata M, Maheshwari A, Bhattacharya S. In vitro maturation in subfertile women with polycystic ovarian syndrome undergoing assisted reproduction. *Cochrane Database Syst Rev* 2013;10:CD006606.
58. Farquhar C, Lilford RJ, joribanks J, Vandekerckhove P. Laparoscopic 'drilling' by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. *Cochrane Database Syst Rev* 2007:CD001122.
59. Yildiz BO. Approach to the patient: contraception in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2015;100:794-802.
60. Stegeman BH, de Bastos M, Rosendaal FR, van Hylckama Vlieg A, Helmerhorst FM, Stijnen T *et al.* Different combined oral contraceptives and the risk of venous thrombosis: systematic review and network meta-analysis. *BMJ* 2013;347:f5298.
61. Halperin IJ, Kumar SS, Stroup DF, Laredo SE. The association between the combined oral contraceptive pill and insulin resistance, dysglycemia and dyslipidemia in women with polycystic ovary syndrome: a systematic review and meta-analysis of observational studies. *Hum Reprod* 2011;26:191-201.
62. Grimes DA, Lopez LM, O'Brien PA, Raymond EG. Progestin-only pills for contraception. *Cochrane Database Syst Rev* 2013;11:CD007541.
63. Maier PS, Mattiello SS, Lages L, Spritzer PM 17-Hydroxysteroid dehydrogenase type 5 gene polymorphism (-71A/G HSD17B5 SNP) and treatment with oral contraceptive pills in PCOS women without metabolic comorbidities. *Gynecol Endocrinol* 2012;28:606-10.
64. Eil C, Edelson SK. The use of human skin fibroblasts to obtain potency estimates of drug binding to androgen receptors. *J Clin Endocrinol Metab* 1984;59:51-5.
65. Pasquali R, Gambineri A. Therapy in endocrine disease: treatment of hirsutism in the polycystic ovary syndrome. *Eur J Endocrinol* 2013;170:R75-90.
66. Spritzer P, Billaud L, Thalabard JC, Birman P, Mowszowicz I, Raux-Demay MC *et al.* Cyproterone acetate

- versus hydrocortisone treatment in late-onset adrenal hyperplasia. *J Clin Endocrinol Metab* 1990;70:642-6. PubMed PMID: 2137832. Epub 1990/03/01. eng.
67. Spritzer PM, Lisboa KO, Mattiello S, Lhullier F. Spironolactone as a single agent for long-term therapy of hirsute patients. *Clin Endocrinol (Oxf)* 2000;52:587-94.
 68. Bruni V, Peruzzi E, Dei M, Nannini S, Seravalli V, Sisti G *et al.* Hepatotoxicity with low- and ultralow-dose flutamide: a surveillance study on 203 hyperandrogenic young females. *Fertil Steril* 2012;98:1047-52.
 69. Ibanez L, Jaramillo A, Ferrer A, de Zegher F. Absence of hepatotoxicity after long-term, low-dose flutamide in hyperandrogenic girls and young women. *Hum Reprod* 2005;20:1833-6.
 70. Moghetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F *et al.* Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. *J Clin Endocrinol Metab* 2000;85:89-94.
 71. Swiglo BA, Cosma M, Flynn DN, Kurtz DM, Labella ML, Mullan RJ *et al.* Clinical review: Antiandrogens for the treatment of hirsutism: a systematic review and meta-analyses of randomized controlled trials. *J Clin Endocrinol Metab* 2008;93:1153-60.
 72. Domecq JP, Prutsky G, Mullan RJ, Sundaresh V, Wang AT, Erwin PJ *et al.* Adverse effects of the common treatments for polycystic ovary syndrome: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2013;98:4646-54.
 73. Christensen SB, Black MH, Smith N, Martinez MM, Jacobsen SJ, Porter AH *et al.* Prevalence of polycystic ovary syndrome in adolescents. *Fertil Steril* 2013;100:470-7.
 74. Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A *et al.* A very large proportion of young Danish women have polycystic ovaries: is a revision of the Rotterdam criteria needed? *Hum Reprod* 2010;25:3117-22.
 75. Codner E, Villarreal C, Eyzaguirre FC, López P, Merino PM, Pérez-Bravo F *et al.* Polycystic ovarian morphology in postmenarchal adolescents. *Fertil Steril* 2011;95:702-6.e1-2.
 76. Vitek W, Hoeger KM. Treatment of polycystic ovary syndrome in adolescence. *Semin Reprod Med* 2014;32:214-21.
 77. Arslanian SA, Lewy V, Danadian K, Saad R. Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. *J Clin Endocrinol Metab* 2002;87:1555-9.
 78. Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L. Metformin to restore normal menses in oligo-amenorrheic teenage girls with polycystic ovary syndrome (PCOS). *J Adolesc Health* 2001;29:160-9.
 79. Ibanez L, Valls C, Ferrer A, cos MV, Rodriguez-Hierro F, de Zegher F. Sensitization to insulin induces ovulation in nonobese adolescents with anovulatory hyperandrogenism. *J Clin Endocrinol Metab* 2001;86:3595-8.
 80. Pasquali R, Antenucci D, Casimirri F, Venturoli S, Paradisi R, Fabbri R *et al.* Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. *J Clin Endocrinol Metab* 1989;68:173-9.
 81. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. *Fertil Steril* 2009;92:1966-82.
 82. Moran LJ, Ko H, Misso M, sh K, Noakes M, Talbot M *et al.* Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. *J Acad Nutr Diet* 2013;113:520-45.
 83. Geier LM, Bekx MT, Connor EL. Factors contributing to initial weight loss among adolescents with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol* 2012;25:367-70.
 84. Bird ST, Hartzema AG, Brophy JM, Etmann M, Delaney JA. Risk of venous thromboembolism in women with polycystic ovary syndrome: a population-based matched cohort analysis. *CMAJ* 2013;185:E115-20.
 85. Baillargeon JP, Iuorno MJ, Nestler JE. Insulin sensitizers for polycystic ovary syndrome. *Clin Obstet Gynecol* 2003;46:325-40.
 86. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R *et al.* Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2013;98:4565-92.
 87. Tfayli H, Ulnach JW, Lee S, Sutton-Tyrrell K, Arslanian S. Drospirenone/ethinyl estradiol versus rosiglitazone treatment in overweight adolescents with polycystic ovary syndrome: comparison of metabolic, hormonal, and cardiovascular risk factors. *J Clin Endocrinol Metab* 2011;96:1311-9.
 88. Mastorakos G, Koliopoulos C, Creatsas G. Androgen and lipid profiles in adolescents with polycystic ovary syndrome who were treated with two forms of combined oral contraceptives. *Fertil Steril* 2002;77:919-27.
 89. Ibanez L, Potau N, cos MV, de Zegher F. Treatment of hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia, and hyperinsulinism in nonobese, adolescent girls: effect of flutamide. *J Clin Endocrinol Metab* 2000;85:3251-5.
 90. Ganie MA, Khurana ML, Eunice M, Gupta N, Gulati M, Dwivedi SN *et al.* Comparison of efficacy of spironolactone with metformin in the management of polycystic ovary syndrome: an open-labeled study. *J Clin Endocrinol Metab* 2004;89:2756-62.
 91. Ganie MA, Khurana ML, Nisar S, Shah PA, Shah ZA, Kulshrestha B *et al.* Improved efficacy of low-dose spironolactone and metformin combination than either drug alone in the management of women with polycystic ovary syndrome (PCOS): a six-month, open-label randomized study. *J Clin Endocrinol Metab* 2013;98:3599-607.
 92. Ibanez L, de Zegher F. Flutamide-metformin plus ethinylestradiol-drospirenone for lipolysis and antiatherogenesis in young women with ovarian hyperandrogenism: the key role of metformin at the start and after more than one year of therapy. *J Clin Endocrinol Metab* 2005;90:39-43.
 93. Ibanez L, Diaz M, Sebastiani G, cos MV, Lopez-Bermejo A, de Zegher F. Oral contraception vs insulin sensitization for 18 months in nonobese adolescents with androgen excess: posttreatment differences in C-reactive protein, intima-media thickness, visceral adiposity, insulin sensitivity, and menstrual regularity. *J Clin Endocrinol Metab* 2013;98:E902-7.
 94. Stabile G, Borrielli I, Arsenio AC, Bruno LM, Benavenga S, Giunta L *et al.* Effects of the insulin sensitizer pioglitazone on menstrual irregularity, insulin resistance and hyperandrogenism in young women with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol* 2014;27:177-82.
 95. Mansfield R, Galea R, Brincat M, Hole D, Mason H. Metformin has direct effects on human ovarian steroidogenesis. *Fertil Steril* 2003;79:956-62.

96. Ibanez L, Ong K, Ferrer A, Amin R, Dunger D, de Zegher F. Low-dose flutamide-metformin therapy reverses insulin resistance and reduces fat mass in nonobese adolescents with ovarian hyperandrogenism. *J Clin Endocrinol Metab* 2003;88:2600-6.
97. Diamanti-Kandarakis E, Mitrakou A, Hennes MM, Platanissiotis D, Kaklas N, Spina J *et al.* Insulin sensitivity and antiandrogenic therapy in women with polycystic ovary syndrome. *Metabolism* 1995;44:525-31.
98. Stener-Victorin E, Baghaei F, Holm G,son PO, Olivecrona G, Lönn M *et al.* Effects of acupuncture and exercise on insulin sensitivity, adipose tissue characteristics, and markers of coagulation and fibrinolysis in women with polycystic ovary syndrome: secondary analyses of a randomized controlled trial. *Fertil Steril* 2012;97:501-8.
99. Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y *et al.* Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes* 2006;55:2256-64.
100. Ladron de Guevara A, Fux-Otta C, Crisosto N, Szafryk de Mereshian P, Echiburu B, Iraci G *et al.* Metabolic profile of the different phenotypes of polycystic ovary syndrome in two Latin American populations. *Fertil Steril* 2014;101:1732-9 e1-2.
101. Sir-Petermann T, Codner E, Maliqueo M, Echiburu B, Hitschfeld C, Crisosto N *et al.* Increased anti-Müllerian hormone serum concentrations in prepubertal daughters of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006;91:3105-9.
102. Sir-Petermann T, Maliqueo M, Codner E, Echiburu B, Crisosto N, Perez V *et al.* Early metabolic derangements in daughters of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2007;92:4637-42.
103. Sir-Petermann T, Codner E, Perez V, Echiburu B, Maliqueo M, Ladron de Guevara A *et al.* Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2009;94:1923-30.
104. Chiavaroli V, Liberati M, D'Antonio F, Masuccio F, Capanna R, Verrotti A *et al.* GnRH analog therapy in girls with early puberty is associated with the achievement of predicted final height but also with increased risk of polycystic ovary syndrome. *Eur J Endocrinol* 2010;163:55-62.
105. Sir-Petermann T, Márquez L, Cárcamo M, Hitschfeld C, Codner E, Maliqueo M *et al.* Effects of birth weight on anti-müllerian hormone serum concentrations in infant girls. *J Clin Endocrinol Metab* 2010;95:903-10.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.