# The polycystic ovary syndrome: a position statement from the European Society of Endocrinology

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## Abstract

Polycystic ovary syndrome (PCOS) is the most common ovarian disorder associated with androgen excess in women, which justifies the growing interest of endocrinologists. Great efforts have been made in the last 2 decades to define the syndrome. The presence of three different definitions for the diagnosis of PCOS reflects the phenotypic heterogeneity of the syndrome. Major criteria are required for the diagnosis, which in turn identifies different phenotypes according to the combination of different criteria. In addition, the relevant impact of metabolic issues, specifically insulin resistance and obesity, on the pathogenesis of PCOS, and the susceptibility to develop earlier than expected glucose intolerance states, including type 2 diabetes, has supported the notion that these aspects should be considered when defining the PCOS phenotype and planning potential therapeutic strategies in an affected subject. This paper offers a critical endocrine and European perspective on the debate on the definition of PCOS and summarises all major aspects related to aetiological factors, including early life events, potentially involved in the development of the disorder. Diagnostic tools of PCOS are also discussed, with emphasis on the laboratory evaluation of androgens and other potential biomarkers of ovarian and metabolic dysfunctions. We have also paid specific attention to the role of obesity, sleep disorders and neuropsychological aspects of PCOS and on the relevant pathogenetic aspects of cardiovascular risk factors. In addition, we have discussed how to target treatment choices based according to the phenotype and individual patient's needs. Finally, we have suggested potential areas of translational and clinical research for the future with specific emphasis on hormonal and metabolic aspects of PCOS.

Published by Bioscientifica Ltd.

## Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of women of reproductive age (1). Its high prevalence has attracted significant public attention ($>1.5\times10^6$ sites dedicated to the syndrome) and, in addition, identification and management of PCOS have been estimated to cost the USA healthcare system $4 billion annually (2). Similar data are not available for Europe. PCOS is a complex endocrine condition, due to its heterogeneity and uncertainty about its aetiology. The diverse nature of PCOS was evident even from the first description of the syndrome by Stein & Leventhal (3), who in their original report described seven women with variable clinical characteristics (i.e. obesity, hirsutism, acne and amenorrhoea) associated with enlarged bilateral polycystic ovaries. Following an international meeting in 1990, held at the U.S. National Institute of Health (NIH), it was recommended that the diagnostic criteria for PCOS should comprise the concomitant presence of anovulation and evidence of hyperandrogenaemia – biochemical, clinical (hirsutism/acne) or both – but without reference to ovarian morphology (4).

In order to provide a more inclusive definition of the syndrome, the report of a meeting of experts at a joint ESHRE/ASRM meeting held in Rotterdam in 2003 proposed that the presence of two of the three criteria (chronic anovulation (CA), hyperandrogenism and polycystic ovaries on ultrasonography) would be sufficient for PCOS diagnosis (5). Nevertheless, disagreements remained as clearly illustrated in a study conducted in 2005, in which it was found that the majority of gynaecologists considered that polycystic ovaries on ultrasound was an essential tool for PCOS diagnosis, whereas the endocrinologist's view was more focused on hirsutism and anovulation (6). In 2006, the Androgen Excess PCOS Society (AEPCOS) suggested a compromise between the two sets of diagnostic criteria, arguing that PCOS is mainly a hyperandrogenic disorder and the existence of hirsutism/acne and/or hyperandrogenaemia constitutes a *sine qua non* for PCOS diagnosis (7). The second criterion essential for the diagnosis according to AEPCOS could be either CA or polycystic ovarian morphology.

The fact that PCOS is often characterised by the presence of insulin resistance and associated hyperinsulinaemia and most of the patients in clinical series are overweight or obese is significant (8). These factors may play an important role in the pathogenesis of androgen excess and the susceptibility to develop earlier than expected glucose intolerance states and type 2 diabetes (T2D) (9). In 2011, the Amsterdam ESHRE/ASMR-sponsored 3rd PCOS Consensus Workshop Group (10) identified different phenotypes, and separated the most classic phenotype, characterised by hyperandrogenism and CA, from those characterised by ovarian dysfunction and polycystic morphology. It was also suggested that major metabolic disorders should be addressed in the clinical workup while defining the PCOS phenotype in each individual patient.

Since PCOS is a very common disorder (the prevalence ranges from 6 to 20% depending on the criteria used), it would be helpful to have unity about the diagnostic criteria (11). Some progress has been made towards that goal by the recommendations of the Expert Panel following the NIH (USA) Evidence-based Methodology Workshop on PCOS in December 2012, whose major results have been summarised and presented very recently (12, 13). While supporting the Rotterdam definition (5) as the most inclusive and appropriate in a global context, it was suggested that a more appropriate, less 'ovary-centric' name for the syndrome should be considered. It is therefore a timely opportunity for the European community of endocrinologists to discuss where future efforts in research and clinical management should be focused.

## The debate on the definition of PCOS

Nowadays, a common perception among medical experts dealing with the syndrome is that the name PCOS constitutes a distraction that impedes progress, and that this name does not reflect the complex interactions that characterise the syndrome (13). Furthermore, the emergence of new definitions with the use of ovarian morphology, besides CA and hyperandrogenism, as diagnostic criteria has increased the phenotypic variety of PCOS presentation. However, the NIH Experts Panel (12) recommended the maintenance of the broad diagnostic criteria of Rotterdam (5), but focused on the need for specific identification of the phenotype of each patient. By using the possible combinations of these criteria, four different phenotypes of PCOS are now identified: i) hyperandrogenism (clinical or biochemical) and CA (H-CA); ii) hyperandrogenism and polycystic ovaries on ultrasound (PCOm) but with ovulatory cycles (H-PCOm); iii) CA and polycystic ovaries without hyperandrogenism

(CA-PCOm); and, finally, iv) hyperandrogenism, CA and polycystic ovaries (H-CA-PCOm). The identification of specific phenotypes in women with PCOS seems to be justified from the metabolic point. In contrast to CA, metabolic abnormalities may dominate the syndrome throughout the subject's lifespan, although no data regarding adolescence are available (1). Several research groups have suggested that the origin of PCOS lies in foetal life and involves the foetal programming of metabolic/endocrine axes, especially carbohydrate metabolism and adrenal secretion (14, 15, 16, 17, 18). Indeed, girls born small for gestational age (SGA) or large for gestational age, these being an indirect index of exposure to stressful intrauterine conditions, manifest a high incidence of PCOS in adolescence (19). Furthermore, in girls with early adrenal androgen secretion clinically disclosed as premature pubarche, that is a different clinical entity with respect to PCOS, several components of PCOS have been found, such as insulin resistance and visceral adiposity, in comparison with their normal peers (20). On the other hand, it has been suggested that a few patients with premature pubarche could develop PCOS later in their life (21). In addition, an increased proportion of these girls develop PCOS in adolescence, indicating a common pathogenetic pathway of these two nosologic entities. Also of interest, girls born SGA who develop premature adrenarche have a significantly higher tendency to develop full-blown PCOS in adulthood compared with other girls who express only one of these two conditions (19). These observations suggest that exposure of a female to harmful events during foetal life and the peripubertal period may considerably affect her metabolic, hormonal and reproductive phenotype.

In addition, some of the available reports also imply that PCOS sequelae continue post-menopause (22, 23, 24, 25), namely in subjects diagnosed with the strict NIH criteria (4). Specifically, the existing unfavourable metabolic/hormonal milieu associated with a cluster of several cardiovascular (CV) risk factors such as oxidative stress, dyslipidaemia, subclinical inflammation and impaired fibrinolysis, is translated to increased CV incidents in these women, compared with their BMI-matched control peers. These findings have been challenged by recent data from the Study of Women's Health across the Nation (SWAN), a longitudinal cohort study aimed at determining the impact of menopause on the cardiometabolic profile. The authors analysed the impact of menopause on the incidence of the metabolic syndrome in women with high levels of androgen and a history of menstrual irregularity (26). Baseline analysis of 2543 pre- and perimenopausal women originally included in the SWAN study indicated that hyperandrogenaemia but not oligomenorrhoea was independently associated with the risk of prevalent metabolic syndrome (27). In the prospective SWAN study, the authors found that among metabolic syndrome-free women at baseline, 497 new cases were identified during 20 249 woman-years of follow-up over 12 years. Women with hyperandrogenaemia and oligomenorrhoea, key features of PCOS according to the NIH criteria (4), developed incident cases of metabolic syndrome at a comparable rate to their counterparts, and there was no significant difference in incidence of self-reported stroke or myocardial infarction by hyperandrogenaemia/oligomenorrhoea status, suggesting that these factors are not associated with worsening of metabolic health after menopause. However, it should be kept in mind that women with the metabolic syndrome present before menopause had been excluded from the study.

With respect to metabolic profile and CV risk factors, several studies suggested that women with PCOS based on the NIH criteria (4) exhibit a more detrimental profile compared with milder phenotypes (10, 11). Usually, women with classic PCOS are characterised by higher body weight, but when comparisons were made between groups matched for age and BMI, it was obvious that the degree of dyslipidaemia, central adiposity, insulin resistance and metabolic syndrome prevalence was significantly higher in women with the classic (or more severe) PCOS phenotype. The prevalence of metabolic syndrome and degree of insulin resistance in the milder phenotype (oligo-anovulatory patients with PCOm but without hyperandogenaemia), although elevated (28), are closer to control subjects than to the other three phenotypes (29). Specifically, women with this phenotype usually display normal insulin sensitivity and a metabolic profile similar to age- and BMI-matched normal women. On the other hand, a recent study evaluating not only CV risk factors but also carotid intima–media thickness reported that in women with PCO and hyperandrogenaemia, the CV risk was lower than that in other classic phenotypes (5). In agreement with the above, Amato *et al*. (30) found that oligomenorrhoea was associated with the visceral adiposity index, a marker of visceral adipose dysfunction, and a CV risk factor. These findings are in disagreement with the general belief that phenotypes with androgen excess have the highest CV risk.

However, one may hypothesise that the above controversial data arise due to the nature of PCOS, since women with hyperandrogenaemia and polycystic morphology may later develop anovulation, especially if they gain weight (18), although in a subgroup of patients the phenotype in itself seems to ameliorate in ageing PCOS women (31). This logical approach underlines the gap in understanding regarding the nature of the syndrome based on available data.

One of the major problems with PCOS definition based on the Rotterdam criteria (5) is the lack of natural history of PCOS. In fact, no consensus exists on how to define this disorder during early and late adolescence, nor during and after menopause (18, 31). In addition, it is not known whether women transfer from one phenotype to another, and specifically from ovulatory to anovulatory PCOS, and how this transition affects their health status in the long term. If the answer to the above question is affirmative, then it can be postulated that women who have presented once with a mild phenotype may at a later stage of their life develop a worse and severe phenotype, with the known adverse sequelae. Although core data answering these questions are thus far not available, it is hypothesised from the pathophysiogical point of view that women can transfer from one phenotype to another depending on their exposure to several factors, such as increment of body weight, dietary intake and exercise habitus (18, 32).

The most astonishing aspect showing the variability of PCOS regarding metabolic derangements is T2D. The current perspective is that women with the syndrome develop carbohydrate metabolism disturbances, such as impaired glucose tolerance (IGT) and T2D over the years (9, 33). A careful examination of available data on which this notion was based shows clearly that this gradual deterioration of glucose is almost only ever observed in obese women with PCOS. Indeed, the prospective studies in which increased susceptibility to IGT and/or T2D has been reported were based on significantly obese patients with very high BMI values ($>30\,kg/m^2$) (9, 33, 34, 35). However, studies conducted in overweight or normal weight patients did not report increased evolution from normoglycaemia to T2D, although occasional cases have been reported (32, 33, 34).

Moreover, it must be borne in mind that although lean women with PCOS display intrinsic insulin resistance, the degree of insulin resistance is not comparable to their obese control peers. Hence, obesity *per se* seems to be the critical risk factor for development of insulin resistance and one may hypothesise that T2D occurrence

in women with PCOS may be an epiphenomenon due to increased body weight, since obesity and PCOS often coincide (33). This hypothesis was, in fact, put forward by several research groups (34, 35, 36, 37), which reported a significantly higher prevalence of PCOS in overweight and obese women compared with their lean peers, although some studies did not confirm this finding (38). In addition, it has been shown that women with PCOS seeking medical advice are significantly heavier that their peers living in the community and dealing with the syndrome without medical assistance (39). This finding may bias medical experts' opinion of the syndrome, since they are dealing with the most serious forms of the disorder.

The design of prospective follow-up studies from adolescence to menopause of women with different PCOS, will provide a definitive answer to the impact of different phenotypes in the metabolic profile. It must be underlined that this issue is of utmost importance given that, with the use of the new criteria and also depending on the population recruited, 20–25% of women carrying a PCOS diagnosis may have ovulatory PCOS, and a percentage ranging from 10 to 20% may suffer from non-hyperandrogenic PCOS (13).

## Aetiology of PCOS: foetal life, birth weight, neonatal and childhood events

There are no certainties about the origin of PCOS (16), and a variety of hypotheses about either the genetic or the environmental origins of PCOS have been postulated. As reported above, the PCOS phenotype can be found from early infancy to puberty, based on predisposing environmental influences and genetic factors (18). There is some evidence that PCOS may partly depend on genetic factors (17). However, it is unlikely that PCOS represents a single gene defect and it is more likely to be polygenic or oligogenic (40, 41). On the other hand, low birth weight and foetal exposure to androgens may contribute to development of the PCOS phenotype (39). In addition, low birth weight is particularly associated with insulin resistance and obesity in adulthood (42). One hypothesis suggested that the clinical features of PCOS may develop as a consequence of genetically determined hypersecretion of androgens by the ovary starting at puberty or very likely long before puberty (42, 43), so that typical clinical and biochemical characteristics of PCOS may become expressed as a consequence of exposure to androgen excess at or before puberty. Through its effect on programming of the

hypothalamo-pituitary unit, hyperandrogenism in foetal life favours excess luteinising hormone (LH) secretion and leads to the development of abdominal obesity and consequent insulin resistance (17). Altered steroid negative feedback regulation of LH together with the compensatory hyperinsulinaemia due to insulin resistance may disrupt ovulatory function, causing anovulation (43). Intrauterine factors with resulting effects on birth weight and possible changes in the intrauterine environment as a function of birth order may also play a role (18). In retrospective analyses, it was demonstrated that, in the subset of girls born SGA, early pubarche, early menarche and PCOS will develop later in their life (18). Intrauterine growth retardation was frequently associated with the development of premature pubarche and hyperinsulinism in girls and functional ovarian hyperandrogenism and disorders of glucose tolerance in adult women (20). Anti-Müllerian hormone (AMH) levels are increased in daughters of women with PCOS in infancy, early childhood and in prepuberty (17). Concerning *in utero* androgenic exposure, lower 3β-hydroxysteroid dehydrogenase 1 and aromatase activities were found in the placentas of women with PCOS (44). Premature adrenarche may lead to the development of at least one subtype of PCOS. Recently, it has been proposed that age at menarche in women with PCOS is influenced by BMI and genetic variants near *LIN28B* (45). Studies of breastfeeding in women with PCOS demonstrated that there are no correlations between DHEAS, testosterone and free androgen index (FAI) in pregnancy with breast size increment or duration of breastfeeding (46).

## Laboratory, potential new biomarkers and ovarian ultrasound

### Laboratory and biomarkers

**Testosterone ▶** Testosterone is the main circulating active androgen, and the total serum testosterone concentration is the first-line recommendation for assessing androgen excess in women (47). Serum testosterone concentrations can also be crucial in the identification of androgen-secreting tumours, although the clinical history with a rapid progression of virilising symptoms is generally helpful to suggest a tumorous source of androgen excess or hyperthecosis. Measuring total testosterone at any time during the menstrual cycle is adequate, since its variations are marginally significant. A simple paradigm for identifying hyperandrogenism in PCOS is reported in Fig. 1. There
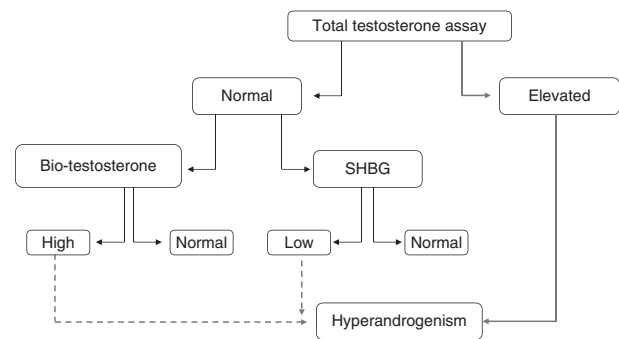


**Figure 1**

Paradigm for identifying hyperandrogenism in PCOS.

is, however, considerable overlap of values with normal healthy control women. This lack of sensitivity is partly due to differences between the assay kits themselves as well as the lack of accuracy of most assay kits at female concentration levels. The vast majority of laboratories performing a total serum testosterone assay use direct methods without extraction before performing an immunoassay (48, 49). In fact, most immunoassays yield values higher than those obtained using gas chromatography coupled with mass spectrometry (GC–MS) or liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) (50, 51), which are recommended as gold standard methods for evaluating steroid hormones (52, 53).

**Free testosterone assays, androstenedione and DHEAS ▶** Free testosterone assays should not be used due to their intrinsic inaccuracy (48). The FAI, calculated by the ratio between total testosterone and sex hormone-binding globulin (SHBG), is possibly the most sensitive measurement for the evaluation of hyperandrogenaemia in PCOS (7) and is largely preferred. There has not been any evidence that measuring androstenedione, the immediate precursor of testosterone that does not bind to SHBG, offers greater specificity or sensitivity compared with total testosterone for identifying hyperandrogenic women with PCOS (7) until recently when a study showed better sensitivity and specificity of androstenedione to diagnose hyperandrogenism in PCOS women compared with testosterone, both steroids being assessed by LC–MS/MS (54). Whether this may apply also to the sub-phenotype of women with ovulatory dysfunction and PCOm should be investigated. Similarly, measurement of DHEAS, an abundant steroid that is mainly from adrenal

source, is not required in routine practice, although when a tumorous source of androgen excess is suspected then DHEAS may be predictive of androgen-secreting adrenal carcinoma (55, 56).

**Androgen assay by mass spectrometry** ▸ For a number of years, LC–MS/MS has amply equalled GC–MS. The emergence of atmospheric pressure chemical ionisation (APCI) and of electrospray ionisation (ESI) has allowed direct coupling of liquid chromatography and mass spectrometry (LC–APCI and LC–ESI respectively) without derivatisation (57). It has recently been shown that the use of extremely high pressures, far higher than with standard LC, produces unrivalled performance in terms of separation. This approach forms the basis for the development of ultra-performance LC, with improved analysis speed, sensitivity and resolution. It is however recognised that LC–MS/MS is not widely used at the moment due to high costs of materials, the special skills required and lack of practicality for large series of assays unless automatic analysis becomes possible. Consideration should be given to making it easier to access to LC–MS/MS based assays, in Europe, for both research and practice.

Steroid profiling has traditionally been performed on 24-h urine samples and, when applied to PCOS, the most consistent finding has been increased 5α-reductase activity with variable results for overall adrenal function and for other enzyme anomalies (58, 59). More recently, the LC–MS/MS methodology has been applied to serum samples from women with PCOS, providing a more sensitive method of characterising androgen excess (60), particularly in anovulatory women with PCOS (61).

**Sex hormone-binding globulin** ▸ Low SHBG has shown excellent diagnostic accuracy for the diagnosis of PCOS in epidemiological studies, even superior to measurements of serum androgen concentrations (62), and low SHBG is a surrogate marker of insulin resistance and androgen excess that predicts the susceptibility to develop metabolic syndrome and gestational diabetes in women with PCOS (63, 64, 65, 66). Overweight and obese women with PCOS are characterised by reduced SHBG, yet this appears to be predominantly due to the excess body fat rather than to the associated insulin resistance and androgen excess (67). Therefore, the data published so far support the concept that SHBG likely only influences the PCOS phenotype indirectly, perhaps by altering the bioavailable androgen levels in the target tissues. Finally, it has been found that polymorphism in the gene encoding SHBG may be associated with reduced SHBG levels in women with

PCOS, independent of the effects of insulin resistance and obesity (68, 69).

**What diagnostic procedures should be favoured in the presence of elevated testosterone levels?** ▸ The differential diagnosis with other causes of raised serum testosterone concentrations should be considered before making a diagnosis of PCOS. Where testosterone is twice the upper normal limit, raising the possibility of an androgen-secreting tumour, it is recommended that a measurement of DHEAS should be obtained together with imaging of the adrenals (computerised tomography or magnetic resonance scans should be preferred) (56). If the DHEAS concentration is normal then the diagnosis of ovarian hyperthecosis, normally associated with insulin resistance, or androgen-secreting ovarian tumour, should be considered. On the other hand, DHEAS may be decreased in the case of sulphatase enzyme defect and in adrenocortical cancer. In ovarian tumours, raised testosterone may be LH-dependent and in some cases can be suppressed by treatment with gonadotrophin-releasing hormone (GNRH) agonist, oestrogen–progestogen or cyproterone acetate (CPA) (70). However, it should be noted that hyperthecosis and ovarian androgen-secreting tumours are both LH-dependent and therefore should be further explored by complementary imaging (71). More rarely, raised total serum testosterone can be associated with a marked elevation of SHBG possibly as the result of use of medication having an oestrogenic effect (tamoxifen and raloxifene), or of hyperthyroidism or liver disease, particularly portal hypertension with primary cirrhosis (57). Where testosterone is just above the normal upper limit, the most likely diagnosis is PCOS. However, a careful screening should be performed for the non-classic form of 21-hydroxylase deficiency (72) by basal or ACTH-stimulated 17 hydroxyprogesterone measurement and, depending on the clinical setting, for Cushing's syndrome by a complete adrenal exploration, including the dexamethasone suppression test.

**What diagnostic approach should be adopted in the presence of normal testosterone levels?** ▸ It is important to recognise that serum testosterone levels may be normal, even in women with hirsutism, but factors that could result in inappropriately low serum testosterone concentrations should be considered. It is recommended that an alternative assay can be used with the consideration of an LC–MS/MS methodology. To account for an effect from low SHBG, a common finding in PCOS patients, SHBG should be measured and with the

testosterone values should be used for calculating free testosterone, using the standard formulae (73) (see Fig. 1). SHBG is typically reduced in the event of excess body fat, metabolic syndrome or familial history of diabetes. It is still debatable if androgen receptor (AR) sensitivity determined by CAG repeat polymorphisms may be informative as a determinant of hyperandrogenism in the presence of normal free testosterone concentrations. That is, reduced AR CAG repeats, while not a genetic marker for PCOS, are associated with higher serum testosterone concentrations (74, 75, 76).

**Screening for T2D and insulin resistance ▶** Fasting plasma glucose and HbA1c are not sensitive methods of screening for T2D in situations of risk, such as PCOS (77, 78), although as a screening tool with adjusted criteria, fasting glucose may have some utility (79). According to the AE-PCOS Society, an oral glucose tolerance test should be performed in all obese women, as well as in lean PCOS women with advanced age (>40 years), in the presence of a personal history of gestational diabetes or family history of T2D (80, 81). Measurements of serum insulin and estimates of insulin resistance are not required for routine clinical management (82).

**Hypersecretion of LH and the LH/follicle-stimulating hormone ratio ▶** Hypersecretion of LH, as the result of increased pulsatility of the GNRH, is a quite common feature of PCOS, particularly in lean women with oligo-amenorrhoea (83). The current consensus did not support the use of a single LH measurement (5) due to its intrinsic variability; LH should be measured in the follicular phase of the menstrual cycle or at random in the presence of amenorrhoea. Because of the pulsatile nature of LH secretion, several measurements may be useful in equiv-ocal cases. It is estimated that over 75% of women with PCOS harbour some degree of dysregulation of the gonadotrophin function (7). Notably, obesity influences LH pulse amplitude but not its frequency (84). These findings are against the use of the LH/follicle-stimulating hormone (FSH) ratio as a criterion for the diagnosis of PCOS. In any case, an increase in LH blood levels is not uncommon in non-obese PCOS women and perhaps in those who are overweight or have mild obesity. Serum LH measurements can also be particularly useful in the differential diagnosis of normogonadotrophic amenor-rhoea, where the most common competing diagnosis is hypothalamic amenorrhoea, associated with an LH concentration lower than that of FSH (85).

**Anti-Müllerian hormone ▶** For the past 10 years, AMH has been recognised as an important marker of the number of small antral follicles. It is not only used to measure ovarian reserve but also reflects the greater number of follicles present in the ovaries with polycystic morphology (PCOm). Serum concentrations of AMH correlate with the antral follicle count and degree of menstrual disturbances (86, 87, 88). The strong association between AMH and follicle count has led some authors to compare the performance of one against the other for the diagnosis of PCOS. However, the results in the current literature are not homogeneous between studies, as demonstrated in a recent compilation (89). Interest-ingly, the serum AMH has been found to correlate with the severity of both hyperandrogenism (90) and oligo-anovulation in women with PCOS (91, 92). Future research is required to explore the predictive value of AMH for the outcome of treatment and in particular for the induction of ovulation (89). Variability of results from studies reporting AMH in women with PCOS can be explained by methodological problems with serum AMH assays. Currently available assays, while better than earlier versions, are soon to be replaced by new kits from several companies. It is therefore impossible at present to propose a consensual and universal diagnostic threshold for serum AMH that is predictive of ovarian dysfunction in PCOS (93).

**Areas in development for laboratory assessment of PCOS ▶** For obesity-related parameters, see the section on CV and metabolic issues relating to PCOS.

**Oestrone ▶** Serum oestrogen concentrations have received relatively little attention in the diagnostic process of PCOS. Although usually considered to be a normal oestrogenaemic state, compared with other causes of amenorrhoea, by aromatisation of excess androgens, it would be expected that an oestrogen parameter would be a potential marker for the syndrome. One group has reported that the combination of serum oestrone and FAI was highly predictive of PCOS status (94).

**Vitamin D ▶** Deficiency of vitamin D is very common in women with PCOS, particularly in those with obesity (95). It has been suggested that vitamin D status may contribute to the development of the metabolic disturbances associated with PCOS, chiefly insulin resistance and glucose intolerance states (95). Current evidence, based on a recent systematic review, supports an inverse association between vitamin D serum levels and body weight and metabolic alterations in PCOS (96, 97).

Deficiency of vitamin D may also affect fertility in women with PCOS. It is established that its receptors are expressed in the ovaries, particularly in the granulosa cells, as well as in the pituitary gland and endometrium (98, 99, 100). With respect to AMH, it is known that, *in vitro*, LH stimulates AMH from the granulosa cells (101), but the main AMH receptors have also been found in the CNS (102, 103). Therefore, it could be suggested that AMH may have an extraovarian function. Interestingly, vitamin D regulates AMH production in different cells (e.g. avian granulosa cells) (104). The AMH gene promoter itself seems to contain a vitamin D response element. In addition, the presence of vitamin D receptors in the hypothalamic preoptic area and in immortalised GNRH-1 cells (105) has been detected. With this background, we expect that in the next few years the potential role of an AMH–vitamin D network will be elucidated.

**Advanced glycation end products** ▸ Advanced glycation end products (AGEs) have received recent attention as possible mediators not only of the metabolic syndrome but also of ovarian physiology. Serum AGE concentrations are raised in lean and obese women with PCOS (106), with localisation of AGEs and their receptors to both theca and granulosa cells (107). A suggested role for the AGE system in ovarian follicular development might help in predicting the outcome of fertility treatments (108).

**Proteomics** ▸ The pursuit of novel biomarkers to define either the aetiology or the complications of PCOS has led to a tentative start of the application of proteomics (109). Publications so far in this field have sampled various tissues in small numbers of women and proposed a panel of possible biomarkers. With plasma being the most easily accessible tissue, the early studies have shown altered expression of acute-phase response proteins and markers of inflammation (110, 111). From omental adipose tissue, proteins related to intermediate metabolism, oxidative stress and differentiation have been described (111, 112). Proteomics analysis of ovarian tissue comparing polycystic and normal morphologies revealed a battery of 69 proteins associated with cellular metabolism, the majority of which were upregulated in PCOS (113).

## Evaluation of ovarian morphology

With the advent of trans-vaginal ultrasonography, assessment of ovarian follicle number has become the main item of polycystic ovarian morphology (PCOm). An increase in ovarian volume (OV), as well as an increased ovarian area, are also considered as accurate markers of PCOm, provided the measurements are carried out on median sections of the ovaries. Nowadays, there is an almost universal consensus on the choice of follicular excess and ovarian enlargement as the main criteria to define PCOm by ultrasound (93).

Establishing the normal values for follicle number per ovary (FNPO), as well as for OV, and especially the setting of accurate thresholds for distinguishing normal ovaries from PCOm, is still the subject of great controversy. With the advent of high-resolution ultrasonography, the threshold of FNPO proposed at the 2003 Rotterdam consensus (5) for the diagnosis of PCOm (i.e. ≥12 follicles measuring 2–9 mm in diameter, mean of both ovaries) (114) is currently met by more than 50% of normal young ovulatory women in some series (115). Most likely, this situation has arisen from the marked improvements in the level of spatial resolution afforded by newer ultrasound scanners. This issue has been revisited recently in two studies comparing PCOS with controls by means of receiver operating characteristic curve analysis and use of well-selected control groups (116, 117). The conclusion of these studies was to suggest raising the diagnostic threshold substantially to ≥19 and to ≥26 follicles per ovary respectively. Compared with 2D estimates, the 3D method seems to detect more follicles per ovary in subjects with PCOm, which is the opposite of that observed when imaging normal ovaries (118, 119, 120). 3D ultrasonography holds promise in the evaluation of PCOm, but further studies are required before recommending its routine use (89).

OV appears to be a good surrogate marker of PCOm although, compared with FNPO, it had less sensitivity for discriminating between patients with PCOm and controls in all the studies comparing both parameters. Therefore, the use of OV for the diagnosis of PCOm is recommended in instances when the image quality does not allow a reliable estimate of FNPO, especially when the transvaginal route is not feasible, such as in adolescent girls (5). Because there is some variability according to age, ethnicity and body weight, the use of in-house reference normal values is highly recommended but, if unavailable, the existing OV ≥10 ml threshold can be used conservatively. The ratio of ovarian stroma to total ovarian size may be a good criterion for the diagnosis of PCOS, with a cutoff value of 0.32 indicating an association with hyperandrogenaemia (121). However, to date there are few studies corroborating the diagnostic potential of this parameter. In general, ovarian stromal volume and total ovarian size are well correlated and, hence, there may not be any

additional value to include stromal size measurements in clinical practice. At present, the lack of uniform data and absence of cutoff values make vascular indices by Doppler impractical for discriminating between PCOm and normal ovarian morphology (93).

## Obesity, body composition and nutrition

Obesity, particularly the abdominal phenotype, is undoubtedly a useful clinical predictor of metabolic abnormalities which can be detected in the early stages of PCOS and, sometimes, it even precedes its development. It is also very frequent in PCOS, although its exact prevalence is unknown, due to the lack of representative population-based data. In fact, most studies performed in this field are cross-sectional and retrospective or, at most, case–control studies. Besides, most of them consist of subjects referred for care (122). Referral to a medical practice can be highly influenced by the degree of patient concern for symptoms, awareness of the disorder and socio-economic status, and all these conditions can be responsible for a referral bias (39). Therefore, a more accurate picture of the association between PCOS and obesity might arise from the studies where PCOS would be detected through the screening of an unselected or minimally biased population. In addition, the studies performed so far do not make it possible to establish the direction of the association between PCOS and obesity, and the causes of this association (66, 105). It is possible that environmental factors such as energy intake, energy expenditure or quality of diet, particularly the amount of AGEs, are somehow involved in PCOS pathogenesis (123), but the lack of rigorous methodology and carefully controlled direct observation of the lifestyle of patients leave the question unresolved. What we actually know from the studies performed so far is that PCOS and obesity are closely associated and that obesity, particularly the abdominal/visceral phenotype, worsens the metabolic and also the reproductive features of PCOS (67). Conversely, it is also possible that androgen excess favours abdominal adiposity from early ages, facilitating insulin resistance (18). Obesity is also strongly associated with the most feared metabolic co-morbidity in PCOS that is T2D, possibly through the well-known association of obesity and insulin resistance. Longitudinal studies demonstrate, in fact, that there is a risk intrinsic to the syndrome to develop T2D and that this risk increases steadily with BMI and is particularly high for BMI over 30 (9, 33, 124). There is also evidence for a specific altered function of the adipocytes in women with PCOS (125). Adiponectin, an adipokine secreted by the adipose tissue, has intrinsic insulin-sensitising activity that is mediated by activation of AMP kinase (126). High molecular weight (HMW) adiponectin is closely related with insulin sensitivity, and low blood levels are associated with obesity and predict T2D. It has been shown that HMW–adiponectin levels are selectively reduced in PCOS, independent of BMI, fat distribution and insulin resistance, and it was suggested that the low levels may also be due to the degree of hyperandrogenaemia through, possibly, an increased action of testosterone on the adipocyte functions (127).

The distribution of fat to abdominal/visceral area is unlikely to be the entire explanation for the metabolic abnormalities observed in PCOS women. However, this distribution together with the amount of body fat contributes significantly to the expression and severity of the PCOS phenotype (128). It has been demonstrated that the adipose tissue of PCOS has an aberrant morphology and function. In particular, adipocytes from women with PCOS are hypertrophic and there is an impaired activity of the sympathetic system in their abdominal fat (129). Altered morphology and function of the adipose tissue are associated with a reduced adipose tissue vascularisation and a consequent hypoxia that stimulates a local low-grade inflammation with an increased production of cytokines, chemokines, adipokines (free fatty acid (FFA), leptin, resistin and visfatin) and a decreased production of adiponectin (129). Interestingly, this chronic low-grade inflammatory state has been associated with the development of local and systemic insulin resistance and, probably through this mechanism, to T2D and to other CV risk factors (130). The cause of the abnormal structure and function of the adipose tissue in PCOS is unclear and much more research has to be done in this exciting area. Available evidence suggests that androgens could be indirectly involved. In fact, androgens stimulate the hypertrophy of adipocytes, by influencing the expression of enzymes and proteins involved in lipid and carbohydrate metabolism, in oxidative stress and in the differentiation of pre-adipocytes into mature adipocytes (131). In addition, androgens increase lipolysis, resulting in an increased release of FFA (132). However, intrinsic defects in adipocyte proliferation and differentiation cannot be excluded and this will certainly be an area of research in the near future. Recently, a link between macrophage activation status and insulin resistance in PCOS women has been found. Namely, it was observed that the high gene expression of *CD11c* (*ITGAX*) along with tumour necrosis factor alpha (TNFα) in subcutaneous adipose tissue were significantly higher in PCOS women.

Moreover, *CD11c* mRNA abundance made a stronger contribution to models predicting TNFα, which has proinflammatory properties that might significantly contribute to the pathogenesis of insulin resistance in PCOS women (133).

## Sleep disorders in PCOS

Limited available data suggest that obstructive sleep apnoea (OSA) is more common in obese PCOS women both in adolescence and during reproductive years, compared with the general population (134, 135). Overall, sleep disordered breathing and daytime sleepiness appear to be two- to threefold increased in obese PCOS patients. Alternatively, normal-weight women with PCOS do not seem to have an increased risk of sleep disorders compared with healthy BMI-matched women (136). BMI, insulin resistance and glucose intolerance are risk factors for the development of sleep disorders in PCOS similar to those reported in non-PCOS studies. Androgen excess might be associated with the presence of OSA in PCOS. The presence of OSA increases cardiometabolic risk in PCOS similarly to non-PCOS women (137), whereas treatment of OSA with continuous positive airway pressure is associated with improvement in cardiometabolic dysfunction of PCOS (138). It should be noted that almost all studies on this topic have a cross-sectional design with a small sample size, making it difficult to establish a mechanistic link between PCOS and OSA. Nevertheless, it seems wise at this moment to screen sleep disorders by clinical questionnaires in obese women with PCOS. In the case of clinical suspicion resulting from these questionnaires, patients should be referred to a centre of sleep disorders for polysomnography and further evaluation.

## Psychological symptoms and heath-related quality of life in PCOS

In addition to the signs and symptoms like hirsutism, acne, irregular menses, infertility and excessive body weight, psychological disorders may have a specific link with PCOS (139, 140, 141, 142) and they may have significant implications on the quality of life (143, 144, 145). Although there are very few available studies, it has been shown that similar psychological profiles exist in both NIH and non-NIH phenotypes of PCOS, which implies that the presence of psychological dysfunction occurs even in milder phenotypes of the syndrome (145), suggesting that psychological function and quality of life should be considered in all women with PCOS.

The assessment of psychological symptoms and quality of life can be performed with appropriate validated questionnaires or structured interviews. The most common questionnaires used to evaluate anxiety, depression and other psychological aspects are the Hospital Anxiety and Depression Scale (HADS questionnaire) (146), the Rosenberg's Self-Esteem Scale (147), the Beck Anxiety Inventory (that evaluates the frequency of anxiety symptoms) (148) and the Beck Depression Inventory (that measures physical, emotional and mental symptoms in depression) (149). A further questionnaire that addresses many areas including depression and anxiety is the Symptom Checklist 90 (SCL-90-R) (150). The choice of each questionnaire largely depends on specialists involved in different studies. The heath-related quality of life (HRQoL) has been investigated by generic questionnaires, such as the Short Form-36 questionnaire (SF-36) (151), the most frequently utilised tool for many diseases besides PCOS, or by a more specific questionnaire validated for PCOS patients, the PCOSQ questionnaire, that involves analysis of emotions, body hair impact, weight, menstrual problems and infertility (152).

Available data show that PCOS status may have significant negative consequences on both the psychological well-being and on the HRQoL, measured by either the SF-36 scores or the PCOSQ (143, 153, 154, 155, 156, 157). Most cohort studies demonstrated that the prevalence of both anxiety (158) and depression (158, 159, 160, 161) may be higher than expected. Another study showed that, compared with controls, a higher lifetime incidence of depressive episodes, social phobia, and eating disorders, and, dramatically, of suicide attempts does occur (161). A recent study has found that depression was related more to infertility while anxiety more to excess weight or obesity (162), whereas other studies have reported that obesity was also independently related to depression (163, 164). Interestingly, one prospective study reported that, after 6 months of combined oral contraceptives (COCs) use, the PCOSQ scores on body hair impact and menstrual problems were significantly improved along with a clinical improvement in hirsutism and menstrual irregularity, although depression and anxiety mean scores and depression rates did not show a significant change (165). Conversely, in obese PCOS women, weight loss has been found to improve depression and quality of life (166).

Whether an objective relationship between hormonal and metabolic profiles and psychological symptoms exists is still controversial. PCOS women with higher anxiety scores were shown to have significantly higher androgen (testosterone or FAI) values than those with lower anxiety

scores, independently of BMI (145, 167). However, other studies did not confirm these findings (163, 168).

Altered stress reactivity has been reported in women with PCOS, as documented by exaggerated ACTH and cortisol stress responses (169), impaired interleukin 6 upregulation after stress (163) and heightened sympathetic nerve activity (170).

Psychological gender and sexuality in PCOS are also a matter of increasing interest, due to the potential impact of high testosterone levels on psychological functions and behaviour. The development of a feminine gender role depends on self-acceptance in all of the aspects of biology, maturity and social role. It was observed that younger PCOS women are more influenced by the biological sphere, particularly worries related to menstrual disorders and infertility. On the contrary, adult PCOS women behave more often as sexually undifferentiated due to a weakened self-assessment in terms of biological maturity and social role (171).

The high prevalence of depression and anxiety in these patients should imply the inclusion of psychological assessment not only in the initial evaluation of women with PCOS but also in their follow-up, after any treatment has been initiated. Much more research should be devoted to the impact of androgens on psychological functions and of obesity and related metabolic alterations. In line with this reasoning, future research should also investigate different aspects of sexuality.

## Cardiometabolic risk factors

### Insulin resistance and endothelial dysfunction

Current epidemiological data suggest increased prevalence of classic and non-classic CV risk factors in women with the different phenotypes of PCOS, according to the Rotterdam definition. Phenotypic variability, particularly ovulatory function and hyperandrogenism, appears to influence CV and metabolic risks the most, as shown by recent studies (54, 172). PCOS and obesity act synergistically to impair insulin sensitivity, thus making insulin resistance highly prevalent in these women. Insulin resistance of the arterial endothelial cells is associated with reduced synthesis and release of nitric oxide (NO), enhanced inactivation of NO after its release from endothelial cells and increased synthesis of vasoconstricting agents leading to increased vascular stiffness and impaired vasodilatory action of insulin, as demonstrated in women with PCOS (173). Moreover, hyperinsulinaemia exerts a direct hypertrophic effect on the vascular

endothelium and the vascular smooth muscle cells and in concert with insulin resistance stimulates endothelin-1 (ET1) production, thus exaggerating endothelial dysfunction (174).

### Role of lipo-oxidative stress

The most prevalent metabolic aberration in PCOS is dyslipidaemia, which is present in 70% of patients, most often represented by hypertriglycaeridaemia and low HDL cholesterol levels and small dense LDL cholesterol (LDL-C) particles – atherogenic dyslipidaemia, typical for the states of insulin resistance (170) while LDL-C seems to be more androgen dependent (175, 176). However, the impact of dyslipidaemia in different PCOS phenotypes is not known. Metabolic abnormalities including dyslipidaemia seem to be transmitted to first-degree relatives of women with PCOS (177) and may deteriorate with obesity (176) and ageing (178). Nevertheless, the influence of BMI on the severity of dyslipidaemia is still controversial and may be influenced by the origin of investigated PCOS women (177, 178). PCOS women in Europe showed differences in lipid values. Namely, while triglycerides were almost universally elevated in PCOS women, in some Caucasian populations HDL was significantly lower and LDL higher in comparison with other populations or reference controls (179, 180). Detailed assessment of the prevalence of different patterns of dyslipidaemia is still needed.

It is supposed that persistent androgen exposure could induce dyslipidaemia via different mechanisms mediated by ARs or the activity of lipoprotein lipase (181). A more atherogenic LDL particle size pattern was observed, suggesting androgen excess modifies LDL early in the life of women with PCOS (182), offering a prolonged period for further oxidative transformation, possibly leading to more atherogenic potential (183). PCOS is associated with oxidative stress characterised by increased production of free radicals followed by decreased serum total antioxidant levels even in non-obese women (184). Some data are indicative of increased oxidative stress from mitochondria of peripheral blood leucocytes (185), but this was not confirmed in mitochondria of muscle tissue (186). It has been shown that several circulating markers of oxidative stress are abnormal in women with PCOS, independent of weight excess (187). Oxidative stress in PCOS may participate in systemic inflammation and together with insulin resistance and consequent hyperinsulinaemia promote ovarian thecal compartment dysregulation and dysfunction of endothelial cells, resulting in hyperandrogenism, anovulation and CV disorders (188).

A contribution of insulin resistance to the disruption of mitochondrial function by impairment of mtDNA in mouse oocytes has been recently suggested (189). This 'transgenerational-effect' could be translated into humans as similar oxidative stress pattern was found in mothers with PCOS and their neonates, implying that mothers' deranged oxidative milieu could be transferred into the future metabolic vulnerability of their offspring (190).

### Role of glyco-oxidative stress: AGEs

AGEs are the products of glycation or glycooxidation of proteins and lipids and increased serum AGEs were reported to be a distinct finding in women with PCOS, independent of obesity, insulin resistance and serum glucose levels (106).

Serum AGEs were closely and positively associated with serum ET1 levels as a marker of endothelial dysfunction in women with PCOS (191). Thus, increased serum AGEs in young PCOS women with no apparent traditional CV risk factors may be involved in pathways of early-onset CV injury. Moreover, the direct correlation of serum AGEs with serum testosterone levels in PCOS women implicates both of these factors in the pathophysiology of reproductive and cardiometabolic aspects of the syndrome (106). It is suggested that AGE dietary interventions or inhibitors might represent an emerging therapeutic approach with significant applications in the metabolic and reproductive consequences of PCOS (192).

### Cardiometabolic risk in PCOS: the heart of the problem

There is an increased prevalence of classic and non-classic CV risk factors in women with PCOS. This is reflected in impaired endothelial function as measured by flow-mediated dilation of the brachial artery, already at an early age and confirmed by the recent meta-analysis (193). Besides the early functional impairment of the vascular wall, the morphologic signs of early atherogenesis as increased intima–media thickness of carotid arteries (C-IMT) are highly prevalent in young women with PCOS (194, 195, 196). Whether the risk translates into increased CV morbidity and mortality has not yet been fully elucidated as appropriate long-term prospective studies are lacking. The data on CV events in PCOS women are inconsistent due to different criteria used to retrospectively confirm the diagnosis of PCOS. Some of the studies including very high numbers of women point towards an increase in either CV morbidity and even mortality in women with irregular menses (197, 198), or

increased incidence of stroke in PCOS women from the UK (199). A prospective study on 32 PCOS patients followed for 21 years did not confirm these data (24). A large study (456, 298 person-years of follow-up) has shown an association between menstrual irregularity and increased age-adjusted risk for CV mortality, which lost significance after adjusting for BMI (200), while the recent meta-analysis has reported a twofold relative risk for arterial disease irrespective of BMI (201).

### Subclinical CV disease in PCOS

Various invasive and more commonly used non-invasive methods, as well as structural markers, were used in patients with PCOS for the evaluation of subclinical atherosclerosis in asymptomatic patients. Endothelial dysfunction was shown to be associated with higher levels of androgens and with insulin resistance (202). This was observed even at very early ages, and with a trend of deterioration of endothelial function from lean to over-weight and obese PCOS women (203). A recent systematic review of the studies has confirmed that C-IMT was thicker in women with PCOS in comparison with controls (204). PCOS women have also been found to present with a greater prevalence and extent of coronary artery calcification than unaffected women, and this was independent of age and BMI (205). Vascular calcifications lead to an increase in arterial stiffness and in pulse wave velocity together with endothelial dysfunction and increased C-IMT, pointing towards early atherogenetic processes driven by insulin resistance and other CV risk factors present in PCOS women without overt CV disease (173). These relationships provide a clue that lifelong exposure to an adverse CV risk profile in PCOS may lead to premature atherosclerosis (206) and that association of CIMT in PCOS is explained only in part by weight and fat distribution and associated risk factors (194).

## Targeting treatment on patient's need: costs, benefits and side effects

### General considerations

The following section summarises all the major therapeutic choices in the treatment of PCOS.

**Lifestyle modifications** ▸ Lifestyle modification, including diet and exercise, is considered a cornerstone of the management of women with PCOS presenting with obesity, particularly the abdominal phenotype.

As reported in a previous paragraph, PCOS is characterised by a vicious circle whereby androgen excess favours abdominal fat deposition which in turn aggravates insulin resistance and compensatory hyperinsulinism, further enhancing ovarian androgen secretion. Hence, therapeutic strategies ameliorating abdominal adiposity and weight excess may inhibit this vicious circle, improving not only the metabolic comorbidities of PCOS but also androgen excess and reproductive aberrations (207, 208, 209, 210, 211). Dietary advice is the major component of lifestyle modification, especially in obese patients. There is little evidence suggesting that the composition of the diet may influence the final outcomes, since weight loss improved the presentation of PCOS regardless of dietary composition in most studies (211). On the other hand, only a few studies have addressed whether exercise training and specific exercise programmes exert beneficial effects on PCOS. Physical exercise may improve fitness, CV, hormonal, reproductive and psychological parameters in patients with PCOS. The type, intensity and frequency of exercise required for treating PCOS are far from being established (208). A recent systematic review and meta-analysis has appraised the evidence regarding the benefits associated with lifestyle modification in women with PCOS (212); however, available data do not allow evaluation of the independent effect of lifestyle modification after accounting for weight loss. Neither does available literature provide long-term follow-up to ascertain benefits on other outcomes such as prevention of diabetes or obesity, fertility or hirsutism (211). In addition, the response to weight loss may be heterogenous. To explore this issue, a follow-up study in obese women with PCOS treated with a long-term lifestyle programmes, consisting of a 1200–1400 kcal/day diet for 6 months, followed by mild calorie restriction and physical activity, showed that weight loss may lead to full recovery of PCOS in more than one-third of women (213). Predictive factors are still unclear, although higher androstenedione levels may be associated with a poorer outcome (213).

In conclusion, exercise training combined with dietary advice aiming at sustained weight loss may ameliorate many aspects of the syndrome in overweight/ obese patients with PCOS. However, there is still not enough evidence to guide specific lifestyle programmes for women with PCOS and predict the response of each patient to weight loss.

**Bariatric surgery** ▶ Lifestyle modification aimed at weight loss should be long-lasting and can be frustrating for both patients and physicians. Moreover, long-term maintenance of a reduced weight is extremely difficult as reflected by the fact that only 15% of the subjects undergoing weight loss interventions maintain their reduced weight (214). In morbidly obese patients, bariatric surgery can be an effective means of weight loss and most women may resolve PCOS signs and symptoms (215). Remarkably, recent evidence-based Australian guidelines suggest a lower BMI threshold for initiation of bariatric surgery in women with PCOS, considering the strong pathogenetic association of the syndrome with obesity (216). Bariatric surgery may in fact prevent or reverse the metabolic syndrome and may also have reproductive benefits (217), it may restore the hypothalamic–pituitary axis, reduce CV risk and even improve pregnancy outcomes. Hence, bariatric surgery should be considered as part of the treatment in morbidly obese PCOS women, especially in those with metabolic syndrome (218, 219, 220)

**Insulin sensitisers and other antidiabetic drugs** ▶ Since insulin resistance and the associated compensatory hyperinsulinaemia are common features, if not an integral part, of the PCOS phenotype, the therapeutic challenge has been expanded to insulin sensitising agents. Metformin is the most widely used insulin sensitiser drug to treat women with insulin resistance and PCOS. The benefits of metformin treatment are believed to target both cardiometabolic disorders and the reproductive abnormalities that characterise the syndrome with modest or null efficacy on hyperandrogenic cutaneous signs (1). The plausible mechanisms of metformin's action include the amelioration of insulin sensitivity in the liver and peripheral tissues with local, direct effects on ovarian steroidogenesis (221). Moreover, metformin appears to ameliorate atherogenic markers, including inflammatory molecules and AGEs acting independently of insulin sensitisation. The latter effect may be exerted directly on the pathways of AGE synthesis and clearance (221).

Thiazolidinediones (TZDs) are another class of insulin-sensitising drugs that have been studied in women with PCOS. Pioglitazone and rosiglitazone, the two currently available TZDs, have been shown to be effective in improving some metabolic (insulin resistance and IGT), hormonal (hyperandrogenaemia) as well as reproductive parameters (ovulation rate and menstrual cyclicity) of PCOS (222, 223). At present there is not sufficient evidence to support the suggestion that TZDs are superior to metformin in metabolic and reproductive aspects of PCOS (222). Most importantly, TZDs may increase body weight due to fluid retention, which is a major concern in

women with PCOS having a high prevalence of obesity (224). Hence, TZDs are not considered as a first-line choice for women with PCOS, but they may be an alternative treatment in insulin-resistant or obese PCOS women who do not tolerate or do not respond to metformin therapy. A specific role for TZDs has been suggested for PCOS women with severe insulin resistant state, often related to a genetic disorder (225). However, there are several caveats in the treatment with TZDs. More specifically, TZDs should not be offered to patients who have any evidence of liver disease or to patients with elevated baseline alanine aminotransferase levels, while regular liver enzyme control is required for patients receiving such treatment. In addition, TZDs should not be prescribed to women desiring pregnancy, because they are category C drugs and may bear a teratogenic risk. Furthermore, regarding rosiglitazone, previous meta-analyses of trials and observational studies suggested that it may increase the risk of heart attack and other adverse CV events as compared with the combination of metformin plus sulphonylurea (226). However, a comprehensive readjudication of the relevant trials has challenged this suggestion, and more recently the FDA has lifted major prescribing and dispensing restrictions for rosiglitazone (227).

Glucagon-like peptide 1 (GLP1) analogues (exenatide and liraglutide) are novel therapeutic options for T2D, acting as incretin mimetics that share similar glucoregulatory properties with the gut peptide hormone GLP1, including glucose-dependent enhancement of insulin secretion (228). Although GLP1 analogues are currently only licensed for the treatment of people with T2D, they represent an attractive option for the treatment of obesity (229, 230), particularly in women with PCOS who display impaired first- and second-phase insulin secretion (231). Exploring this perspective, Elkind-Hirsch et al. (232) undertook a prospective 24-week pilot study in 60 obese oligo-ovulatory women with PCOS, randomised to receive either metformin or exenatide or a combination of both. Menstrual cycle frequency, the primary study endpoint, as well as ovulatory rates, hormonal parameters and metabolic markers were significantly improved in all treatment groups and to a significantly greater degree in the group taking the combined treatment, compared with the metformin-treated group (232). Another preliminary report has suggested that another GLP1 analogue, liraglutide, may have an add-on effect on weight loss in obese women with PCOS who had lost <5% body weight during a 6-month pre-treatment with metformin (233).

In brief, changes in diet and lifestyle remain the primary choice in the management of reproductive and

metabolic and CV sequelae in overweight and obese women with PCOS. Metformin is the primary insulin sensitising drug to be used as an adjuvant to general lifestyle modification in patients who have IGT or, of course, overt T2D. Furthermore, accumulating evidence shows that, in a subset of women with PCOS, metformin treatment may be effective for the improvement of reproductive function irrespective of insulin resistance and glucose intolerance (33). Since metformin exerts beneficial metabolic, endocrine and ovarian effects acting by tissue-specific mechanisms, this insulin sensitiser seems to confer a global therapeutic benefit including not only cardiometabolic aspects but also reproductive aspects of PCOS. However, much more research should be performed to identify those women who may derive advantages from metformin. The use of TZDs should be reserved only for patients who are intolerant or refractory to metformin, while the potential therapeutic efficacy of GLP1 analogues in obese PCOS women should be further explored.

**Use of oral contraceptives and progestins in management of PCOS ▸** COCs have an important place in the management of both menstrual disorders and androgen excess symptoms. Progestins, usually given cyclically, also have a role in the management of menstrual dysfunction. Although COCs remain the mainstay of the pharmacological therapy of PCOS, even low dose pills may contribute to metabolic alterations and are linked to an increased risk of CV events (234, 235). These data have raised major concerns over the long-term safety of this treatment, although, much to the contrary, data in PCOS patients did not show significant worsening of insulin resistance, glucose tolerance and lipid profiles during treatment with antiandrogenic COCs (236). The most widely prescribed COCs contain ethinylestradiol (EE) at doses ranging from 20 to 35 μg and an antiandrogenic progestin. Although both second- and third-generation COCs are equally effective in controlling menses, it is advisable to avoid COCs with higher oestrogen doses or those containing 19-nor derived progestins, which are modified androgens. Such COCs may be associated with more adverse effects on the CV risk, which may be inherently increased in women with PCOS, particularly the obese ones (237). Although there is no evidence that COCs containing EE doses lower than 35 mg exert a significant influence on plasma glucose concentrations and insulin secretion (238), androgenic progestins may worsen insulin resistance and lipid profile (239). In addition, COCs may aggravate low grade inflammation (240).

Another concern raised by recent data on the use of COCs is the potential increase of risk of venous thromboembolism. Even low dose COCs containing antiandrogenic progestins, such as drospirenone, have been associated with an increased risk of venous thromboembolic events among women in the general population and, particularly, women with PCOS (241, 242). Overall, physicians should consider the increased risk of metabolic abnormalities, CV events and venous thromboembolism when prescribing contraceptive therapy to women with PCOS.

**Menstrual regulation ▶** Menstrual irregularity in the form of oligomenorrhoea or prolonged and/or heavy vaginal bleeding is common in women with PCOS. In those patients who do not wish to conceive, a low-dose combined COC is the most convenient form of treatment (1). Cyclical progestins (typically micronised progesterone 100–200 mg daily or medroxyprogesterone acetate 10 mg daily for 10–14 days/month) are a reasonable alternative, particularly in women who do not need the pill for contraceptive purposes. In severely oligomenorrheic patients, treatment is strongly advised, because of the long-term risks of unopposed endometrial exposure to oestrogen (endometrial hyperplasia and cancer).

**Hirsutism and acne ▶** COCs are also important in management of hirsutism, acne and androgenetic alopecia. The major mechanism of action is the suppression of LH-mediated ovarian androgen production together with markedly increasing SHBG concentrations that, by sequestering testosterone, reduce free testosterone concentrations even to below the normal range. In more severe cases, anti-androgen therapy is indicated. The most commonly used anti-androgen in Europe is CPA. This also has progestogenic activity and can be combined with EE to provide cycle control in addition to the amelioration of hyperandrogenic symptoms. This treatment is equally effective for the treatment of acne, although other options such as antibiotics and retinoic acid derivatives may be considered. CPA, flutamide, finasteride or spironolactone can be added to any COC to amplify the anti-antiandrogenic activity (243, 244, 245, 246) although, except CPA, all other antiandrogens are still not registered for these indications, even though their well-defined activity on dermatological signs of androgen excess has been documented, as summarised in the following paragraph.

**Anti-androgens ▶** Hyperandrogenaemia/hyperandrogenism is an integral part of PCOS and an imperative criterion for the diagnosis of PCOS (4, 5, 7). We strongly believe that androgen excess is the major abnormality in PCOS. Normal androgen blood levels in some women do not mean that PCOS may be associated with normal androgen secretion. In fact, technical reasons might be responsible for inaccurate testosterone levels (48, 49, 57), as discussed in the previous paragraph on 'Laboratory and biomarkers'. In addition, recent data have supported the suggestion that simultaneous measurement of serum testosterone and androstenedione may represent a useful tool to predict metabolic issues in PCOS women (54). Moreover, hyperandrogenism is closely related to ovulatory dysfunction and follicle excess, which might be a marker of an 'occult' hyperandrogenism, as demonstrated by a principal component analysis by Dewailly *et al.* (247). Accordingly, hyperandrogenism could still be a major abnormality in this phenotype. Most patients seek medical advice because of hirsutism, which is the most common clinical manifestation of hyperandrogenaemia. In addition, PCOS is the most prevalent diagnosis among patients presenting with hirsutism (248, 249). Therefore, hyperandrogenaemia is a major target of therapeutic management of PCOS (250). Since hirsutism negatively influences the psychological well-being of young women (see previous paragraph), antiandrogen treatment improves not only their physical appearance but also their psychological situation and quality of life (245). Antiandrogens should be used only with concomitant contraception to avoid foetal male pseudohermaphroditism in the event of unplanned pregnancy. At least 9–12 months are required in order to decide whether or not the antiandrogen treatment is effective in the amelioration of hirsutism (249, 251, 252, 253). The factors to be considered in the selection of antiandrogen drugs include the severity of hirsutism, the cost and the effectiveness of the drug, side effects and the associated clinical features/manifestations of the patient (245, 246).

Spironolactone is an aldosterone receptor as well as an AR antagonist. Doses between 50 and 200 mg are generally effective for the treatment of hirsutism, while the preferred dose is 100 mg/day. It is accepted as a safe antiandrogen drug although it may cause several side effects, particularly in higher doses (breast tenderness, menstrual disturbances, headache or polyuria) (243, 245, 246).

CPA is a steroidal antiandrogen. The recommended dose of CPA for the treatment of hirsutism is 50 or 100 mg/day for 10 days/cycle. However, studies comparing various dosages of CPA demonstrated that low and high doses of CPA have similar effects in decreasing the hirsutism scores. Consequently, the most commonly used dosage is 2 mg CPA combined with 35 mg EE. CPA may

result in several side effects including liver toxicity, headache, weight gain, breast tenderness, loss of libido, oedema and mood changes (243, 244, 252).

Flutamide is a non-steroidal antiandrogen. A low dose (≤250 mg/day) is as effective as a higher dose (500 mg/day) in the treatment of hirsutism (246, 254, 255). Although serious liver toxicities have been reported with high doses of flutamide (750–1500 mg/day), available data have shown no evidence of hepatotoxicity in hyperandrogenic girls or young women receiving low-dose flutamide (62.5–250 mg/day) for up to 54 months (245, 254, 255). However, liver function tests should be monitored regularly during the treatment.

Finasteride is not actually an antiandrogen but a 5α-reductase inhibitor that inhibits the local conversion of testosterone into dihydrotestosterone before the latter binds to the nuclear AR in target tissues. Finasteride 5 mg daily is a safe drug that is effective for the treatment of hirsutism, similarly to antiandrogens (256).

Drospirenone, a relatively new progestin, has anti-androgenic and antimineralocorticoid features. Although it is weaker than the other antiandrogens in the treatment of hirsutism, drospirenone has been widely used as a combination with EE in women with PCOS. Available studies have reported that a 6-month treatment with the drospirenone-containing COC is effective in improving hirsutism in women with PCOS (245, 257). The potential benefits offered by this improved pharmacological profile of drospirenone have translated into a positive effect on body weight in clinical trials of women with PCOS (258).

Overall, antiandrogen drugs may be used alone or combined with another antiandrogen, an insulin sensitiser (metformin) or with an oral contraceptive pill. The combined treatments are generally more effective than one drug alone. In particular, a combination of an antiandrogen and COCs with metformin may lead to more beneficial metabolic effects than monotherapy with either an antiandrogen or a COC (259, 260, 261, 262). There are sufficient data in the literature to confirm that low doses of antiandrogens are as effective as high doses of antiandrogens, so the lowest effective dose of an anti-androgen agent should be used to avoid dose-related side effects and reduce costs.

## Targeting treatment based on a phenotypic approach

The therapeutic management of the syndrome should consider the heterogeneity in PCOS phenotypes.

Therefore, a careful individualised approach is required to follow-up these women throughout their life.

## Therapeutic approach to the metabolic phenotype

Recent advances using non-targeted metabolomic approaches (263) have indicated that obesity is the major determinant of the metabolic heterogeneity of patients with PCOS. When considered as a group, both lean and obese women with PCOS have increased circulating insulin levels relative to those of serum glucose compared with healthy lean and obese women (264). Such a finding strongly suggests a certain degree of hepatic (central) insulin resistance irrespective of obesity, since increased insulin levels in conjunction with normal hepatic insulin sensitivity would necessarily lead to hypoglycaemia. However, the metabolomic profiles of non-obese patients with PCOS were consistent with suppression of lipolysis and increased glucose utilisation in peripheral tissues, clearly indicating preserved insulin sensitivity in peripheral tissues such as skeletal muscle and adipose tissue (262). The profiles of obese patients with PCOS, on the contrary, indicate the existence of both central and peripheral insulin resistance (263). Hence, substantial metabolic heterogeneity, strongly influenced by obesity, underlies PCOS and the possibility that hyperinsulinaemia may occur in the absence of universal insulin resistance in non-obese women with PCOS should be considered when designing diagnostic and therapeutic strategies for the management of this prevalent disorder. Therefore, the presence of obesity is of capital importance not only for the association of PCOS with metabolic disorders, but also for the management of the syndrome. In this regard, every effort should be made to prevent obesity and abdominal adiposity in non-obese women with PCOS. Lifestyle modification such as maintenance of regular physical activity, dietary counseling in pre-obese women and proactive promotion of smoking cessation should be routinely advised to non-obese patients. The latter is especially important because smoking is associated with abdominal adiposity and potentiates the unfavourable effects of current drug therapies for PCOS on blood clotting tests and endothelial function (264). The same approaches should be applied to obese women with PCOS, in whom some anti-obesity drugs might be considered. Unfortunately, currently available anti-obesity drugs are seldom useful in the long-term. For PCOS patients presenting with grades 2 and 3 obesity, bariatric surgery may be especially useful, because the marked weight loss usually attained after such procedures

usually resolves not only the metabolic disorders of PCOS but also PCOS itself, restoring ovulatory function and fertility (see previous paragraph). It is noteworthy that the onset of metabolic disorders, such as IGT, diabetes, dyslipidaemia and even hypertension, occurs at earlier ages in women with PCOS compared with the general population. Other than the need for a more aggressive approach towards adequate control of these and other CV risk factors, the drug treatment of metabolic disorders is not specific for patients with PCOS, although the use of insulin sensitisers and statins may secondarily ameliorate some of the symptoms of PCOS (265, 266). Finally, it should be stressed that there is no solid evidence supporting that the presence of metabolic complications should influence the choice of treatment for PCOS. On the one hand, insulin sensitiser drugs are inferior to COCs for the control of PCOS signs and symptoms (265). Modern third generation COCs containing an antiandrogenic compound are not associated with significant adverse effects on the metabolic profile of PCOS patients and might even exert beneficial effects on their lipid profiles and adipokine secretion (1).

## Therapeutic approach to the hyperandrogenic phenotype

It is likely that in endocrinology departments the great majority of women with PCOS are seen because of hirsutism as PCOS accounts for 70–80% of patients with hirsutism (250), therefore the hyperandrogenic phenotype is the most common form of PCOS in endocrinology outpatient clinics. The choice of treatment should be defined according to age and the specific phenotype of the individual patient. Among others, selected classes of patients who may require specific attention in defining the therapeutic plan are i) adolescent girls, ii) normal weight hirsute women with PCOS, iii) obese hirsute women with PCOS, iv) hirsute women with PCOS seeking pregnancy, v) hirsute women with PCOS and menopause and vi) hirsute women with PCOS and glucose intolerance states. Review articles focused on treatment of hirsutism in women with PCOS have recently been published and may be referred to for more detailed information (248). The treatment of hirsutism may take advantage of different strategies, even including lifestyle changes in the presence of excess weight or obesity. In fact, all these interventions may be helpful in lowering high androgen levels and consequently ameliorating the severity of hirsutism (246). In most cases, the hirsutism score may improve by more than 50% and testosterone blood values

may reach the normal range. However, the therapeutic plan should be prolonged for a long period of time in order to maintain weight loss (245, 246).

Pharmacological treatment is preferred in women who do not desire pregnancy. Pharmacological agents consist of COCs and antiandrogens. For most hyperandrogenic women with PCOS, COCs are suggested as the first-line drugs, unless contraindicated, for most hyperandrogenic women with PCOS (1, 245). If there is a contraindication for the use of COCs, antiandrogens may represent the first choice. For many decades, CPA combined with EE has been used as the most common agent for the treatment of hirsutism and its efficacy has been proved in a long series of clinical studies. Finasteride is a very safe drug and can produce seminal efficacy, although some studies found that it may be less effective when compared with other antiandrogens. Drospirenone may be combined with EE, but its activity is inferior to that of CPA (245, 246). Flutamide has significant clinical efficacy in the treatment of hirsutism in the long term (251, 266), although some concern has been raised because of its potential hepatotoxicity (see previous paragraph). It is important to note that antiandrogens are usually used in combination with COCs, especially in severe hirsute patients. The combination of CPA (2 mg) + EE (35 mg) and spironolactone or finasteride have been found to be more effective than one each of these drugs alone (261, 267, 268). On the other hand, antiandrogens may also be used alone when COCs are contraindicated, but this should be advised only after ensuring safe contraception, even if non-hormonal, is established. Metformin is not effective in the treatment of hirsutism (1).

Additional treatments of hirsutism are cosmetic procedures. They may be particularly effective as individual therapy in controlling mild and localised hirsutism, but can also be recommended as an adjuvant to pharmacological therapy in cases of clinically moderate to severe hirsutism, according to each woman's wishes. Electrolysis and the newer methods, such as laser therapy and intense pulse light, are folliculytic treatments that may result in permanent amelioration of hirsutism, although they are operator dependent and may be associated with local side effects (269, 270). Their main advantages are related to their rapid effectiveness and, possibly, to a relative permanence of hair removal. However, it should be considered that a recent Cochrane review of hair removal methods found little evidence of effectiveness for some of these techniques, with only alexandrite and diode lasers being able to reduce hair by

∼50% in a few months (270). Unfortunately, there are no long-term follow-up studies and, in many cases, these procedures frequently require multiple therapeutic sessions. In some cases of mild hirsutism localised on the face, an alternative may be the topical application of a 13.9% eflornithine (an irreversible inhibitor of L-ornithine decarboxylase) cream, which may reversibly slow facial hair growth in many hirsute women. However, it does not remove hair (271).

### Therapeutic approach to the reproductive phenotype

Infrequent or absent ovulation is the predominant cause of reproductive dysfunction in women with PCOS. Abnormal endometrial function has been reported but this is usually secondary to anovulation (e.g. endometrial hyperplasia due to unopposed oestrogen stimulation). The main therapeutic issues for women with PCOS and reproductive dysfunction are therefore treatment of infertility or else menstrual regulation in those women who do not desire pregnancy.

**Diet and lifestyle ▶** Weight reduction alone may result in spontaneous ovulation in overweight or obese women with PCOS, and it is recommended that diet and lifestyle changes (including increasing daily exercise) should be the first-choice treatment in overweight and obese women with PCOS (211, 272). Even in women who do not ovulate spontaneously following weight reduction and lifestyle modifications, the response to induction of ovulation is likely to be improved by these measures, although there have been reports of pregnancies after nine or more months of treatment (273). In fact, excess weight or obesity has an adverse effect on ovulation rate and both elevated androgens and high LH negatively affect responsiveness to treatment (273, 274, 275, 276).

**Induction of ovulation ▶** The objective of agents to induce ovulation is to increase serum FSH levels, to stimulate follicle development, in women with PCOS who typically have sub-optimal serum FSH concentrations. This can be achieved either by raising endogenous levels of FSH (as with the use of anti-oestrogens or aromatase inhibitors) or by giving exogenous FSH by daily injection. The oestrogen receptor antagonist, clomiphene citrate (CC), remains the treatment of first choice for induction of ovulation in women with PCOS (263) and typically results in ovulation in 75–80% of cases. This is usually given as a 5-day course at a starting dose of 50 mg/day at the onset of menses or a progestagen-induced withdrawal bleed. It is wise to monitor the first cycle of treatment using ultrasound scans so that the dose can be adjusted if there is no response (or if too many follicles develop). If ovulation is successfully induced, it is conventional to continue treatment with CC for up to 6–12 months (272). A more recently proposed alternative to CC or other anti-oestrogens are aromatase inhibitors. Initial studies have reported effective induction of ovulation but progress to routine therapeutic use has been delayed by early concerns about safety. We are now waiting for the results of large randomised control trials (RCTs) and so the use of aromatase inhibitors for induction of ovulation cannot yet be recommended, even though initial results from previous studies are encouraging (277).

Management of CC-resistant patients (those who fail to ovulate) is difficult, as is the choice of treatment in those who ovulate but have not conceived within 6 months. In many centres, the treatment of choice in CC non-responders is low-dose FSH (272), with careful ultrasonographic monitoring of the ovarian response. In specialist centres, the use of low-dose FSH has been successful in maintaining the multiple pregnancy rate at a minimum (272, 277). Data from an RCT suggest that laparoscopic ovarian diathermy may be as effective as low-dose FSH in inducing ovulation, but adjunctive therapy with CC and/or FSH was also required after surgery in about two-thirds of cases (278, 279, 280, 281).

Metformin has been reported to improve ovulation rates in women with PCOS when given alone or together with CC (1, 282) and has been widely used in women with oligo- or anovulation. However, it is questionable whether the small improvement in ovulation rate in women given metformin alone is independent of the weight loss that can occur in some patients during metformin treatment (1, 283). Results of the initial clinical trials suggested that addition of metformin improves the efficacy of CC treatment but subsequent data from large, prospective, double-blind, randomised control trials have shown no benefit of metformin alone or in combination with CC in terms of live birth rates (284, 285).

**Menstrual regulation ▶** Menstrual regulation may be indicated in those women who do not wish to conceive. Many patients suffer occasional distressing episodes of prolonged and/or heavy vaginal bleeding. Even in patients without menstrual symptoms, treatment may be advisable because of the long-term risk of unopposed oestrogen (endometrial hyperplasia and cancer). A low-dose COC (containing EE, 20–35 µg) may be the most convenient

form of treatment, although cyclical progestogen is a reasonable alternative, particularly in women who do not need the pill for contraceptive purposes. For details of oral contraceptive use in women with PCOS, see section 'Use of oral contraceptives and progestins in management of PCOS' above and recent Endocrine Society clinical practice guidelines (1).

## Future directions of research in PCOS

This paper expresses an overall perspective of endocrinologists in Europe on PCOS, aiming to help clinicians in the management of this complex and multifaceted disorder. At the same time, it clearly underlines the need for clinical and translational research in many areas of uncertainty, in order to provide an adequate rationale for the diagnostic work-up, for the treatment choices, according to the age and needs of each patient, and on the prevention of dysmetabolic consequences. Further research is also needed to define the aetiological aspects of PCOS, which should include not only genetic and, probably, epigenetic factors, but also early events responsible for the development of different phenotypes. In turn, this effort could favour preventive strategies, anyhow feasible before or during adolescence. Ethnic differences in the clinical manifestations of PCOS require specific attention, due to the genetic background and the effects of environmental factors, often related to the development of obesity and associated metabolic comorbidities. Diagnosis of PCOS might also be potentially improved by the use of new biomarkers of androgen excess and ovarian dysfunction. In this context, we believe that it is time for the development of a more objective method to define and quantify hirsutism in the different parts of the body, possibly with the collaboration of dermatologists. An important area of future basic and clinical research is also represented by the study of impact of androgen excess and the androgen–oestrogen imbalance on metabolism. This could be particularly relevant for the development of therapeutic strategies for prevention of T2D and, possibly, CV events, not only during adult age but extended to well after the menopause. In this regard it is important to consider that the significantly higher propensity of obese women with PCOS to develop T2D may disclose specific pathophysiological mechanisms related to the dangerous effect of the sex hormone imbalance on insulin secretion and action, through still poorly defined molecular mechanisms. The psychoneurological consequences of androgen excess also require much more consideration in the diagnostic work-up and in the management of women with PCOS. New methods addressing infertility are also under intensive research activity worldwide. Finally, we clearly recommend separately including the different possible PCOS phenotypes when planning clinical studies including heterogeneous phenotypes or interventional therapeutic trials targeted at the treatment of hyperandrogenism, infertility or associated metabolic comorbidities.

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