CHAPTER

26

Polycystic Ovary Syndrome

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous and still largely unexplained disorder, whose etiology remains largely a mystery. It is the most common endocrinopathy in reproductive aged women affecting 5%–8% [1], though with changes in the diagnostic criteria the prevalence has also nearly doubled. Among women with anovulation, PCOS is the cause in 55%–91% of cases [2]. PCOS was first described by Stein and Leventhal in 1935 and was first diagnosed in situ based on enlarged ovaries by pelvic examination with a history of amenorrhea and hirsutism [3]. The ovary was subsequently evaluated histologically to prove abnormal morphology, and the ovary was largely felt to be the prime culprit of the syndrome [3]. Assays confirm increased androgen excretion and circulating levels.[4]

The discovery of hyperinsulinemia and decreased sensitivity to insulin in women with PCOS led to a de-emphasis on the ovary as a diagnostic criterion. Instead, PCOS was recognized as an endocrinopathy of multifactorial etiology. This concept was summarized in a 1990 National Institutes of Health—National Institute of Child and Human Development (NIH-NICHD) consensus conference on PCOS [5]. The most widely accepted diagnostic criteria are the 2003 Rotterdam criteria, which were subsequently endorsed by the European Society for Human Reproduction and Embryology and the American Society of Reproductive Medicine [6,7]. Diagnosis is based on the presence of 2 of the 3 of oligo/amenorrhea, biochemical or clinical findings of hyperandrogenism, or polycystic ovaries, while excluding other disorders, including Cushing's Syndrome, Atypical Congenital Adrenal Hyperplasia, androgen-secreting tumors, thyroid disorders, and other disorders causing oligo/anovulation [7]. Subsequently, an Evidence-Based Methodology Workshop sponsored by the NIH in 2012 upheld the Rotterdam Criteria [8] as did several subsequent guidelines including the Endocrine Society PCOS guidelines [9]. It is important to note that these diagnostic criteria remain largely the result of expert consensus.

DIFFERENTIAL DIAGNOSIS OF POLYCYSTIC OVARIAN SYNDROME

The differential diagnosis of PCOS is broad as many disorders mimic the phenotype making it particularly important to understand the differential diagnosis and ensure these are ruled out prior to making the diagnosis. The differential diagnosis for PCOS is included in Table 1. It is imperative to perform a thorough history and physical exam to assist in the appropriate diagnosis of PCOS.

HYPOTHYROIDISM

Thyroid disorders are among the most common endocrinopathies in the general population and therefore a common potential mimicker of PCOS. It is well documented in the literature to support that women with hypothyroidism have increased ovarian volume and cystic changes of the ovary [10]. The increase in thyrotropin-releasing hormone (TRH) leads to an increase in thyroid-stimulating hormone (TSH) and prolactin. TSH and prolactin then lead to PCO morphology by changing the ratio of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and increasing dehydroepiandrosterone sulfate (DHEAS) from the adrenal gland, thereby causing oligo/anovulation and polycystic appearing ovaries. With treatment of the hypothyroidism and returning to euthyroid state, there is resolution of the PCOS morphology and normalization of the hypothalamic-pituitary-ovarian (HPO) axis [11]. Serum TSH should be evaluated to evaluate the potential for hypothyroidism, and treatment should be initiated until euthyroid and the potential diagnosis of PCOS can then be evaluated.

HYPERPROLACTINEMIA

Hyperprolactinemia is attributed in up to 20% of those presenting with amenorrhea [12] and can present with

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TABLE 1 Differential Diagnosis for PCOS

1. Hypothyroidism
2. Hyperprolactinemia
3. Late-onset CAH
4. Androgen-secreting tumors
5. Cushing's syndrome
6. Other causes

signs of hirsutism, infertility, ovarian cysts, alopecia, and acne [13–15]. Hyperprolactinemia also can present with galactorrhea a clear difference in phenotype from PCOS. Prolactin levels should be evaluated in all patients presenting with oligo/amenorrhea [16]. The normal range of prolactin is between 5 and 20 mg/mL, and the patient should be counseled not to perform strenuous exercise, avoid breast stimulation and intercourse, and have the test performed first thing in the morning to avoid a false positive test result. A thorough medication history should be performed as certain medications such as antipsychotics can cause hyperprolactinemia [17], and with persistent elevations, subsequently an magnetic resonance imaging (MRI) of the hypothalamus/pituitary should be performed looking for a prolactin-secreting macroadenoma versus microadenoma and less common hypothalamic abnormalities. Treatment may consist of a dopaminergic agonist or in some cases surgical excision.

NONCLASSICAL CONGENITAL ADRENAL HYPERPLASIA

Nonclassical congenital adrenal hyperplasia (NC-CAH) is a term referring to the milder form of adult-onset CAH. The CYP21A2 (21-hydroxylase) gene is the most commonly mutated gene in humans. It is tightly linked to the HLA locus on the short arm of chromosome 6, a frequent site of genetic recombination [18]. Nonetheless, it remains a rare disorder in the larger population. It is estimated to be present in less than 1% of unselected hirsute women. CAH occurs with the highest frequency in the U.S. population among Native Americans in Alaska. Those with high carrier status include Ashkenazi Jews, Mediterranean decent, Middle Eastern, and Indian [19,20]. A lack of 21 hydroxylation leads to an accumulation of 17-hydroxyprogesterone and progesterone and therefore a lack of cortisol synthesis proximal to the defective enzyme. This leads to adrenocorticotropic hormone (ACTH) levels increasing, resulting in overproduction and accumulation of cortisol precursors. These, in turn, are shunted to androgen biosynthetic pathways, causing excessive production of androgens, resulting in androgen excess and in some cases virilization, and not salt wasting as with classical CAH. Roughly 55% will have oligomenorrhea, 80% hirsutism, 40% polycystic ovaries, 33% acne, and 12% infertility mimicking the PCOS phenotype with some key differences [21–23]. It is important to note PCOS rarely presents with virilization as can happen with NC-CAH, i.e., temporal balding, frontal hair recession, clitoromegaly, deepening of the voice, breast atrophy, or changes in body contour, but rather presents with androgenic alopecia (thinning of the crown region) and acne.

NC-CAH can be screened for with a fasting 17-hydroxyprogesterone (17-OHP) level. A value less than 4 ng/mL is considered normal if obtained in the morning and follicular phase of the menstrual cycle [24]. Values above the upper limit of normal should be given an ACTH stimulation test (the gold standard) by giving 0.25 mg of Cortrosyn, a synthetic form of ACTH. A second sample is subsequently collected 60 min later with 17-OHP values generally, less than 10 ng/mL deemed normal. A genetic analysis has suggested that mutations are likely to be identified on both alleles when the ACTH-stimulated 17-OHP value exceeds 1500 ng/dL [18].

ANDROGEN-SECRETING TUMORS

The most common androgen-secreting tumor in a premenopausal woman is a Sertoli-Leydig cell tumor (SLCT), a rare sex cord stromal tumor, which causes rapid virilization with a prevalence of 0.5% of all ovarian neoplasms [25]. Elevated levels of testosterone and androstenedione are seen in up to 80% of patients with SLCT accompanied by hyperplasia of the gonadal stroma [26]. Any large ovarian tumor can produce androgens indirectly by causing hyperplasia of the surrounding normal stroma (i.e., benign cystic teratomas, dysgerminomas, epithelial tumors).

Adrenal tumors are rare, with an estimated incidence of two cases per one million persons per year, which are equally likely to be benign and malignant. They are usually caused by benign cortisol-secreting adenomas that cause suppression of ACTH secretion, leading to ACTH-dependent enzyme expression reduction, resulting in attenuation of androgenic steroidogenesis [27].

CUSHING'S SYNDROME

Cushing's syndrome is a rare, but serious condition marked by hypercotisolism most commonly secondary to a pituitary ACTH-secreting adenoma, but can be

secondary to an adrenal adenoma [28,29]. Significant overlap exists between PCOS and Cushing's syndrome including hirsutism, acne, alopecia, polycystic ovaries, and oligo/amenorrhea, but with key differences [30,31]. Cushing's syndrome also presents with moon facies, buffalo hump, abdominal striae, supraclavicular fat pads, and hypertension, which is not a common amalgam of signs among patients with PCOS.

It is imperative to consider screening patients with these symptoms for Cushing's syndrome prior to a diagnosis of PCOS secondary to the serious long-term effects of hypercotisolism, including increased rate of diabetes, osteoporosis, and mortality, with the ability to treat/cure Cushing's syndrome [32]. After treatment for Cushing's syndrome and resolution of the hypercortisolism, there is typically resolution of the presenting symptoms including, polycystic ovaries, oligo/amenorrhea, infertility, and insulin resistance [29]. Screening for Cushing's syndrome can be completed with an overnight dexamethasone suppression test or a 24-h urine free cortisol level collection.

OTHER CAUSES

Hyperthecosis is a syndrome characterized by the presence of luteinized ovarian theca cells in the ovarian stroma and not related to the preantral follicles, the key difference between hyperthecosis and PCOS [33]. Hyperinsulinemia is postulated to be a potential cause of hyperthecosis by altering the molecular type of LH secreted from the pituitary leading to a higher bioactive form causing an overstimulation of the ovarian theca cells [34,35]. Exogenous androgen use or doping with steroids, increasingly more common in athletic women, may also create a PCOS phenotype, as can excessive androgen replacement in pre- or postmenopausal women. Hirsutism and virilization in pregnancy can be secondary to *hyperreactio luteinalis* or fetoplacental sources, such as aromatase deficiency within the placenta leading to hyperandrogenism [36,37].

DIAGNOSIS AND CLINICAL STIGMATA OF POLYCYSTIC OVARY SYNDROME

Since first being described in the 1930s by Stein and Leventhal there have been many definitions for PCOS. They first noted an amalgam of symptoms including obesity, hirsutism, and chronic anovulation, but since then we have adopted both biochemical and radiographic imaging to assist in diagnosis [3]. Since then there have been three primary recommended definitions. The first criteria were established in 1990 by the National Institute of Health (NIH) and established a recommended diagnostic criteria as hyperandrogenism and/or hyperandrogenemia, menstrual dysfunction, and exclusion of other known disorders [38]. The second, and most commonly used diagnostic criteria were established in 2003 entitled the Rotterdam criteria and were defined as any two of the three following: oligo and/or anovulation, clinical and/or biochemical signs of hyperandgrogenism, and polycystic ovaries [7]. Since then the Androgen Excess and PCOS (AE-PCOS) society convened and established another recommended set of diagnostic criteria as hyperandrogenism (hirsutism and/or hyperandrogenemia), and ovarian dysfunction (oligoanovulation and/or polycystic ovaries), and exclusion of other androgen excess or related disorders [39]. As noted in the introduction, the Rotterdam criteria are the most commonly utilized diagnostic criteria, though it is recommended to always clearly define the criteria used in research studies.

There has been much debate about the significant increase in diagnosis of PCOS since the Rotterdam criteria were established and prompted the AE-PCOS society to evaluate data to ensure the appropriate diagnosis of PCOS as it carries with it a lifetime of increased health risk, which will be discussed later in this chapter. As more research into the syndrome is undertaken and our understanding advances, so too the definition will likely continue to evolve.

IDENTIFICATION OF POLYCYSTIC OVARIES

Polycystic ovary is a misnomer in the name of the syndrome (i.e., *polycystic ovary* syndrome) as it is but one of the possible signs to establish a diagnosis but alone is insufficient to identify pathology in a women. Polycystic ovaries are ubiquitous and are often found in normal women of younger age [40]. This morphology is primarily established through transvaginal ultrasound and has been defined based on the Rotterdam criteria as the presence of at least 12 follicles measuring 2–9 mm within a single ovary or ovarian volume greater than 10 mL. The AE-PCOS society has modified this to attempt to more accurately diagnose those with PCOS as our ultrasound technology can more accurately delineate between small follicles and preantral follicles and recommend at least 25 preantral follicles measuring between 2 and 9 mm, with no change to the ovarian volume cutoff of greater than 10 mL [41]. This has been colloquially described as a "black pearl necklace" secondary to the often times peripheral location of the preantral follicles with increased amounts of central ovarian stroma. The change in diagnostic criteria based on PCOM has been shown to change the diagnosis of almost one-fifth of women diagnosed based on the previous diagnostic criteria, but with

a paucity of prospective data showing the clinical advantage of this change [42].

The PCOM has been incorrectly identified to be synonymous with the diagnosis of PCOS, but this is confusing to those not educated on the complex nature of the syndrome as almost 30% of women with normal menses and no evidence of hyperandrogenism have the clinical morphology, as well as other disease entities having a strong association with the PCOM [43–46]. Reports have suggested that polycystic ovaries *per se* may identify a group of women with some further stigmata of reproductive and metabolic abnormalities found in the endocrine syndrome of PCOS [47,48], but the data are inconsistent [49]. It is important to note that not all women with the endocrine syndrome of PCOS have polycystic appearing ovarian morphology. However, polycystic ovaries appear to be an independent risk factor for ovarian hyperstimulation syndrome (OHSS) after ovulation induction [50,51], and thus it makes sense to document the morphology of the ovary in infertile patients seeking fertility treatment, but little conclusive evidence exists to support that the PCOM independently has any significant risk to subsequent health [41].

With the identification of anti-mullerian hormone (AMH) as a product of the granulosa cells of small ovarian follicles, preantral and antral follicles, there has been increasing documentation of elevated levels in women with PCOS with good correlation with antral follicle counts on ultrasound [52,53]. This has led some groups to seek using AMH levels to help diagnose the syndrome in lieu of ultrasound morphology. However, a similar problem as with polycystic ovary morphology is present as many younger normal women have elevated AMH levels, so likely age-related cutoffs are needed [54].

HYPERANDROGENISM

Hyperandrogenism can be documented based on clinical stigmata of androgen excess, such as by the presence of acne, hirsutism, or androgenic alopecia or by biochemical confirmation of circulating androgens. Ethnic, and presumably underlying genetic, differences in population may result in the presence of hyperandrogenenemia without clinical signs of hyperandrogenism [55].

HYPERANDROGENEMIA

Both the adrenal glands and ovaries contribute to the circulating androgen pool in women. The adrenal preferentially secretes weak androgens such as DHEA or its sulfated "depot" form DHEA-S (up to 90% of adrenal origin). These hormones, in addition to androstenedione (often elevated in women with PCOS), may serve as prohormones for more potent androgens such as testosterone or dihydrotestosterone (DHT). The ovary is the preferential source of testosterone, and it is estimated that 75% of circulating testosterone originates from the ovary (mainly through peripheral conversion of prohormones by liver, fat, and skin, but all through direct ovarian secretion). Androstenedione, of both adrenal (50%) and ovarian (50%) origin, is the only circulating androgen that is higher in premenopausal women than in men, yet its androgenic potency is only 10% of testosterone. DHT is the most potent androgen, although it circulates in negligible quantities and results primarily from the intracellular 5alpha-reduction of testosterone. In the past, measurement of 3alpha-androstanediol glucuronide, a peripheral metabolite of DHT, was used as a circulating marker of androgen excess in the skin (hirsutism and acne), but its clinical use is negligible.

Thus, circulating testosterone levels are the androgen of choice to measure, and their circulating levels may offer better discrimination between a control population and the affected population with PCOS. A 14% overlap in elevated androgen levels was noted between women with PCOS and a prospectively recruited cohort of cycling control women [56], vs a 20%–30% overlap of polycystic ovaries in a normal population [43,44]. A circulating total testosterone level was found to be the best hormonal correlate of the combined syndrome of hyperandrogenic chronic anovulation and polycystic ovaries [57]. Many prefer either a free testosterone or a bioavailable testosterone level because that better reflects the suppressive effects of hyperinsulinemia on sex hormone-binding globulin (SHBG) [58]. The current clinical practice guidelines from the Endocrine Society recommend the use of elevated total, bioavailable, or free serum testosterone level for the diagnosis of PCOS [9]. Assays are reproducible and eliminate any observer bias in identifying women with androgen excess; however, given the interassay variability, it is difficult to assign a uniform and specific level of circulating testosterone, which is the cutoff for diagnosing PCOS and you should refer to your labs upper limit of normal [59]. Although there is movement toward assay of sex steroids using LC/MS/MS technology which tends to have levels less than ELISA methods. However, good correlations and similar precision have been noted between these assays [60].

HIRSUTISM

Hirsutism is defined as excess body hair in undesirable locations, and as such is a subjective phenomenon that makes both diagnosis and treatment difficult. Most commonly, hirsutism is associated with PCOS tends to be an androgen-dependent, midline-predominant hair growth.

The pilosebaceous unit (PSU) is the common skin structure that gives rise to both hair follicles and sebaceous glands and is found everywhere on the body except the lips, palms, and soles. Before puberty, the body hair is primarily fine, unpigmented vellus hair. After puberty and stimulated by the increased androgens, some of these hairs (mainly midline hair) are transformed into coarser, pigmented terminal hairs. A similar mechanism may explain the increase in acne with puberty, with increased sebum production by the sebaceous glands. One of the central paradoxes is that androgens can exert opposite effects (vellus to terminal, terminal to vellus), depending on the site of the hair follicle [61]. It is important to note that factors other than androgen action may contribute to the development of hirsutism. Hyperinsulinemia, which accompanies many benign forms of virilization, can also stimulate the PSU either directly or indirectly by contributing to hyperandrogenemia.

Hirsutism and acne, however, are heterogeneous and common disorders, similar to polycystic ovaries. It is estimated between 50% and 74% of women with hirsutism may have PCOS [1,62]. Hirsutism is also not invariably present in a woman with PCOS with values quoted as high as 76% [1,63]. There are, for instance, ethnic differences in target tissue sensitivity to circulating androgens and intracellular androgens [64], such that marked androgen excess may not manifest as hirsutism [55]. Methodology of the assessment of hirsutism and response to treatment have been poorly validated [65].

Hirsutism scores are notoriously subjective [66], though further evaluation has shown an acceptable agreement among well-trained independent observers [67]. Even the most frequently utilized standard of subjective hirsutism scores, the modified Ferriman-Gallwey score, relies excessively on nonmidline, nonandrogen dependent body hair to make the diagnosis [68], though this is considered the gold standard [69]. This method scores 9 areas of the body and assigns a score of 0–4 for each area. A sum of these 9 scores with a value greater than 8 is generally used as the cutoff indicative of hirsutism, though ethnic differences make this poorly generalizable.

In a large multicenter clinical trial in women with PCOS, 50%–60% of the 400 women prospectively identified to have hyperandrogenemic chronic anovulation had no evidence of hirsutism [70]. Also, hirsutism is frequently idiopathic and accompanied by normal circulating androgen levels [64], although other studies with more thorough examination have shown idiopathic hirsutism to be rare (<10% of a hirsute population) [71]. For the sake of diagnosis of PCOS, hirsutism is felt to reflect the presence of increased androgens, but the severity of hirsutism is also not correlated with the androgen concentrations making this common presenting complaint problematic, and further research is required to assess a more specific diagnostic criteria for hirsutism.

CHRONIC ANOVULATION

In the broadest definition, PCOS has been identified with the World Health Organization (WHO) type 2 ovulatory dysfunction, or normoestrogenic anovulation. Although chronic anovulation may be the sine qua non of the syndrome, only a small percentage of women with PCOS are completely amenorrheic. Most are oligomenorrheic and experience varying intervals of vaginal bleeding. The cause of this vaginal bleeding may be physiologic (postovulatory withdrawal bleed) or pathologic. How infrequent should the menstrual bleeding be to qualify as "chronic anovulation" and how do you classify persistent anovulatory bleeding? There is no consensus here, but general guidelines are 6–8 spontaneous episodes of vaginal bleeding per year and cycle intervals greater than 35 days is considered prolonged. The baseline endogenous ovulatory frequency is unknown in an untreated PCOS population, but the ovulation rate in the largest multicenter randomized controlled trial in women with PCOS to date demonstrated an almost 30% ovulatory frequency in the placebo-treated arm, indicating either a significant placebo effect and/or high endogenous rate [70]. Also, as women with PCOS age they tend toward more frequent ovulation and normalization of the menstrual cycle [72].

INSULIN RESISTANCE

The decrease of insulin resistance in women with PCOS compared to appropriate controls (~35%–40%) is of a similar magnitude to that seen in type 2 diabetes and is independent of obesity, glucose intolerance, increases in waist-hip-girth ratio, and differences in muscle mass [73]. This synergistic negative effect of obesity and PCOS on hepatic glucose production is an important factor in the pathogenesis of glucose intolerance in PCOS. One of the most common prevailing theories about the etiology of type 2 diabetes proposes that the primary pathogenetic defect is peripheral insulin resistance, resulting in compensatory hyperinsulinemia. Over time there is beta cell dysfunction, leading to inadequate secretion of insulin and ultimately to beta cell exhaustion, and the development of frank type 2 diabetes. There is now a relatively substantial body of literature confirming beta cell dysfunction in PCOS, although as in diabetes, there is still considerable debate regarding the primacy of the defects and their worsening over time [74]. Basal insulin levels are increased, and insulin secretory response to meals has been shown to be reduced in women with PCOS [75].

There are data supporting hyperinsulinemia as a potential cause and treatment strategy of hyperandrogenemia

found in PCOS, though studies still have not determined if hyperinsulinemia leads to hyperandrogenemia or the exact opposite. Hyperinsulinemia leads to a decrease in sex hormone-bindings globulin (SHBG) and therefore increased circulating androgens [76,77]. This theory is further validated in studies that have attempted to correct hyperinsulinemia and observe the downstream effects, which have shown improvement. The largest study to date evaluating correction of hyperinsulinemia showed an improvement in ovulation rate and clinical signs of hirsutism [70]. A large meta-analysis showed an improvement of induced ovulation with treatment of the insulin resistance with metformin with an odds ratio of 3.88 [78]. In a Cochrane database systematic review evaluating multiple types of insulin sensitizing agents, they showed these medications were successful in improving insulin sensitivity and reduced hyperandrogenemia [79]. This was also found in those patients independent of obesity suggesting PCOS alone, and not obesity, is the cause of hyperinsulinemia [80,81].

DETECTION OF INSULIN RESISTANCE IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

Total body insulin sensitivity can be assessed by the euglycemic glucose clamp technique [82]. Here, exogenous insulin is infused to produce the desired insulin concentration. Glucose is infused simultaneously to maintain euglycemia. At steady state, the amount of glucose infused is equal to the amount of glucose utilized by the tissues and can be used as an index of sensitivity to insulin. The more glucose that is infused, the greater the sensitivity to insulin and vice versa. Total body insulin sensitivity can also be assessed via a modification of the intravenous glucose tolerance test known as frequently sampled intravenous glucose tolerance test (FSIGT) [83]. In this test, a bolus injection of glucose is given and blood is very frequently sampled for glucose and insulin levels. Minimal model analysis is then applied to the glucose and insulin levels obtained, and an insulin sensitivity index is derived. The precision of the model can be improved by enhancing second phase insulin secretion with an intravenous injection of the insulin secretagogue tolbutamide or of insulin, 20 min after the glucose bolus. Minimal model estimates of insulin sensitivity are highly correlated with euglycemic clamp determinations of insulin action [83]. The FSIGT has been well validated in women with PCOS [80]. This test is substantially less labor intensive and costly to perform than the euglycemic clamp, but it still remains impractical and expensive in a clinical setting. Both of these tests have little benefit as diagnostic tests to identify insulin resistance in a PCOS population. A third option is the glucose to insulin ratio, which has been shown to have both adequate sensitivity (95%), specificity (84%), positive (87%), and negative (94%) predictive values for detecting insulin resistance in obese women with PCOS and is easily attainable [84]. A value the G/I ratio ratio of less than 4.5 is viewed as abnormal and indicative of insulin resistance. Another homeostatic test that is frequently utilized to assess insulin resistance in women with PCOS is the HOMA-IR [85]. This is calculated as fasting insulin (microU/L) $\times$ fasting glucose (nmol/L)/22.5. There are no accepted cutoffs for abnormal with this test.

ORAL GLUCOSE TOLERANCE TESTING AND HEMOGLOBIN A1C

Oral glucose tolerance testing (OGTT) allows for the diagnosis of clinically recognized categories of glucose tolerance, including impaired glucose tolerance (IGT) and type 2 diabetes. Recent studies have suggested that the prevalence rates of glucose intolerance are as high as 40% in women with PCOS when the less stringent WHO [86] (2-h glucose $\geq$140 mg/dL with a fasting glucose of $<$126 mg/dL) criteria are used [87–89]. These studies are of interest because they have shown nearly identical rates of IGT and type 2 diabetes among a diverse cohort, both ethnically and geographically as well as from different investigational groups. This would suggest that these abnormalities may represent a universal characteristic of women with PCOS, at least those diagnosed on the basis of hyperandrogenic chronic anovulation.

Fasting glucose levels are poor predictors of glucose intolerance risks in PCOS because nearly 40% of women with PCOS with IGT had normal fasting glucose values. This is supported by the limited studies of hemoglobin A1c (HbA1c) in women with PCOS, which tend to be normal even in women with PCOS with IGT [70,90]. Although diagnosing diabetes by fasting glucose using the 1997 ADA criteria may detect a more severe form of diabetes than the WHO criteria [91], this category of women with normal fasting glucoses and glucose intolerance may be exactly the subset of women (young and otherwise healthy) for whom more intensive early intervention can prevent long-term complications, such as the development of diabetes [92].

HbA1c is a hematologic test used to detect the average glycemic index of a red blood cell over a 3-month period. It is currently used as a measure of chronic glycemic control in diabetes treatment, but also in the diagnosis of prediabetes (HbA1c $>$ 5.7%) and diabetes mellitus (HbA1c $>$ 6.5%) as recommended by the American Diabetes Association. Several studies have been performed to see if HbA1c is a good marker for insulin resistance in those patients with PCOS, secondary to its widespread

availability, absence of need for fasting, and average over a longer period of time. Several studies have shown inferiority in comparison to the 2-h OGTT in detecting insulin resistance, and therefore the OGTT should be used over the HbA1c at the current time [93,94].

ANTIMULLERIAN HORMONE AND POLYCYSTIC OVARIAN SYNDROME

Anti Mullerian hormone (AMH) is secreted by the granulosa cells of small antral follicles and is important in folliculogenesis [95]. With the increased number of antral follicles found in PCOS, it is therefore not surprising women with PCOS have a fivefold higher concentration of AMH compared to ovulatory women [96]. This significant increase in AMH concentration is important because of the potential implications AMH has on folliculogenesis. AMH is felt to inhibit folliculogenesis by decreasing FSH concentrations and arrest developing antral follicles in the 2–5 mm size, and subsequently decrease the likelihood of ovulation [97–99].

AMH has been implicated as a possible target for treatment secondary to its role in many aspects of the clinical and biochemical role in those with PCOS. It has been implicated as a potential contributor to insulin resistance [100], infertility/response to varying fertility treatments [101–104], and hyperandrogenism [105,106]. In a systematic review, AMH has been positively correlated with PCOS severity and has been suggested to be added to the diagnostic criteria for PCOS with good predictive value [107]. AMH as a target for antibodies to improve the sequelae of PCOS is a hypothesis that is yet to be tested, but has potential based on prior data. It is not yet assuredly ascertained whether AMH is a cause, or the downstream effect on many of the reported PCOS morbidities, but will be interesting once human trials begin.

CLINICAL SEQUELAE OF POLYCYSTIC OVARY SYNDROME

Infertility and Chronic Anovulation

The most common reason that women with PCOS present for medical care is because of infertility, caused by chronic anovulation. This chronic anovulatory state is felt to be multifactorial in nature: the granulosa cells are more sensitive to LH at a smaller size [108], suppression of FSH leads to inhibition of follicle maturation [109], hyperinsulinemia enhances granulosa cell response to LH [110], obesity, and AMH as stated prior. As a general rule, PCOS women represent one of the most difficult groups in which to induce ovulation both successfully and safely. Many women with PCOS are unresponsive to clomiphene citrate (CC) and human menopausal gonadotropins (hMGs), and this is exacerbated by the underlying obesity. On the other end of the spectrum are women with PCOS who over respond to both of these medications. Women with PCOS are at especially increased risks of OHSS, a syndrome of massive enlargement of the ovaries and transudation of ascites into the abdominal cavity that can lead to rapid and symptomatic enlargement of the abdomen, intravascular contractions, hypercoagulability, and systemic organ dysfunction [111]. They are also at increased risk for multiple pregnancy. In addition, there is emerging evidence that baseline hyperinsulinemia may contribute to the increased OHSS risk [112,113].

Gynecologic Cancer

Endometrial cancer is the most commonly diagnosed invasive gynecologic cancer in women in the United States. Case series and meta-analysis have identified women with PCOS at high risk for developing endometrial cancer and often at an early age [114–119], suggesting a large public health risk. A large systematic review demonstrated a nearly threefold increase in lifetime risk of endometrial cancer for those with PCOS at 9%, compared to the baseline population risk of 3% [120]. But there is actually little solid evidence to link PCOS and endometrial cancer particularly because of the lack of prospective studies and the heterogeneity in diagnosis and confounders, such as obesity and criteria used for diagnosis, in and among studies [121]. A recent case-control study from Australia demonstrated a fourfold increased risk of endometrial cancer with patient's with PCOS and including a subanalysis controlling for obesity [122]. They found a more than double increased risk, but this was not statistically significant still leaving the association up for debate [122]. As more evidence and studies are developed, it would seem an association exists because of the overlap between PCOS stigmata and endometrial cancer risk factors, but the exact mechanism is not perfectly understood. This lack of understanding and exact connection should not deter a practitioner from preventative therapies, early evaluation for hyperplasia, and applying appropriate treatment strategies for hyperplasia and cancer diagnosis.

The mechanism by which women with PCOS may be at increased risk for endometrial hyperplasia, and endometrial cancer is thought to be chronic stimulation of the endometrium with weak, but bioactive estrogens, combined with the lack of progestin exposure. This condition known as unopposed estrogen is perhaps the clearest hormonal risk factor for endometrial cancer [123]. PCOS women have been shown to be normoestrogenic, perhaps

even hypoestrogenic with elevated levels of estrone [124]. A Scandinavian study looked at a group of both premenopausal and postmenopausal women with endometrial carcinoma and found hirsutism and obesity in both groups of cases compared to controls [125]. In the younger group, they additionally noted a recent history of anovulation and infertility, two of the most common presenting complaints of women with PCOS (in addition to hirsutism and obesity) [126]. Endometrial hyperplasia has often been noted in association with anovulation and infertility, common symptoms of PCOS [116,127,128]. There are no systematic prospective studies of the prevalence of endometrial hyperplasia/neoplasia in a population with PCOS. Other gynecologic cancers have been reported to be more common in women with PCOS, including ovarian [129] and breast cancer [130], but well-designed studies are still desired and further studies have not been consistent in these findings [119].

Type 2 Diabetes Mellitus

Since the 1920s, it has been known that patients with signs of hyperandrogenism (and PCOS) are at an increased risk for development of type 2 DM. This is felt to be consistent with the commonly found hyperinsulinemia and insulin resistance in women with PCOS, as this is a well-established pathway to development of Type 2 DM [131]. It has been shown that 40% of women with diabetes of reproductive age can be directly attributed to PCOS [132] with an average conversion rate of 1%–5% per year and as high as 10% conversion rate per year in the Latina population with a history of gestational diabetes [133].

Retrospective studies looking at diabetes prevalence over time have generally noted an increased prevalence with age in women with PCOS. Studies from Scandinavia have shown increased rates of type 2 diabetes and hypertension compared to controls [134]. This study used a combination of ovarian morphology and clinical criteria to identify women with PCOS and found that 15% had developed diabetes compared to 2.3% of the controls [134]. A case-control study of PCOS in the United States has shown persistent hyperinsulinemia and dyslipidemia as women PCOS age, although androgen levels tend to decline in older women with PCOS [135]. In a thin Dutch population, although the overall prevalence of self-reported diabetes was 2.3%, in women with PCOS aged 45–54 years ($n = 32$), the prevalence of diabetes was four times higher ($P < 0.05$) than the prevalence of this condition in the corresponding age group of the Dutch female population [136].

Adult women with PCOS have glucose intolerance rates of 40% (as defined by prevalence of either IGT or type 2 diabetes as diagnosed by a 2-h glucose value after a 75 g OGTT) [87,88]. Data suggest that adolescents may have soaring rates of glucose intolerance [137], compared with adults, which appears to be mirrored in the adolescent population with PCOS [138]. Studies of large cohorts of women with PCOS have demonstrated that the prevalence rates of glucose intolerance are as high as 40% in women with PCOS when the less stringent WHO criteria are used [87,88]. These studies are of interest because they have shown nearly identical rates of IGT and type 2 diabetes among a diverse cohort, both ethnically and geographically as well as from different investigational groups.

In a long-term prospective observational study, it was found that in an Italian cohort of women with PCOS were 2.6 times more likely to have diabetes and a prevalence of 6.8 times that of aged matched controls in the middle-aged population, suggesting a worsening likelihood over time [139]. In this same study, they found that obesity is an independent risk factor for development of diabetes [139], consistent with prior published data [140]. This is also consistent with prior data suggesting obese patients with PCOS have a higher likelihood of DM2 compared with the lean PCOS phenotype, which was not highly associated with risk of progression to DM2 and obesity and PCOS are synergistic in risk of progression [141]. PCOS is now classified as a nonmodifiable risk factor for the development of diabetes by the American Diabetes Association [142] and periodic screening using the 2 h 75 g OGTT should be utilized for diagnostic screening because of the high prevalence of conversion to IGT and DM2 with PCOS [143].

Cardiovascular Disease

Both PCOS and cardiovascular disease (CVD) are common in women, but is CVD more common (and at an earlier age) in women with PCOS? The metabolic profile noted in women with PCOS is similar to the insulin-resistance syndrome, a clustering within an individual of hyperinsulinemia, mild glucose intolerance, dyslipidemia, and hypertension [144]. There is prolific literature identifying obesity, dyslipidemia, glucose intolerance, diabetes, and hypertension as risk factors for CVD in women with PCOS [145–150].

However, the metabolic syndrome and PCOS remain distinct. When women with the metabolic syndrome are studied for reproductive stigmata of PCOS, they are no more likely to have polycystic ovaries than are other segments of the population, and less than half have a history of oligomenorrhea [151]. Nor do all women with PCOS have the metabolic syndrome. In some countries, most women with PCOS (~80%) are nonobese [152], and as many as 50% of obese women with PCOS may not have documented insulin resistance by intensive

testing [84]. There is actually little published evidence, even with long-term study follow-up, supporting a link between PCOS and increased premature cardiovascular morbidity or mortality [153]. The best evidence for an association between PCOS and CVD may be billing-based analyses lining up ICD diagnosis codes with a variety of cardiovascular events and diagnoses, documenting increased risk among women with a diagnosis of PCOS compared to controls [154].

This brings to light the difficulty of studying the CVD risk for patients with isolated PCOS compared with the increased comorbidities associated with the PCOS. The AE-PCOS Society sought to answer this conundrum and give recommendations based on their findings. In a rigorous evaluation of the currently available studies, which only included systematic reviews comparing PCOS with control patients and strict exclusion criteria of the study designs, they determined what modifiable factors placed patients with PCOS at increased risk stratified by at risk or at high risk. Women with PCOS and obesity (particularly abdominal obesity), tobacco use, dyslipidemia, hypertension, insulin resistance, and family history of premature vascular disease (<55 years old for a male relative or <65 years old for a female relative) are at risk [147]. While those with metabolic syndrome (defined as BP ≥135/85 mm/Hg, waist circumference ≥88 cm in the non-Asian population; ≥80 cm in East/South Asian women, elevated fasting glucose ≥100 mg/dL, reduced HDL-C ≤50 mg/dL, and elevated triglyceride values ≥150 mg/dL)m, type 2 DM, or overt vascular or renal disease are at high risk [147]. These were subsequently endorsed by the American Association of Clinical Endocrinologists [155].

Based on these findings, the AE-PCOS Society recommends all patients with PCOS have waist circumference and body mass index (BMI) determined at every visit, a complete lipid profile completed at diagnosis and every 2 years thereafter, a 2-h 75 g OGTT and repeated every 2 years, blood pressure checked at every visit, and assessment for depression/anxiety [147]. These recommendations for screening for CVD have also been incorporated into the Endocrine Society Guidelines, though the recommended follow up rescreening period is longer [9]. These varying guidelines highlight the need to periodically clinically reassess patients with PCOS for metabolic disease.

Treatment of PCOS

When a diagnoses of PCOS is made, it is important to keep in mind the patient's desires and indication for presentation to the clinician's office. The different treatment strategies are best thought of through two separate perspectives. The first being to optimize short- and long-term health based on comorbidities as described above. It is common patient's desire weight loss, optimization of average glucose, improved hirsutism and acne, and decreased potential for long-term negative effects regarding cancer and cardiovascular health. The other common cause for presentation is secondary to infertility and chronic anovulation, as this is the most common cause of infertility affecting 1 in every 15 women [1]. By separating fertility management and symptomatic management, it improves patient understanding and clarifies goals.

GENERALIZED TREATMENT STRATEGIES OF POLYCYSTIC OVARY SYNDROME

Lifestyle Modification

Obesity has become an epidemic in our society and contributes substantially to reproductive and metabolic abnormalities in PCOS and there is no evidence supporting an improvement in this association. A significant proportion of women with PCOS are obese [156] and a large emphasis should be placed on counseling these women, particularly in the adolescent period, on weight loss to improve metabolic and long-term sequelae [157]. Insulin resistance, accompanied with obesity, are independent risk factors for progression to diabetes and heart disease and exacerbated by the PCOS phenotype and therefore imperative to address this population as early as possible to potentially optimize outcomes [158].

Unfortunately, there are no effective treatments that result in permanent weight loss, and it is estimated that 90%–95% of patients who experience a weight decrease will relapse [159]. For obese patients with hirsutism, weight loss is frequently recommended as a potential benefit. Weight loss has been show to increase SHBG through improved insulin sensitivity, which lowers bioavailable androgen levels. Weight loss and exercise have been shown to improve ovulation rates in observational and randomized studies. In one study, about 50% of these women who lost weight experienced improvement in their hirsutism [160,161]. There have been few studies on the effect of exercise alone on insulin action in hyperandrogenic women [162]. It is reasonable to assume that exercise would have the same beneficial effects on insulin action in women with PCOS as women with type 2 DM [163–165]. A large issue with weight loss in this patient population is it may be more difficult for obese women with PCOS to comply with exercise recommendations due to joint issues [166]. Caloric restriction and other adjuvant therapies may be most effective for achieving weight loss as one RCT with meal replacements, or list at demonstrated 6%–7% weight loss in a 16-week period [167].

Ovarian Suppressive Therapies

Women with hyperandrogenemia, stigmata of hirsutism and acne, and those with increased risk of endometrial cancer would theoretically benefit most from this form of therapy. Suppressing the ovary has been achieved with oral contraceptives, depot progestins, or GnRH analog treatment.

OCPs have been the mainstay of therapy for many years in patients with PCOS. Oral contraceptives inhibit ovarian steroid production by lowering gonadotropins and raise SHBG through their estrogen effect, thus further lowering bioavailable testosterone. They may also inhibit DHT binding to the androgen receptor and 5α-reductase activity and increase hepatic steroid clearance. These myriads of actions contribute to improving hirsutism. There are theoretical reasons for choosing an oral contraceptive using a less androgenic progestin or one with specific androgen antagonistic properties, but few studies show a clinical difference between different types of progestins. Although several oral contraceptive pills, including a triphasic oral contraceptive containing norgestimate, have been shown to improve acne and have received an FDA indication for this treatment, other pills also appear to offer similar results. In a double-blind randomized controlled trial evaluating the therapeutic effects of three different types of oral contraceptive pills showed equivalent findings after 6 months, but started to show differences after 12 months of treatment, with cyproterone acetate showing stronger antiandrogen effects, drosperenone next, and desogestrel the least suggesting a potential for ideal decision making in decision for OCP pill, but with no difference in metabolic paramaters [168].

There have been a paucity of data directly evaluating OCPs and other stigmata of PCOS, but there have been concerns raised regarding the potential that OCPs may actually worsen insulin resistance and therefore worsen the metabolic effects in women with PCOS and worsen long-term survival and progression to diabetes and CVD [169]. In obese women with PCOS, OCPs have been shown to worsen glucose tolerance and exacerbate components of the metabolic syndrome (systolic blood pressure and serum triglycerides). Interestingly, concurrent lifestyle modification with weight loss abrogates any metabolic impact of OCPs. Further prospective data are required prior to removing OCPs as the mainstay of therapy for patients with PCOS [167].

A GnRH agonist may further lower circulating androgens, but comparative trials have not shown a greater benefit and result in unacceptable bone loss and other reported side effects. Glucocorticoid suppression of the adrenal also offers theoretical benefits, but deterioration in glucose tolerance is problematic for women with PCOS, and long-term effects such as osteoporosis are a significant concern.

Metformin

Metformin is a biguanide that works primarily by suppressing hepatic gluconeogenesis, but it also improves insulin sensitivity in peripheral tissue. Metformin was initially FDA approved for the treatment of type 2 DM, but has been used clinically for many years in patients with PCOS. In a large systematic review, Metformin has been shown to be effective in reducing fasting insulin concentrations, fasting glucose concentrations, total testosterone, and systolic blood pressure (not diastolic). There was, however, no evidence on any effect on decreasing BMI, SHBG, cholesterol, or triglycerides [170]. Metformin should therefore be used to decrease the potential for progression to DM2, as well as to decrease CVD with improvements in inflammatory markers, and atherogenic profiles [171]. While metformin generally shows mild improvements in multiple aspects of the PCOS phenotype, its overall benefits as a first line or solo agent to treat women with PCOS is relatively minor.

Thiazolidinediones

Thiazolidinediones are peroxisome proliferator-activated receptor (PPAR) agonists and are thought to improve insulin sensitivity through a post receptor mechanism. It is difficult to separate the effects of improving insulin sensitivity from that of lowering serum androgens because any "pure" improvement in insulin sensitivity can raise SHBG and thus lower bioavailable androgen. In a large multicenter trial, troglitazone has been shown to have a dose-response effect in improving ovulation and hirsutism [70]. This appeared to be mediated through decreases in hyperinsulinemia and decreases in free testosterone levels. Troglitazone has subsequently been removed from the worldwide market because of hepatotoxicity. Pioglitazone and rosiglitazone are still available. Although these medications are effective in improving insulin sensitivity, glycemic control, menstrual regularity, and ovulation rates without the significant hepatotoxicity seen with troglitazone [172], the side effect profiles of these drugs are concerning. There is often weight gain, which appears to be a class effect as well as more serious side effects such as increased cardiovascular events with rosiglitazone and bladder cancer with pioglitazone. Thus, the general unfavorable risk-benefit ratio does not favor use of these drugs for PCOS.

Treatment of Hirsutism

Many of the aforementioned generalized treatments are also applicable to the treatment of hirsutism. Most medical methods, while improving hirsutism, do not produce the dramatic results patients desire. In general, combination therapies appear to produce better results

than single-agent approaches; responses with medical therapies often take 3–6 months to notice improvement; and adjunctive mechanical removal methods are often necessary. The Endocrine Society recommends starting with monotherapy with either OCPs or antiandrogens [173]. If the patient is not satisfied with the results after 6 months or longer of monotherapy, then the combination of OCPs and antiandrogens can be utilized. Mechanical hair removal is also an option with laser/photoepilation and eflornithine cream can be added for a more rapid response. The Endocrine Society specifically recommends against flutamide (a nonsteroidal antiandrogen with high risks of teratogenicity), topical antiandrogen therapy, insulin-lowering medications, and GnRH agonists (unless a severe form of hyperandrogenemia exists, such as hyperthecosis) [173].

Spironolactone, a diuretic and aldosterone antagonist, also binds to the androgen receptor with 67% of the affinity of DHT [174], making it a relatively potent aldosterone antagonist. Spironolactone exhibits a dose-dependent response and has been shown to be mildly effective in decreasing Ferriman-Gallwey scores in a meta-analyses of randomized controlled trials [175]. Spironolactone is teratogenic and poses a risk of feminization in a male fetus if the patient were to conceive while utilizing this medication. It has other mechanisms of action, including inhibition of ovarian and adrenal steroidogenesis, competition for androgen receptors in hair follicles, and direct inhibition of 5α-reductase activity. It is a potassium-sparing diuretic, and therefore can exacerbate hyperkalemia, and should be used cautiously in patients with renal insufficiency. It has also been successfully utilized for the treatment of acne [176,177]. An additional concern is potential interactions with other medications that may have similar effects. These include progestins such as cyproterone acetate or drospirenone, commonly found in many oral contraceptive formulations that have similar antimineralocorticoid activity.

Ornithine decarboxylase is necessary for the production of polyamines, which are necessary in hair growth. Eflornithine, a potent and irreversible inhibitor of ornithine decarboxylase, has been found to be effective as a facial cream against hirsutism and has been approved by the FDA for this indication. As shown in a randomized double-blind controlled trial, eflornithine does not remove hair, but does reduce the rate of hair growth and improve appearance and quality of life [178]. It is given as a 13.9% cream of eflornithine hydrochloride and applied to affected areas twice a day for a minimum of 4 h each. In clinical trials, 32% of patients showed marked improvement after 24 weeks compared to 8% of those treated with a placebo; benefit was first noted at 8 weeks. Unfortunately, the effects are not permanent and return of pretreatment hirsutism was noticeable at 8 weeks after discontinuing the therapy. It is a pregnancy category C medication that is well tolerated. A variety of adverse skin conditions occur in 1% of those studied.

Mechanical hair removal (e.g., shaving, plucking, waxing, depilatory creams, electrolysis, and laser vaporization) can control hirsutism, and these methods are often utilized by women. Laser vaporization works by damaging the hair using the principle of selective photothermolysis with wavelengths of light well absorbed by follicular melanin and pulse durations that selectively thermally damage the target without damaging surrounding tissue. Patients with dark hair and light skin are ideal candidates, and it appears to be most effective during anagen, the active growth phase of hair follicles during which the hair root is rapidly dividing.

TREATMENT OF INFERTILITY

Lifestyle Modification

As with all aspects of PCOS, obesity and insulin resistance are at the center. Obese women have decreased fecundity, as well as complications within pregnancy and delivery. Obese women had increased rates of stillbirth, gestational diabetes, hypertensive disorders, cesarean delivery, operative vaginal deliveries, prolonged labor, shoulder dystocia, fetal neural tube defects, fetal cardiac defects, and neonatal death [179,180]. Weight loss is considered the first-line therapy in infertility in the obese patient population with PCOS. Weight loss has been shown in case series and observational studies to improve ovulatory rates and, more importantly, live-birth rates [181]. It is encouraging that even a nominal and realistic weight loss goal of 5%–7% significantly improved ovulation and live-birth rates [167,182]. In a large randomized trial of preconception interventions weight loss as opposed to OCPS led to significantly higher ovulation rates with PCOS (around 40%) and a trend toward higher live-birth rates [167]. When compared to immediate treatment with clomiphene, preconception weight loss not only improved ovulation rates by 40% but also improved live-birth rates by two- to threefold [181]. This implies that the quality of ovulation is enhanced by weight loss yielding higher fecundity per ovulation. However, another large multicenter study found that a less intensive weight loss intervention in anovulatory women did not improve live-birth rates after subsequent infertility treatment [183].

Clomiphene Citrate

CC has traditionally been the first-line treatment agent for infertility in women with PCOS, although it has now been replaced by letrozole. Clomiphene is a nonsteroidal agent and a member of a large family of

triphenylethylene derivatives, which includes tamoxifen. It is a racemic mixture of two isomers, zuclomiphene (longer acting) and enclomiphene (potent inducer of ovulation). Clomiphene has a long half-life; only 51% of the oral dose is excreted after 5 days, and the zu isomer can be detected in the serum for up to 1 month after treatment. Clomiphene is thought to work as a selective estrogen receptor modulator (SERM), acting as an estrogen antagonist at the hypothalamic-pituitary axis and stimulating GnRH secretion. In a large RCT, ovulation rates were found to be 49%, pregnancy rates of 30%, and live-birth rates of 23% over six ovulation induction cycles [184]. CC is typically well tolerated, but hot flushes, headaches, and visual complaints have all been reported [185]. Roughly 50% of women with PCOS do not respond to CC. Clomiphene has recently been unavailable in many countries, which is likely increasing use of letrozole, which remains available.

Letrozole

Letrozole is an aromatase inhibitor whose primary action is suppression of estrogen production, thereby decreasing the negative feedback of estrogens in the hypothalamus leading to increased GnRH production and FSH secretion and subsequent ovarian follicular development. Letrozole also has a significantly shorter half-life than clomiphene, so it is no longer present at the time of implantation. Letrozole has become more widespread and common in its use in the PCOS patient population for ovulation induction and is considered by many to be first-line therapy. In a large multicenter RCT, there was found to be a 13% absolute increase in ovulation rate and 8% increase in live-birth rate for Letrozole compared with CC with a relative rate increase for live birth over 40% without other significant negative outcome measures [186]. This was confirmed in a large systematic review and meta-analysis where the effect size of letrozole over clomiphene was even great approaching 70% [187]. Many people have already replaced Letrozole with CC for ovulation induction in the PCOS patient population, and there are data to support improved ovulation induction outcomes compared to gonadotropins as well [188]. Compared to clomiphene, letrozole is associated with greater decreases in luteal phase estradiol levels and increases in progesterone levels mimicking a more physiologic ovulatory cycle.

Gonadotropins

Gonadotropins are also frequently utilized in both step-up and step-down regimens to induce ovulation in women with PCOS, although the use of low-dose step-up FSH regimens have resulted in a markedly decreased rate of OHSS and multiple pregnancy rate [189]. In one of the largest trials of gonadotropins in women with PCOS to date, women were randomized to a conventional method of ovulation with more aggressive dosing and increases in FSH dosing compared to a low-dose protocol; higher pregnancy rates were achieved with the low-dose protocol (40% vs 20% for the conventional arm) [190]. There were fewer cases of multiple pregnancy and ovarian hyperstimulation in the low-dose arm and a higher percentage of monofollicular ovulation (74% vs 27%) [190]. Low-dose therapy with gonadotropins offers a high rate of monofollicular development (50% or greater) with a significantly lower risk of OHSS leading to cycle cancellation or more serious sequalae [190–194]. A Cochrane review reports a reduction in the incidence of OHSS with FSH compared to hMG in stimulation cycles without the concomitant use of a GnRH agonist and a higher overstimulation rate when a GnRH agonist is added to gonadotropins (odds ratio: 3.15, 95% CI: 1.48–6.70) [195]. Despite theoretical advantages, urinary-derived FSH preparations did not improve pregnancy rates when compared to traditional and cheaper hMG preparations; their only demonstrable benefit was a reduced risk of OHSS in cycles when administered without the concomitant use of a GnRH agonist. A meta-analysis found no studies of adequate power to confirm the benefit of pulsatile GnRH agonist to induce ovulation in PCOS [196].

Laparoscopic Ovarian Drilling

Ovarian drilling is the method by which small perforations are made within the ovary surgically. The mechanism of action is not completely understood, but it has been postulated the decrease in ovarian follicles decreases the negative feedback on FSH leading to upregulation in the hypothalamus and therefore a return to ovulation. In a Cochrane database systematic review, there was found to be a 34% live-birth rate, which was found to be equivalent to CC, gonadotropins, or letrozole [197]. With no significant advantages of surgical management for patients with PCOS in regard to pregnancy rates, miscarriage rates, or live-birth rates laparoscopic ovarian drilling has largely been replaced by medical therapies. There is the potential benefit for long-term return to ovulation and therefore decrease need for recurrent fertility treatments, but with the increased surgical risk this should be individualized [198].

In Vitro Fertilization

In vitro fertilization is the process by where controlled ovarian hyperstimulation occurs in order to have multiple mature oocytes, which are ultimately retrieved

through a minor surgical procedure whereby the ovarian cysts are aspirated, and the oocytes are collected and fertilized within the lab. The fertilized embryo is then placed within the endometrial canal for implantation. Comparatively, women with PCOS have a better prognosis for conception and live birth with IVF than other etiologies of infertility and this advantage holds on into the 40's of a women's age [199]. Controlled ovarian hyperstimulation increases the risk of OHSS particularly in the PCOS patient population [200]. This has led from a shift away from the long GnRH agonist protocols with increased risk of OHSS in women with PCOS to the shorter GnRH antagonist protocols. GnRH antagonist protocols allow for the use of alternate triggers other than hCG to induce ovulation. This include the GnRH agonist trigger [201] and more recently the use of kisspeptin [202]. However, the latter remains experimental.

Another innovation to decrease the risk of OHSS after controlled ovarian hyperstimulation is the decision to electively freeze all viable embryos, known as "freeze all" and transfer the embryos after ovarian recovery. This has been shown in observational studies and RCTs to improve outcomes. One large RCT of fresh vs frozen embryo transfer noted a marked decrease in the OHSS rate among the elective frozen transfer group along with an improve live-birth rate, mediated largely through decreased pregnancy loss [203]. However, the frozen embryo transfer group had an increased rate of preeclampsia for unknown reasons.

POLYCYSTIC OVARY SYNDROME: A LIFETIME DISORDER?

The concept of PCOS as a reproductive disorder limited to the years between menarche and menopause has been shattered by its association with insulin resistance and its sequelae. This has led to the expansion of PCOS to a lifetime disorder, with premenarchal and postmenopausal phenotypes. In 1962, Neel was the first who argued for the existence of "thrifty" genes, which would have been preferred during human evolution [204]. Thrifty genes promoted an insulin-resistant phenotype, which utilized and stored energy efficiently, a survival benefit in the feast-or-famine world of the hunter/gatherer. The immense resources of the Human Genome Project have led to massive searches for genes that cause Type 2 DM and obesity. Although many candidate genes in type 2 DM and obesity have been identified, pundits would claim that the lack of success in identifying specific mutation (except in rare, severe phenotypes) argues against a purely genetic cause for these conditions in the larger population.

This belief has led to the development of the alternate hypothesis, that we are creatures of our environment, with the intrauterine environment exerting the greatest impact on our subsequent metabolic phenotype. The Barker hypothesis proposes that the source of insulin resistance lies in a harsh intrauterine milieu [205]. This milieu environmentally imprints our future metabolic fate. For those exposed to a nutritionally restricted womb, decreased fetal growth and birth weight are the result. The intrauterine famine results in imprinting a thrifty phenotype, where the anabolic effects of growth factors must be blunted in a nutrient-limited environment, one that ceases at birth. For instance, in the skeletal muscle, glucose is shunted to the development of more vital organs. They remain forever resistant to the effects of insulin, forming the basis for developing type 2 DM and CVD in later life [206]. Studies by the proponents of this theory have shown that adults with low birth weight have an increased prevalence of IGT and type 2 DM [207] and have stigmata of the insulin-resistance syndrome [208].

Premature pubarche, defined as the early appearance of pubic hair, has been postulated as an early expression of PCOS in prepubescent girls [209]. Adolescent girls with premature pubarche have been noted to be hyperinsulinemic [210] and to have an increased androgen response to a GnRH agonist [211]. Baseline DHEAS and androstenedione levels at diagnosis or premature adrenarche correlated with 17-OHP values after GnRH agonist stimulation, suggesting that functional ovarian hyperandrogenism is more common in these girls (same gene for CYP17 is expressed in both the adrenal gland and ovary). This sign of increased CYP17 activity is therefore the first phenotypic manifestation of this abnormality in both glands.

These studies suggest that hyperinsulinemia plays a role in the hyperandrogenism found in premature pubarche and PCOS [212–214]. This hyperinsulinemia has been observed throughout all stages of puberty and cannot be explained by an increase in BMI when compared to normal subjects [215]. In one study, 45% of girls with a history of premature pubarche had ovarian hyperandrogenism [211]. These girls have subsequently developed chronic anovulation at the age of menarche [216]. Further low birth weight among girls with premature pubarche appeared to exacerbate the PCOS phenotype [217].

At the other end of the reproductive spectrum, both menstrual irregularity [72] and hyperandrogenemia [218] appear to normalize as women with PCOS approach their late thirties and early forties. Evidence also suggests that polycystic ovaries may be more prevalent in younger women with PCOS, and that these also resolve with age [49]. Thus, many of the reproductive stigmata may resolve before menopause, leading to the difficulty discussed as follows of linking long-term sequelae such as endometrial cancer and CVD back to an earlier phenotype.

GENETIC ETIOLOGY

While there are clearly genetic components to PCOS, they have been difficult to entangle. It shares many facets with other complex genetic diseases such as type 2 diabetes or heart disease, in which the genetic component is a strong contributor to risk, but without a clear hierarchy of critical genes identified. There are several difficulties in conducting genetic research in the search for genes that cause or contribute to the PCOS phenotype. PCOS is a heterogeneous disorder with controversy about diagnostic criteria. There are likely multiple potentially completely unrelated disorders that lead to a common phenotype. An example of this is late onset congenital adrenal hyperplasia which share many phenotypic features with PCOS but is the result of an autosomal recessive defect. PCOS is associated with infertility and low fecundity. Thus, it is rare to find large pedigrees with multiple affected women with whom to perform classic genetic analyses such as linkage analysis. This further reinforces the likelihood that mendelian patterns of inheritance significantly contribute to the disorder. PCOS also has an age/reproductive maturity onset and eventual senescence. Assigning phenotypes based on hyperandrogenism or anovulation to premenarchal girls and postmenopausal women is thus difficult as differences may not have appeared or have vanished. Similarly ovarian suppressive therapies such as OCPs can normalize hyperandrogenism and oligomenorrhea. Although a male phenotype has been postulated, beginning first with the early onset male pattern androgenic alopecia, there are no rigorously established clinical or biochemical features that can be used to identify "PCOS males." This makes formal segregation analysis and genetic linkage studies more difficult. The lack of animals that spontaneously develop a PCOS-phenotype, especially mice, precludes the use of powerful tools of genetic mapping.

FAMILY STUDIES: PHENOTYPIC VARIATION

The foundation of genetic studies is the evidence that disease clusters in families. None of the existing family studies of PCOS convincingly establishes a mode of inheritance, but there is clearly a complex multifactorial genetic component in inheritance [219]. The diagnostic criteria used to assign affected status differed among the studies, as did the methods with which the status of first- and second-degree relatives was ascertained. By and large, ovarian morphology determined from tissue biopsy, direct visualization, or diagnostic imaging, in association with menstrual disturbances and evidence for hyperandrogenism, has been used in most studies as the criteria for diagnosing PCOS in probands. Despite the heterogeneity in study design and the inability to obtain comprehensive phenotype information to permit a formal segregation analysis, collectively the existing literature strongly suggests the clustering of PCOS in families.

Twin studies support a genetic component to PCOS with increased prevalence of the symptoms of PCOS among twin sisters, with increased concordance of symptoms in monozygotic twins over dizygotic twins [220,221]. Many of these traits including hirsutism and oligomenorrhea show high concordance suggesting a high heritability to them. These studies are supported by studies of first-degree relatives of women with PCOS.

Nearly 50% of sisters of women diagnosed with PCOS had elevated total or bioavailable testosterone levels, suggesting that hyperandrogenemia is a dominant trait. The contribution of genetics to blood androgen levels was substantiated in a large population study [222]. Of 93 women diagnosed with PCOS, an evaluation of first-degree relatives found that 35% of mothers and 40% of sisters were also able to be diagnosed with PCOS [223]. First-degree female relatives also have an increased prevalence of insulin resistance and metabolic syndrome [224]. These reproductive and metabolic abnormalities are found to a lesser extent in male relatives [225]. In an evaluation of the male siblings of those diagnosed with PCOS found elevated levels of DHEAS [226], as well as insulin resistance [227]. Premature male balding has commonly been recognized as a male phenotypic feature [228].

Several groups have noted an increased incidence of cardiovascular events in parents of women with PCOS that exceed expected population prevalences [229,230]. These studies require replication, but they do provide further concern about CVD clustering in these families.

GENOME-WIDE ASSOCIATION STUDIES AND NEXT-GENERATION SEQUENCING IN PCOS

There have now been a number of high-quality Genome-Wide Association Studies (GWAS) performed in developed countries throughout the world including China [231], the United States [232], and Europe [233]. These studies have consistently identified significant association with genes that fit into our understanding of the pathophysiology of PCOS such as the LH receptor, the FSH gene and the insulin receptor. They have also identified genes not previously identified as potential candidate genes such as the THADA and DENND1A genes. DENND1A encodes a protein associated with receptor-mediated cell surface signaling, potentially affecting

gonadotropin and insulin signaling, but it has also been found in the cell nucleus such that it may also regulate genes. Expression of a variant of this gene in normal human thecal cells increases androgen production and knockdown of the gene suppresses androgen production [234], suggesting a potential novel target to treat PCOS.

These studies have provided independent replication of genetic association in distinct populations (i.e., both Han Chinese and Northern European Caucasians), suggesting that common genetic variants contribute to the PCOS phenotype across the racial spectrum. Despite utilizing a variety of phenotyping techniques and varying definitions of PCOS, including some subjects who self-identified as having PCOS, genetic associations were replicated, implying that there is some genetic commonality to all the diagnostic criteria. Further these studies all support a heritable component to PCOS, though the common variants identified to date explain only a tiny portion of the heritability of PCOS and individual variants contribute little.

Recently, there has been increased interest in performing next-generation sequencing to find rare variants that may provide a substantial heritable component to complex disorders, but may not be fully recognized in a GWAS. Such sequencing of the AMH Gene in women with PCOS has identified a small subset of women who have variants that may lead to increased levels of inactive AMH, which may lead to a PCOS phenotype [235].

While these studies have shed new light on PCOS, they have not to date resulted in either genetic diagnostic tests, predictive pharmacogenomics markers for drug response or adverse events or new treatments.

CONCLUSION

PCOS is a common endocrinopathy that still largely remains a mystery. It is a disorder of unexplained hyperandrogenic chronic anovulation and clearly heterogeneous in etiology; however, many women are noted to have profound peripheral resistance to insulin-mediated glucose uptake and associated metabolic abnormalities. Despite evidence of familial clustering of both reproductive and metabolic abnormalities, no clear or overwhelming molecular or genetic mechanism has been identified to date to explain most cases. Women with PCOS present with infertility, menstrual disorders, hirsutism, and signs of hyperandrogenism. They are at increased risk for type 2 diabetes and display multiple risk factors for endometrial cancer and CVD, although further well-designed studies are necessary to document event risk over the lifetime. Randomized studies of infertility treatment have helped establish the primacy of oral ovulation induction agents as first-line agents, but there is a paucity of well-designed trials to guide chronic or long-term treatment of women with PCOS. Treatment tends to be symptom based, and the search continues for a single therapeutic agent, which will resolve both reproductive and metabolic abnormalities.

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