DIAGNOSIS AND MANAGEMENT OF POLYCYSTIC OVARY SYNDROME

Nadir R. Farid · Evanthia Diamanti-Kandarakis
Editors

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Editors
Nadir R. Farid
The London Endocrine Clinic
21 Wimpole Street
London WIG 8GG
farid@thelondonendocrineclinic.com

Evanthia Diamanti-Kandarakis
University of Athens Medical School
Athens, Greece
akandara@otenet.gr

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Preface

Consulting citation trends in PubMed for key words or phrases is instructive as to the interest of the scientific community in the subject and by inference its currency and likely importance. In the 5 years between 1985 and 1989 there were 91 papers on PCOS. The rate escalated rapidly in the next half-decades to 727, 1642, and 4355, 2005–July 2008. Insulin resistance was more topical and references reference to the term increased rapidly: 1995–1999, 8517, 2000–2004, 21,828 and in 2005–July 2008 only rose to 48,914.

The observation that most women with PCOS are insulin resistant has been an important turning point. From being predominantly the domain of gynaecologists as an ovarian disorder, PCOS was thereafter recognized as a heterogeneous metabolic disorder with polycystic ovaries as part of its manifestations. We have since learnt that not all women with PCOS are insulin resistant, apparently this predominates in those who suffer from both hyperandrogenism and anovultion. Some women with PCOS who have apparently normal serum insulin levels are nevertheless show increased ovarian androgens secretion at those levels of insulin.

With the mounting epidemic of insulin resistance, many doctors in practice encounter women who are symptomatic, have more or less regular cycles, are sub-fertile, are at most slightly hyperandrogenemic and without PCOS ovarian morphology . There is no consensus on how to manage these women. We also do not know the rate at which untreated mild PCOS becomes more severe or that at which women satisfying the criteria of The Androgen Excess Society (AES) who are not anovulatory become so and apparently acquire insulin resistance.

PCOS, a complex multigenic disorder, can have major impact on quality of life with depressive tendencies, infertility, obesity, manifestations of hyperandrogenism as well as long term increased risks for diabetes, the metabolic syndrome non-alcoholic fatty liver and cardiovascular disease. These and other issues are expertly laid out in the pages of this book, as is their management. The intuitive expectation that PCOS is associated with increased risk for cardiovascular disease is supported by strong surrogate evidence, although outcome studies are lacking. And for now at least an increased risk for women who had PCOS and have entered the menopause is tentative.

The authors, all international experts in their areas of contribution, clear lay out the problem, offer practical advice in the management of various aspects of the syndrome and raise question where current knowledge is incomplete or new data is necessary.

The idea of this book arose out of a consensus among the contributing authors and many others we consulted with that knowledge about the diagnosis, health implications and up-to-date management of PCOS among community doctors and trainees is at best incomplete and at most spotty. This book is an effort to redress that deficit.

That this book has materialized at all is thanks to the efforts of Ms Laura Walsh who worked hard to find a niche for this book in the ranks of what now become one of the largest publishing houses in the world. Mrs Maureen Tobin kept us all on track, and has done a wonderful organizational job...all very quietly.

It was an immense pleasure for me to work again with Maureen and Laura on a new successful project.

Nadir R. Farid

Contents

Contributors

Jean-Patrice Baillargeon
Associate Professor, Departments of Medicine and Physiology/Biophysics, Director, Program of Endocrinology and Metabolism, University of Sherbrooke, Sherbrooke, QC J1H 5N4, Canada, Jean-Patrice.Baillargeon@USherbrooke.ca

Thomas M. Barber, MA (Hons), MBBS, MRCP (UK)
Research Fellow and Specialist Registrar, Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK, tom.barber@drl.ox.ac.uk

Susan J. Barter, MBBS, MRCP, DMRD, FRCR
Consultant Radiologist, Addenbrookes Hospital University Trust, Hills Road, Cambridge CB2 2QQ, UK, suebarter@btinternet.com

Salvatore Benvenga, MD
Master on Childhood, Adolescent and Women's Endocrine Health, University of Messina, Programma di Endocrinologia Molecolare Clinica, A.O.U. Policlinico G. Martino, Padiglione H, 4 piano, 98125 Messina, Italy, s.benvenga@me.nettuno.it

Susan Calhoun, PhD
Sleep Research and Treatment Center, Department of Psychiatry, Pennsylvania State University College of Medicine, Hershey, PA, USA, scalhoun@hmc.psu.edu

Enrico Carmina, MD, PhD
Professor of Medicine, Professor of Endocrinology and Head of Endocrine Unit, Department of Clinical Medicine, University of Palermo, Via delle Croci 47 90139 Palermo, Italy, enricocarmina@libero.it

Charikleia D. Christakou, MD
Endocrine Section of the 1st Department of Medicine, University of Athens, Medical School, Athens, Greece, cchristak@med.uoa.gr

Colin J Davis
Consultant Obstetrician and Gynaecologist, St Bartholomews Hospital London, London, UK, cdavisobgyn@aol.com

Evanthia Diamanti-Kandarakis, MD
Endocrine Section of the 1st Department of Medicine, University of Athens, Medical School, Athens, Greece, akandara@otenet.gr

Brad Eilerman, MD
Division of Endocrinology, Department of Medicine, University of Cincinnati College of Medicine, The Vontz Center for Molecular Studies, Cincinnati, OH 45267, USA

Nadir R. Farid, MD
The London Endocrine Clinic, 21 Wimpole Street, London WIG 8GG, farid@thelondonendocrineclinic.com

Neoklis A. Georgopoulos, MD
Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Patras Medical School, Greece, neoklisg@hol.gr

Sabrina Gill, MD
Division of Endocrinology & Metabolism, St. Paul's Hospital and University of British Columbia, Vancouver, BC, Canada, SGill@providencehealth.bc.ca

Ettore Guastella
Department of Obstetrics and Gynecology, University of Palermo, Palermo, Italy, ettore_guastella@libero.it

Janet E. Hall, MD
Reproductive Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, jehall@partners.org

Robert A. Hegele, MD
Director, Blackburn Cardiovascular Genetics Laboratory, Scientist, Vascular Biology Research Group, Robarts Research Institute, 406 - 100 Perth Drive, London, Ontario, Canada N6A 5K8, hegele@robarts.ca

Melissa J. Himelein, PhD
Professor of Psychology, University of North Carolina at Asheville, Licensed Psychologist, Center for Applied Reproductive Science, Johnson City, TN, & Asheville, NC, USA, himelein@unca.edu

Roy Homburg, FRCOG
Professor of Reproductive Medicine, VU University Medical Centre, 1007MB Amsterdam, the Netherlands; IVF, Department of Obstetrics and Gynecology, Barzilai Medical Center, Ashkelon, Israel, r.homburg@vumc.nl

Tisha R. Joy
Robarts Research Institute, 406 – 100 Perth Drive, London, Ontario, Canada N6A 5K8, tjoy@uwo.ca

Eleni Kandaraki, MD
Second Department of Obstetrics and Gynecology, Division of Human Reproduction, Aristotle University of Thessaloniki, Greece, akandara@otenet.gr

Heather A. Kenna, MA
Department of Psychiatry and Behavioral Sciences, Stanford University, School of Medicine, Stanford, CA, USA, hkenna@stanford.edu

Alexandra Lawrence, MD, MRCOG
Subspeciality Trainee in Gynaecological Oncology, Hammersmith Hospital, Du Cane Road, London, W12 0HS, UK, AlexandraC.Lawrence@imperial.nhs.uk

Kate Marsh, BSc, M Nutr Diet, Grad Cert Diab Edn & Mgt
Advanced Accredited Practising Dietitian (AdvAPD) and Credentialled Diabetes Educator (CDE), The PCOS Health & Nutrition Centre, Sydney, Australia, kate@nnd.com.au

W. Peter Mason, MD
Gynaecological Oncologist, Chairman North West London Network, Hammersmith Hospital NHS Trust, London, UK, wpetermason@aol.com

John E. Nestler, MD
Division of Endocrinology and Metabolism, Virginia Commonwealth University, Richmond, VA 23298-0111, USA, nestler@hsc.vcu.edu

Dimitrios Panidis
Second Department of Obstetrics and Gynecology, Division of Human Reproduction, Aristotle University of Thessaloniki, Greece, panidisd@med.auth.gr

Natalie L. Rasgon, MD, PhD
Department of Psychiatry and Behavioral Sciences Stanford University, School of Medicine, Stanford, CA, USA, nrasgon@stanford.edu

Pasquali Renato
Division of Endocrinology, Department of Internal Medicine, S. Orsola-Malpighi Hospital, University Alma Mater Studiorum, Via Massarenti 9, 40138, Bologna, Italy, renato.pasquali@aosp.bo.it

Manfredi Rizzo
Department of Clinical Medicine, University of Palermo, Via delle Croci 47, 90139 Palermo, Italy, mrizzo@unipa.it

Marzieh Salehi, MD
Division of Endocrinology, Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA, Marzieh.Salehi@uc.edu

Rehan Salim, MD, MRCOG
Assisted Conception Unit, University College London, London, UK, Rehan.Salim@uclh.nhs.uk

Paul Serhal, MBBS, MRCOG
Assisted Conception Unit, University College London, London WC1X 8LD, Paul.serhal@uclh.nhs.uk

Susmeeta T. Sharma, MBBS
Department of Internal Medicine, University of Florida, Gainesville, FL, USA, susmeetasharma@yahoo.com

Pascale G. Stemmle, BS
Department of Psychiatry and Behavioral Sciences, Stanford University, School of Medicine, Stanford, CA, USA, pascale@stanford.edu

Emma Stevenson, MD
School of Psychology and Sports Sciences, Northumbria University, Newcastle Upon Tyne, NE1 8ST, UK, e.stevenson@unn.ac.uk

Samuel S. Thatcher, MD, PhD
Director, Center for Applied Reproductive Science, Johnson City, TN and Asheville, NC, USA

Yaron Tomer, MD, FACP
Division of Endocrinology, University of Cincinnati College of Medicine, The Vontz Center for Molecular Studies, Cincinnati, OH 45267, USA, tomery@UCMAIL.UC.EDU

Agathocles Tsatsoulis, MD, PhD, FRCP
Professor of Medicine/Endocrinology, Department of Endocrinology, University of Ioannina, Ioannina, 45110, Greece, atsatsou@uoi.gr

Evert JP van Santbrink, MD, PhD
Senior consultant in Reproductive Medicine, Division of Reproductive Medicine , Department of Obstetrics and Gynecology, Erasmus Medical Center, Rotterdam, the Netherlands, e.vansantbrink@erasmusmc.nl

Alexandros N. Vgontzas, MD
Sleep Research and Treatment Center, Department of Psychiatry, Pennsylvania State University College of Medicine, Hershey, PA 17033, USA, avgontzas@hmc.psu.edu

Nectaria Xita, MD
Department of Endocrinology, University of Ioannina, Ioannina, 45110, Greece, nxita@yahoo.com

Part I
The Syndrome

Chapter 1
Clinical Manifestations of PCOS

Pasquali Renato

1.1 Introduction

The PCOS is the commonest hyperandrogenic disorder in women and one of the most common causes of ovulatory infertility, with an estimated prevalence of 4–7% worldwide [1]. Over the years, after the first description by Stein and Leventhal in 1935 [2], its definition has been re-addressed several times. In 1990 the National Institutes of Health (NIH) established new diagnostic criteria, based on the presence of hyperandrogenism and chronic oligo-anovulation, with the exclusion of other causes of hyperandrogenism such as adult-onset congenital adrenal hyperplasia, hyperprolactinemia and androgen-secreting neoplasms [3]. The inclusion of ultrasound morphology of the ovaries as a further potential criterion to define PCOS was proposed by the Rotterdam consensus conference, which established that at least two of the following criteria – oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries (PCO) at ultrasound – are sufficient for the diagnosis [4]. More recently, the fundamental role of hyperandrogenism has been pointed out [5]. However, PCOS compromises other pathological conditions that strongly modify the phenotype and play a dominant role in the pathophysiology of the disorder, including insulin resistance and hyperinsulinemia, obesity and metabolic disorders, all favouring, together with androgen excess, an increased susceptibility to develop type 2 diabetes mellitus (T2DM) and, possibly, cardiovascular diseases (CVD). PCOS by itself may also have some genetic component as documented by familial aggregation and recent genetic studies [1]. All the clinical features may however change throughout the lifespan, starting from adolescence to postmenopausal age. Therefore, PCOS should be considered as a lifespan disorder, although the specific phenotype of PCOS in postmenopausal women is still poorly defined [6]

1.2 How to Approach the Patient

Most patients do not know anything about the definition of PCOS, with some exceptions. They go to the doctor because of their health problems, which are sometimes relevant only for cosmetic reasons, particularly in young women. Major concerns in asking for the doctor's help are represented by (i) clinical signs of androgen excess, particularly hirsutism; (ii) menses irregularities, including amenorrhea; (iii) unexplained infertility; or (iv) obesity and related features. Doctors should be aware that all these problems are often differently perceived on an individual basis, and that the patient may be greatly involved in the solution of one of them and relatively disinterested in the others, depending on age, cultural background and perceived importance of clinical features. Accordingly, affected women may refer to different specialists, such as dermatologists, gynaecologists or endocrinologists. Nonetheless, each doctor should try to evaluate all the signs and symptom of the patient in a holistic clinical approach, in order to make a diagnosis and select appropriate treatment, when needed.

P. Renato (✉)
Division of Endocrinology, Department of Internal Medicine, S. Orsola-Malpighi Hospital, University Alma Mater Studiorum, Via Massarenti 9, 40138, Bologna, Italy
e-mail: renato.pasquali@unibo.it

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1.3 Clinical Picture of PCOS

The clinical evaluation should include a complete medical history and physical examination, and consideration of differential diagnosis. Although diagnostic criteria for PCOS for research studies have been proposed, it should be remembered that many patients may not meet the strict criteria reported above, therefore requiring blood testing and other diagnostic procedures. In addition, the definition does not cover all features of PCOS, with particular emphasis on excess body weight and metabolic disorders. Therefore, in an individual patient, the history should always naturally begin with the symptoms and signs that are causing most concern to the patient. Fundamental methodological aspects to consider should be

(a) if a patient has signs of androgen excess (such as hirsutism), does she have irregular menses and anovulation?
(b) if a patient has irregular menses, particularly during adolescence, does she have hyperandrogenic signs, or should these alterations be related to androgen excess, if necessary investigated by blood tests?
(c) if a patient is infertile, should androgen excess conditions (clinically evident or biochemically defined) be investigated?
(d) how to define the frequent presence of excess body weight in relation to the previously reported problems?
(e) how to frame metabolic alterations from a pathophysiological and therapeutic perspective?
(f) given the potential genetic background of PCOS, how useful is the family history to define the diagnosis?

If these aspects are appropriately taken into consideration, the clinical diagnosis of PCOS is not complex and does not require extensive laboratory evaluation, provided a differential diagnosis with other overlapping conditions responsible for androgen excess, menses irregularities, infertility, obesity and metabolic disturbances is performed.

Past medical history should also include a knowledge of prior ovarian surgery that could impact current hormonal and menstrual status, and prior records of an abdominal procedure may provide information as to the appearance of the uterus and/or ovaries. A complete history of prior therapies must be documented, including topical treatments for acne and hirsutism that are likely to influence the appearance of the skin over time. In some cases, it may be apparent that the symptoms of PCOS merely became evident because a woman has recently discontinued oral contraceptive pills that had masked the symptoms. At the other extreme, new onset androgenic symptoms could also be explained by the recent use of topical testosterone creams for the treatment of low libido or vulvar dermopathies. The medication list may also reveal prior treated conditions that the patient had not recognized which might be related to variability in their menstrual cycles or weight profiles. Finally, acne is known to be caused by certain medications, including azathioprine, barbiturates, corticosteroids, cyclosporine, disulfiram, halogens, iodides, isoniazid, lithium, phenytoin, psoralens, thiourea and vitamins [7]. Finally, a list of all cosmetic therapies is necessary for the interpretation of physical findings; topical and other treatments of hirsutism and acne will in fact influence the clinical manifestations of these conditions.

1.4 Family History

Several studies have documented an increased risk of PCOS in sisters and daughters of women with PCOS, so the history provides an opportunity to identify new cases of PCOS. Hirsutism, acne, menstrual irregularity, early cardiovascular disease, obesity and T2DM are all potential indicators of a familial tendency towards the PCOS [8]. A family history of infertility and/or hirsutism may also indicate disorders such as non-classic congenital adrenal hyperplasia. The presence of symptoms that are very different from those of other family members may increase the level of concern for a more pathologic explanation for the menstrual defects or the androgenic symptoms. The family history of metabolic defects and CVD is also an opportunity to quantify the risk of the patient.

1.5 Evaluation of Clinical Hyperandrogenism

Hyperandrogenic signs and symptoms are the hallmark characteristic of PCOS. Most women with PCOS have clinical evidence of hyperandrogenism, which includes hirsutism, acne, oily skin and, sometimes, male pattern balding or alopecia. Rarely, virilizing symptoms may be present, such as increased muscle mass, deepening of the voice, or clitoromegaly, although these findings should prompt a search for an underlying ovarian or adrenal neoplasm, or classic form of previously undiagnosed congenital adrenal hyperplasia. The age at onset, the rate of progression and any change with any treatment or with fluctuations in weight or skin problems should be determined.

In general, hirsutism is the most representative sign of clinical hyperandrogenism. It is defined as excess terminal (thick, pigmented) body hair in a male distribution, which usually starts during pubertal development or right after it, although not infrequently it may manifest in the adult age. It can also be expressed earlier, even during mid-childhood, where it can be associated in a mild form with a premature adrenarche characterized by the appearance and progressive development of pubic and/or axillary hair [9]. Typical areas of androgen-dependent terminal hair are face (particularly upper lip and chin); around the nipples and the breast area; and the abdomen, along the linea alba. Rapid and progressive worsening of hirsutism, or a later age of onset, suggest the possibility of ovarian or adrenal tumour, although they could even follow suspension of previous treatments or changes in weight.

Acne is typically the first manifestation of hyperandrogenism after menarche, in the teenage years. The typical acne lesions vary in increasing order of severity (see: *Physical examination*), which are highly dependent on previous topical, systemic and cosmetic treatments. A familiar prevalence of acne may be present.

Androgenic alopecia may also occur. Terminal hair growth is age dependent, and it may not be apparent until the early twenties after several years of exposure to excess androgens. Male pattern hair loss tends to present even later, in the later twenties and beyond**.** Androgenic alopecia may be graded by well-known subjective methods (see: *Physical examination*).

1.6 Evaluation of Menstrual Irregularity and Chronic Anovulation

Anovulation is undetectable in childhood, whereas in the perimenarcheal phase, adolescent women exhibit a transient state of anovulation, characterized by accentuated 24-hour LH levels [10]. However, making a correct clinical diagnosis of ovarian dysfunction at this age represents a difficult task and another 2 or 3 years may be needed. In fact, the menstrual cycle is rather long and variable during the first few years after menarche, and the establishment of regular ovulatory cycles is a slow process in physiological conditions. Using sequential progesterone measurements, it has been shown that more than 80% of the cycles are anovulatory during the first year after menarche, 60% during the third year and 25% after the sixth year are still anovulatory [10]. On the other hand, there are data supporting the finding that anovulatory pubertal or post-pubertal girls may have higher testosterone, androstenedione and LH levels than their ovulatory counterparts [10]. These young girls therefore appear to be characterized by endocrinological features resembling PCOS, although it cannot be excluded that even “physiological” anovulation during and after puberty may be associated with transient hyperactivity of the hypothalamic-pituitary-gonadal axis leading, in turn, to increased androgen production. Moreover, in early puberty, ovarian hyperandrogenism is rarely detected, but it becomes more common after the age of 14–15 years. On the other hand, the persistence of the high LH level profile in hyperandrogenic adolescent girls may be responsible for anovulation and therefore for irregular menses [11]. This should be appropriately considered in clinical practice. In fact, the typical clinical manifestations of PCOS occurring at puberty and adolescent age include irregular menses, particularly oligomenorrhea, increased LH levels and signs of androgen excess, such as hirsutism or acne.

The menstrual irregularity of PCOS typically manifests in the peripubertal period, although some women may apparently have regular cycles at first and subsequently develop menstrual irregularity in association with weight gain. Menses irregularities include mild or severe oligomenorrhea (cycle length more than 35–40 days) or amenorrhea (no cycles for 6 or more consecutive months).

In addition, anovulation is very common in the presence of mild oligomenorrhea, but also when normal cycles are present [12]. Some cycles may be associated with dysfunctional bleeding. Endometrial atrophy may be present in some women with PCOS who have prolonged amenorrhea, which may be related to androgen excess.

Chronic anovulation is one of the most important criteria in the diagnosis of PCOS. Some women with hyperandrogenic symptoms appear to have regular menstrual cycles, which necessarily require at least one or two assessments to document ovulation, at least in adult women. In the majority of women with severe oligomenorrhea or amenorrhea, chronic anovulation is usually present. However, occasional ovulation may occur, particularly in women with less severe oligomenorrhea. Ovulation can be easily detected by measuring progesterone levels in the luteal phase, at approximately days 20–22 after cycle onset. Appropriate hormone levels suggesting an adequate luteal phase are 6–8 ng/mL.

Additional information in a young or adult woman suspected to have PCOS should be obtained: age of menarche, presence of symptoms of ovulation or of premenstrual symptoms (ovulatory pain, premenstrual discomfort, breast tenderness), previous pregnancies or abortion and particularly oral contraceptive (OC) use [12]. Most young women, in fact, have a history of long-term OC use, often with different preparations, and this may have masked or delayed the recognition of menstrual dysfunction or hyperandrogenic symptoms [6]. In women presenting while taking OC, blood testing or pelvic ultrasounds should not be performed until they have discontinued OC use for at least three months. Notably, a sudden onset of menstrual dysfunction should raise the consideration of other aetiologies. Obviously, in the presence of recent unexplained amenorrhea, pregnancy should be excluded by appropriate testing. Moreover, weight- and exercise-related causes, hyperprolactinemia, subclinical or overt thyroid dysfunction, particularly in young women, should be investigated. Premature ovarian failure should also be suspected in adult women with unexplained amenorrhea.

1.7 Evaluation of Infertility

PCOS is a common cause of counselling in infertility clinics. Infertility was already included in the original description of PCOS by Stein and Leventhal [2]. Infertility related to PCOS is typically not difficult to diagnose due to the associated menstrual irregularity and anovulation. The primary cause is chronically irregular ovulation, leading to a reduced number of ovulations and unpredictable timing. However, some women do not receive the diagnosis of PCOS until they are being evaluated for infertility. The presence of PCOS does not rule out other abnormalities, so that male factor infertility and tubal patency must still be assessed. If the patient is at risk for metabolic defects, these must be screened for and treated as appropriate, to minimize pregnancy complications related to diabetes in particular. An increased rate of early pregnancy loss in PCOS may be an additional cause of infertility, but the mechanism of this is poorly understood [13]. A reduced rate of conception relative to the rate of ovulation after therapy with clomiphene citrate and exogenous gonadotropins is also well known; by contrast there are data suggesting that women diagnosed with polycystic ovaries (PCO) at ultrasound may be more likely to hyperstimulate in response to ovulation-inducing medications [7]

1.8 Evaluation of Overweight and Obesity

From the earliest descriptions of PCOS, obesity has been a prominently recognized clinical feature. Thus, some clinicians mistakenly fail to consider the PCOS diagnosis in lean women. However, several recent population-based studies of PCOS indicate that obesity is not a universal feature, with 30–70% of women with menstrual dysfunction and evidence of hyperandrogenism not being obese, depending on geographical areas and ethnicities [1]. Some recent data support the evidence that prevalence of PCOS may increase with increasing BMI [14].

Overweight and obesity, as well as different patterns of body fat distribution, can be easily assessed by anthropometric measures (see *Physical examination*). In adolescent girls, weight gain often precedes the onset of menses abnormalities [6]. In addition, a careful weight history should be performed, focusing on factors

influencing weight gain, and on changes in clinical hyperandrogenic signs (hirsutism, etc.), menses and ovulation and, if pertinent, on fertility in relation to previous weight fluctuations. Major stressful events should also be investigated, since they may precede weigh loss or gain. Finally, previous dietary treatments or eating disorders should be investigated. Birthweight and subsequent catch-up should also be recorded, when data are available (particularly in adolescent and young women), with the help of parents and obstetric charts. This information can in fact help to understand the pathophysiological development of PCOS.

Obesity has profound effects on the clinical, hormonal and metabolic features of PCOS, which largely depend on the degree of excess body fat and on the pattern of fat distribution [15]. In massively obese women, the prevalence of PCOS may be much higher than expected [16]. A higher proportion of obese PCOS women complain of hirsutism and other androgen-dependent disorders, such as acne and androgenic alopecia, in comparison to normal-weight women. Moreover, obese PCOS women are characterized by significantly lower sex hormone binding globulin (SHBG) plasma levels and worsened hyperandrogemia (particularly total and free testosterone, and androstenedione) in comparison with their normal-weight counterparts. The androgen profile can be further negatively affected in PCOS women by the presence of abdominal body fat distribution with respect to those with the peripheral phenotype, regardless of BMI values [15].

Menstrual abnormalities can also be more frequent in obese than normal-weight PCOS women. Reduced incidence of pregnancy and blunted responsiveness to pharmacological treatments to induce ovulation may also be more common in obese PCOS women [17]. A decreased efficiency of assisted reproductive technologies (ART) has also been demonstrated, with the consequence that in some countries, e.g., the United Kingdom, obese women with a BMI greater than 35 are not entitled to ART through the National Health System until they have reduced their body weight by appropriate therapeutic strategies [18].

Lipodystrophic states are rare disorders in which PCOS should also be ruled out [19].

1.9 Additional Information: Insulin Resistance, Metabolic Syndrome, T2DM and Risk for CVD

Metabolic abnormalities are very common in PCOS [20,21] and should always be investigated. Other than PCOS status per se, a positive family history for T2DM, obesity, dyslipidaemia and CVD is common. Second, the presence of the abdominal pattern of fat distribution should be considered, this condition being a clinical sign of dysmetabolic disorders and cardiovascular risk. Insulin resistance can also be present in otherwise normal weight PCOS women, and most of them tend to have an android shape. Acanthosis nigricans may be a valuable sign of insulin resistance.

Most patients consult the doctor after they have *undergone* laboratory *tests* or other diagnostic procedures; therefore, their careful evaluation should be part of the first clinical approach. Confirmation of insulin resistance can be obtained by simple biochemical tests, based on the ratio between glucose and insulin blood concentration, in both fasting and glucose-stimulated condition. However, they are relatively inaccurate on *an* individual basis [22]; reference tests, such as the euglycemic hyperinsulinemic clamp technique and the frequent-sampling intravenous glucose tolerance *test,* are reserved *for* research purposes. In the presence of normal fasting glucose values, fasting insulin levels can however predict in by approximately 80% insulin resistance measured by the clamp tech*n*ique [23].

Approximately half of PCOS patients have the metabolic syndrome, which can be clinically suspected in the presence of abdominal obesity, although an abdominal fatness pattern can be present even in normal weight women [23]. According to the National Cholesterol Education Program Expert Panel (NECP/ATPIII) criteria [24], the threshold values for waist circumference should be 88 cm in women, whereas the International Diabetes Federation (IDF) more recently adjusted the threshold according to the different ethnicities, and in Europeans it should be 80 cm [25]. A relatively but significantly small increase of arterial blood pressure can be found in PCOS women, particularly if they are overweight or obese. Values of systolic and diastolic blood pressure higher than 130 mmHg and 80 mmHg, respectively, can further suggest the metabolic syndrome. A biochemical evaluation of fasting glucose, triglyceride and HDL-cholesterol blood levels are however needed to confirm the diagnosis.

Because women with PCOS have an increased risk of insulin resistance and T2DM [26], it is important to assess the specific risk factors in each patient. In addition to weight, which is a major factor that increases the risk of diabetes, a history of glucose intolerance during pregnancy also increases the risk of later diabetes. The risk can be increased especially in women with a first-degree relative with T2DM [26].

The prevalence of non-alcoholic fatty liver disease (NAFLD), a benign condition of ectopic fat deposition and non-alcoholic steatohepatitis (NASH), is increased not only in obesity or the metabolic syndrome, but also probably in women with PCOS [27]. It appears reasonable to inquire about symptoms and risk factors for liver disease, including family history and alcohol ingestion. Liver dimensions are usually increased in these conditions and can be determined by physical examination.

There is a great debate as to whether women with PCOS are susceptible to a significant risk for CVDs [28]. In the last few years, a growing amount of data has been published showing that states of insulin resistance such as T2DM, obesity (particularly the abdominal phenotype) and PCOS are characterized, among other well-defined factors, including hormonal and metabolic alterations, by impaired coagulation and fibrinolysis, anatomical and functional endothelial injury and vascular dysfunctions, and a state of subclinical inflammation, which overall represent independent risk factors for CVDs. Retrospective studies have however not confirmed a higher prevalence of myocardial infarction or stroke in PCOS [29]. Nevertheless, a careful clinical examination of the cardiovascular system should be always performed, particularly in adult premenopausal and particularly postmenopausal women with previously diagnosed PCOS.

1.10 The Impact of Obesity on Insulin Resistance, Metabolic Syndrome, T2DM and Risk for CVD

Obese PCOS women are invariably more insulin resistant than their insulin resistant normal weight counterparts, and they may have more severe fasting and glucose-stimulated hyperinsulinemia. Although it is commonly accepted that both obesity and PCOS status (i.e. androgen excess) have an additional deleterious effect on insulin sensitivity, specific mechanisms have still not been adequately defined and could even be different among obese and non-obese PCOS women [15,30]. In the presence of obesity, studies performed to investigate insulin secretion in relation to the magnitude of ambient insulin resistance have however shown that there is a subset of PCOS women exhibiting a significant impairment of β-cell function. Interestingly, β-cell dysfunction has been particularly found in those women who had a first-degree relative with T2DM, so that a heritable component of β-cell secretion in families of women with PCOS has been suggested [31].

Worsening insulin resistance in the long term may represent an important factor in the development of glucose intolerance states (including impaired glucose tolerance and T2DM) in PCOS women, particularly in the presence of obesity [30]. This rarely occurs in those with normal weight [20], which suggests that obesity may represent a indispensable prerequisite.

Although PCOS per se may be associated with alterations of both lipid and lipoprotein metabolism, the coexistence of obesity usually leads to a more atherogenetic lipoprotein pattern, characterized by lower HDL cholesterol and higher triglyceride blood concentrations. Therefore, it is not surprising that the prevalence of the metabolic syndrome is significantly more common in obese PCOS women. It is, however, still unclear whether the increased prevalence of other risk factors for CVD reported in PCOS women may depend on the presence of obesity.

1.11 Sleep Disorders

Recent studies have shown that PCOS women may have an increased risk of the obstructive sleep apnoea syndrome (OSAS), diagnosed either by questionnaire or by overnight polysomnography [32]. This sleeping disorder is much more common in the presence of obesity. Thus, women with PCOS should be questioned about signs and symptoms of OSAS. Such symptoms include habitual snoring, nocturnal restlessness and daytime sleepiness.

1.12 Diet History and Food Intake

Dietary habits and history should be helpfully used in every patient with hyperandrogenism, infertility and metabolic disorders, such as obesity, and particularly in those with PCOS. These can be obtained with the help of dieticians or using standardized questionnaires. Excess energy and fat intake can be found in PCOS patients, although contradictory data have been reported [15]. Notably, a potential role of advanced glycation end-products (AGEs), known to be implicated in the atherosclerotic process and correlated with molecular damage, oxidative stress and endothelial cell activation, has been recently emphasized in the pathophysiology of insulin resistance associated with PCOS [33]. Since AGEs are present in many foods, it is expected that this will be extensively investigated in future research. Although a clear role of dietary factors has not yet clearly been defined in the pathophysiology of PCOS, it has been clearly demonstrated that changes in lifestyle towards a healthy diet may significantly improve not only body weight and fat distribution in otherwise affected obese women, but also menses and fertility, besides metabolic disturbances [15].

1.13 Psychological Aspects and Quality of Life

A few recent studies have evaluated the quality of life in women with PCOS and have begun to document the adverse psychological and health impacts of this condition. This can be performed using specific questionnaires adapted to PCOS and investigating different domains, such as emotions, body hair, weight, infertility and menstrual problems [34]. Moreover, studies using psychological questionnaires to investigate obsessive-compulsive behaviour, interpersonal sensitivity, depression, anxiety, aggression and psychoticism have shown a significant prevalence of these problems [35]. The extensive use of these questionnaires could improve the clinical assessment of patients with PCOS and provide effective treatment based on personal complaints rather than on a doctor's targets. This is particularly relevant in improving patient compliance and avoiding over-treatment in otherwise healthy women.

1.14 PCOS After Menopause

PCOS after menopause still represents an undefined endocrinological entity. In normal women, the transition to postmenopause involves not only a decrease in ovarian oestrogen formation but also a reduction of ovarian androgens [6]. Little is known about what happens to ovarian morphology and androgen production in women with PCOS after menopause. In one study analyzing a group of postmenopausal women, it was found that 42–44% of them had morphological ultrasound features consistent with PCO, and the comparison between the two groups showed that postmenopausal women with PCO had higher serum concentrations of testosterone and triglycerides than postmenopausal women with normal ovaries [36]. These findings strongly resemble PCOS features and indicate that this disorder is probably higher than expected in postmenopausal women. On the other hand, it should also be considered that hyperandrogenism appears to partly resolve before the menopause in women with PCOS [6]. In fact, one study found that total and non-SHBG-bound testosterone levels were reduced by approximately 50% among women aged 42–47 years with respect to 20–42 years of age and remained stable in women older than 47 years of age [37]. When PCOS women were compared to controls, testosterone levels were similar between the two groups in the age range of 42–47 years, whereas they were significantly higher in PCOS women than controls under or above this range. The assumption that hyperandrogenism tends to improve during late fertile age in PCOS women may explain the tendency of women with PCOS to cycle regularly as they grow older. These preliminary studies emphasize the need for further research, with particular emphasis on the role of androgen excess in the pathophysiology of metabolic and cardiovascular diseases [38], which are dramatically increasing in postmenopausal women. In a recent study [39] aimed at evaluating the risk of CV events in 390 postmenopausal women enrolled in the NIH–NHLBI sponsored Women's Ischemia Syndrome Evaluation (WISE) study, it was found that a total of 104 women had

clinical features of PCOS defined by a premenopausal history of irregular menses and current biochemical evidence of hyperandrogenemia. These women were found to be more often diabetic ($p<0.0001$), obese ($p=0.005$), more frequently had the metabolic syndrome ($p<0.0001$) and more angiographic coronary artery disease (CAD, $p=0.04$) compared to women without clinical features of PCOS. These data emphasize that identification of postmenopausal women with clinical features of PCOS may provide an opportunity for prevention of CVD events.

1.15 Physical Examination

1.15.1 Anthropometry

It is important to measure height (metres) and weight (kilograms), calculate body mass index (BMI) (kg/m^2) and assess body fat distribution by waist, hip and the waist-to-hip ratio (WHR) [40] at baseline and during follow-up. Waist circumference may add additional information as to the cardiovascular risk profile for individual women. In addition to truncal obesity, a buffalo hump and supraclavicular fat deposition may suggest the presence of Cushing's syndrome.

1.15.2 Skin

Hirsutism is defined as excess terminal (thick pigmented) body hair in a male distribution, and it is commonly noted on the upper lip, chin, periareolar area of the breast, in the midsternum and along the linea alba of the lower abdomen. There is substantial ethnic variability in hirsutism; Asian women, for example, often have a lesser degree of hirsutism [7]. Hirsutism should be distinguished from hypertrichosis, the excessive growth of androgen-independent hair which is vellus, prominent in non-sexual areas, and most commonly familial or caused by systemic disorders (hypothyroidism, anorexia nervosa, malnutrition, porphyria and dermatomyositis) or medications (phenytoin, penicillamine, diazoxide, minoxidil or cyclosporine). The most widely used semi-quantitative method for estimating hirsutism is the Ferriman and Gallway score [41]. However, recent studies support the concept that hair growth on the face may be more relevant than in other parts of the body [42]. By means of this score, the efficacy of treatment can be easily quantified and followed-up.

Typical acne lesions include blackheads, whiteheads, inflammatory lesions, severe pustular lesions and scars, in increasing order of severity. Acne can be graded according to different stages [43], which are highly dependent on previous topical, systemic and cosmetic treatments. Obviously, evaluation and monitoring of therapy in women with PCOS is mandatory, although there are no controlled studies.

Androgenic alopecia may be graded by well-known subjective methods, such as the Ludwig score [44]. More sophisticated information can be obtained with the help of dermatologists, who are confident with much more extensive diagnostic methods, including pulling and weighing hairs in a defined region, standardized photographs and assessing hair density in defined regions of the scalp.

Other skin findings that should be sought include seborrhea, acanthosis nigricans, and striae, thin skin, or bruising, which suggests possible Cushing's syndrome. Acanthosis nigricans is particularly relevant in the clinical evaluation of PCOS. As reported above, this is a common finding in women with PCOS, particularly in those with obesity. It can be found on the nape of the neck and in the axillary region, and sometimes in other parts of the body (elbows, folds of the skin, hands, etc.). Its presence may represent a skin marker of insulin resistance and the metabolic syndrome. Its presentation may, however, be poorly defined, and clinical skin examination may be very insensitive for detecting acanthosis nigricans, as documented by a study comparing clinical staging with histological examination [45].

1.15.3 Reproductive System

A complete reproductive system examination should be conducted at the time of diagnosis and in follow-up examinations as appropriate to the initial findings and progression of symptoms. The breast exam should include a specific assessment of atrophy (potential evidence of significant hyperandrogenemia), and galactorrhea, as well as the mandatory assessment for pathologic masses. The external genitalia should be examined for evidence of clitoromegaly, which should prompt a search for androgen-producing neoplasms or undiagnosed class 21-hydroxilase deficiency. The examination should also verify that the internal genitalia (vagina, uterus and ovaries) are present. Otherwise, an evaluation for other rare causes of amenorrhea and hyperandrogenism (ex testicular feminization) must be considered.

Pelvic ultrasound may assist in the physical examination and therefore the diagnosis of PCO should be performed according to the criteria described by the Rotterdam Consensus Conference [4], unless updated.

1.15.4 General

PCOS is a systemic disorder that requires a complete physical investigation, from head to toe in an objective search for abnormalities. Skill in physical diagnosis is acquired with experience, but it is not merely technique that determines success in eliciting signs, and it reflects a way of thinking more than a way of doing. Previous paragraphs have focussed particular attention on anthropometry, signs of androgen excess and a systematic evaluation of the reproductive system. Arterial blood pressure should always be measured, and a careful investigation of the cardiovascular system should be performed. The abdominal examination should include assessment of hepatic size (to evaluate possible hepatic enlargement due to NAFLD), as well as palpation for adrenal and pelvic masses, if possible. Other skills depend on the specific phenotype.

1.15.5 Differential Diagnosis

The diagnosis of PCOS is often a diagnosis of exclusion. Other causes of hyperandrogenism include hyperprolactinemia, drugs (danazol and androgenic progestins, valproate), non-classic congenital adrenal hyperplasia, Cushing's syndrome and androgen secretion (ovarian or adrenal) tumours.

The differential diagnosis of acne includes acne rosacea (which generally responds to antibiotic therapy and is not a typical feature of PCOS), acne fulminans (which is most common in adolescent males and is associated with fever, arthalgias and leukocytosis), the SAPHO syndrome (defined as synovitis, acne, pustulosis, hyperostosis and osteitis and requires referral for systemic therapy).

Other causes of menstrual dysfunction need to be considered, including pregnancy, ovarian failure, outflow track obstruction and hypothalamic amenorrhea, in the appropriate clinical context.

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Chapter 2
The Menstrual Cycle in PCOS

Sabrina Gill and Janet E. Hall

Polycystic ovary syndrome (PCOS) is defined as a syndrome of ovarian dysfunction along with the cardinal features of hyperandrogenism and polycystic ovary morphology in the absence of other explanatory endocrinopathies [1]. The etiology of PCOS is multifactorial and complex with hyperinsulinemia, abnormal ovarian steroidogenesis, and neuroendocrine abnormalities playing significant interactive roles. The vast majority of patients have menstrual irregularities and recent studies have indicated that those with menstrual cycle dysfunction also tend to be more hyperandrogenic and hyperinsulinemic [2, 3]. This chapter will review the integration of ovarian, hypothalamic and pituitary factors that occur in normal menstrual cycles; will discuss the variable patterns of menstrual dysfunction in patient with PCOS; and will review what is known about the potential etiology of ovarian dysfunction in PCOS.

2.1 The Normal Menstrual Cycle

The normal menstrual cycle is divided into two stages (Fig. 2.1) – the follicular phase begins with day 1 of menses and is noted by the emergence of a cohort of follicles that develop in response to rising levels of FSH. In normal women, a single follicle from this cohort will develop into a dominant follicle and ovulate in response to the mid-cycle LH surge (MCS). The luteal phase begins after ovulation when hormonal events prepare the endometrium for implantation should conception occur.

Neuroendocrine axes regulate and integrate neural and hormonal information and translate these signals to physiological actions that impact the synthesis and secretion of different hormonal systems. Neuroendocrine regulation of the menstrual cycle involves a complex integrated network of feedback mechanisms between the hypothalamus, pituitary, and target organs. The hypothalamic-pituitary-gonadal (HPG) system is comprised of the gonadotropin-releasing hormone (GnRH) producing neurons of the hypothalamus, the pituitary gonadotropes which secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and the ovary which responds to gonadotropin secretion with follicular development and ovulation and with secretion of estradiol, progesterone, and the gonadal peptides, inhibin A and inhibin B. Ovarian steroid and non-steroidal hormones, in turn, modulate the hypothalamic and pituitary components of the reproductive axis [for review see ref. 4].

2.2 Dynamics of Hypothalamic Secretion During the Normal Menstrual Cycle

Frequent blood sampling studies with measurement of LH as a marker of GnRH secretion and the use of pharmacological probes, such as GnRH antagonists, have been utilized to evaluate the physiology of GnRH secretion

S. Gill (✉)
Division of Endocrinology & Metabolism, St. Paul's Hospital and the University of British Columbia, Vancouver, BC, Canada
e-mail: SGill@providencehealth.bc.ca

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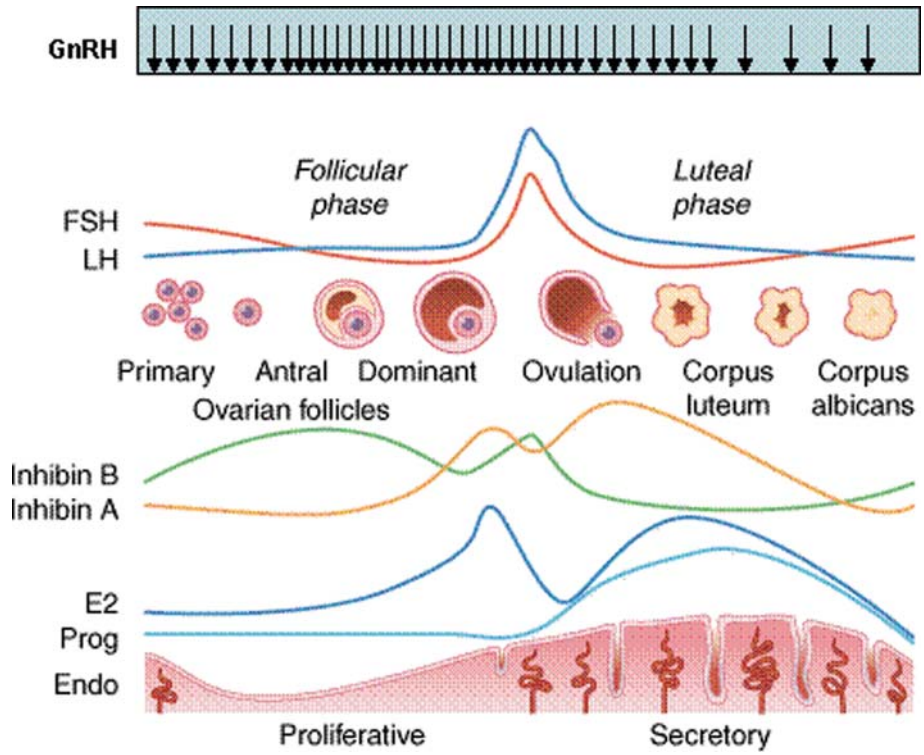

Fig. 2.1 Hormonal dynamics of the normal menstrual cycle. Adapted from [4]

in studies in women with normal menstrual cycles [4] and in women with PCOS [5]. In normal women, the frequency of pulsatile GnRH secretion is dynamically regulated across the menstrual cycle (Fig. 2.1). The transition from the end of one cycle to the beginning of the next is marked by an increase in pulsatile LH/GnRH secretion from the luteal phase frequency of one pulse every four hours to a pulse of every 90 minutes in the early follicular phase. During the mid-follicular phase, LH pulse frequency increases to one pulse per hour, and this frequency is maintained through the MCS. After the MCS and ovulation, the GnRH pulse generator slows down to one pulse every 90 minutes, followed by a further decline to one pulse every four hours during the late luteal phase. The luteal phase decrease in GnRH pulse frequency is secondary to rising progesterone levels in the presence of estrogen. Although gonadal steroid levels fluctuate less dramatically because of prolongation of their half-life due to binding to sex hormone binding globulin (SHBG), progesterone concentrations can fluctuate dramatically in the mid and late luteal phases (from 2.3 to 40.1 ng/mL) in response to the relatively infrequent pulses of LH [6].

Changes in the frequency of pulsatile GnRH secretion across the menstrual cycle are important because of the effect of GnRH pulse frequency on the differential regulation of pituitary LH and FSH synthesis and secretion. At slow GnRH pulse frequencies, GnRH receptor (GnRHR) concentrations on gonadotrope cell surfaces are relatively low with activation of a single signal transduction pathway stimulating expression of α-subunit, LHβ, and FSHβ. Faster GnRH pulse frequencies increase GnRHR concentrations resulting in greater activation of the signal transduction pathway and stimulation of a second signal transduction pathway that specifically inhibits FSHβ gene expression [7]. Thus, slow frequencies of pulsatile GnRH stimulation of the gonadotrope result in increased synthesis of FSH while faster GnRH pulse frequencies favor the synthesis and secretion of LH.

2.3 Feedback During the Normal Menstrual Cycle

FSH levels rise 3-fold in the early follicular phase in response to release from the negative feedback effects of estradiol and probably inhibin A (Fig. 2.1). FSH release is further facilitated by the increase in GnRH pulse frequency that occurs with the late luteal phase decline in progesterone [4]. The luteal-follicular rise in FSH is critical for initiation of folliculogenesis and the beginning of a new cycle of follicle development. With recruitment and early development of a new cohort of follicles, estradiol and inhibin B increase, inhibiting FSH. This mid-follicular phase decrease in FSH is important for ensuring that only a single follicle emerges as dominant and reaches maturity. While the initial increase in estradiol inhibits GnRH, LH, and FSH secretion, the exponential rise in estradiol that subsequently occurs with growth of the dominant follicle exerts a positive feedback effect on gonadotropin secretion and LH levels rise 10-fold. Ovulation occurs within 36 hours after the midcycle LH surge. LH levels subsequently decrease and reach a nadir by the late luteal phase. Progesterone secretion begins with luteinization of the theca-granulosa cells which is induced by the LH surge, reaching peak concentrations in the mid-luteal phase. The corpus luteum also secretes estrogen and inhibin A, which follows a similar pattern to that of progesterone.

2.3.1 Normal Folliculogenesis

At the level of the ovary, growth factors, such as stem cell growth factor, basic fibroblast growth factor, growth differentiation factor-9 (GDF9), and anti-mullerian hormone (AMH or MIS), regulate recruitment of primordial follicles for growth [8]. The selected follicles proliferate in response to the luteal-follicular rise in FSH. FSH also improves survival of granulosa cells and recruitment of a dominant follicle. With selection and development of the follicles, secretions of inhibin B, estradiol, and subsequently inhibin A combine to inhibit FSH secretion. Local positive factors (such as insulin-like growth factor), which promote growth and inhibit apoptosis of follicles, and negative factors (such as AMH), which decreases granulosa cell sensitivity to FSH and inhibits aromatase activity, play roles in selective negative differentiation of the remaining follicles allowing a single-dominate follicle to emerge [4, 9]

2.4 Menstrual Dysfunction in PCOS

Normal menstrual cycles range between 25 and 35 days due to variability in the length of the follicular phase in different women. In PCOS, 60–80% of patients present with menstrual irregularities with fewer than nine menstrual periods per year [10]. In some patients, menses occur very infrequently or not at all while in 5–10% of PCOS women, more frequent bleeding and menorrhagia may occur. Importantly, not all episodes of vaginal bleeding follow ovulation. Anovulatory bleeding has been reported in up to 20% of women who report normal menstrual cycles [11], and measurement of progesterone may be required. For this reason, current guidelines for the diagnostic criteria of PCOS specify oligo- or anovulation rather than oligo- or amenorrhea [1]. The pathophysiology of PCOS is multifactorial with dysregulation of gonadal and adrenal steroidogenesis, abnormal neuroendocrine regulation and insulin resistance.

2.5 Gonadal Steroids in PCOS

The polycystic ovary is characterized by an increased number of antral follicles and an increase in the mass of theca cells surrounding each follicle. Serum levels of inhibin B are higher in PCOS reflecting an increase in the number of antral follicles while the reported decrease in dimeric inhibin B production per follicle is consistent with a decrease in the number of granulosa cells per follicle and arrested folliculogenesis [12]. Inhibin does not

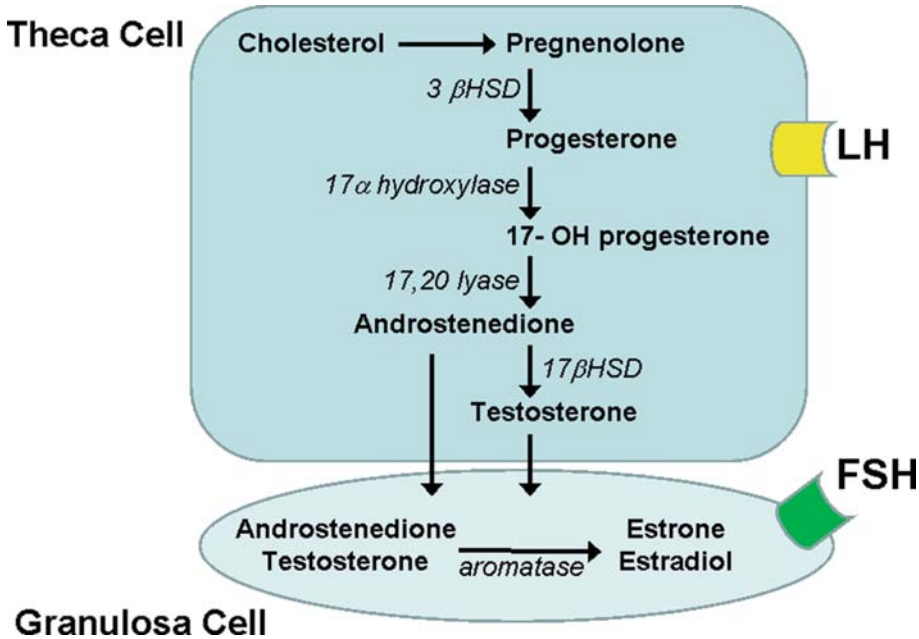

Fig. 2.2 Ovarian steroidogenesis in the theca and granulosa cells

fully account for the relative suppression of FSH compared to LH in women with PCOS, but higher LH levels may suppress inhibin B [13].

Ovarian steroidogenesis requires the coordinated activities of the theca cells, which synthesize androgens from cholesterol under the control of LH and granulosa cells, which aromatize these androgen precursors to synthesize estrogens under the control of FSH (Fig. 2.2). Theca cells in patients with PCOS exhibit defects in a number of enzymes in the steroidogenic pathway, such as 17-hydroxylase and 17,20-lyase, that may contribute to increased ovarian androgen production [14].

Ovarian 17-hydroxyprogesterone hyper-responsiveness to hCG or a GnRH agonist in women with PCOS is indicative of the increased sensitivity of the ovary to LH stimulation [15] and likely reflects the combination of theca cell hyperplasia, the increased number of antral follicles and steroidogenic pathway dysregulation. In addition, studies showing that suppression of LH either acutely using a GnRH antagonist or chronically using a GnRH agonist results in decreased testosterone levels support the importance of the increased LH in the etiology of ovarian hyperandrogenism in PCOS [16].

There is evidence that adrenal hyperandrogenemia may also play a role in biochemical hyperandrogenemia in some patients with PCOS and girls with premature pubarche, and exaggerated adrenarche are at higher risk of development of PCOS in adulthood [17].

2.6 Follicular Development in PCOS

Follicular development in PCOS is abnormal for two reasons. First, in the ovarian hyperandrogenemia environment, there is a 6-fold increase in the number of primary growing follicles (2–5 mm). Androgens promote preantral and antral follicle development by increasing proliferation and sensitivity of theca and granulosa cells to gonadotropins and inhibiting apoptosis [18]. In the theca cells, there is upregulation of steroidogenic enzymes, such as 3β-hydroxysteroid dehydrogenase, and 17α-hydroxylase/17,20 lyase, with increased androgen and progesterone secretion [14]. The inhibins, particularly inhibin A, may also increase LH-induced androgen production in thecal cells [19].

In addition to the increase in follicular development in PCOS, the appropriate process of follicular arrest with selection and development of the dominant follicle is inconsistent. These patients lack the dynamic modulation of FSH that characterizes the normal luteal-follicular transition and is responsible for orderly recruitment of follicles into the growing pool. Serum and follicular fluid AMH levels are also higher in women with PCOS [18] potentially decreasing aromatase activity and inhibiting development of the dominant follicle. Finally, premature exposure of the granulosa cells to LH also leads to inhibition of cell proliferation and follicle growth resulting in poor follicular development [20].

2.7 Neuroendocrine Abnormalities in PCOS

While abnormalities at the level of the ovary itself are clearly important, the neuroendocrine abnormalities in PCOS also contribute to abnormal follicular development. LH levels are elevated in comparison to FSH resulting in 94% of women having an elevated LH/FSH ratio [5]. The LH amplitude response to GnRH is exaggerated and GnRH pulse frequency is increased in PCOS at approximately one pulse per 50–60 min [21]. Furthermore, recent studies have also shown an increase in the overall amount of GnRH secreted that is similar in magnitude to the increase in pulse frequency [21]. As discussed above, this pattern of GnRH secretion favors the synthesis and secretion of LH over FSH. Spontaneous ovulation transiently improves the abnormal LH/FSH ratio in PCOS [5]. However, studies have shown that the sensitivity to progesterone-induced slowing of GnRH pulse frequency in women with PCOS is less than in normal women [22]. There has been considerable controversy regarding whether the neuroendocrine abnormality in PCOS is a primary abnormality or is secondary to other factors. However, reversal of abnormalities in progesterone-induced slowing of pulse frequency by androgen receptor blockade suggests that it is due at, least in part, to secondary mechanisms.

In normal adolescents, menstrual irregularities are not uncommon for several years after menarche. However, irregular menstrual cyclicity may persist ultimately leading to the diagnosis of PCOS. In peripubertal girls with hyperandrogenemia, there is early evidence of neuroendocrine abnormalities, including an increased LH/FSH ratio, and a faster frequency and higher amplitude of LH pulses [22]. It has been hypothesized that hyperandrogenemia in adolescence may lead to reduced sensitivity of the GnRH pulse generator to progesterone-induced slowing resulting in an increase in the LH to FSH ratio, impairment of ovarian folliculogenesis and augmentation of hyperandrogenemia.

In women with PCO morphology and regular ovulatory cycles, gonadotropin dynamics are identical to those in normal ovulatory women [23]. Thus, PCO morphology in an abnormal gonadotropin environment is required for development of menstrual dysfunction in PCOS. Testosterone levels are higher in this population than in women with normal ovarian morphology, independent of any abnormalities in gonadotropin secretion, possibly due to the increased ovarian thecal mass with PCO morphology.

In anovulatory women with PCOS, correction of abnormal FSH dynamics by reducing the negative feedback effect of estrogen using estrogen receptor blockers or aromatase inhibitors or more directly through administration of exogenous gonadotropins or pulsatile GnRH does not universally correct the ovulatory defect. With all treatment modalities, improved ovulation is negatively affected by hyperandrogenemia, high BMI, and insulin resistance [24, 25]. Such factors impact both locally at the ovary and on the neuroendocrine axis.

2.7.1 Impact of Hyperandrogenemia

Androgens appear to influence folliculogenesis through effects both at the hypothalamus and directly at the ovary. Animal studies support the hypothesis described above that the increased GnRH pulse frequency that is characteristic of PCOS is related to hyperandrogenemia [26]. In animal studies, prenatal androgen exposure is associated with increased LH pulsatility and decreased sensitivity to progesterone-induced slowing of GnRH pulses and increased GABAergic drive on GnRH neurons [22]. At the level of the ovary, androgens interrupt ovulation by providing a negative environment for egg release [27].

2.7.2 Impact of BMI

Obesity is prevalent in PCOS, occurring in approximately 30–60% of patients and is negatively associated with the success of ovulation induction. BMI and percent body fat are negatively correlated with LH and in very obese women with PCOS, the LH/FSH ratio may be relatively normal [5]. There is no effect of BMI on either the amount or frequency of GnRH secretion in women with PCOS [21] indicating that obesity does not exert its effect on LH secretion at the hypothalamic level. The decrease in LH responsiveness to GnRH as a function of obesity supports a direct effect of factors relating to BMI at the pituitary level as does the increase in clearance of LH, which is proposed to be due to changes in the isoforms of LH secreted [27]. Leptin, which is secreted by adipocytes and is regulator of appetite and energy homeostasis, is higher in women with PCOS and inversely related to LH levels [28] suggesting that it may mediate the effect of BMI on LH secretion. Other potential mediators include ghrelin which is also inversely related to BMI, and hyperinsulinemia, which is discussed further below. At the level of the ovary, inhibin B is inversely related to BMI [29] suggesting that follicular development is also negatively impacted by obesity.

2.7.3 Impact of Hyperinsulinemia

Insulin resistance to glucose uptake is observed in approximately 50–75% of lean and obese women with PCOS [30]. Hyperinsulinemia is positively associated with anovulation and hyperandrogenemia. Importantly, reduction of insulin resistance and insulin levels with weight loss, metformin or thiazolidenediones improves spontaneous follicular development, ovulation, and hyperandrogenemia [31]. Hyperinsulinemia and/or insulin resistance may play a role at multiple levels.

Hyperinsulinemia secondary to peripheral insulin resistance has important effects at the level of the ovary, synergizing with LH in stimulation of androgen synthesis in the granulose cell [32]. Hyperinsulinemia is associated with lower levels of inhibin B, and there is evidence that high concentrations of insulin result in premature differentiation of granulosa cells and follicular arrest. While the effects of medications that improve insulin resistance and decrease peripheral insulin levels are most obvious in obese women, the significant response of lean women with PCOS to insulin sensitizers is consistent with additional in vitro evidence of a direct effect of insulin-sensitizing agents on ovarian steroidogenesis [33] and supports the hypothesis that abnormalities in insulin signaling [30] may play a role in disordered menstrual cycle dynamics in women with PCOS, independent of BMI.

Insulin receptors are present on the pituitary and hypothalamus. Unlike anti-androgen agents, metformin failed to have a significant impact on the sensitivity of the GnRH pulse generator on gonadal steroid feedback [34]. The role of insulin at the pituitary is controversial. However, recent studies suggest that in women with PCOS, insulin suppresses the LH response to GnRH and may be responsible, at least in part, for the inverse relationship between LH and BMI in PCOS [35]. Finally, insulin decreases hepatic production of sex-hormone biding globulin, resulting in elevated bioavailable androgens [30].

2.8 Summary

Menstrual cycle dysfunction is common in PCOS due to disordered folliculogenesis and anovulation and may present as oligoamenorrhea, amenorrhea, or dysfunctional bleeding. The degree of menstrual dysfunction is highly variable between patients and is generally more marked in association with higher androgen and insulin levels and a higher BMI. Menstrual dysfunction in PCOS is attributed to multiple factors: neuroendocrine abnormalities, ovarian dysregulation of steroidogenesis and insulin resistance, each contributing at various levels to impact folliculogenesis and ovulation. Intervention at various levels has been shown to improve and promote appropriate follicle development and fertility.

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