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Report of the international symposium: polycystic ovary syndrome: first Latin-American consensus

Editor's Choice

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SUMMARY

During the last years, numerous consensuses have been held in different countries in order to review the data concerning diagnosis and treatment and their relationship with the ethnic origin, social status and lifestyle of women with Polycystic Ovary Syndrome (PCOS). This study describes the conclusions concerning diagnostic criteria and the appropriate treatment of women with PCOS reached during the International Symposium Polycystic Ovary Syndrome, First Latin-American Consensus held in Buenos Aires, Argentina on 4th and 5th May 2009 to be applied in South American.

Review Criteria

- Review of the conclusions concerning diagnostic criteria and the appropriate treatment of women with Polycystic Ovary Syndrome reached during the International Symposium.
- Review of pertinent medical literature.

Message for the Clinic

Polycystic Ovary Syndrome is a heterogeneous pathology, frequently associated with type 2 diabetes, cardiovascular or hepatic disease and obesity. Physicians should consider that, as smaller for gestational age, low-birth weight, precocious pubarche are factors related to the onset of Polycystic Ovary Syndrome, it is important to follow girls in these groups.

Introduction

Polycystic ovary syndrome (PCOS) is most commonly defined according to the proceedings of an expert conference sponsored by the National Institute of Health (NIH) in April 1990, which noted the disorder as having (i) hyperandrogenism and/or hyperandrogenemia, (ii) oligoovulation and (iii) exclusion of known disorders. Alternatively, another expert conference held in Rotterdam in May 2003 defined PCOS by two of the following three features, after the exclusion of the related disorders: (i) oligoovulation or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism or (iii) polycystic ovaries. In fact, the Rotterdam 2003 consensus expanded the NIH 1990 definition creating two new phenotypes: (i) ovulatory women with polycystic ovaries and hyperandrogenism and (ii) oligoanovulatory women with polycystic ovaries, but without hyperandrogenism. In 2006, the Board of Directors of the Androgen Excess and PCOS (AE-PCOS) Society reviewed all the current data concerning the phenotype of PCOS to clarify what phenotypes constitute PCOS.

PCOS is one of the most common endocrinological diseases (5–10%) encountered in premenopausal women (1,2). It has been described that hyperinsulinaemia, frequently associated with PCOS, increases both the risk of cardiovascular diseases and the development of diabetes mellitus (3). Although, during the last decade several clues have emerged from human and animal studies, little is known about the aetiology and pathophysiology of PCOS. During the last years, numerous consensuses have been held in different countries to review the data concerning the diagnosis and treatment and their relationship with the ethnic origin, social status and lifestyle of women with PCOS. This study describes the conclusions concerning diagnostic criteria and the appropriate treatment of women with PCOS reached during the International Symposium Polycystic Ovary Syndrome, First Latin-American Consensus held in Buenos Aires, Argentina on 4th and 5th May 2009.

Phenotypes in women with polycystic ovary syndrome

Based on the available data and using the 2003 ESHRE/ASRM criteria and the AE-PCOS Society Task Force (4–7), the diagnosis of PCOS may be assigned to patients presenting four different phenotypes (Table 1):

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Table 1 Phenotypes in polycystic ovary syndrome

Type	Characteristics	Related parameters
1	Hyperandrogenism Chronic anovulation Polycystic ovaries (*Classical PCOS. Type 1 represents the 90% of PCOS cases*)	Androgen levels, body weight, insulin resistance and cardiovascular risk are elevated
2	Hyperandrogenism Chronic anovulation Normal ovaries (*Clinical presentation of Type 1, relatively uncommon*)	Androgen levels, body weight, insulin resistance and cardiovascular risk are elevated
3	Hyperandrogenism Ovulatory cycles Polycystic ovaries (*Ovulatory PCOS*)	Androgen levels and cardiovascular risk are elevated Insulin resistance is moderated Body weight is between normal values
4	No hyperandrogenism Chronic anovulation Polycystic ovaries (*Mild form of PCOS, in discussion*)	No hyperandrogenism or insulin resistance Body weight is between normal values

Based on the available data and using the 1990 NHI; 2003 ESHRE/ASRM criteria and the AE-PCOS Society Task Force (4–7). PCOS, polycystic ovary syndrome.

• Clinical and/or biochemical hyperandrogenism, chronic anovulation and polycystic ovaries (*classical PCOS*).
• Clinical and/or biochemical hyperandrogenism, chronic anovulation, but normal ovaries.
• Clinical and/or biochemical hyperandrogenism, polycystic ovaries, but ovulatory cycles (*ovulatory PCOS*).
• Chronic anovulation, polycystic ovaries, but no clinical and/or biochemical hyperandrogenism.

The first phenotype is the most common and the most severe and is usually associated to obesity, more severe insulin resistance and metabolic disorders. It includes 90% of patients with classical PCOS (NIH diagnostic criteria). The second phenotype is relatively uncommon and has not been well studied, although it seems to be very similar to the first phenotype in its clinical presentation. The last two phenotypes appear to represent mild forms of PCOS. In particular, patients with ovulatory PCOS have been well studied. These patients present the same features of patients with classical PCOS (hyperandrogenism, polycystic ovaries, insulin resistance and increased cardiovascular risk factors), but insulin resistance and metabolic alterations are less severe. In addition, body weight is higher in patients with classical PCOS, thus suggesting that environmental factors may act as modifiers, possibly favouring the development of more severe forms of PCOS.

On the other hand, there are few data on the fourth phenotype, and there is a controversy on whether these patients should be considered part of the PCOS spectrum, as increased ovarian androgen production is considered essential for the development of the syndrome. AE-PCOS Society has suggested that this particular phenotype should be kept apart until more data are available. However, some recent reports have shown that patients with polycystic ovaries and chronic anovulation, but not hyperandrogenism present features similar to those of other PCOS patients (4,5,8). In fact, these patients have: higher luteinizing hormone (LH) and LH/follicle stimulating hormone (FSH) ratio and slightly higher insulin levels than healthy women and may be considered affected by a particular, milder form of PCOS (5,8). However, it is important to differentiate this subgroup of PCOS from patients with hypothalamic hypogonadism, who may present polycystic ovaries. It is suggested that a progesterone test should be performed before diagnosing PCOS in patients with no hyperandrogenism. Probably, only genetic studies may either confirm or exclude that all these different phenotypes are part of the same disorder.

The AE-PCOS Society also concluded that the PCOS phenotype is determined by the age of the women and consequently with the endocrine status (5). In summary, the PCOS phenotype is determined by the body mass index (BMI), the ethnic origin, the social status, the lifestyle of the patients and can therefore change through the reproductive age.

Low-birth weight, precocious pubarche and their relationship with the onset of polycystic ovary syndrome

Precocious pubarche (PP) is defined as the appearance of pubic hair before the age of 8 years in girls and 9 years in boys (9). Most cases of PP are because of an early and isolated maturation of the zona reticularis of the adrenal gland. The relationship between low-birth weight at birth (LBW) and PP remains controversial and it appears that the ethnic origin is involved. LBW has been found to be associated with increased dehydroepiandrosterone sulphate levels and PP in Spanish and Australian girls (10–17), but not with other populations (18,19).

Evidence indicates that LBW and PP are associated with endocrine-metabolic abnormalities including hyperinsulinaemia and dyslipidaemia of prepubertal onset, and functional ovarian hyperandrogenism and ovulatory dysfunction at adolescence (12,14–17,20). Genetic and environmental factors acting in complex multifactorial pathways underlie this sequence (13–15). The usual hypothesis proposed to explain the development of these long-term alterations involves the adaptive response to in-uterus malnutrition and modifications thereof; called 'Fetal Origins' (21).

In summary, considering that PP is associated with ovarian hyperandrogenism, decreased insulin sensitivity and LBW (12,20), PP is taken as an early marker of future pathologies during adult life (12–16,22).

Menarche marks the end of skeletal growth and the beginning of reproductive life. In girls, the onset of puberty is inversely related to the rate of progression through puberty (23). The less prepuberal height gain is usually compensated by more puberal growth. However, in girls who have experienced LBW followed by rapid catch-up growth in infancy, this compensatory mechanism fails and there is a higher secretion of suprarenal androgens (24). On the other hand, in a recent experience (25), it has been reported that PP represents a common and usually benign sign. However, as 26% of the patients studied had a pathological underlying condition, the authors suggest that all children with PP should be evaluated by a paediatric endocrinologist.

Among the factors contributing to PP, it has been reported that girls with PP show: (i) altered androgen receptor gene CAG repeat polymorphism (26); (ii) increased methylation of androgen receptor (27); (iii) increased levels of insulin and insulin growth factors (28,29); (iv) decreased insulin sensitivity during prepuberal and puberal stages (30); (v) reduced aromatase activity as a consequence of the CYP 19 gene variation (31); and (vi) alterations in other genes involved in steroidogenesis, such as 21-hydroxylase, 3-beta-hydroxysteroid dehydrogenase type 2, 5-alpha reductase and 11-beta-hydroxysteroid dehydrogenase (32).

In summary, the relationship between LBW and the development of PP or PCOS during the adult life remains controversial. It is clear that the genes related to steroidogenesis are altered in girls with PP. However, it appears that obesity – as a compensatory mechanism of LBW during the first years of life – represents a more determinant factor which could favour the onset of PCOS in genetically predisposed girls. Considering that PCOS phenotype can change through the reproductive age, together with the fact that girls and adolescents show a heterogeneous ovarian morphology, it appears that the diagnosis of PCOS should be made after the age of 18 years.

Low-birth weight and smallness for gestational age as early markers of insulin resistance

In 1989, Barker et al. (33) reported that the systolic blood pressure during adult life was inversely related to birth weight. This association – independently of the gestational age – was attributed to reduced foetal growth and was the first evidence that the intrauterine environment influences both the blood pressure and cardiovascular diseases during the adult life. Later studies have demonstrated a strong association between LBW and the development of insulin resistance during the adult life (34–37). It appears that foetal programming for adaptation to an adverse intrauterine environment results in a rapid response to glucose metabolism characterised by lower insulin sensitivity in the uterus. Retrospective studies demonstrated that men and women with LBW can develop the syndrome X – *the small-baby syndrome* – during the adult life (35). Syndrome X is characterised by hypertension, dyslipidaemia, central obesity and type 2 diabetes, associated with excessive food intake (35).

Relationship between LBW and syndrome X

The glucose-insulin-IGF-I is the predominant axis involved in the nutrient availability to the foetus (38). In fact, under-nutrition is related to decreased IGF-I levels (39), and small-for-gestational age (SGA) newborns have significantly lower levels of both IGF-I and IGF-binding protein-3 (IGFBP-3) than appropriate for gestational age (AGA) newborns (40). Moreover, postnatal IGF-I concentrations increase slowly in childhood and predict the velocity of height during the subsequent year in healthy

children (41). In infants with intrauterine growth retardation, low cord IGF-I levels normalise rapidly after birth, but remain significantly reduced in children with intrauterine growth retardation at 2 years (40). At older ages, mean IGF-I levels are low in SGA children of both short and normal statures compared with healthy children born AGA (42–44). Recently, it has been reported that the alterations of the IGF-IGFBP-3 axis persist in adult life (45). Although the origin of this persisting alteration of the IGF-IGFBP axis in adulthood needs to be elucidated, its potential contribution to the long-term metabolic and cardiovascular complications associated with foetal growth restriction is accepted.

In summary, the foetal adaptation to an adverse intrauterine environment results in lower insulin sensitivity (an insulin resistance status) that affects the IGF-IGFBP axis. Then, this 'adaptative mechanism' results in the onset of the syndrome X during the adult life.

Development of polycystic ovary syndrome throughout life

Manifestations of PCOS can negatively impact different tissues throughout life (Table 2). The intrauterine microenvironment influences the foetal development and the future endocrine response during the adult life (33–35,37). Hyperinsulinaemia is a compensatory mechanism in response to insulin resistance (46). Paradoxically, the ovarian tissue responds to hyperinsulinaemia excessively leading to altered steroidogenesis, hyperandrogenism and anovulation (47,48). Therefore, hyperinsulinaemia impairs oocyte development (49).

On the other hand, the excess of androgens can alter other tissues. In fact, hyperandrogenism induces detrimental effects on endometrium resulting in miscarriage (50–52). It has also been reported that an excess of androgens can induce hyperplasia and endometrial cancer (53,54). In addition, a negative correlation between both androstenedione and testosterone and uterine glycodelin A levels has been reported in women with PCOS (50,51) and in early pregnancy (55,56). On the other hand, anovulatory PCOS patients have alterations in uterine vascularisation (57). In fact, the pulsatility and the resistance index of the uterine artery (two measures of blood impedance) are higher in PCOS patients compared with healthy controls and are detrimental factors for endometrial receptivity (58). The endometrial tissue is not only the target for steroid hormones but also for insulin, and the insulin receptor is cyclically regulated in normo-ovulatory women (59). Recently, it has been reported that insulin regulates endometrial stromal differentiation (decidualisation) (60). Therefore, hyperinsulinaemia downregulates hepatic IGFBP-1, resulting in elevated free IGF-I in the circulation (61).

In summary, elevated oestrogen (without the opposing effects of progesterone in the absence of ovulation), hyperinsulinaemia, elevated free IGF-I and androgens contribute to endometrial dysfunction, infertility, increased miscarriage rate, endometrial hyperplasia and endometrial cancer common in women with PCOS.

Finally, it has been reported that insulin acts as an embryotoxic agent. Preimplantatory mouse embryos exposed to high concentrations of IGF-1 or insulin undergo extensive apoptosis via down-regulation of the IGF-1 receptor (62).

In summary, hyperinsulinaemia, hyperandrogenism and the alterations of their related parameters are involved in the altered responses to steroidogenesis and ovulation, endometrial function, oocyte development and blastocyst implantation.

Table 2 Polycystic ovary syndrome development throughout the life

Tissue and function	Effect
Oocyte development	Hyperinsulinaemia affects oocyte cumulus and consequently oocyte capacity (49)
Foetal development	Intrauterine hyperandrogenism influences the foetal development and the future endocrine response during the adult life (33–37)
Endometrial uterine tissue	Hyperandrogenism induces detrimental effects of the endometrial response resulting in miscarriage (50–52)
Uterine tissue	Hyperandrogenism induces hyperplasia and endometrial cancer (53,54)
Uterine gland secretions	Hyperandrogenism decreases glycodelin A secreted by uterine glands during early pregnancy inducing miscarriage (50,51,55)
Uterine vascularisation	Anovulatory PCOS patients have detrimental blood impedance of uterine artery (57) and poor uterine receptivity (58)
Endometrial decidualisation	Hyperinsulinaemia impairs endometrial stromal differentiation (60)
Embryo	Hyperinsulinaemia acts an embryotoxic agent (62)
Cardiovascular risk	Women with PCOS show increased cardiovascular risk (8,66–69)

PCOS, polycystic ovary syndrome.

Androgen excess and cardiovascular risk

Cardiovascular diseases represent a major cause of death in most developed countries; however, the onset of cardiovascular diseases (including coronary heart disease, cerebrum-vascular disease and peripheral vascular disease) is somewhat delayed in women than in men (63). Considering that this delay is of about 10–15 years, the Third Report of the National Cholesterol Education Program in the Adult Treatment Panel III, 2002 (64) defined 55 and 45 as the risk-factor age in women and men respectively. It has been suggested that differences in the hormonal status and mainly in androgen levels may explain this gender disparity (65). Consistently with this hypothesis, it has been shown that women with PCOS have a higher cardiovascular risk despite their age (8, 66–69) (Table 2). In fact, at the age of 20–30, women with PCOS have increased cardiovascular risk and this finding has been consistently confirmed across several geographic areas and ethnic groups (70). About 50–60% of young patients with PCOS show dyslipidaemia, low levels of high density lipoprotein (HDL)-cholesterol and increased levels of low density lipoprotein (LDL)-cholesterol and triglyceride (71,72). Moreover, non-classical serum markers of cardiovascular risk (such as C-reactive protein and homocysteine) are increased and early signs of clinical and subclinical atherosclerosis (carotid intima-media thickness, coronary artery calcium) are also altered in PCOS patients (73).

However, whether the greater cardiovascular risk found in women with PCOS is because of excess of androgens needs to be addressed. In fact, PCOS women present insulin resistance, hyperinsulinaemia and abdominal obesity, all of which cause increased cardiovascular risk (3). To clarify this point, Rizzo et al. (74) studied the prevalence of several cardiovascular risk factors (including obesity, insulin resistance, dyslipidaemia and elevated levels of C-reactive protein) in hyperandrogenic women with different phenotypes: women with classical PCOS, ovulatory PCOS or idiopathic hyperandrogenism. The authors found that serum androgen levels were similar in all groups, but that patients with idiopathic hyperandrogenism did not show increased cardiovascular risk factors. In addition, the women with the classical PCOS phenotype (with higher BMI and increased prevalence of insulin resistance) showed dyslipidaemia, elevated levels of C-reactive protein and early signs of atherosclerosis. The authors also reported pathological values of carotid intima-media thickness in 15% of young women with classical PCOS, but not in those with idiopathic hyperandrogenism.

In summary, all these findings led to conclude that androgens have a limited role in cardiovascular risk in women with PCOS and that other features of the syndrome (including obesity, insulin resistance and abdominal obesity) seem to play a major role in cardiovascular risk of women with PCOS.

Need for hepatic evaluation in the polycystic ovary syndrome

Non-Alcoholic Fatty Liver Disease (NAFLD) is the new scientific name for simple hepatic steatosis, a common disorder of the liver function that in the USA has the highest incidence (45%) in Hispanic women (75). Although NAFLD is considered a benign disorder, 10% of patients develop liver injury and necro-inflammation (non-alcoholic steatohepatitis or NASH) and up to 20% of NASH subjects may progress to more advanced liver disease (liver cirrhosis or cancer) (76). It has been reported that increased circulating inflammatory factors and low circulating adiponectin may trigger the appearance of NASH in patients with NAFLD (77,78).

Obesity and metabolic syndrome are the main causes of NAFLD (79) and some components of metabolic syndrome, as low HDL-cholesterol levels and hypertriglyceridemia, are common in patients with fatty liver. Moreover, NAFLD is considered to be the hepatic consequence of metabolic syndrome (80). Considering that PCOS is the most common cause of metabolic syndrome, it is not surprising that NAFLD is very common in patients with this ovarian disorder (81). It has been reported that 41–55% of PCOS women show NAFLD (81–83). Although women with PCOS show increased levels of inflammatory factors and low adiponectin concentration (84,85) – both risk factors for NASH – no data on prevalence of NASH and liver cirrhosis in PCOS are available.

In summary, data presented in the Symposium led to conclude that: (i) PCOS patients with obesity and/or metabolic syndrome should be screened for NAFLD; (ii) liver sonography should be performed and patients with NAFLD aminotransferase levels should be assessed; (iii) increased levels of liver enzymes may suggest the progression to more severe liver diseases; (iv) several issues regarding the association between PCOS and liver diseases need to be clarified (particularly the risk of PCOS patients to develop NASH); (v) in patients with NAFLD it is essential to treat metabolic syndrome by improving lifestyle (diet and exercise); (vi) patients with increased liver enzymes need a special care and, in some instances, insulin-sensitising drugs may be

needed; (vii) if after improving body weight and metabolic alterations, increased liver enzyme levels persist, a liver biopsy is recommended.

Treatments in the polycystic ovary syndrome

Lifestyle modifications

It has been established that obesity – one of the risk factors for reproductive failure – has to be corrected prior to treatment initiation. It is well known that obesity is associated with anovulation (86), pregnancy loss (87) and late pregnancy complications (pre-eclampsia, gestational diabetes) (88). Obesity in PCOS is linked to failure or delayed response to the various treatments including clomiphene citrate (CC) (89–91), gonadotropins (92,93) and laparoscopic ovarian diathermy (94). Weight loss is associated with improved spontaneous ovulation rates in women with PCOS (86,95), and spontaneous pregnancy has been reported after losing as little as 5% of initial body weight (96). Therefore, the weight loss prior to conception improves live birth rate in obese women with or without PCOS (95).

The treatment of obesity may include lifestyle therapy (diet and exercise), pharmacological treatment and bariatric surgery (97–99). In summary, weight loss is considered the first-line therapy in obese women with PCOS seeking pregnancy (Table 3).

Clomiphene citrate treatment

The aim of ovulation induction for women with anovulatory PCOS is to restore fertility and achieve a singleton live birth. This premise is difficult to reach in patients with PCOS as multiple intrinsic factors associated with the syndrome induce ovarian hyperstimulation. The associated factors to be considered are obesity, hyperandrogenemia, hyperinsulinaemia and insulin resistance (91).

Table 3 Treatment of polycystic ovary syndrome

Treatment	Application
Lifestyle modification	Obesity is associated with anovulation (86)
Weight loss and exercise are considered as the first-line therapy before treatments	Obesity in PCOS is linked to the fail of treatments (I89–93)
	Weight loss is associated with improved spontaneous ovulation rates in women with PCOS (86,95)
	Spontaneous pregnancy after losing 5% of initial body weight (96)
Clomiphene citrate (CC)	CC blockades the negative feedback mechanism of FSH
Is the first choice to induce ovulation	It is recommended 50–250 mg CC per day for 5 days starting from 2 to 5 days of spontaneous or induced bleeding
	Successful ovulation is 70–85% and pregnancy 40–50% (101–104)
Insulin-sensitising agents	Insulin-sensitising drugs (metformin is the most common) induces ovulation by improving insulin sensitivity (107,118,119)
Is the second choice to induce ovulation in women who did not respond to CC	Metformin is a safe effective and non-teratogenic agent (belongs to the group B drug), increases pregnancy rates and avoids miscarriage (112–117,120)
Gonadotrophins	The significantly higher hyperstimulation rate, the associated risk of multiple pregnancies and the cost do not currently justify the routine use of gonadotrophins (122–129)
This treatment is based on the concept that initiation and maintenance of follicle growth depend on FSH concentrations	
Statins	Statins produce several beneficial effects over and above the reduction in plasma cholesterol levels. In addition, it has been suggested that simvastatin treatment may also lead to improvement of hypothalamo-pituitary function (130,131)
Statins improve endocrine/clinical aspects of PCOS by regulating the lipid profile. The mechanisms of action on inhibition of testosterone levels are related to inhibition of the mevalonate pathway	
Aromatase inhibitors	The treatment with aromatase inhibitors may be considered in a subset of PCOS patients who are clomiphene citrate resistant or those who, after discussion of risks and benefits, are not candidates for clomiphene citrate, gonadotropins or GnRH analogues (132–135)
They have ovulation-inducing effects by inhibiting androgen-oestrogen conversion	
Laparoscopy ovarian surgery (LOS)	The main indication for LOS is CC resistance in women with anovulatory PCOS. LOS also may be recommended for patients who persistently hypersecrete LH, either during natural cycles or in response to CC, because it may reduce LH secretion (136–138)
In vitro Fertilisation (IVF)	After failure of weight reduction, anti-oestrogen therapy, LOS and ovarian stimulation, then, IVF could be the final option (139,140,142)

PCOS, polycystic ovary syndrome.

Speakers of the Symposium are in agreement that CC must be the first choice to induce ovulation in anovulatory women with PCOS. The administration of 50–250 mg CC per day for 5 days starting from day 2 to 5 of spontaneous or induced bleeding is recommended. 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. Successful ovulation has been reported in 70–85% of treated women, resulting in a pregnancy rate of 40–50% (100,101). If no ovulation is induced after three consecutive treatments of increased doses of CC, this group of patients is considered as 'clomiphene-resistant'.

The mechanism of CC action is not entirely known, but it is thought to involve the blockade of the negative feedback mechanism that results in increased secretion of FSH. There is no agreement in the rates of ovulation and pregnancy reached after CC treatment, but it is accepted that the conception rate alone is 22% (102–104).

It has also been studied that a group of PCOS patients who were able to ovulate after CC treatment showed early miscarriage as a consequence of a failure in the implantatory process (105). In this group of PCOS patients, CC induced aberrant endometrial β_3 integrin expression and the failure of the down-regulation of progesterone receptor during the window of implantation (105).

Insulin-sensitising agents

It has been largely demonstrated that insulin resistance contributes to oligoovulation and infertility (106–108). In addition, insulin resistance likely contributes to the increased incidence of gestational diabetes among women with PCOS and may also contribute to the increased incidence of early miscarriage reported for the syndrome (109–111).

Numerous studies now confirm that administration of the insulin-sensitising drug metformin improves insulin sensitivity in women with PCOS and leads to an increased frequency of ovulation and improved fertility (107,112–120, among others) (Table 3).

It is agreed that to understand the role of metformin for the treatment of infertility in PCOS, it is critical to note the distinctly different pharmacological characteristics of metformin vs. CC, the standard drug for ovulation induction. CC is specifically a fertility drug that acts directly to induce ovulation and carries up to 10% chance of multiparity, and its onset of action is rapid (100,101). In contrast, metformin is a drug that affects metabolism and acts indirectly to induce ovulation by reducing the circulating concentration of insulin (107). The onset of action of metformin is slower and gradual: up to 6 months of treatment with metformin may be needed to clinically improve ovulation (118). Given the differences in action and pharmacodynamic properties, CC would be expected to be more effective than metformin in *rapidly* inducing ovulation in women with PCOS. Therefore, in a woman who desires pregnancy immediately, a rapidly acting induction agent such as CC would be most appropriate.

On the other hand, a recent meta analysis of 17 rigorously conducted studies performed between 1996 and 2007 that included 1639 subjects documented that ovulation is improved in many women with PCOS when treated with metformin (119). Menstrual cyclicity and ovulation improve in approximately 69% of women with PCOS treated with metformin, with 88% of responders achieving normal menstrual cyclicity. It thus seems reasonable to first use metformin to treat in a woman with PCOS with a longer time line for pregnancy, as metformin does not carry the risk of multiparity associated with CC. If after six or more months of treatment with metformin ovulation has not improved, treatment with CC remains an option.

Combined treatment of clomiphene citrate plus metformin

It remains controversial whether metformin should be added to CC to enhance ovulation and fertility. The meta analysis noted earlier reported that the addition of metformin to CC significantly increases both the ovulation and pregnancy rates in women with PCOS and 'showed a favourable effect of the combination therapy over CC alone for live births', although not statistically significant. In addition, the results of the recent multicenter Pregnancy in Polycystic Ovary Syndrome (PPCOS) Trial evidence that the addition of metformin to CC improves ovulation (121). It is noteworthy that, in the PPCOS Trial, adding metformin to CC increases the cumulative ovulation rate from 49.0% to 60.4% (CC alone vs. the combination of the two drugs respectively; $p = 0.003$), thus confirming the beneficial effect of metformin on ovulation induction with CC. The improvement in the ovulation rate is not associated with a statistically significant increase in the live birth rate (22.5% with CC alone vs. 26.8% with a combination of the two drugs; $p = 0.31$). However, this 4.3% higher live birth rate in the combination group may have represented a true benefit of metformin that the study was underpowered to detect.

There are several possible explanations that can account for the fact that the addition of metformin to CC increases ovulation rate, but not the live birth rate. While there are several possibilities, a straightfor-

ward explanation is the fact that not every cycle of ovulation induction is equivalent. Induction with CC often results in the recruitment and ovulation of multiple follicles per cycle, whereas induction with metformin typically results in ovulation of a single follicle per cycle. Ovulation of multiple follicles per cycle increases the odds of conception, but with a risk of multiparity. This idea is supported by the observation that in the PPCOS Trial, the multiparity rate was 6% (including one set of triplets) in the CC alone group compared with a rate of 0% in the metformin group. Interestingly, the multiparity rate in the combination group was intermediate at 3%, suggesting that the addition of metformin to CC may have reduced the number of follicles recruited per cycle, without adversely affecting the live birth rate because of the increase in cumulative ovulations. Unfortunately, ultrasounds were not obtained in the study, and the possible utility of adding metformin to CC to decrease multiple births remains untested and speculative.

Finally, for those women with a short-term, but not immediate desire for pregnancy, consideration should be given to pretreatment with metformin prior to adding CC as appropriate. This approach may offer two advantages. First, as the onset of action of metformin is gradual, pretreatment with metformin for two or more months prior to adding CC may be associated with rates of ovulation and live birth higher than the treatment with the two drugs simultaneously. Second, a major problem of pregnancy in PCOS is that the women are frequently obese, decreasing the efficacy of CC and increasing the risks of pregnancy-related complications, such as gestational diabetes and pre-eclampsia. While controversial, metformin may facilitate weight loss in some women, especially when coupled with diet and exercise. Pretreatment for several months with the combination of metformin, calorie-restricted diet and exercise may result in patients with less overweight in whom induction of ovulation with CC is likely to be more successful and in whom the risk for pregnancy-related complications is reduced.

Given the above observations, the conclusions were that metformin remains an important therapeutic option in the pharmacological treatment of infertility in PCOS, and its use should not be restricted to women with glucose intolerance, as recommended by the ESHRE/ASRM consensus statement. Taking into consideration the different time lines for achieving pregnancy among women with PCOS, it has been suggested that:

- In women with PCOS who desire a rapid establishment of pregnancy, CC should be the first-line agent.
- In women with PCOS for whom pregnancy is a goal at a more distant time (> 6 months), initial treatment with metformin, combined with diet and exercise, is an option to induce ovulation.

Gonadotropin treatment

Ovulation induction using gonadotropin therapy is based on the physiological concept that initiation and maintenance of follicle growth may be achieved by a transient increase in FSH above a threshold dose to generate a limited number of developing follicles. For this reason, the risk to use gonadotropins to induce ovulation in women with PCOS is the multiple follicle development (122,123).

The recommended starting dose of gonadotropin is 37.5–50.0 IU/day. The duration of gonadotropin treatment should not exceed six ovulatory cycles. Intense ovarian response monitoring is required to reduce complications. Currently, two low-dose regimens are used: *Step-up regimens*: They are on the principle of a stepwise increase in FSH supply to determine the FSH threshold for follicular development. To further reduce the risk of ovarian hyperresponsiveness, the duration of the initial dose of FSH is extended (from 7 to 14 days) and the weekly dose increase is reduced (from 100% to 50% of the dose), leading to the so-called 'chronic low-dose regimen (124). *Step-down regimen*: This regimen is designed to achieve the FSH threshold through a loading dose of FSH with a subsequent stepwise reduction as soon as follicular development is observed in the ultrasound (125). It remains controversial whether a step-up or a step-down regimen is more appropriate. It has been reported that both achieve similar high rates of monofollicular development (126) and by the other side that a step-up regimen is safer in terms of monofollicular development (127). In addition, it appears that a step-down regimen requires more experience and skill compared with a low-dose step-up regimen (128). Recently, a combined approach of sequential step-up and step-down regimens has been shown to help reduce the risk of over-response (129).

In summary, the significantly higher hyperstimulation rate, the intense ovarian response monitoring required to reduce complications, 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 (Table 3).

Statins

As it was discussed in a previous section, at the age of 20–30, women with PCOS have increased cardiovascular risk independently of geographic areas and

ethnic groups (8,66–70). About 50–60% of young patients with PCOS show dyslipidaemia, low levels of HDL-cholesterol and increased levels of LDL-cholesterol and triglyceride (71,72). Several beneficial effects produced by statins over and above the reduction in plasma cholesterol levels, the so-called 'pleiotropic effects' of statins, have been described. In a prospective, crossover trial of 48 women with PCOS, it has been recently reported that statin simvastatin improves endocrine/clinical aspects of PCOS by regulating the lipid profile (130). The mechanisms of action of simvastatin on inhibition of testosterone levels are related to inhibition of the mevalonate pathway (130). In addition, it has been suggested that simvastatin treatment may also lead to improvement of hypothalamo-pituitary function (130). However, Rizzo et al. (131) reported that statins had a lower impact on atherogenic lipoprotein phenotype showing a moderate beneficial effect. In summary, statins represent a new tool of treatment of PCOS directed to improve the lipid profile and markers of systemic inflammation.

Aromatase inhibitors

Evidence suggests that non-steroidal aromatase inhibitors, letrozole and anastrozole, may have ovulation-inducing effects by inhibiting androgen-oestrogen conversion. Select trials with aromatase inhibitors have demonstrated efficacy for increased endometrium thickness and ovulation and pregnancy rates when used in CC resistant or treatment-naïve patients (132). Letrozole appears to be a suitable ovulation-inducing agent in PCOS women with CC failure and was found to be most effective when baseline oestradiol level > 60 pg/ml (133–135).

Conclusions: further trials comparing aromatase inhibitors with CC are necessary before aromatase inhibitors can be recommended routinely for ovulation induction in women with PCOS and infertility. However, aromatase inhibitors may be considered in a subset of this population, specifically women who are CC resistant or those who, after discussion of risks and benefits, are not candidates for CC, gonadotropins or GnRH analogues.

Laparoscopic ovarian surgery

Laparoscopic ovarian surgery (LOS) was developed from the traditional wedge resection to modern-day minimal access techniques, usually using laparoscopic ovarian diathermy, known as 'ovarian drilling' (136). The main indication for LOS is CC resistance in women with anovulatory PCOS. LOS may also be recommended for patients who persistently hypersecrete LH, either during natural cycles or in response to CC, because LOS may reduce LH secretion. In addition, LOS may be useful in anovulatory women with PCOS who need laparoscopic assessment of their pelvis or who live too far away from a hospital for the intensive monitoring required during gonadotropin therapy. In approximately 50% of LOS-treated women, adjuvant therapy is required. In these women, the addition of CC can be considered after 12 weeks if no ovulation is detected (137). The addition of FSH should be considered after 6 months (137). Five randomised controlled trials compared the effectiveness of LOS with that of gonadotropins for women with CC-resistant PCOS and did not show a difference in ongoing pregnancy rate or live birth rate (137,138).

In summary, LOS achieves unifollicular ovulation with no risk of ovarian hyperstimulation syndrome or high-order multiparity. Intensive monitoring of follicular development is not required after LOS. LOS is an alternative to gonadotropin therapy for CC-resistant anovulatory PCOS. The risks of surgery are minimal and include the risk of the laparoscopy itself, adhesion formation and destruction of normal ovarian tissue. Irrigation with an adhesion barrier may be useful, but there is no evidence of efficacy from prospective studies.

Assisted reproduction techniques: *in vitro* fertilisation

In principle, anovulation is not an indication for *in vitro* fertilisation (IVF). The logical therapy for women with PCOS is the induction of ovulation. The main complication of ovulation induction is the occurrence of a 10% multiple pregnancy rate, especially after the use of gonadotropin therapy. For this reason, the use of gonadotropins may be questioned (139). After failure of weight reduction, the different discussed treatments or LOS, it may be argued that induction of ovulation with exogenous gonadotropin therapy should be omitted and replaced by ovarian stimulation and IVF (140). By using IVF with single-embryo transfer, the risk of multiple pregnancies is markedly reduced (141). In women with PCOS who do have associated pathologies, IVF is indicated, such as in case of tubal damage, severe endometriosis, preimplantation genetic diagnosis and male factor infertility (142) (Table 3).

Conclusions

- The diagnosis of PCOS may be assigned to patients presenting four different phenotypes (Table 1).
- Data on the fourth phenotype are few and there is a controversy whether these patients should be considered part of the PCOS spectrum, as ovarian

androgen increased production has been considered essential for the development of the syndrome.

• The AE-PCOS Society has suggested maintaining the fourth phenotype separated until more data are available.

• Probably, only genetic studies may either confirm or exclude that all these different phenotypes are part of the same disorder.

• PCOS phenotype could change through the reproductive age.

• The relationship between LBW and the development of PP or PCOS during the adult life remains controversial and it appears that the ethnic origin is involved. However, genes related to steroidogenesis are altered in girls with PP.

• Considering that PCOS phenotype could change through the reproductive age, together with the fact that girls and adolescents show heterogeneous ovarian morphology, it appears that the diagnosis of PCOS in girls with LBW and/or PP has to be made after 18 years.

• As hyperinsulinaemia, hyperandrogenism and the alterations of their related pathways are involved in the altered responses to steroidogenesis, ovulation, endometrial function, oocyte development and blastocyst implantation, the polycystic syndrome may develop throughout all the reproductive life.

• Although several cardiovascular risk factors (including obesity, insulin resistance, dyslipidaemia and elevated C-reactive protein plasma levels) are present in hyperandrogenic women with different phenotypes of PCOS, the role of androgens on cardiovascular risk in women with PCOS still needs to be fully addressed.

• PCOS patients with obesity and/or metabolic syndrome should be screened for NAFLD.

• PCOS women present both risk factors for NASH and increased inflammatory factors, such as low adiponectin levels; however, no data are available on the prevalence of NASH and liver cirrhosis in PCOS.

• Lifestyle therapy which comprises diet and exercise is necessary prior to selection of the adequate treatment.

• Weight loss is recommended as the first-line therapy in obese women with PCOS seeking pregnancy.

• The recommended first-line treatment for ovulation induction remains to be the anti-oestrogen CC.

• Metformin use in PCOS should not be restricted to women with glucose intolerance.

• Given the differences in action and pharmacodynamic properties, CC is expected to be more effective than metformin in rapidly inducing ovulation in women with PCOS.

• 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. Essentially to decrease the cardiovascular risk described in PCOS patients.

• The treatment with aromatase inhibitors may be considered in a subset of PCOS patients who are CC resistant or those who, after discussion of risks and benefits, are not candidates for CC, gonadotropins or GnRH analogues. However, further trials comparing aromatase inhibitors with CC are necessary before aromatase inhibitors can be recommended routinely for ovulation induction in women with PCOS and infertility.

• LOS is a modern resection technique known as 'ovarian drilling'. The main indication of LOS is CC resistance in women with anovulatory PCOS, patients who persistently hypersecrete LH and patients who need laparoscopic assessment of their pelvis.

• After failure of weight reduction, anti-oestrogen therapy, LOS and ovarian stimulation, IVF could be the final option.

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