Polycystic Ovary Syndrome

Current and Emerging Concepts

Lubna Pal
David B. Seifer
Editors

Second Edition

Springer

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ISBN 978-3-030-92588-8 ISBN 978-3-030-92589-5 (eBook)
https://doi.org/10.1007/978-3-030-92589-5

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Dedication for Lubna Pal

To my late mother, Dr. Jahanara Pal, Brigadier (Retd.), Army Medical Corps, Professor Emeritus, Armed Forces Medical College, Pakistan, and my late father Dr. Khawaja Muhammad Mohsin Pal, Major General (Retd.), Army Medical Corps, Pakistan)—for the life lessons of selflessness, commitment, empathy, and humility, for your passionate belief in human will and potential, and for your absolute commitment to excellence and equality—you have been inspirational at every step of the way. To my brothers, Dr. Khawaja Muhammad Inam Pal (Professor of Surgery, The Agha Khan University Hospital, Karachi, Pakistan) and Shakeel Pal, for decades of companionship, comradery, support, and for the precious memories— looking forward to spending more time catching up in years ahead! To my husband, Dr. Sohail Kayani, for his unconditional support— I could not have been where I am without you. To my sons, Jehanzeb Kayani and Teimur Kayani—you reinvigorate my faith in humanity—as you negotiate your own careers in the field of

medicine, may your journeys be exciting, challenges be surmountable, your companions be sincere and compassionate, and the ride so worth it. To the many teachers, mentors, and trainees I have had the privilege to learn from—thank you for the invaluable lessons that have contributed to my growth as a clinician, researcher, and teacher. To my patients— thank you for the privilege of participating in your care and for the invaluable learning in the art of medicine.

Dedication for David B. Seifer

"May the love of my art inspire me at all times…" Maimonides

To the memory of my parents and to honor the members of my family who have provided inspiration along my journey: my mother, for her passion, intellectual curiosity, and the pursuit of knowledge; my father, for his mindset to challenge the norm while being respectful of others; my brother for his quest of a physical challenge and his embrace of the joy of life; my wife for her commitment to excellence and her moral compass; and our two wonderful sons for inspiring us to be the best versions of ourselves and who are our legacy for the future.

Preface

Since its initial description in 1935, medicine's approach to polycystic ovary syndrome (PCOS) has been an iterative assessment with an ever-evolving understanding of this prevalent yet complex entity. Successions of revolutionary advances in fields of genetics, cell biology, and technological evolutions have allowed increasing clarity on the complexity of an entity that is ubiquitous, and the exact underpinnings of which remain largely elusive 85 years since its recognition.

The first edition of *Polycystic Ovary Syndrome: Current and Emerging Concepts* was published in 2014 with an overarching goal of familiarizing a diverse spectrum of readership (learners, practitioners, scholars, and researchers) with the core concepts relating to the pathophysiology, and of enhancing preparedness of practitioners from across disciplines in caring for reproductive age women with PCOS. It is the enthusiasm with which the first edition was embraced globally and the strides that the field of PCOS has witnessed in a span of just 7 years that have fueled our efforts to bring to the enthusiasts the most up-to-date information on what is known about the complex entity called PCOS.

The second edition stands enriched, not just by a meticulous review and revision of the 19 original chapters (comprising the first edition) but also by inclusion of additional 8 chapters that will introduce our readers to the unravelling mysteries and contemporaneous knowledge in the field. The overall format of the text follows a paradigm that is similar to the first edition. Part I (Chaps. 1, 2, and 3) provides an overview of the epidemiology of PCOS while stressing on similarities and distinctions in presentation between adults and adolescents. In Part II (Chaps. 4, 5, 6, 7, 8, and 9), the readers are systematically introduced to processes that are recognized as relevant to the pathophysiology of PCOS. Practicing clinicians will find the information assembled in Parts III (Chaps. 10, 11, 12, 13, and 14), IV (Chaps. 15 and 16), and V (Chaps. 17 and 18) of value in individualizing optimal care for women with PCOS. Less appreciated, yet highly consequential risks and societal burden of PCOS diagnosis are addressed in Part VI (Chaps. 19, 20, 21, and 22). In Part VII, Chaps. 23, 24, and 25 examine some emerging concepts in the field of molecular biology and their relevance to the pathophysiology of PCOS; Chap. 26 introduces the readers to the newer classes of therapeutic agents in the field of diabetes that may hold potential for benefit of women with PCOS, and the last Chap. 27 aims to underscore to the medical and research communities against complacency in our overarching responsibilities to the community.

We are indebted to each one of our expert contributors, as it is through their generosity of intellect and time that we are able to present, in the second edition of *Polycystic Ovary Syndrome: Current and Emerging Concepts*, a contemporaneous review on the topic of PCOS.

The final work product is a state-of-the-art presentation of our current understanding of PCOS. It is directed to and written for healthcare professionals with a range of expertise. This would include any healthcare professional interested in obtaining an in depth understanding of this constantly evolving syndrome which impacts a portion of women of almost any age, culture, involving a variety of organ systems which display a myriad of clinical phenotypes and presentations.

It has been our privilege to have had this opportunity of bringing together this team of experienced practitioners and scientists. It is our hope that this work will be as meaningful to the clinicians as it is to the researchers who are attempting to better understand the complexities of this disorder, and that our collective efforts will directly benefit the multitude of women with PCOS.

New Haven, CT, USA

Lubna Pal
David B. Seifer

Acknowledgments

We are indebted to the contributing authors, as this endeavor would not have been possible without their intellectual generosity and commitment—it has been our privilege to have had this opportunity of working with each one of you!

We are grateful to both our mentees and mentors in the collective acquisition and dissemination of knowledge, processes which build the foundation of academic medicine.

Our sincere thanks to the publisher's developmental editor, K. Sheik Mohideen—— your diligence, patience, and perseverance have been invaluable in helping us bring this project to a successful completion.

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Part I

Epidemiology of Polycystic Ovary Syndrome

Diagnostic Criteria and Epidemiology of PCOS

1

Alexandria M. Freeman, Heather R. Burks, and Robert A. Wild

Key Points

- PCOS is considered the most common endocrine disorder among reproductive-age women.
- The three recognized sets of criteria for PCOS diagnosis include the NIH criteria, the Rotterdam criteria, and the Androgen Excess and Polycystic Ovary Syndrome Society criteria.
- The Rotterdam criteria is the most widely used for diagnosis. It defines PCOS as the presence of at least two of the following: ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology.
- Other potential diagnoses including thyroid disorders, hyperprolactinemia, congenital adrenal hyperplasia, and Cushing's syndrome should be ruled out prior to diagnosing PCOS.
- The prevalence of PCOS depends upon the diagnostic criteria used. Rotterdam is most inclusive, followed by the Androgen Excess and Polycystic Ovary Syndrome Society criteria. The NIH criteria are the most strict, and therefore prevalence of PCOS is lowest.
- PCOS does not represent a single entity but occurs on a spectrum of heterogeneous disorders represented by a variety of different phenotypes of PCOS.
- Acne and hirsutism may be the presenting symptoms of PCOS and should prompt a thorough evaluation.
- Women with PCOS have an increased rate of many major cardiovascular risk factors: obesity, insulin resistance, metabolic syndrome, dyslipidemia, type 2 diabetes, and liver disease.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_1

- Women with PCOS are at an increased risk for additional chronic disorders such as depression and endometrial cancer.
- Identifying PCOS allows for screening and implementation of preventive strategies in order to minimize overall health risk in this population.

Diagnostic Criteria for Polycystic Ovary Syndrome

Since 1935, when Stein and Leventhal originally described the combination of oligo-ovulation and hyperandrogenism [1], the polycystic ovary syndrome (PCOS) has undergone multiple iterations of diagnostic criteria. Initially, description of the syndrome was based upon case reports. Over time, as new and better evidence became available, multiple efforts have been made to better characterize this syndrome to increase appreciation of this complex entity.

Clinicians worldwide may now choose between three major sets of *diagnostic criteria* to arrive at a diagnosis of PCOS. The first set of relatively stringent criteria was outlined at the National Institutes of Health (NIH) in Bethesda, Maryland, in 1990 but has largely been replaced in clinical practice by the *Rotterdam criteria*. A task force sponsored by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) met in Rotterdam, the Netherlands, in 2003 to review the available data and proposed a revision to the 1990 NIH diagnostic paradigm, hence the inception of the Rotterdam criteria. More recently, in 2009, the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society outlined its own set of criteria. It is important to appreciate that the subtle heterogeneities within the various diagnostic criteria utilized by investigators impacts upon the reported prevalence of PCOS in a given population.

The NIH meeting in 1990 was the first international conference on PCOS, and the guidelines that resulted from this meeting were based largely on expert opinion rather than the results of analytic studies [2]. The criteria set forth included (1) chronic anovulation and (2) clinical or biochemical signs of hyperandrogenism. Both criteria *must* be present, and other diagnoses *must* be excluded to allow reaching a diagnosis of PCOS. Once this initial step was taken to clearly define the syndrome, in ensuing years, better analytic studies revealed additional information subsequently evaluated by the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group to revise the original NIH proposed set of diagnostic criteria.

The Rotterdam consensus includes three diagnostic criteria and states that *any two of the three* must be present in order to make the diagnosis [3]. The revised criteria include (1) oligo- or anovulation, (2) clinical or biochemical signs of hyperandrogenism, and (3) polycystic appearing ovaries (PCO) on imaging. Other disorders *must*, of course, be excluded, including 21-hydroxylase-deficient non-classic congenital adrenal hyperplasia (NC-CAH), Cushing's syndrome, and androgen-secreting tumors as well as commoner entities such as thyroid dysfunction and hyperprolactinemia (Table 1.1). The addition of morphological appearance of

Table 1.1 Tests to rule out other potential diagnoses

Differential diagnoses	Tests to differentiate from PCOS
Cushing's	Dexamethasone suppression test 24-hour free urine cortisol
Congenital adrenal hyperplasia	17-hydroxyprogestrone
Thyroid disorders	TSH, T3, T4
Hyperprolactinemia	Prolactin
Premature ovarian failure	FSH, AMH
Androgen-secreting tumor	DHEAS, testosterone

polycystic ovaries identifies two additional phenotypes not previously included in the diagnosis: women with ovulatory dysfunction and polycystic ovaries but without hyperandrogenism and ovulatory women with hyperandrogenism and polycystic ovaries; deeper explorations reveal that these subcategories within PCOS identified based on the Rotterdam diagnostic criteria manifest subtle but distinct hormonal and metabolic milieu when compared to cases of PCOS identified based on the more stringent NIH criteria. The stated rationale for incorporating these additional phenotypes included the recognition that PCOS does not represent a single entity but rather occurs on a spectrum of heterogeneous disorders, as well as the associated long-term health risks such as of type 2 diabetes mellitus and cardiovascular disease, commonly encountered in women diagnosed with PCOS. The Rotterdam consensus statement advocated widening the inclusion criteria to avoid missing patients with the potential for these increased health risks. The addition of polycystic morphology evolved based on improving ultrasound resolution.

The most recent set of diagnostic criteria was compiled by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society in 2009 [4]. Their expert opinion review reexamined the key recognized features of PCOS, including menstrual dysfunction, hyperandrogenemia, clinical signs of hyperandrogenism, and polycystic ovarian morphology. Each feature was examined for its appropriateness for inclusion as a defining criterion, based on a thorough review of existing literature. A slightly modified version of the criteria for the diagnosis of PCOS emerged: (1) hyperandrogenism, including hirsutism and/or hyperandrogenemia; (2) ovarian dysfunction, including oligo-anovulation and/or polycystic appearing ovaries; and (3) exclusion of other androgen excess or related disorders. The AE-PCOS criteria also acknowledge that related disorders of hyperandrogenism must be excluded but allow that the clinician may take into account the prevalence of these differential diagnoses when deciding what tests to order. Disorders to consider in the differential diagnosis of PCOS include androgen-secreting neoplasms, Cushing's syndrome, 21-hydroxylase-deficient congenital adrenal hyperplasia, thyroid disorders, hyperprolactinemia, and premature ovarian failure. Similar to the NIH criteria, androgen excess is a necessary component of the diagnosis by AES criteria. Therefore, the phenotype of ovulatory dysfunction and PCO alone—permissible under Rotterdam—does not qualify for a diagnosis of the syndrome by AES criteria. The combination of menstrual dysfunction and PCO, in the absence of features of hyperandrogenism or evidence of hyperandrogenemia, has, in fact, been shown to have

the most similar anthropometrics, hormonal profile, and metabolic risks to the control subjects. The AES consensus criteria for defining PCOS are thus more inclusive than the NIH version but less so than the Rotterdam criteria.

Anti-Müllerian hormone (AMH) has recently been proposed as a surrogate marker for the diagnosis of PCOS. Indeed, AMH levels correlate independently with both PCO morphology and androgenic profile [5]. Although a cutoff value is not agreed upon, a level of 4.7 ng/mL has a specificity of 79.4% and sensitivity of 82.8% in diagnosing PCOS in symptomatic women [6]. Some propose AMH be used as a substitute for ovarian morphology on ultrasound. This would be especially useful in a setting where ultrasound is not readily available. When used in addition to ultrasound, it may also identify more cases of PCOS than ultrasound findings alone [7]. AMH may not identify all phenotypes of PCOS equally but does show promise for a new possible objective test for PCOS. Another parameter proposed as an adjunct to PCO morphology is an assessment of the ovarian stromal volume, measured as a ratio of the stromal area to total area of the ovary (S/A ratio). Although this S/A ratio performed well when discriminating between women with and without PCOS, and correlated with androgen levels, it has not been adopted as part of any of the existing diagnostic criteria [8, 9]. In addition, follicle count per ovary is suggested as a better ultrasonographic marker for diagnosis of PCOS [10].

Patients may initially present to a multitude of potential providers prompting a diagnosis of PCOS. Some may present as early as adolescence; however diagnosis can be difficult as menstrual irregularities and acne are common during this time. Many women present to obstetrician-gynecologists with oligo/anovulation or infertility; however, they also may present to a dermatologist with acne or hirsutism. It is prudent that primary care providers are able to recognize the symptoms of PCOS as it is associated with other health disorders. Diagnosis provides an opportunity to implement appropriate screening and prevention strategies for these women. As women are diagnosed in their reproductive years, it is important to note PCOS is associated with an increased risk for obstetric complications including gestational diabetes and hypertensive disorders [11]. Research has also shown a strong association with PCOS and non-alcoholic fatty liver disease among other significant chronic medical conditions [12].

Determination of hyperandrogenism in females can be problematic, both during clinical and biochemical assessment. Laboratory assays for androgens were initially designed for detection in males and have been calibrated accordingly. For example, total testosterone assays are typically calibrated for normal male levels, the lower end of which is 250 ng/dL. The upper end of normal female total testosterone ranges between 45 and 80 ng/dL (inter-laboratory differences exist, and clinicians should familiarize themselves with the assay range for the laboratories serving their patient population). Some take great pains to analyze normal women as reference and use extraction and chromatography and focus on specificity; others do not. Both the above specified values are well below the fifth percentile for the assay detection range, where assay results may become unreliable; notably, calibration studies have not been done to develop a commercial female assay. An additional diagnostic dilemma is that the reporting of clinical hyperandrogenism is examiner-dependent

and can be subjective. While a standardized tool such as the Ferriman-Gallwey score can help objectify evaluation, this method has been shown to have good intra-observer reliability but poor inter-observer reliability [13]. Furthermore, a universal application of such tools across all ethnic groups may discount the normal ethnic variation in the appearance of body hair.

Inclusion of ultrasonographic evidence of PCO morphology into the definition of PCOS is controversial. The various sets of criteria place different degrees of emphasis on an isolated phenotypic PCO component not uncommonly encountered in the general reproductive-age population; the NIH criteria do not address ovarian morphology, the Rotterdam criteria in 2003 include PCO as a phenomenon distinct from menstrual irregularities, and the AES lumps ovarian morphology into an "ovarian dysfunction" category along with oligo-anovulation and requires only one or the other to suffice as a diagnostic criterion. It is important to appreciate that PCO morphology is not specific to PCOS and can be found in 20–30% of the general population of women 20–25 years of age; isolated PCO therefore should not be considered an indication of the syndrome in the absence of menstrual irregularities, infertility, or complaints of hirsutism [14].

In some ways, efforts to agree on diagnostic criteria are artifactual. There continues to be controversy and lack of complete agreement for what elements constitute optimal criteria for PCOS diagnosis, in part because of the natural clinical desire to move to discreet categorical criteria for the ease of diagnosis. In truth, there is a continuum of presentation from those persons minimally affected, with regular menses and only mild excess of androgens to those who have a unilateral PCO, to those who manifest more severe grades of androgen excess. Efforts to include hyperandrogenemia as diagnostic criteria will remain inadequate until the sensitivity of androgen assays is better refined because of our current inability to accurately quantify circulating androgens in women.

Prevalence of Polycystic Ovary Syndrome: Regional and Ethnic Variation

Although the prevalence of PCOS in any specified population is dependent upon the diagnostic criteria used, there is regional and ethnic variation. While most reports on the prevalence of PCOS range between 2 and 20%, the chosen diagnostic criteria are recognized to influence the determined prevalence. A retrospective birth cohort in Australia found a prevalence of 8.7% using NIH criteria, 17.8% using Rotterdam criteria, and 12.0% using AES criteria [15] (Table 1.2). A similar prevalence pattern was found in Turkey, where 6.1% met NIH criteria, 19.9% met Rotterdam criteria, and 15.3% met AES criteria [16]. In Iran the estimated prevalence of PCOS was 7% based on the NIH criteria, 15.2% using Rotterdam criteria, and 7.92% using AES criteria [17]. In North America, most estimates of the general population in the United States range from 4 to 8% in the literature, although most of this information comes from an unselected population of white and black women in the southeast region [18, 19]. Mexican-American women have a higher prevalence, reportedly as

Table 1.2 Relative population prevalence of PCOS (%) based on individual diagnostic criteria

	Diagnostic criteria		
	NIH[a]	Rotterdam[b]	AES[c]
March et al. [11]	8.7	17.8	12.0
Yildiz et al. [12]	6.1	19.9	15.3
Mehrabian et al. [13]	7.0	15.2	7.9

[a]National Institutes of Health international conference 1990
[b]Task force sponsored by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), 2003
[c]Androgen Excess and Polycystic Ovary Syndrome Society diagnostic criteria 2009

high as 13% [20]. Interestingly, the estimated prevalence of PCOS among women in Mexico is 6%, only half of that found in their counterparts in the United States [21]. These discrepancies highlight not just an ethnic diversity in the prevalence of the disorder but also the significance of lifestyle in the occurrence of PCOS. Likely prevalence is underestimated.

In India, PCOS is reported among 9% of adolescents [22]. Among Indian women 15–35 years of age evaluated at a rural gynecology clinic, 13% presented with menstrual irregularities, half of which were found to have PCOS, estimating the prevalence to be around 6.5% [23]. In Sri Lanka, a similar prevalence of 6.3% was noted among women age 15–39 [24]. In Iran, the prevalence of PCOS is reported as 8.5% out of a sample of reproductive-aged women selected for participation in the Tehran Lipid and Glucose Study [25]. A Greek study on the island of Lesbos found a prevalence of 6.8% [26]. The overall prevalence of PCOS among a population of urban indigenous Australian women, using NIH criteria, was 15.3% [27]. A study in the United Kingdom found the prevalence to be 8% using stricter NIH criteria, while 26% of their population met Rotterdam criteria, illustrating the differences seen when using different diagnostic criteria. In Spain, a population of Caucasian women presenting spontaneously for blood donation was found to have a prevalence of 6.5% [28]. A meta-analysis published in 2016 reported an overall prevalence of PCOS at 6% using NIH criteria and 10% using Rotterdam or AES criteria [29]. By any measure, PCOS is one of the most prevalent endocrine disorders worldwide, with obvious regional and ethnic variation.

Excess in facial and body hair and intractable acne are common reasons for women to seek evaluation with subsequent unmasking of PCOS. Rates of hirsutism vary among ethnic groups. In the United States, the reported rates are similar between black and white women (around 5%) [30], but in Kashmir, India, the prevalence is much higher at 10.5% [31]. Among women with hirsutism, up to one-third have an underlying diagnosis of PCOS. Around 27% of women presenting with acne were found in one study to have undiagnosed PCOS, compared to 8% of controls [32]. Patients presenting with acne resistant to standard treatment have an even higher rate, near 50% [33]. Among adolescents with irregular menses, after a 6-year follow-up period, 62% continued to have irregular menses, 59% of whom were diagnosed with PCOS. In other words, approximately one-third of the original adolescent population with irregular menses was diagnosed with PCOS within the study period [34].

Summary

PCOS is considered the most common endocrine disorder among reproductive-age women and is characterized by a chronic course, with features that suggest varying combinations of reproductive functional deficits (such as ovulatory dysfunction or PCO morphology) and androgen excess (such as acne and hirsutism). The diagnosis of PCOS is based on well-defined criteria, and currently there are three major sets of diagnostic criteria available for utilization in clinical practice. Regional prevalence of PCOS can vary depending on the diagnostic criteria utilized as well as the ethnicity studied. Women with isolated symptoms of acne, hirsutism, and irregular menstrual cycles should be offered targeted screening. Beyond the symptom burden relating to PCOS that adversely impacts quality of life, and perhaps more clinically significant, is the higher prevalence of several medical comorbidities in the PCOS population that have been extensively covered in additional chapters in this text. Identifying PCOS and screening for these adjunct disorders will allow for timely institution of preventive strategies aimed at minimizing the overall health risk in this population.

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Prevalence, Presentation, and Diagnosis of PCOS in Adolescents

2

Tania S. Burgert and Emily Paprocki

Key Points

- Diagnosis of PCOS poses unique challenges given overlapping symptoms of puberty.
- Diagnostic criteria for PCOS are in the pediatric and adolescent population less well characterized compared to the adults.
- Some menstrual irregularity is common within the first 1–2 years of onset of menarche; however menstrual disturbances may be the earliest sign of PCOS in adolescents and merit evaluation.
- Hyperandrogenemia is an important feature of PCOS diagnosis in adolescence.
- Equilibrium dialysis remains the most sensitive indicator of measuring free testosterone, while total testosterone is best measured using high-performance liquid chromatography coupled with tandem mass spectrometry. Normative data for circulating levels of total and free testosterone, and for free androgen index in adolescent population, are lacking.
- Clinical hyperandrogenism may be difficult to interpret in adolescents. Moderate to severe inflammatory acne during peri-menarche should be evaluated as a sign of clinical hyperandrogenism.
- Similar to adults, relevance of insulin resistance to the pathophysiology of PCOS also holds true for the adolescent population.
- Early diagnosis and treatment can improve menstrual symptoms, body composition, and cardio-metabolic profile in patients with PCOS.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_2

- If clinical suspicion persists but a diagnosis cannot be made in pediatric or adolescent age, the patient should be considered at risk for PCOS, treated symptomatically, and reevaluated after reproductive maturity is attained.
- Per 2018 international guidelines, consideration for pelvic ultrasound to assist in arriving at a diagnosis of PCOS should be deferred, at the minimum, until 8 years post-menarche.

Introduction

Until recent years, the diagnosis of polycystic ovary syndrome (PCOS) was reserved for adult women presenting with sub/infertility tied to irregular menses/anovulation and hirsutism/hyperandrogenism. With increasing awareness of multifactorial diseases in younger populations, PCOS has become a more frequent consideration among clinicians caring for pediatric and adolescent patient populations. However, transferring adult diagnostic criteria for PCOS to the adolescent population has proven to be most challenging, mainly due to overlapping symptoms of normal puberty. In puberty, central axis immaturity and physiologic insulin resistance are often coupled with anovulation and mild acne/hirsutism, mimicking a PCOS phenotype. In many cases, the differentiation to bona fide PCOS lies merely in the degree of clinical and biochemical expression. However, puberty is the first test of ovarian handling of insulin and gonadotropin stimuli and therefore offers an opportunity for early diagnosis of this clinical condition that may have lifelong health implications. Genetically predisposed adolescents may exhibit an exaggerated ovarian response to physiologic and non-physiologic (e.g., obesity) stimuli at puberty, with an ensuing hormonal cascade that is well recognized in the context of PCOS (Fig. 2.1).

In the past, an overlap in symptoms of physiologic puberty and PCOS had led to a diagnostic hesitation, likely leaving young women under-evaluated and under-treated until reproduction is desired. Recent recommendations from the 2018 international evidence-based guideline, however, encourage early diagnosis if possible. If the diagnosis cannot be made but clinical suspicion remains, the patient should be considered at risk for PCOS, treated symptomatically, and reevaluated after reproductive maturity has been reached [1, 2]. Given the potential medical implications of untreated PCOS such as metabolic syndrome/type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), infertility, and endometrial cancer, early detection and treatment can have multiple health benefits. As with most conditions in the pediatric age group, very few studies have been conducted looking at the long-term benefits of early interventions for adolescent PCOS. However, data are available for a group of non-obese adolescents who developed PCOS after being diagnosed with premature adrenarche, a common condition recognized as heralding PCOS. For this group of adolescents, Ibanez and de Zegher have extensively studied the effect of a combination of treatments that target hyperandrogenism and insulin resistance [3, 4]. It appears that early diagnosis and treatment improves menstrual symptoms, body composition, and, most importantly, cardio-metabolic profile in patients with PCOS.

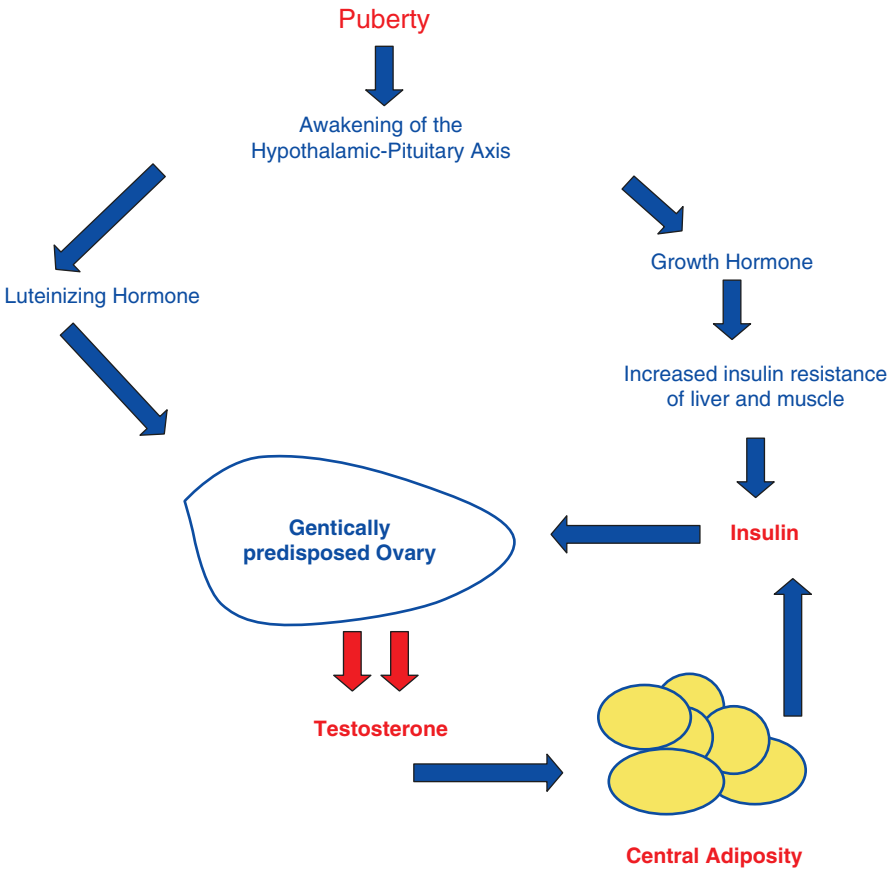

Fig. 2.1 Hormonal paradigm in pubertal PCOS

Prevalence of PCOS in Adolescence

Even though PCOS is a highly prevalent inheritable condition in adult women, reported prevalence in adolescents varies greatly, given the heterogenicity of diagnostic criteria applied over the past decade. A recent large meta-analysis reports that depending on the diagnostic criteria used, the prevalence of PCOS in adolescents is 11.0% (Rotterdam criteria), 3.4% (NIH criteria), and 8.0% (Androgen Excess and PCOS Society criteria) [5].

Diagnosis of PCOS in Adolescence

In general terms, PCOS is defined by a constellation of signs and symptoms, after other organic causes have been excluded. Such conditions include adrenal disorders (i.e., late-onset congenital adrenal hyperplasia), thyroid disorders, primary or secondary ovarian insufficiency, hyperprolactinemia, androgen-producing tumors, and

pregnancy. The first unifying approach to *adult* PCOS was proposed during a 1990 consensus meeting at the National Institutes of Health (NIH). The so-called NIH criteria require the presence of both ovulatory dysfunction (OD) and clinical and/or biochemical hyperandrogenism (HA). In 2003 another PCOS consensus workshop held in Rotterdam, the Netherlands, broadened the definition to allow ultrasound evidence of polycystic ovarian morphology (PCOM) to substitute for either OD or HA. Hence, additional "non-classic" phenotypes (C and D, Table 2.1) of *adult* PCOS were acknowledged [2, 6, 7].

It is important to note, although these phenotypes exist within the spectrum of adult clinical manifestation; currently only the B phenotype is recommended to make the diagnosis in adolescents.

A. OD+ PCOM+ HA – complete phenotype
B. OD + HA – classic phenotype – same as NIH phenotype and recommended for adolescent diagnosis
C. HA + PCOM (ovulatory phenotype)
D. OD + PCOM (non-androgenic phenotype)

Over the years expert opinion questioned the validity of all PCOS subtypes, and therefore Rotterdam criteria were increasingly substituted by the 2009 Androgen Excess and PCOS Society criteria which required the presence of hyperandrogenism plus one of two signs of disturbed ovarian physiology: either OD or ultrasound evidence of PCOM.

However, the first PCOS genome-wide association studies (GWAS) in the Han Chinese in 2012 [8] and the following PCOS GWAS in Europeans in 2015 [9, 10] suggested that similar genetic underpinnings were present in both classic and non-classic phenotypes of PCOS. This was further confirmed in a 2018 PCOS GWAS meta-analysis [11]. As a consequence of the GWAS findings, Rotterdam criteria were resurrected by the scientific community, and the 2018 international evidence-based guideline for PCOS recommended using Rotterdam criteria for more inclusive diagnosing. **However, Rotterdam criteria are currently not recommended to replace the NIH criteria for diagnosing PCOS during adolescence.** This is mainly due to the concern that immature ovaries during adolescence may often appear polycystic on ultrasound and could erroneously be classified as ovaries with PCOM. Research is currently underway to delineate the normal appearance of ovaries for the first 2 years post-menarche so that pathologic ovarian morphology can be differentiated from normal ovarian morphology during the pubertal transition.

Table 2.1 Clinical manifestations of individual phenotypes

	Clinical manifestations		
Phenotype	**Ovulation dysfunction**	**Hyperandrogenism**	**Polycystic ovaries**
A: Complete	x	x	x
B: Classic	x	x	
C: Ovulatory		x	x
D: Non-androgenic	x		x

NIH Criteria Applied to Adolescents

Ovulatory Dysfunction

While menarche is the first sign of an intact hypothalamic-pituitary-ovarian (HPO) feedback loop, the HPO system may take up to 5 years to mature fully [12]. Therefore, "physiological" anovulation has become the main assumption in examining adolescents with menstrual irregularity. In support of this assumption is the description of 244 unselected post-menarchal girls (mean age 15.2 years) of which 51% had menstrual irregularity that was not consistently associated with other features of PCOS [13]. However, this study applied a narrow definition of a regular menstrual pattern (cycles were considered abnormal if they varied by >4 days from month to month). It is therefore conceivable that the authors' designation of a "normal" cycle was too narrow in its application to adolescents. Interestingly, a prospective follow-up study found that 62% of women diagnosed with menstrual disturbance during adolescence were still irregular in their mid-20s and 59% of those fulfilled criteria of PCOS [14]. Furthermore, a large Finnish Cohort of adolescent girls with irregular menses by self-report had higher testosterone levels than their eumenorrheic counterparts, indicating that irregular menses at age 15–16 years may denote a non-physiologic state [15]. The authors suggested that a regular menstrual pattern at age 15–16 years excludes the risk of hyperandrogenemia with a specificity of 72%. Interestingly, irregular menstrual cycle patterns at age 15 were more predictive of oligo-amenorrhea at age 18 than hormonal profiles [16].

The 2018 international PCOS guidelines extensively reviewed the available literature on menstrual cycle regularity in adolescents and, based on available evidence and expert opinion, created an easy-to-follow reference for assessing peri-menarchal menstrual irregularity (Table 2.2). The recommendations allow for diagnosis of OD as early as 1 year post-menarche. Furthermore, primary amenorrhea by age 15 years old or >3 years post-thelarche is considered consistent with ovulatory dysfunction.

Table 2.2 Abnormal menstrual patterns in adolescents based on years post-menarche

	1st year post-menarche	2nd year post-menarche	3rd year post-menarche	4th year post-menarche
Menstrual pattern	Any menstrual pattern, including stretches of amenorrhea, is considered normal during the 1st year post-menarche	Irregular cycles and skipping of cycles are still considered normal	Cycles should now be more regular every 21–45 days between menstrual periods	Adult menstrual cycle with intervals of 21–35 days (at least 8 cycles a year) should be established
Interpretation	PCOS cannot be considered	If periods are >90 days apart, the adolescent should be evaluated for PCOS	If periods are >45 days apart or <21, the adolescent should be evaluated for PCOS	If periods are >35 days apart or <21, the adolescent should be evaluated for PCOS

Hyperandrogenism

Today, most clinicians who diagnose PCOS in adolescents based on NIH criteria heavily rely on evidence of biochemical hyperandrogenism in the form of elevated total or free testosterone [17]. Still, actual comparable testosterone cutoffs across laboratories remain elusive, since laboratory assays vary widely between laboratories and normative data for testosterone levels in adolescents are lacking [18]. General consensus views a total testosterone >40–50 ng/dL and a free testosterone >1.4–1.7 nmol/L as suggestive of hyperandrogenism [4].

Clinical hyperandrogenism is difficult to interpret in adolescents. Acne, for example, has always been attributed to pubertal hormone changes. However, the 2018 international evidence-based guideline recommends consideration of moderate to severe inflammatory acne during peri-menarche as a sign of clinical hyperandrogenism [1]. Hirsutism on the other hand is a more established marker of clinical hyperandrogenism in adolescence, although it can be less pronounced during this time as it may take time for hyperandrogenemia to affect the hair follicles. Therefore, the development of any facial hair or progressive facial hair during adolescence should prompt a complete evaluation for clinical hyperandrogenism and hyperandrogenemia. Based on the international PCOS guideline, the modified Ferriman–Gallwey (F-G) scoring system can be used to denote clinical hyperandrogenism. The scoring system is based on additive points for hair in body areas such as the upper lip, chin, chest, upper arm, upper and lower back, inner thigh, and lower and upper abdominal area. A score of 4–6 suggests hirsutism, depending on ethnicity [1].

Even though clinical hyperandrogenism was recently endorsed by the international guidelines to stand independently of biochemical assessment, biochemical hyperandrogenism has traditionally been recommended in the diagnosis of adolescent PCOS [19]. The most sensitive indicator of biochemical hyperandrogenemia is the determination of free testosterone through equilibrium dialysis [20]. However, this method is not widely available and is technically complex and expensive. Alternatively, the free androgen index (FAI) correlates well with free testosterone measured through equilibrium analysis and can be calculated from measures of total testosterone and SHBG [19, 21]. The best methods for evaluation of total testosterone are methods using high-performance liquid chromatography coupled with tandem mass spectrometry. These are ideal for routine clinical measurements such as diagnosing PCOS [22]. Still, methodological challenges remain when obtaining accurate testosterone measurements. Studies comparing different methodologies have shown poor precision at lower testosterone levels (commonly seen in women) and significant variability between the methods. The specific mechanism causing imprecision at lower levels is unknown but thought to be due to interfering substances causing inaccurate measurements [22]. Assay interference from other steroid molecules with a similar structure and other technical aspects of the assays can be challenging [17]. Additionally, adolescent normative data for total testosterone, free testosterone, and FAI are lacking. However, very high total testosterone levels in any laboratory above 200 ng/dL may suggest the presence of an ovarian androgen-secreting tumor and would warrant further imaging evaluation [23].

For complete androgen evaluation in adolescents with possible PCOS, a measure of adrenal androgen precursors such as 17-OH progesterone and DHEA-S is important to rule out variants of late-onset congenital adrenal hyperplasia [24] as well as to assess the contribution of adrenal androgen production to the clinical picture [20]. Further evaluation is warranted for a 17-OH progesterone level above 200 ng/dL. An elevation of DHEA-S above 800 μg/dL should prompt further investigation into an adrenal androgen-secreting neoplasm [23]. All androgen hormones should be measured in the morning, preferably in the follicular part of the menstrual cycle [24].

Pelvic Ultrasound Evaluation

Ultrasound is not recommended for diagnosing PCOS in adolescents. The 2018 international guidelines recommend employing ultrasound at the earliest, 8 years post-menarche. This is mostly due to lacking data in regard to normal ovarian morphology during peri-menarche. Furthermore, the preferential application of the transabdominal scanning technique over the transvaginal approach in most adolescents may limit the transferability of visual criteria established for the transvaginal approach. However, with improved ultrasound technology (Fig. 2.2), this should become less of a concern for future transabdominal studies [25, 26]. Therefore, continued interest in exploring the utility of ultrasound in adolescents with PCOS remains, and we recently reported that markers of reproductive dysfunction correlated not only with ovarian size but also small follicle number in adolescents with

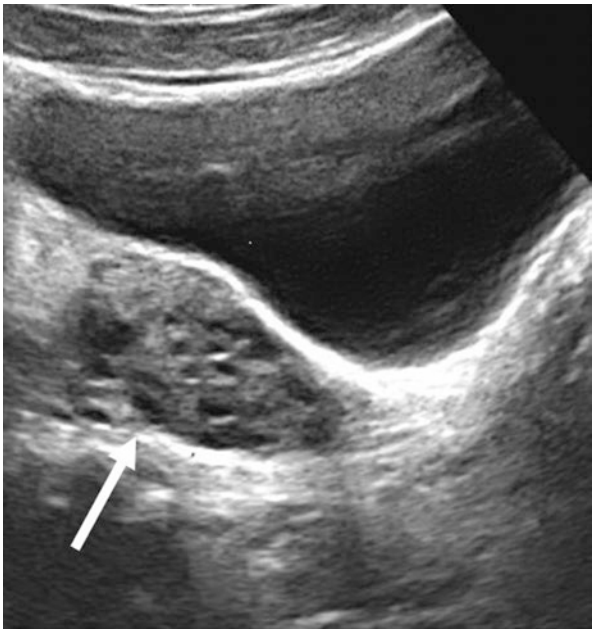

Fig. 2.2 Transabdominal ultrasound

PCOS [27]. Another study from Denmark, examining transabdominal ultrasound in over 300 adolescents, found that the median ovarian volume in normally menstruating adolescents 3 years post-menarche was between 6 and 7 cm^3 [28]. These findings establish that as early as 3 years post-menarche, ovaries appear similar in size to adult normal standards. While enlarged ovarian volume (>10 cm^3) is one of the transvaginal ultrasound criteria of polycystic ovarian morphology in adult women (see Fig. 2.2), some consider the stromal to ovarian ratio more PCOS specific than the overall ovarian size assessment. A recent cross-sectional ultrasound examination of large cohort of normo-cyclic adolescents in Italy found that an increased stromal/ovarian area ratio was a more consistent observation in 10–16% of the subjects which could possibly differentiate normal variation from pathology [29].

Transabdominal ovarian ultrasound from a young postmenarcheal adolescent (11 years old, 6 months) obtained from an ongoing study examining ovarian morphology early post-menarche. We note excellent resolution of ovarian features, notably small antral follicles.

The white arrow is pointing to the image of the ovary.

Table 2.3 provides an overview of aspects to consider when diagnosing PCOS during adolescence.

Comorbidities of PCOS

Even though obesity and insulin resistance are not part of the PCOS definition, they are closely linked morbidities that tie into perpetuating the clinical phenotype and ultimately are responsible for the development of T2DM, the most serious metabolic and cardiovascular risk in PCOS. Other metabolic risk factors such as dyslipidemia, hypertension, impaired glucose metabolism, and non-alcoholic fatty liver disease (NAFLD) either pave the road to T2DM or are found at the time of diagnosis as concomitant comorbidities.

Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is common in obese youth and is associated with deteriorating glucose metabolism [30]. Studies show that in adult women with PCOS, there is a 2.5-fold increase in NAFLD [31]. Part of this risk is due to the increased insulin resistance and obesity seen in PCOS. Androgen excess is also thought to play a role in the development of NAFLD. Total testosterone and free androgen index have both been shown as independent predictors of NAFLD in women with PCOS [31]. Screening liver enzymes should be obtained at diagnosis of PCOS. Risk factors suggesting continued monitoring of liver functions include a rising body mass index (BMI), dysglycemia, and new diagnosis of obstructive sleep apnea. Persistently elevated serum levels of AST and/or ALT that are greater than two times the upper limit of normal warrant further evaluation by a hepatologist [32]. Liver biopsy remains the gold standard for diagnosing NAFLD. Hepatic

Table 2.3 Evaluation for PCOS in adolescent population

Assessment	Considerations	Implications
Supporting PCOS		
Clinical	Menstrual disturbance of oligomenorrhea, primary or secondary amenorrhea. Physical examination of acanthosis nigricans, central weight distribution, facial hair/hirsutism Family history of PCOS, infertility, gestational diabetes, type 2 diabetes	
Laboratory	Elevation of morning total testosterone, free testosterone (low sex hormone-binding globulin)	>200 ng/dL suggestive of ovarian androgen-secreting tumor
Imaging	Transabdominal ultrasound indicated if testosterone >200	
Ruling out other conditions		
Laboratory	Morning 17-hydroxyprogesterone	>200 ng/dL suggestive of late-onset congenital adrenal hyperplasia
	Dehydroepiandrosterone-sulfate	Adrenal hyperandrogenism/>800 μg/dL suggestive of adrenal tumor
	Thyroid stimulating hormone	Thyroid disorder
	Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2)	Elevated FSH and LH and low E2 levels suggest primary ovarian insufficiency. Normal to low FSH, LH, and low E2 suggest hypothalamo-pituitary dysfunction
	Urine/serum human chorionic gonadotropin	Rule out pregnancy
	Prolactin	Prolactinoma or drug-induced cause for elevated prolactin
	Urinary cortisol assessment/ dexamethasone suppression test	Rare conditions such as Cushing's syndrome are not routinely ruled out but should be considered if clinical suspicion arises (e.g., adolescent with elevated blood pressure)

ultrasound and MRI are additional diagnostic tools. Evaluation with these methodologies is limited due to the expensive and invasive nature of these tools. Therefore, in a study of 108 overweight and obese adolescents with PCOS, a risk factor predictor for the development of NAFLD in adolescent PCOS was proposed [33]. Magnetic resonance imaging was used to quantify hepatic fat fraction. Through logistic regression and candidate predictor variables, a risk index using BMI percentile, waist circumference, ALT, and SHBG showed an accuracy of 81%, sensitivity of 91%, and specificity of 70% for diagnosing NAFLD in a validated cohort.

Prediabetes and Diabetes

The most important cardiovascular risk-enhancing condition in PCOS is T2DM. Also, prediabetes, as defined by either abnormal fasting or abnormal

glucose tolerance during a 2-hour standard oral glucose tolerance testing with a 75-gram glucose load (OGTT), is not only an antecedent to T2DM but in and of itself a cardiovascular disease risk factor [34]. In adolescent medicine, obesity, a family history of T2DM, and the presence of acanthosis nigricans on skin examination are the red flags that prompt thorough testing of glucose metabolism in adolescents. However, adult studies have demonstrated that prediabetes and T2DM can be present in non-obese women with PCOS and, therefore, the diagnosis of PCOS in and of itself should prompt a metabolic evaluation [35].

In adolescents with PCOS, data on the prevalence and presence of prediabetes and T2DM are still emerging. Furthermore not many studies distinguish the prevalence of prediabetes/T2DM between obese and non-obese adolescents with PCOS. In the few studies examining adolescents with PCOS, the data on disturbance in glucose metabolism vary between 6% and 33% and are often confounded by the overwhelming presence of obesity [36–38]. In a study in Indian adolescents with PCOS, where obesity is less confounding but there is more of a heritable risk of insulin resistance and diabetes, impaired glucose metabolism was present in 9.7% [39]. In one of our examinations of glucose metabolism in adolescents with PCOS, we found that impaired glucose metabolism was present in adolescent PCOS across the BMI spectrum [40] (Fig. 2.3).

Similar findings were reported in a contemporaneous study conducted in Chinese youth with PCOS. In this study, 14% of non-obese adolescents and 25% of obese

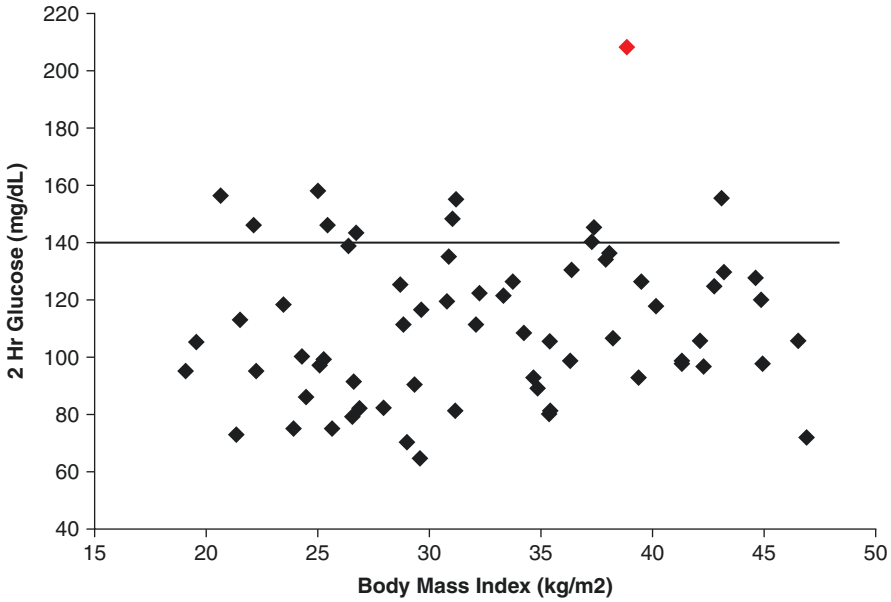

Fig. 2.3 Impaired glucose tolerance (IGT), as defined by a 2-h stimulated serum glucose ≥140 mg/dL, occurred across the spectrum of BMI in adolescents with PCOS. One obese adolescent met criteria for type 2 diabetes, based on 2-h serum glucose >200 mg/dL (in red)

adolescents with PCOS had IGT on OGTT, while no diagnoses were made in the control subjects [41]. A more recent study from Turkey examining insulin resistance in adolescents with PCOS found that compared to fasting analyses of insulin resistance, examination of OGTT was significantly more likely to detect insulin resistance [42]. Further supporting a prediabetes risk in PCOS are the results of a series of studies examining metabolic and other hormonal features in pubertal daughters of women with PCOS. Compared to BMI-matched controls, daughters of women with PCOS have significantly higher 2-hour insulin levels during OGTT than matched controls at all stages of puberty [43].

Indeed, an OGTT may further direct the diagnostic process for a clinician examining the adolescent patient with symptoms of PCOS. This was illustrated in a recent case of an adolescent with normal BMI (23.7 kg/m2), who was given the diagnosis of PCOS based on clinical and biochemical hyperandrogenism with irregular menses. Fasting labs revealed a normal HbA1c of 5.6% and a normal fasting glucose of 80 mg/dL [44]. However, OGTT revealed a 2-hour blood sugar of 199 mg/dL and a corresponding insulin level of 1480 uIU/ml (<50 uIU/ml). These results led to the diagnosis of type A insulin resistance, a genetic condition with a defect in the insulin receptor gene. The condition would have been missed if OGTT had not been performed and insulin levels not been examined. While severe insulin resistance syndromes such as type A insulin resistance are generally considered rare conditions, they are of heterogeneous origin (genetic and acquired) with variable clinical expression and therefore are often mis- or underdiagnosed in practice [45].

Insulin resistance and T2DM are intimately linked to the PCOS condition; other cardiovascular risk factors may mainly be a function of the adiposity often seen in PCOS. The metabolic syndrome, for example, is a constellation of metabolic disturbances that is associated with cardiovascular disease. The most common definitions require at least three of the following criteria: (central) obesity, dyslipidemia (related to either triglycerides or HDL), hypertension, or impaired glucose metabolism. In the general adolescent population, metabolic syndrome is strongly tied to the degree of obesity [46]. Studies in adults have found that metabolic syndrome occurs more frequently and at younger age in women with PCOS [47, 48]. Therefore, metabolic screening should be implemented in overweight adolescents with PCOS.

Mood Disorder

The prevalence and severity of depressive and anxiety symptoms are increased in PCOS. Clinicians taking care of adolescents with PCOS should screen for depressive and anxiety symptoms at the time of PCOS diagnosis and consider repeat screening throughout routine follow-up. Recommended screening tools include standardized mental health assessment questionnaires such as the PHQ-3, PHQ-9, and CESD-20 [49]. A recently published study assessed psychiatric disorders, health-related quality of life, and self-esteem in adolescents with PCOS. A total of 28 adolescents with PCOS ages 13–18 years and 31 age- and sex-matched healthy peers were recruited. The psychiatric diagnosis rate was significantly higher in the

Table 2.4 Metabolic considerations after diagnosis of PCOS in an adolescent population

	Considerations	Implications
Laboratory	Fasting glucose, insulin, and 2-h oral glucose tolerance test with 75 g of glucose, HbA1c	Examine for prediabetes, diabetes, insulin resistance
	Liver function tests	Screening for non-alcoholic fatty liver disorder
	Fasting lipid profile	Examine for dyslipidemia as knowledge may impact on treatment choice, e.g., caution is advised when using oral contraceptive regimen in the setting of moderate to severe triglyceridemia
Mental health screening	(PHQ-3, PHQ-9)	Screening for depression, anxiety, suicidality

PCOS group than in the control group, and percentage of PCOS patients with more than one psychiatric disorder was 25%. In the PCOS group, the most common single disorder was major depressive disorder (21%); however, the sum of the anxiety disorders (including generalized anxiety disorder, separation anxiety, social anxiety, etc.) was 42% [50].

Given the metabolic implications that go hand in hand with the diagnosis of PCOS, additional evaluation may be considered, as outlined in Table 2.4.

Summary

While the manifestations of PCOS during adolescence may be similar to those seen in the adults, diagnostic dilemmas abound and diagnostic criteria are less studied in this population compared to the adults. A timely diagnosis of PCOS in the afflicted adolescent allows an opportunity of not only successfully addressing the burdensome symptoms but also initiating preventative strategies aimed at mitigating long-term sequelae of this chronic disorder.

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Polycystic Ovarian Syndrome: A Diagnosis of Exclusion

3

Mira Aubuchon

Key Points

1. PCOS is one of the most common of reproductive disorders. Though prevalence estimates are heterogeneous owing to population type, sampling bias, phenotype criteria, and assessment methodology.
2. PCOS diagnosis includes at least two of three of the following: hyperandrogenism, chronic anovulation, and sonographic polycystic ovarian morphology, for which serum anti-Müllerian hormone level may be a proxy.
3. Although laboratory and imaging methodology have evolved, none are perfectly sensitive for PCOS diagnosis, and it remains necessary to exclude other conditions with similar presenting features.
4. These exclusionary conditions closely mimic PCOS but require distinct screening, management and counseling and thus must be ruled out prior to establishing the diagnosis of PCOS.
5. Non-classical congenital adrenal hyperplasia (NCAH) can be screened with a serum 17-hydroxyprogesterone level, with phenotype that results from compound heterozygous mutations and impacts offspring via autosomal recessive genetic transmission.
6. Virilization with markedly elevated ovarian and/or adrenal androgen levels should raise suspicion for androgen-secreting tumor.
7. Cushing's syndrome screening should be initiated in a patient with suspected PCOS if symptoms of cortisol excess are present, using salivary, urinary, or stimulated serum testing, results of which may indicate a neoplastic process.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_3

8. Exogenous androgen exposure may be elicited by history-taking with attention to possible dietary supplement contamination, iatrogenic pro-androgenic medication effects, intentional use, or passive transfer.
9. Thyroid hypo- and hyperfunction as screened by TSH level can lead to reproductive dysfunction, reversible upon achievement of euthyroid state.
10. Etiologies for hyperprolactinemia include neoplastic and pharmacologic but may also be due to physiologic factors related to sample collection or to multimeric prolactin isoforms.
11. Functional hypothalamic amenorrhea (FHA) diagnosis requires exclusion of structural and endocrine conditions such as androgen excess but in fact may actually coexist with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a common chronic condition with implications for morbidities, both in short-term (e.g., subfertility and pregnancy-related complications) and long-term risks (e.g., type 2 diabetes, cardiovascular disease, depression, poor quality of life, and overall mortality) [1]. A prompt diagnosis allows opportunities for early institution of preventive strategies for prevention of sequelae. However, diagnosing PCOS can be challenging, as the symptoms do overlap with a number of disorders that themselves require specific treatment. No single symptom, examination finding, or laboratory data is diagnostic of this disorder. Rather, arriving at a diagnosis requires a systematic approach aimed at excluding the differential diagnoses that could account for the patient's presentation; the diagnosis of PCOS is thus one of systematic exclusion.

Table 3.1 lists the common alternative etiologies that could mimic PCOS along with the recommended laboratory screening.

Prevalence and Epidemiology of PCOS

There is considerable heterogeneity in estimates of PCOS prevalence owing to population type, sampling bias, phenotype criteria and assessment methodology (see next section), suppressive medications, and comorbidities including obesity [2]. For example, a large global meta-analysis found that 19% of unselected women and 50% of referred patients had the PCOS phenotype consisting of hyperandrogenic anovulation with sonographic polycystic morphology, whereas for other less morbid phenotypes, the prevalence of PCOS was higher in unselected women compared to referral patients; the prevalence estimates increased with obesity particularly in referral populations [2]. PCOS estimation is challenging at reproductive age extremes, with the few published studies in adolescence reporting very low <1% prevalence [3], while perimenopause is associated with improvements in oligomenorrhea and biochemical markers which may lead to underdiagnosis [4].

Table 3.1 Exclusionary diagnoses and screening for PCOS

Condition	Initial screening
Hypo and hyperthyroidism	TSH – anytime of day
NCAH	17OHP 8 am, early follicular phase if cycling otherwise random, after overnight fast
Hyperprolactinemia	Prolactin timing initially as convenient but if elevated redraw at 2–3 hours post-awakening after 8–12-hour fast and 2-day abstain sexual stimulation
Androgen-secreting tumor	Testosterone, DHEAS – 8 am, early follicular phase if cycling otherwise random
Cushing's syndrome	(If clinically indicated): Nighttime salivary cortisol ×2, 24-hours urinary free cortisol ×2 over 2 weeks, or dexamethasone suppression test (11 pm dexamethasone 1 mg, 8 am serum cortisol and ACTH)
Exogenous androgen exposure	History-taking
FHA	Estradiol, FSH, LH, AMH
PCOS	History and exam, above labs, pelvic US

NCAH: non classic congenital adrenal hyperplasia; *FHA*: Functional hypothalamic amenorrhea; *17OHP*: 17 hydroxy progesterone; *TSH*: Thyroid stimulating hormone; *AMH*: Antimullerian hormone; *US*: Ultrasound; *ACTH*: Adrenocorticotropic hormone; *DHEAS*: Dehydroepiandrosterone sulfate; *PCOS*: Polycystic ovary syndrome

Diagnostic Criteria for PCOS (Clinical Manifestations, Laboratory, and Imaging)

PCOS may include hyperandrogenism, chronic anovulation, and sonographic polycystic ovarian morphology, once other etiologies have been excluded. Hyperandrogenism, either clinical or biochemical, is required for diagnosis of PCOS by the 1990 National Institutes of Health criteria and the 2006 Androgen Excess PCOS criteria, in contrast to the 2003 Rotterdam criteria, which allows for any two of three [5–7]. The Rotterdam criteria were affirmed by international consensus in 2018, and the methodology of diagnosis was further defined in this guideline and can be accessed from the website https://www.monash.edu/medicine/sphpm/mchri/pcos/guideline [1, 8]. Although obesity, luteinizing-hormone elevations, and insulin resistance are important PCOS markers, these are not required for diagnosis [1].

Clinical hyperandrogenism can manifest with hirsutism, acne, or alopecia [1]. Hirsutism is the most important measure of clinical hyperandrogenism for PCOS diagnosis and manifests as excess midline-distributed terminal hair, in contrast to softer vellus hair which may be similarly distributed, that is optimally assessed with a standardized visual scaling score such as the Ferriman-Gallwey score ≥4–6 with thresholds preferably adjusted for ethnicity; assessment may be limited in the setting of self-treatment however [1]. The Endocrine Society recommends evaluating all women with an abnormal hirsutism score with an early morning total and free testosterone level [9]. Severe acne is associated with biochemical hyperandrogenism, but there is no universally accepted and standardized acne diagnostic scale or

scoring threshold for PCOS diagnosis [1]; in adolescents, at least ten or more facial lesions are suggested for diagnosis [10]. Alopecia can be assessed via standardized criteria but is poorly associated with hyperandrogenemia, and the predictive value for PCOS is uncertain [1]. Virilization with rapid onset/progression of hyperandrogenic symptoms may indicate non-PCOS neoplastic process [1] (see on androgen secreting tumors).

Biochemical hyperandrogenism is best assessed when hirsutism is absent and hormonal contraceptives have been discontinued for several months, utilizing calculated free or bioavailable serum testosterone or liquid chromatography-mass spectrometry (LCMS)-measured total and/or free testosterone; dehydroepiandrosterone sulfate (DHEAS) and androstenedione may be additionally considered when testosterone levels are non-diagnostic [1]. Salivary testosterone and urinary testosterone glucuronide are not recommended [9]. Threshold upper levels for serum androgens are non-standardized, but generally PCOS-associated hyperandrogenemia is mildly elevated beyond laboratory-established threshold ranges [1]. For study purposes, serum testosterone ≥0.5 ng/ml has been reported as a threshold for hyperandrogenemia [11]. Reference ranges for androgens are ideally stratified by age and pubertal status, and marked elevations above threshold levels may indicate etiologies other than PCOS [1]. In cycling women, testosterone level is most predictive when drawn in the morning, when levels are diurnally the highest, and during the early follicular cycle phase [8, 9].

Cycle lengths <21 days or >35 days, or the report of fewer than eight menses per year with onset or persistence past 3 years post-menarche, all suggest ovulatory dysfunction or chronic anovulation [1]. However, eumenorrhea with absent ovulation as measured with serum progesterone is present in 20% of PCOS patients [6].

Ultrasound PCOS diagnosis thresholds were updated for 8 MHz bandwidth endovaginal transducers to ≥20 follicles 2–9 mm per ovary (previously ≥12 follicles) and/or ovarian volume ≥10 ml as measured either vaginally or abdominally in the absence of other pathophysiological ovarian change [1]. Ultrasound is not mandatory for diagnosis when other clinical and/or biochemical criteria are met [1].

Antimullerian hormone (AMH) is of increasing interest as a biomarker of PCOS, though not currently incorporated into the diagnostic criteria. Serum AMH among infertile women positively correlates with serum total testosterone [12], with mean AMH 3.9 ng/ml noted in the highest testosterone quartile (mean ≥0.36 ng/ml) [12], while another group found mean AMH 8.58–11.45 ng/ml among women with hyperandrogenemia with higher levels in the presence of PCOM. AMH ≥35 pmol/l (≥4.9 ng/ml) may serve as a proxy for polycystic ovary sonographic morphology (PCOM) [13], while another investigation found mean AMH 11.19 ng/ml among PCOM [11]. Limitations of AMH include overlapping threshold levels for different populations and non-standardized assay methodology.

Updated guidelines affirmed routine laboratory exclusion of thyroid disorders, hyperprolactinemia, and congenital adrenal hyperplasia, and further evaluation depending on clinical presentation for hypogonadotropic hypogonadism, Cushing's disease, and androgen-secreting tumors (Table 3.1) [1].

Diagnosis in adolescents uses similar menstrual criteria as adults when more than 3 years post-menarche and affirms use of cycle lengths <21 days when >1 year post-menarche, while suggesting longer duration of oligomenorrhea (>45 days) in patients who are 1–3 years post-menarche [10]. In adolescents, diagnosis of PCOS is discouraged within 1 year of menarche, and pelvic ultrasound is not recommended within 8 years of menarche [10]. Biochemical and clinical hyperandrogenic diagnostic criteria are similar for adolescents and adults, but regardless of clinical severity, any adolescent complaint of unwanted hair should be addressed to minimize negative psychosocial impact [10].

Congenital Adrenal Hyperplasia

Ten percent of the world's female population has hyperandrogenism, and 4.2% of hyperandrogenic women have non-classical congenital adrenal hyperplasia (NCAH) [14]. NCAH is estimated to impact 1:1000–1:2000 Anglo-Saxons and 1:100 Ashkenazi-Jewish and certain ethnic groups within sub-continent India, North Africa, and the Middle East; it is relatively uncommon among Black women [14, 15]. While classical salt-wasting or simple virilizing CAH can be detected neonatally, NCAH often is unrecognized until later childhood or adulthood [14] and may be asymptomatic [16]. Both classical and NCAH are caused by autosomal recessive genetic aberrations for enzymes (genes) involved with the adrenocorticotropic hormone (ACTH)-regulated adrenal steroid pathway, most commonly affecting 21-hydroxylase (CYP21A2) 90–95% of the time [15], less commonly 5–8% 11β-hydroxylase (CYP11B1) [15], and rarely 3β-hydroxysteroid dehydrogenase (HSD3B2).

Presenting NCAH symptoms in children include premature pubarche prior to age 10 and menstrual irregularities in adolescents [14]. The majority of female adult NCAH are due to 21-hydroxylase enzyme deficiency, featuring hyperandrogenic symptoms and cardiometabolic dysfunction similar to that seen in PCOS [14, 17]. Alopecia is present in 2–8% of NCAH and clitoromegaly in 6–20%, and polycystic sonographic ovary morphology has inconsistently been reported as well [14]. Nearly half of NCAH women report menstrual/ovulatory disturbances, and nearly a third report fertility difficulties with either infertility and/or pregnancy losses [14, 17]. Infertility in CAH has been attributed to anovulation and impact of chronic progesterone exposure on cervical mucus and endometrium [14]. CAH due to 11β-hydroxylase deficiency presents in genetic females at median age 1 year, and the phenotype is typically classical genital ambiguity or simple virilizing associated with hypertension; adult NCAH presentations appear to be rare [15]. 3β-hydroxysteroid dehydrogenase is associated with no to mild genital ambiguity in genetic females, owing in part to minimal ovarian testosterone production during the critical period (8–12 gestational weeks) of fetal sexual differentiation [18]. However, due to intact expression of the isoform HSD3B1 in peripheral (extragonadal, extra-adrenal) tissues, the gonadotropin rise that occurs after age 8 leads to elevated testosterone, DHEAS, and precocious puberty in 80% of 3BHSD2

deficiency cases; a case series also reported menstrual irregularity and PCOS phenotype [18].

Classical or simple virilizing CAH is associated with a severely deficient or nonfunctional adrenal enzyme leading to decreased or absent adrenal cortisol with subsequent reduced negative feedback on the anterior pituitary and subsequent compensatory increased adrenocorticotropic hormone (ACTH) secretion, leading to hypertrophy of the adrenal fasciculata and reticularis with an accumulation of precursor hormones proximal to the deficient enzyme that are themselves androgenic or converted into androgens [14]. In contrast, NCAH-associated androgen excess often occurs in the setting of normal adrenocorticotropic hormone (ACTH) levels and fairly normal cortisol response to ACTH [14]. Suggested mechanisms for this phenomenon include inefficient mutated NCAH enzyme kinetics that lead independently of ACTH to increased precursor protein [14].

21-hydroxylase converts progesterone into deoxycorticosterone, and it converts 17-hydroxyprogesterone into 11-deoxycortisol [16]. In NCAH-associated 21-hydroxylase deficiency, 17OHP levels are typically normal at birth [16], but this precursor eventually rises to excess as it is converted both to androstenedione, via 17 and 20 lyase and through a "backdoor" pathway, and to dihydrotestosterone peripherally in target tissues [14, 16]. Elevated adrenal precursor progesterone in NCAH may amplify the disruptive impact of androgen excess on the hypothalamus, exaggerating the gonadotropin release hormone (GnRH) pulse frequency and thereby luteinizing hormone (LH) hypersecretion, leading to ovarian androgen excess as well as to polycystic ovarian morphology [14].

11β-hydroxylase catalyzes the biosynthesis of cortisol and corticosterone from 11-deoxycortisol and 11-deoxycorticosterone [15]. Cortisol within the adrenal fasciculata layer is also derived from the conversion of corticosterone by 17-hydroxylase [16]. With CYP11B1 deficiency, the elevated precursor hormones additionally to 17OHP include 11-deoxycortisol and 11-deoxycorticosterone, of which the latter contributes to hypertension [15].

3β-hydroxysteroid dehydrogenase converts the Δ5 steroids pregnenolone; 17-hydroxy pregnenolone; dehydroepiandrosterone (DHEA) into, respectively, the Δ4 ketosteroids progesterone; 17-hydroxyprogesterone; and androstenedione within the adrenal gland [18]. Within the ovary/gonad, 3β-hydroxysteroid dehydrogenase also converts androstenediol into testosterone [18]. With HSD3B2 deficiency, the excess precursor hormones include 17-hydroxypregnenolone and DHEA and is a less androgenic milieu due the absence of backdoor precursors compared to the precursors observed with 21- and 11β-hydroxylase deficiencies [18].

The clinical picture of NCAH overlaps considerably with PCOS, and both entities may have similarly elevated testosterone and DHEAS levels [17]. In a hirsute patient with a family history or from a high-risk ethnic group, NCAH screening should be performed regardless of normality of testosterone levels [9] (Table 3.1). NCAH screening should be obtained in the morning during the follicular phase of the menstrual cycle when the endogenous ACTH is maximal and there will be no interference of ovulation on progesterone levels [19, 20]. For oligo or amenorrheic patients, random day for serum screening is appropriate [9]. An overnight fast is

preferred, given that food intake may affect the hypothalamic-pituitary-adrenal axis [21]. Either a basal or cosyntropin-stimulated 17OHP level >10 ng/ml (30 nmol/L) preferably measured by LCMS is diagnostic of NCAH and suggests that a severe loss-of-function enzyme genetic mutation may be present [14]. Suggested thresholds vary for basal 17OHP level, but if levels are >1.7 ng/ml to >2 ng/ml (5.1 nmol to 6 nmol/l), then a stimulated level is indicated [14]. About 20% of PCOS patients may have similar basal 17OHP levels as CAH, so stimulation testing is then appropriate [17]. Stimulation is performed by administering 250 mcg of cosyntropin intravenously and measuring serum 17OHP at baseline, 30 minutes and 60 minutes [14]; androstenedione may also be measured at each time point [16]. Positive screening results with 17OHP most commonly indicate 21-hydroxylase deficiency though if 11β-hydroxylase deficiency is clinically suspected, elevated 11-deoxycortisol is diagnostic [15]. However, normal basal or stimulated 17OHP levels do not exclude the presence of heterozygous NCAH carrier states [14, 16] or the rare instance of 3β-hydroxysteroid dehydrogenase deficiency, which may be screened for additionally with serum elevated levels of 17-hydroxypregnenolone and DHEA or an elevated 17-hydroxypregnenolone/cortisol ratio using LCMS [18].

Classical CAH and NCAH most commonly arise from inactivating mutations within the 21-hydroxylase (CYP21A2) gene located within the short arm of chromosome 6 that decrease the activity of the 21-hydroxylase enzyme [22]. Completely non-functional 21-hydroxylase activity is associated with deficient aldosterone and cortisol biosynthesis and manifests as classical CAH with salt wasting and genital ambiguity, while a severely reduced functioning enzyme (having 1–2% of full 21-hydroxylase activity) will lead to simple virilizing non-salt-wasting CAH with preserved aldosterone production which is typically grouped under the classical designation [22]. The functional CYP21A2 gene is in close proximity to the non-functional CYP21A1P pseudogene, making it vulnerable to acquiring pseudogene sequences due to misalignment of the recombining homologous DNA strands during meiosis or rarely due to uniparental disomy [22]. Nonsense and frame shift mutations within either coding or non-coding (examples include promotor and steroidogenic factor binding regions) regions from large deletions or duplications are associated with classical CAH, while missense mutations of a few nucleotides are associated with both classical and NCAH [14, 22]. Although hundreds of mutations have been described, a small number of them account for the majority of CAH and NCAH cases, and those mutations are the focus for molecular genetic testing [22]. Missense mutations of CYP21A2 associated with NCAH include P30L, P453S, R339H, R369W, I230T, and V281L [22], but P30L has also been associated with the simple virilizing form CAH [14].

11β-hydroxylase and aldosterone synthase, respectively, are encoded by the genes CYP11B1 and CYP11B2, located in close proximity within the long arm of chromosome 8; neither has a pseudogene in contrast to CYP21A2 [15]. Mutations of CYP11B1 are commonly missense and typically associated as mentioned previously with severe classical or simple virilizing phenotypes, though a few including R448P are associated with NCAH [15]. 3β-hydroxysteroid dehydrogenase deficiency is so uncommon, affecting fewer than 1 in 100,000 live births, that only the

classical CAH form has been genotyped and primarily consists of missense mutations [18].

Most CAH patients are compound heterozygotes of loss-of-function mutations with variably affected alleles and typically have cosyntropin-stimulated 17-hydroxyprogesterone levels >15 ng/ml (45 nmol/l) [14]. Classical CAH will result if both alleles are severely affected leading to a non-functioning enzyme, while NCAH with its reduced activity (20–60% activity) enzyme typically reflects a mild mutation in one allele and either a mild or more frequently a severe mutation in the other allele [14, 22]. Usually the phenotype will reflect the mild mutation when the other allele has a severe mutation [16]. Rarely, mild-allele homozygosity for CYP21A2 can lead to classical CAH, as in the case of double mild mutations it presents both cis and biallelic in a so-called double homozygote finding [23]. Hemizygous states can also lead to CAH if one allele is pathogenic and the other allele is deleted [22]. Genetic transmission to offspring is autosomal recessive; heterozygotes with either a mildly or severely affected allele and an unaffected second allele are typically asymptomatic [22]. Genetic counseling should be offered to CAH patients [14].

Since the last edition of this chapter, molecular genetic screening for CAH has become widely available, to evaluate for the most common pathogenetic variants. For example, the multiplex ligation-dependent probe amplification (MLPA) assay is used to detect deletions and duplications of the CYP21A2 and CYP21A1P by detecting copy number variation using a single pair of primers and oligonucleotide probes with progressive increasing lengths [22]. Caution is warranted, however, for several reasons. First, normal polymorphisms may be reported that do not affect enzyme protein production such as D183E which is present in the CYP21A1P gene and K102R, S268T, and N493S which are within the CYP21A2 gene [22]. Second, if there are two pathogenic variants detected from a patient, it is not possible without having the parents also be genotyped to know whether the variants are present in trans (each on opposite alleles) and therefore likely to have a phenotype as opposed to cis (both variants are on same allele) in which case the patient would be a heterozygous carrier [22]. It is also important to consider the polymerase chain reaction (PCR) gene amplification methodology, as it is preferable to amplify CYP21A2 rather than the pseudogene in order to improve sensitivity to detect CYP21A2 variants [22]. There may also be family-specific variants that may be missed on the molecular testing [14].

With classical CAH, treatment is titrated to serum levels, with goals of 17OHP <1000 ng/dl and androgen levels within the age-specific reference range, stress-dosing with glucocorticoids is used liberally, and mineralocorticoid treatment is required in salt-wasting phenotypes [16]. Treatment of NCAH depends on the symptoms and is different from classical CAH. Generally glucocorticoids in NCAH should be used sparingly to avoid glucocorticoid-associated morbidity including metabolic disease and Cushing's syndrome [14, 16]. Glucocorticoids may be appropriate for pre-pubertal and peri-pubertal girls with rapidly advancing bone age or

early pubarche, undergoing major surgery or post-trauma, or with cosyntropin-stimulated serum cortisol level <18 mcg/dl (<497 nmol/l) [14]. Additionally children may benefit from growth hormone to optimize their height in cases of advanced bone age and gonadotropin-releasing hormone analogs to ameliorate precious pubarche [16, 18]. However in NCAH adults, there is a lack of data to support routine supraphysiological steroid loading prior to surgery or post trauma, and such loading should be reserved for abrupt discontinuation of chronic maintenance steroids [14].

Androgen excess symptoms in adult women respond well to ovarian androgen suppression using oral contraceptives, cyclic progestins, GnRH analogs, and peripheral androgen blockers; glucocorticoids are less effective [9, 14]. Spironolactone anti-androgen treatment for NCAH appears to be safe from an anti-mineralocorticoid standpoint, though it is recommended to avoid during conception as it may impair male fetal virilization [14]. CAH-associated infertility associated with anovulation can be treated with ovulation induction similar to that used for PCOS, while glucocorticoids including hydrocortisone 20–25 mg daily or prednisone 2.5–5 mg daily may be added to further ameliorate androgen and/or progesterone excess [14]. Recurrent pregnancy loss attributed to CAH may be treated with hydrocortisone or prednisone which are inactivated by the placenta [14]. Proponents of prenatal use of dexamethasone which crosses the placenta, suppresses fetal pituitary ACTH, and reduces fetal genital virilization note that post-natal or childhood genitoplasty is associated with sexual and urinary morbidity [16]. Opponents of prenatal dexamethasone therapy cite concerns with fetal intellectual development, and this treatment is considered experimental by the Endocrine Society [14, 16]. Bilateral adrenalectomy has also been performed for refractory CAH, medication-induced Cushing's syndrome, or intolerance to medical therapy, with overall good outcomes reported [24].

Androgen-Secreting Tumors

Androgen-secreting neoplasms are a rare cause of androgen excess in reproductive women, and thus much of the published knowledge comes from case reports which primarily describe adrenal and ovarian origins, coexisting with or mimicking other androgen excess disorders. Compared to PCOS, androgen-secreting neoplasms are more frequently associated with virilization including severe hirsutism, alopecia, clitoromegaly, profound hyperandrogenemia which may include serum total testosterone >200 ng/dl, and severe cardiometabolic derangements, along with failure of medical management to improve hirsutism, hyperandrogenemia, or hyperglycemia [25]. During pregnancy, placental aromatase offers substantial fetal protection from maternal androgen excess; however, incidences of female fetal virilization have been reported in settings of excessive maternal levels of non-aromatisable

androgens, such as seen in rare cases of maternal 11-oxygenated C19 androgen producing adrenal tumor [26] and with benign pregnancy luteomas [27] have been reported.

Not all androgen-secreting tumors present with rapid progression or marked serum androgen elevation [9]. If the index of suspicion is high, dynamic testing can be considered including testosterone assessment following administration of human chorionic gonadotropin (hCG) which may indicate an ovarian source; alternatively, invasive site-specific venous sampling can be performed for assessing sex steroid levels which can also lateralize the site of involvement for surgical excisional planning [25]. Ovarian androgen-secreting tumors of various histologies have been reported in both pregnant and nonpregnant states including the aforementioned luteoma [27] and granulosa cell, Brenner, and Leydig cell tumors. In patients presenting with signs and symptoms of virilization, with evidence of markedly elevated circulating levels of testosterone but with normal DHEAS levels, and with nonspecific findings on ovarian ultrasound, selective ovarian venous sampling should be considered to help guide surgical decisions [25, 28, 29].

During embryogenesis, adrenal and gonadal tissues migrate together and adrenal tissue remnants may subsequently be found on the broad ligament or rarely the ovaries [30]. Under conditions of sustained ACTH stimulation, these remnants, called ectopic adrenal rest tumors, can proliferate and be endocrinologically active [30]. Adrenal rest tumors undetected by CT imaging may be detected by positron emission tomography-CT utilizing tagged 11C-metomidate, which has high affinity for 11β-hydroxylase overexpressed by adrenal rest tumors [30]. Affected patients with adrenal rest tumors with NCAH may have failure of or inadequate androgen suppression by glucocorticoids [30]. Adrenal tumors that autonomously produce 17OHP may also be misdiagnosed initially as NCAH, with the correct diagnosis made after failure of glucocorticoids to suppress 17OHP, absence of NCAH mutation on genotyping, adrenal mass identified on CT imaging, and restoration of glucocorticoid suppression post-adrenal resection [31] DHEAS levels >700 μg/dL merit evaluation for adrenal tumor [9].

Cushing's Syndrome

Cushing's disorders are characterized by cortisol excess, in which terminology indicates etiology. Cushing's disease arises from functional ACTH-secreting pituitary tumors, while Cushing's syndrome encompasses endogenous pituitary or nonpituitary neoplasms which may be ACTH dependent or independent, as well as exogenous/iatrogenic sources. These are uncommon, with an incidence of 3 per million for Cushing's syndrome annually [32] and 1.2–2.4 cases per million annually for Cushing's disease [33]. Severe Cushing's syndrome is associated with considerable morbidity and mortality stemming from cardiometabolic, infectious, thromboembolic, and neurocognitive disease [34, 35]. The Endocrine Society recommends initiating screening for Cushing's syndrome in the presence of plethoric

facies, proximal muscle weakness, easy bruisability, reddish-purple striae >1 cm, features unusual for age including hypertension and osteoporosis, and adrenal incidentalomas [34].

Clinical features and comorbidities significantly overlap in Cushing's disorders and PCOS, including hirsutism, biochemical hyperandrogenemia, menstrual disturbances, and type 2 diabetes [36]. PCOS is associated with increased tissue cortisol synthesis from biologically inactive cortisone in the adipose tissue and ovarian luteinized granulosa cells due to increased reductase activity of 11β-hydroxysteroid dehydrogenase isoform 1, although serum and late-night salivary diurnal variation of cortisol are normal, in contrast with Cushing's syndrome [37, 38]. Among 230 US patients with Cushing's disease, 79% were female and 43% had PCOS [39]. The mechanism for disordered menstruation in Cushing's syndrome may relate to hypercortisolism-associated hypogonadotropic estrogen deficiency [40]. Total circulating androgen levels in Cushing's syndrome may be normal although low sex hormone-binding globulin may contribute to relative hyperandrogenism [40].

International consensus recommends evaluating a suspected PCOS patient for Cushing's syndrome only if clinical features are suggestive [8], and PCOS is not part of the Endocrine Society's Cushing's syndrome screening algorithm [41]. Indeed, hypercortisolism screening for the presenting symptom of hirsutism revealed zero cases of Cushing's syndrome among 105 screened patients [42]. However, subclinical Cushing's syndrome was found in a case series including 26 reproductive age women with Cushing's disease of whom 13 were initially misdiagnosed with PCOS [36]. Androgen levels may be helpful to distinguish Cushing's syndrome from PCOS, perhaps due to hypercortisolism-induced direct hypothalamic gonadotropic inhibition and ovarian androgen suppression [43]. Total testosterone <1.39 nmol/L (<40 ng/dl) had high sensitivity and positive and negative predictive values to distinguish Cushing's syndrome from age- and weight-matched PCOS patients, while bioavailable testosterone >0.24 nmol/L (>6.9 ng/dl) had good specificity to rule out Cushing's syndrome [43]. However, it remains to be seen as to whether such subclinical or mild Cushing states would lead to worsened morbidity and mortality in contrast to overt Cushing's syndrome [34]. Further complicating diagnosis is that certain medications including medroxyprogesterone acetate, commonly utilized for endometrial protection in PCOS, have been rarely reported to induce iatrogenic Cushing's syndrome characterized by simultaneous adrenal cortical insufficiency and reduced androgen levels [44].

The Endocrine Society recommends first-line screening modalities for Cushing's syndrome to include late-night salivary cortisol, urinary free cortisol, or serum cortisol following low-dose dexamethasone suppression [45]. All have very high sensitivity and specificity but poor positive predictive value necessitating additional confirmatory testing [46]. A positive screening test should be confirmed with a different positive test to diagnose Cushing's syndrome, with concordant positive screening results followed by additional diagnostic testing to identify the etiology, which includes an assessment of ACTH dependence and location of cortisol overproduction [47]. In ACTH-independent Cushing's syndrome, endogenous ACTH levels will be suppressed, suggesting an adrenal or other ectopic source of

autonomous excess cortisol production, while for ACTH-dependent Cushing's syndrome, ACTH levels will be inappropriately normal or elevated which suggests a pituitary origin typically neoplastic [47]. It should be noted that dopamine agonists including cabergoline and bromocriptine may suppress ACTH levels [47].

Patients with positive Cushing's screening may be referred to specialty centers to localize the autonomous source of either the ACTH or cortisol using either targeted pituitary MRI (>6 mm adenoma is suggestive of pituitary source) or adrenal imaging or dynamic testing with the corticotropin-releasing hormone test and/or desmopressin test (due to aberrant vasopressin v2 receptors, pituitary corticotroph adenomas respond more strongly to this), and if necessary venous sampling of the inferior petrosal sinus or adrenal vein may be employed [48]. Seventy percent of Cushing's syndrome cases arise from ACTH-dependent pituitary tumors and 25% from ACTH-independent adrenal pathology (hyperplasia, adenoma, and carcinoma), and the remainder are ectopic of variable ACTH dependence [49]. Patients with discordant results on screening in whom post-test Cushing's suspicion remains high can be observed and retested after a 6-month interval if symptoms persist [50].

Five percent of cortisol circulates as biologically active free cortisol in plasma, with the other 95% bound to proteins and inactive [51]. Salivary cortisol is in equilibrium with plasma free cortisol, is not influenced by rate of saliva production, and shows the same diurnal variation as serum cortisol with late-night nadir [51]. Although positive predictive values are poor, late-night salivary cortisol as measured by enzyme immunoassay with an abnormal elevated threshold of ≥3.3 nmol/L (≥1.2 ng/ml) has 97.5–99.8% negative predictive value for, respectively, ACTH-independent and ACTH-dependent neoplastic Cushing's syndrome [35]. Typically at least two samples on different nights are collected, as etiologies for non-neoplastic late-night salivary elevated cortisol results include exogenous corticosteroid use (including inhaled or topical), chronic kidney disease, alcohol or tobacco use, disordered sleep-wake cycle, acute excitement or stress, or inappropriate sampling time [35]. Testing is typically performed after discontinuing hormonal contraception, though one study found late-night salivary cortisol levels in 12 healthy women taking oral contraceptives were similar to 42 healthy unmedicated women [52]. Traditionally the salivary cortisol sample is collected between 2300 and 2400 hours, but more flexible timing at typical bedtimes 2025–2400 hours produces similar results [53]. The sample is collected by chewing on a specialized collection device for 2–3 minutes and can be stored at room temperature for up to a week or frozen at −20 °C for longer storage before analysis [51]. Sampling is ideally performed 2 hours or longer after eating, drinking, smoking, or teeth-brushing [38].

For urinary free cortisol, at least two samples are collected each over 24 hours beginning with an empty bladder over a 2-week span, samples should be refrigerated for analysis using high-performance liquid chromatography (LC) or LC associated with tandem mass spectrometry, and values >4 fold of the upper range of

normal suggest Cushing's syndrome [41, 45, 54]. Drugs including fenofibrate and carbamazepine may spuriously increase urinary free cortisol [45]. Although all screening methods perform comparably, urinary free cortisol had a 17% false negative rate in comparison with late-night salivary cortisol in one study [54]. Due to estrogen-mediated increased cortisol-binding globulin (CBG) hepatic release which increases total serum cortisol, urinary free cortisol rather than serum testing has been suggested for Cushing's screening during pregnancy or with use of estrogen-containing contraceptives, but late-night salivary cortisol can also be considered [46, 50, 52]. Progesterone secretion during normal pregnancy, however, displaces cortisol from CBG and may contribute to higher free cortisol levels [52]. In patients with renal failure, the dexamethasone suppression test (DST) is favored over urinary free cortisol screening [50].

For the low-dose overnight DST, patients self-administer 1 mg dexamethasone orally at 23:00 (11 pm) and the following morning at 08:00 have plasma ACTH and serum cortisol measured; normal results are when both levels are suppressed [50, 55]. Threshold elevated serum cortisol level for a positive test is >50 nmol/L (≥18.2 ng/ml), and suppressed morning ACTH suggests autonomous cortisol secretion probably from an adrenal source [55]. Serum assays measure total cortisol (free and bound) which as previously mentioned is elevated in the presence of estrogen, leading to possible false-positive dexamethasone suppression testing [50]. Other drugs may either facilitate (anti-epileptics, alcohol) or inhibit (fluoxetine, cimetidine, diltiazem) dexamethasone clearance and thus create false-positive or false-negative results, respectively [50]. Medical conditions including kidney disease and obesity have also been associated with false-positive DST [55]. Co-measurement of serum dexamethasone has been suggested, as a threshold level of dexamethasone >3.3 nmol/L is necessary for endogenous cortisol suppression [55].

Treatment of Cushing's syndrome includes lifelong surveillance and is aimed at curbing comorbidities including infection, thromboembolism, and psychosis and surgical resection of neoplastic disease whenever feasible [34]. Adjunctive therapies for poor surgical candidates or non-curative surgery include mifepristone (glucocorticoid receptor antagonist and anti-progestin), ketoconazole (steroidogenesis inhibitor), metyrapone (11β-hydroxylase inhibitor), dopamine agonists (ACTH inhibitor), pasireotide (somatostatin receptor agonist), and radiation [34].

Exogenous Androgens

History-taking is of paramount importance since standard assays do not detect unsanctioned anabolic steroids including stanozolol, oxandrolone, methandrostenolone, methenolone enanthate, and nandrolone which women have used for body fitness, body building, or sexual functioning purposes and are associated with masculinizing effects including loss of menstruation, acne, voice changes, and genital enlargement [9, 56]. Inadvertent passive transfer of prescribed androgens used by a

close contact has also been reported to cause androgen excess presentation [57]. Androgen metabolites may also be present in commercial dietary supplements [9]. Certain medications may also cause hirsutism including valproic acid which raises plasma testosterone levels [9].

Idiopathic Hyperandrogenism

Patients may display hyperandrogenic symptoms in the absence of triggers and with normal menstrual function and biochemical testing, referred to as idiopathic. Although heterozygous carriers of CYP21A2 are considered to be asymptomatic, one study found higher carrier frequency among patients with idiopathic hyperandrogenism compared to controls [58]. Patients with idiopathic hyperandrogenism were found in one study to have altered skin mRNA expression of interleukin 6, sulfatase, and HSDB2 expression and in another study to have reduced expression of aromatase and paradoxically low 5α reductase expression, all of which suggest disordered local control of androgen secretion within peripheral tissues [59, 60].

Abnormal Thyroid Functioning

Thyroid and gonadal health are closely related in ways that influence the development of features that may mimic PCOS. Thyroid disease has a variable presentation which may be asymptomatic (even when biochemically overt) or symptomatic with non-specific complaints including fatigue, weight changes, and temperature intolerances [61]. Women with thyroid dysfunction may additionally note menstrual dysfunction commonly hypomenorrhea and/or oligomenorrhea and biochemically may have increased testosterone and androstenedione levels [62]. Hyperthyroid patients may have LH elevations and absent LH surges, while hypothyroid patients have a blunted LH response to GnRH possibly due to TRH-stimulated prolactin increase [62]. Gonadotropin aberrations in thyroid disease were normalized with restoration to euthyroid state [62]. Therefore, screening for thyroid disorders is appropriate for evaluation of a patient suspected to have PCOS as well as hyperprolactinemia and/or galactorrhea (see next section).

Clinical stigmata of overt hypothyroidism may include goiter, coarse dry skin, alopecia, eyelid edema, and decreased deep tendon reflexes [63, 64]. Biochemically, elevated TSH >4.49 mU/L and free T4 below the laboratory's reference range are consistent with overt hypothyroidism; suppressed TSH <0.45 mU/L and free T4 above the reference range indicate overt hyperthyroidism whereas abnormal TSH with normal range free T4 is referred to as subclinical disease [65]. In an unselected population, TSH was normal in 93%, out of which 95% had normal free T4 level [65], while others have reported that undiagnosed overt hypothyroidism is less common at 0.5% [66]. Existing guidelines recommend a two-step screening approach that begins with testing serum TSH level, and if abnormal proceeding with repeat testing of TSH along with checking freeT4; including both tests in a single initial

step leads to unnecessary free T4 tests [64, 65]. However, if suspicion is high for central (secondary) intracranial hyper or hypothyroidism, other autoimmune disorders are present, or if overt stigmata are present including goiter, heart failure, arrhythmia, coronary artery disease, or fractures, further workup is appropriate [65]. Typically levels are drawn irrespective of time of day, but it should be noted that TSH levels exhibit diurnal variation, peaking in the late afternoon/evening [64, 67]. Falsely abnormal TSH and free T4 results may arise in patients taking biotin supplements, with anti-mouse heterophile antibodies or with other autoimmune conditions that may raise the level of macro complexes of TSH bound to anti-TSH antibodies [64, 68]. Low TSH and variable free T4 levels may also be observed in normal pregnancy and with use of medications including glucocorticoids, metformin, and heparin [68].

Patients with normal TSH levels toward the limits of the normal reference range (4.0–4.5 mU/L and 0.5–0.99 mU/L) merit closer surveillance, as one study found a higher risk over the next decade of developing, respectively, overt hypothyroidism or hyperthyroidism [69].

Approximately 5% of women in the general population have subclinical thyroid disease [66]. Although subclinical thyroid disease has inconsistently been linked to cardiovascular disease, thyroid treatment does not appear to mitigate these outcomes, and thus there are no specific recommendations for asymptomatic community thyroid screening [61, 66].

Hypothyroidism is most commonly primary as in arising in the thyroid, typically from autoimmune chronic lymphocytosis (Hashimoto's) [64]. Nearly all primary hypothyroidism is due to Hashimoto's so it is not recommended to additionally measure thyroid antibodies (antithyroid peroxidase and antithyroglobulin) solely for diagnosis [64]. It is also not recommended to draw total T3 or free T3 as those are typically normal with hypothyroidism, and thyroid imaging is not recommended unless a thyroid nodule is palpable [64]. Trisomy 21, monosomy X, iodine deficiency, use of medications including lithium and amiodarone, and the presence of other autoimmune conditions including celiac disease, vitiligo, type 1 diabetes, and adrenal insufficiency are associated with a higher rate of primary hypothyroidism [64]. Diagnosis of central secondary hypothyroidism is more challenging, as TSH may be normal, but the finding of a low reverse T3 level may distinguish this condition from non-thyroidal illness [64]. Risk factors for secondary/central hypothyroidism include tumors, intracranial surgery or trauma, and use of medications including glucocorticoids [64]. Left untreated, hypothyroidism can progress to life-threatening myxedema coma, characterized by hypotension, bradycardia, seizures, and hypothermia [64]. Standard therapy for overt hypothyroidism is oral levothyroxine, which has a half-life of 5–7 days and is initiated at 1.6 mcg/kg according to body weight that corresponds to a BMI 24–25 kg/m^2 (not actual weight) for the patient's height and with steady state levels anticipated in 5–6 weeks [64]. Lower initial levothyroxine doses 25–50 mcg should be utilized for age >60 and adjusted every 6–8 weeks with titrating dose to TSH level for primary and free T4 for central/secondary hypothyroidism [64]. Absorption of levothyroxine, a sodium salt, is inhibited by proton pump inhibitors and facilitated by taking on an empty stomach separated at least 4 hours from intake of soy, calcium, and iron [64]. Levothyroxine

(LT4) is converted peripherally into triiodothyronine (T3), but some patients may benefit from the addition of synthetic LT3 to their regimen or treatment may consist of desiccated powdered porcine thyroid containing both T3 and T4 used cautiously to prevent thyrotoxicosis from rapidly absorbed T3 [64]. Replacement thyroid dose requirements increase with estrogen use due to increased T4 binding protein, pregnancy, malabsorption syndromes, and gastritis [64].

Symptoms of overt hyperthyroidism include sweating, palpitations, heat intolerance, fatigue, dyspnea, and weight loss; autoimmune Graves' disease is also associated with eye pain and swelling and rash on the shins, while iatrogenic or infectious subacute thyroiditis may present with sore throat, fever, or neck pain [68]. Exam may reveal tachycardia, lid lag, and goiter; Graves' disease additionally presents with inflammatory eye findings, diffuse goiter, clubbing of fingers, and pretibial myxedema, while subacute thyroiditis displays thyroid tenderness, and toxic solitary or multinodular goiter is associated with characteristic palpation findings [68]. Total T3 may be checked to evaluate for T3 thyrotoxicosis if the free T4 is normal in the setting of suppressed TSH, but free T3 is not recommended due to concerns with assay accuracy [68]. If exam findings are not definitive for hyperthyroidism etiology, serum thyrotropin receptor antibodies (TRAb) should be obtained and if elevated indicate Graves' disease [68]. If TRAb is non-diagnostic or there is a palpable thyroid nodule, radioactive iodine uptake testing and thyroid imaging with ultrasound are recommended [68]. To treat hyperthyroidism, propylthiouracil (PTU) is recommended during the first trimester of pregnancy due to lower teratogenicity risk, but due to associated hepatotoxicity with PTU, methimazole is favored overall based on its potency, toxicity profile, and easier dosing with 5–20 mg daily for free T4 within twice the upper limit or normal and 30–40 mg daily for free T4 higher than three times the upper normal range [68]. Half will have remission with 12–18 months of treatment, with the remainder requiring several years of treatment; refractory cases may benefit from radioactive iodine ablation of the thyroid or thyroidectomy [68].

Hyperprolactinemia

Endogenous feedback loops promote physiologic prolactin elevation during pregnancy and postpartum to support successful lactation. Infant suckling reduces hypophysial pituitary stalk dopamine, thereby releasing the anterior pituitary lactotrophs from tonic inhibition and allowing increased prolactin secretion by the anterior pituitary that during pregnancy reach 150–300 ng/ml by term [70, 71]. The increased prolactin additionally induces a state of reproductive quiescence through suppression of GnRH pulsatility, thus limiting LH pulse frequency and amplitude with resulting hypogonadism [72]. Prolactin receptors are typically absent among GnRH neurons, so prolactin suppression occurs indirectly, likely mediated by kisspeptin [72]. Kisspeptin neurons in the arcuate nucleus of the hypothalamus are potent promotors of GnRH pulsatility, and their activity is inhibited by prolactin acting through prolactin receptors within the kisspeptin neurons [73]. GnRH

pulsatility can be restored during hyperprolactinemia by exogenous kisspeptin and in animal prolactin receptor knockout models, and ovulation may also be restored with the exogenous administration of pulsatile GnRH [72, 73]. Physiologic promotors of prolactin secretion include coitus, exercise, sleep, stress, pregnancy, high-protein diet, and lactation [70, 74–76].

There are rare case reports of prolactin deficiency <5 ng/ml associated with alactogenesis, but in contrast to hyperprolactinemia, prolactin deficiency has not been associated with altered gonadal states or infertility [73, 77]. Pathologic prolactin excess leading to hypogonadism and low bone density may arise from central (hypothalamic, stalk, or pituitary) neoplasm, infection, or trauma; systemic including chest wall trauma, renal or hepatic insufficiency, seizure disorder, untreated thyroid disorder, or cranial irradiation; and pharmacologic etiologies including estrogen, estrogen-containing contraceptives, antidepressants, antipsychotics, antihistamines, antihypertensives, anticonvulsants, opiates and analogs, and dopamine antagonists [70, 78]. Prolactin is a pleiotropic hormone, such that hyperprolactinemia may reduce bone density both indirectly by inducing both hypogonadism and osteoclastic receptor activator of nuclear factor KB ligand (RANKL) and directly by inhibiting bone osteoblasts [73, 77]. Pituitary tumors or adenomas called prolactinomas are the leading cause for hyperprolactinemia, whereas in non-neoplastic cases, the etiology is commonly drug/pharmacology-related [70, 73, 74]. Most prolactinomas occur sporadically rather than inherited familially, with an incidence rate in Sweden of 1.6 per 100,000 people [79]. Seventy-five percent of pituitary adenomas in women are prolactinomas [78].

The prevalence of hyperprolactinemia in asymptomatic controls in one study was 1.4% [80]. Ten to 25% of women with oligo/amenorrhea have hyperprolactinemia [75]. Among women with amenorrhea and galactorrhea, 75% had hyperprolactinemia [75], and conversely among women with hyperprolactinemia, 50% report galactorrhea [78]. Women with self-reported androgen excess had a PCOS prevalence of 82%, while hyperprolactinemia prevalence was similar to healthy controls [80]. Among patients with hyperprolactinemia (≤100 ng/ml), however, oligo and amenorrhea were observed in 56%, androgen excess in 51%, and polycystic ovary morphology in 62% [81]. Similar to PCOS, prolactin excess has been associated with infertility, hyperinsulinemia, and metabolic disease [82, 83], though hyperprolactinemia is not associated with increased rates of diabetes, cardiovascular disease, or all-cause mortality [73]. Prolactin promotes hair growth at the hair follicle, and hyperprolactinemia has been, albeit inconsistently, associated with hyperandrogenemia and hirsutism [73]. Furthermore, metformin which is used for PCOS treatment may also normalize prolactin levels from hyperprolactinemia [82], and conversely dopamine agonist treatment for hyperprolactinemia improves glucose control and metabolic parameters [83] and in one study reduced DHEA-S levels [73]. Due to overlapping clinical features with PCOS and prolactin excess, laboratory screening must be utilized to distinguish these conditions.

Diurnal rise of prolactin occurs during sleep, but the change is minimal compared to waking hours, and thus serum prolactin can initially be obtained when convenient [67, 70], but if subsequent levels are needed, those are recommended to

be drawn after fasting and at least 2–3 hours from awakening [76]. Laboratory threshold of prolactin elevation is typically considered as >25 ng/ml (μg/L) [70, 74]. Levels >250 ng/ml are often and >500 ng/ml almost always associated with macroadenomas >10 mm [70, 78]. Medications, stress (venipuncture), exercise, chest wall surgery or piercings, and breast stimulation all may raise prolactin levels to typically <100 ng/ml, but higher levels have been observed with risperidone, metoclopramide, and phenothiazines [70, 74, 75]. The antipsychotics haloperidol and risperidone are particularly potent inducers of hyperprolactinemia due to their exclusive dopamine receptor affinity and lack of blood-brain barrier crossing, while milder or absent prolactin elevations are associated with atypical antipsychotics including aripiprazole, clozapine, olanzapine, quetiapine, and ziprasidone [84]. After discontinuation of the antipsychotic, half of patients have normalization of the prolactin levels within 4 days [75]. Among women taking the SSRI drug fluoxetine, 22% developed hyperprolactinemia <40 ng/ml [75]. Estrogen upregulates prolactin gene expression in pituitary lactotrophs [77], and estrogen-containing oral contraceptives have inconsistently been associated with mild hyperprolactinemia in up to 30% of users [75]. Opiates, alcohol, cocaine, and marijuana may also raise prolactin levels [75]. Untreated overt or subclinical primary hypothyroidism may be associated with mild hyperprolactinemia due to elevated thyrotropin release hormone that is reversible with achievement of euthyroid state [75].

Identifying and ameliorating or discontinuing (if prudent) triggers as well as confirming an elevated level with a repeat prolactin level is advisable particularly when the level is <80 ng/ml [74, 75]. Further evaluation of elevated prolactin may include serum evaluation using polyethylene glycol precipitation to detect the presence of elevated macroprolactin which does not require further evaluation; normal macroprolactin level is <10% compared to the active monomeric prolactin comprising 65–85% of the total circulating prolactin [70, 74, 75]. Although 58% of asymptomatic prolactin elevations were due to macroprolactinemia, 10% have asymptomatic monomeric hyperprolactinemia, while 39% of macroprolactinemia patients were symptomatic [75]. A normal prolactin level in a symptomatic patient with a large pituitary tumor on imaging may indicate the presence of a false-negative result due to a hook effect, whereby high prolactin levels saturate the assay antibodies and may be revealed upon serial dilution of 1:100 [70, 74]. Preferably prolactin screening is utilized for symptomatic cases, but if asymptomatic with an incidental prolactin level <50 ng/ml, observation with annual prolactin surveillance may be appropriate [75]. If both the macroprolactin and pituitary imaging are normal, persistently elevated prolactin >200 ng/ml has been associated with assay interference from heterophile, anti-animal, rheumatoid factor, other autoimmune endogenous antibodies, non-specific antibodies, and supplements containing biotin [74].

Symptomatic patients with unexplained persistent hyperprolactinemia regardless of severity are advised to undergo a pituitary MRI with gadolinium contrast to evaluate for an adenoma [70, 74]. Because the pituitary is a small organ ~8–9 mm in height and because prolactinomas are commonly microadenomas <10 mm, detection by MRI requires high resolution (1–2 mm slices without a gap), high signal/

noise ratio, rapid image acquisition time, gadolinium intravenous bolus contrast, and dynamic images preceding and immediately subsequent to contrast administration [85]. Microprolactinomas typically show hypointensity which is mild on non-contrast T1 images but more prominent on T1 immediately post-contrast; if contrast is contraindicated, T2 images may aid in identification and may be helpful in differentiating an adenoma from a cyst [85]. T2 images are particularly helpful for macroprolactinomas ≥10 mm in identifying tumor margins, and both T1 and T2 may detect associated optic chiasm, suprasellar space or cavernous sinus involvement, and hemorrhage [85].

Macroprolactinomas are associated with prolactin levels >250–500 ng/ml, microprolactinomas with levels ≤250 ng/ml, and non-functioning adenomas with levels <100 ng/ml, though in a significant minority prolactin levels are disassociated with tumor size [75]. The aforementioned hook effect should be suspected in the setting of symptoms and ≥3 cm tumor size but inappropriately normal prolactin levels [75]. Patients with symptoms of headaches and visual changes may additionally be screened with visual field testing if there is suspicion of optic chiasm compression by a large tumor [70]. Prolactin is co-secreted in 15–25% of growth hormone-producing pituitary adenomas, so measurement of IGF-1 is advised particularly when acromegaly and/or a macroadenoma are present [75, 78, 79].

Though microprolactinomas have an indolent course with 3–6.9% progression to macroadenoma size, consequences of hypogonadism can be significant; 70% of patients with prolactinomas have osteopenia [77]. Therefore, treatment of macroprolactinoma is appropriate either with estrogen support or with dopamine agonists [77, 78, 86]. Spontaneous resolution of untreated hyperprolactinemia has also been reported with microprolactinomas, with higher likelihood for prolactin <150 ng/ml and minimal to no menstrual dysfunction [77]. Dopamine agonist medications are the mainstay of prolactinoma treatment, of which the most frequently used are bromocriptine and cabergoline with the goals of euprolactinemia, tumor shrinkage (particularly macroprolactinomas), and eugonadism [86]. Dopamine agonists bind to inhibitory G protein lactotroph cell D2 receptors leading to reduced prolactin gene expression and cell proliferation along with tissue apoptosis [87]. Cabergoline is used preferentially due to higher D2 receptor affinity, longer half-life, better side effect profile, higher rates of euprolactinemia, and higher likelihood of resolution of hypogonadal symptoms and galactorrhea as compared to bromocriptine [86]. Initial dosing ranges for cabergoline, which has a 63–69 hour half-life, are 0.25 mg/week to 3 mg/week, while bromocriptine, with a 5–6 hour half-life, is initiated at 2.5 mg/day to 15 mg/day [86, 87]. Cabergoline in doses >3 mg per day used for Parkinson's disease has been associated with cardiac valvulopathy, raising concerns for chronic lower doses used for neuroendocrine tumor treatment [88]. However, a large study found no evidence of cardiovascular compromise between users of a median cabergoline dose of 2 mg weekly and nonusers over a median 27 months of observation [88].

With dopamine agonist therapy, 90% of microprolactinomas and 70–80% of macroadenomas will shrink by >50% [87]. Once tumor shrinkage to this degree occurs, the dopamine agonist dose may be decreased until the prolactin level

remains normal at the lowest dose, and at that point consideration may be given to discontinuing the dopamine agonist [87]. Within 1 year of medication discontinuation, hyperprolactinemia may recur in 85% of pretreatment macroadenomas and 60% of microadenomas, with improved prognosis for remission in the setting of tumor disappearance and nadir prolactin level <5 ng/ml during treatment [87]. Surgical treatment with transsphenoidal resection alone or in combination with medication may be appropriate in cases of invasive tumor, visual field disruptions, and pharmacologic treatment failure [78, 86].

Maternal prolactin does not cross the placenta, and the growth rate during pregnancy of microprolactinomas is 2.4% vs untreated macroadenomas which grow in 16.4% [77, 86]. Therefore, dopamine agonists may be discontinued during pregnancy in the setting of a microadenoma but may be beneficial for macroadenomas with neuro-ophthalmologic symptoms [86]. Although dopamine agonists may cross the placenta, adverse pregnancy outcomes are reported to be similar in users compared to nonusers [77]. It is unclear whether dopamine agonists in the doses used for hyperprolactinemia will lead to exacerbation of psychiatric illness in those taking antipsychotic medications but caution appears warranted [89]. Treatment may be individualized; estrogens rather than dopamine agonists, coordination with a psychiatric consultant to determine if a prolactin-sparing antipsychotic such as aripiprazole may be utilized, or surgery rather than pharmacologic treatment for macroprolactinomas may be appropriate [89].

The lactational method of contraception is associated with <10% pregnancy rate in the first 6 months postpartum, decreasing to <2% if amenorrheic [90]. The median time from delivery to the first postpartum bleed was 248 days [91], and menstruation returned in ~40% of exclusively breastfeeding women at 6 months postpartum [90]. Prolactin levels vary greatly among exclusively breastfeeding women but appear to peak in the first 2 weeks postpartum at a mean of 270 ng/ml and then fall by 8 weeks to 177 ng/ml [92]. Lactating PCOS women have lower post-suckling prolactin levels compared to control lactating women by 8 weeks postpartum, though levels became similar between groups after weaning [93]. Serum gonadotropin levels were similar among exclusively breastfeeding PCOS and non-PCOS mothers at 4 weeks postpartum, but by 8 weeks postpartum, significantly increased LH pulsatility and decreased FSH levels were noted in the lactating PCOS mothers, and these differences persisted after weaning [93].

Prolactin has physiologic effects on corpus luteum maintenance, either excess or deficient prolactin states have been associated with impaired corpus luteum function [94]. Therefore, even if a patient is eumenorrheic, it may be reasonable to address hyper-monomeric-prolactinemia infertility or recurrent miscarriage with encouragement of weaning if still breastfeeding or with prolactin-lowering medications [94, 95]. Typically dopamine agonists are discontinued within 6 weeks of pregnancy diagnosis, though both cabergoline and bromocriptine have been safely used during pregnancy particularly for progressively enlarging macroadenomas [95–97]. One study found that bromocriptine 2.5–5 mg daily continued until 10 weeks gestation reduced miscarriages among women with recurrent pregnancy loss [95].

Functional Hypothalamic Amenorrhea (FHA)

FHA, formerly called stress-induced amenorrhea, is present in 1–4.5% of reproductive age women [98, 99] and has been found in 30% of women presenting to infertility clinics in the United States [100]. In contrast to idiopathic or congenital isolated hypothalamic hypogonadism, FHA diagnosis includes both secondary oligo/amenorrhea plus a triggering event. Extreme psychosocial stress including trauma, divorce, unexpected death of a loved one, or abuse; weight loss; increased energy expenditure in the form of vigorous exercise; and decreased energy intake or disordered eating are all acknowledged triggers for FHA [99]. However FHA may commonly present with similar severities of psychosocially stressful life events and normal weights relative to control women, suggesting that FHA may have underlying sensitivities leading to disordered neurobiological responses [99, 100]. In one study of FHA, >90% had secondary amenorrhea at a mean age 21, and nearly 30% reported delayed puberty at >age 15 [99]. The Endocrine Society established the following criteria for FHA to include cycle lengths repetitively >45 days or amenorrhea >3 months; hypoestrogenism <184 pmol/L that may be confirmed with a failure to bleed after progestin challenge; the presence of a triggering event such as weight loss, vigorous exercise, or stress; and the exclusion of a structural pituitary abnormality by brain MRI [98]. Failure to bleed is poorly associated with estradiol <146.9 pmol/L [98]. Only 6% of FHA women bled at ultrasound measured endometrial thickness of ≤1.5 mm, suggesting value of endometrial thickness as a good prognostic indicator of progestin challenge induced withdrawal bleed [98].

Hypoestrogenism increases fracture risk in FHA, particularly with anorexia nervosa (AN) where patients have altered microstructure of cortical and trabecular bone, reduced bone density, and reduced bone strength [101]. This may be offset by weight-bearing exercise but only partially so, as oligomenorrheic exercisers had higher bone density than AN but had similar rates of fracture as AN and reduced distal tibia and spine bone densities as compared to both eumenorrheic exercisers and eumenorrheic control women [101]. Exercise was not protective for non-weight-bearing areas, as oligomenorrheic exercisers had reductions in distal radius bone density and strength compared to controls, and this decline was similar to AN [101].

FHA shares several clinical and biochemical characteristics with PCOS, and FHA itself is also a diagnosis of exclusion after evaluating for hyperprolactinemia, androgen excess, thyroid disorders, and ovarian failure [99, 101]. AMH levels are frequently increased compared to controls for both PCOS and FHA, 30–40% of FHA women have PCO ultrasound morphology, 40–57% bleed with progestin withdrawal challenge [98], and 19% without PCO ultrasound morphology had either hirsutism or hyperandrogenemia [102]. Certain characteristics may be helpful to distinguish these conditions. Amenorrhea with ≤3 cycles/year particularly with low body mass index (BMI) is more common in FHA than PCOS, which is more typically associated with oligomenorrhea, >3 cycles per year, and higher BMI [98, 99]. BMI <21.2 had 100% sensitivity to differentiate FHA from PCOS [98]. Aggregate data from several studies suggested the following discriminatory thresholds to distinguish FHA from PCOS: LH <5.36 IU/L, AMH <41.3 pmol/L (<5.78 ng/ml),

antral follicle count <16, and total testosterone <1.26 nmol/L (<36.3 ng/dl) with levels below the thresholds favoring FHA [98]. Although 11% of PCOS have disordered eating behaviors compared to 7.6% of controls, this is dwarfed by the 50% prevalence in FHA [98]. Women with FHA are also more likely than those with PCOS to report stressful life events, 59% versus 26.6%, respectively [98].

Five percent of FHA cases meet criteria for PCOS, having both polycystic ultrasound morphology and hyperandrogenism, and a small percentage of those may go on to develop overt PCOS following menstrual recovery and FHA resolution, suggesting a masking effect of FHA-induced low LH levels [98]. Like PCOS, FHA displays altered GnRH dynamics that lead to anovulation and menstrual dysfunction, but these manifestations have underpinnings distinct from PCOS. During the normal luteal-follicular transition, FSH rises, followed by negative feedback by dominant follicle-produced estradiol on hypothalamic kisspeptin and GnRH expression to maintain low LH levels during the early follicular phase which allows proper dominant follicle development and subsequently activates a different set of kisspeptin/GnRH neurons with positive estrogen feedback to promote an optimal mid-cycle GnRH and LH surge [100]. Studies of cynomolgus monkeys, which menstruate similarly to humans, suggest that the key driver of FHA-associated pathology is norepinephrine (NE) [100]. Even mild weight-neutral stress induces an excess of brain NE in both unaffected and FHA monkeys but which in FHA-prone monkeys blocks the estrogen-associated negative feedback and leads to higher kisspeptin and GnRH-stimulated LH and disrupted FSH secretion in the luteal transition and early follicular phases followed by low progesterone levels in the mid-luteal phase and amenorrhea; progesterone levels, ovulation, and menstruation were restored to normal in the FHA monkeys by administration of the NE blocker reboxetine [100]. Genetic predisposition may account for the inter-individual heterogeneity of disordered responses to the same triggers, as nearly 30% of FHA patients have other affected family members, and a higher number of heterozygous rare sequence variants have been identified with FHA women relative to controls for GnRH-associated genes [99]. While GnRH-associated genes have been traditionally associated with more severe loss-of-function congenital hypothalamic hypogonadal disease, FHA gene alterations may be associated with variably disordered GnRH function that increases sensitivity to stress [99].

Given that FHA is secondary to a trigger, it is most desirable to reverse the condition by elimination of the trigger. When there is an identifiable trigger, prognosis for FHA resolution is good; 83% of FHA cases are reversible, even with a median FHA duration of 7.5 years [99]. When the trigger for FHA is weight loss, eventual restoration of menstrual function may require both regain of weight that is higher than the pre-loss weight by 2.2 kg or ≥90% of ideal body weight that is sustained for 2 years [98]. Therefore, it is typically necessary to utilize medication either during or in lieu of trigger elimination.

To optimize bone health, FHA patients are encouraged to use calcium and vitamin D supplements with a goal of serum 25OH vitamin D levels in 32–50ng/ml range [101]. Combination oral contraceptives are used for bone density maintenance in FHA but importantly do not increase bone density, perhaps due to the first pass effect and suppression of insulin growth factor 1, which has trophic effects on

bone [101]. Transdermal estradiol is beneficial for bone density improvement, but it remains to be seen as to whether fracture rates are improved [101].

As mentioned earlier, pharmacologic correctives for FHA may some day transcend animal models and include NE blockers such as reboxetine or atomoxetine, though this is speculative at this time [100]. Although brain serotonin levels are increased in FHA monkeys, the serotonin reuptake inhibitor citalopram did not improve hypothalamic, serum, or menstrual FHA manifestations [100]. Administration of investigational kisspeptin and kisspeptin receptor agonists to women with FHA is associated with exaggerated and sustained follicular phase LH and FSH elevations, so this may represent a future therapeutic pathway [103]. FHA women exhibit normal LH response to GnRH administration [98], and pulsatile GnRH given every 90 minutes either subcutaneously or using an intravenous pump induced ovulation in 79% of treated FHA patients with low rates of hyperstimulation syndrome more typically associated with exogenous gonadotropin stimulation [104]. Although the GnRH infusion pump is approved by the Food and Drug Administration, it is not currently available in the United States [105].

Hyperandrogenic Insulin Resistance with Acanthosis Nigricans (HAIR-AN) Syndrome

HAIR-AN syndrome may either be a distinct entity or an extreme sub-phenotype of PCOS and is present in 5% of hyperandrogenic women [106]. Much of the literature is limited to case reports of both adolescent and adult patients. The etiology may be acquired or inherited through mutations of the insulin receptor gene [106–108]. Affected patients have markedly elevated insulin that leads to the characteristic velvety pigmented cutaneous patches of acanthosis nigricans and extremes of ovarian androgen excess with associated morbidities of obesity, cutaneous disfigurement, and depression [106]. Management of HAIR-AN is challenging as metformin and oral contraceptives are largely ineffective, though lifestyle and diet recommendations are similar to PCOS [106, 107]. The GLP-1 analog liraglutide 1.8 mg/day was found to improve insulin sensitivity, fat mass, menstrual cyclicity, and hirsutism despite no changes in weight in a case series of five HAIR-AN patients [107]. GnRH agonist therapy consisting of leuprolide 11.25 mg intramuscular monthly injections led to decreased serum testosterone, reduced hirsutism, and decreased ovarian size in a 16 year old with an insulin receptor gene mutation refractory to other therapies though morbidity due to hypoestrogenism is of concern [108].

Hyperreactio Luteinalis

The largest case series to date of hyperreactio luteinalis reported on ten patients who presented with enlarged multicystic ovaries size 8.5–20 cm found incidentally at cesarean section for nine women and 3 months postpartum for one woman. Two women had preeclampsia, and three had hirsutism or elevated testosterone [109]. A

review of the medical literature revealed 40 cases of associated hyperandrogenemia, but serum testosterone levels were not predictive of maternal virilization [110]. Histology was benign and revealed numerous cystic follicles and markedly luteinized ovarian theca interna cells, suggesting a hypersensitivity to maternal hCG and not a neoplastic condition [109]. None of the women in this case series were virilized although other reports found maternal virilization rates of 30% [110]. Ovarian enlargement and hyperandrogenemia typically resolve postpartum; oophorectomy may be indicated if malignancy is suspected or to treat acute benign complications such as torsion [109, 110]. This is an unlikely scenario to encounter but may be part of the differential diagnosis of hirsutism in the postpartum period, and for other life stages, it illustrates the importance of pregnancy screening in the workup for PCOS.

Summary

PCOS is the most common cause of hyperandrogenic anovulation among reproductive-aged women. Early diagnosis allows the opportunity of timely interventions aimed at reducing long-term morbidity in this population. A variety of potentially serious disorders can, however, mimic the clinical presentation of PCOS. Despite a patient meeting well-defined clinical criteria, the diagnosis of PCOS is one of exclusion after systematic evaluation to rule out common endocrinopathies that can masquerade as PCOS (Table 3.1). A detailed understanding of these exclusionary conditions, which have morbidities and management strategies distinct from PCOS, supports the underpinnings of the PCOS diagnostic strategy.

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Part II

Pathophysiology, Endocrine, and Metabolic Milieus of Polycystic Ovary Syndrome

Recent Advances in the Genetics of Polycystic Ovary Syndrome

4

Michelle R. Jones and Mark O. Goodarzi

Key Points

- Genome-wide association studies in large sample sizes have identified, with high confidence, about 20 susceptibility loci for PCOS.
- Robust susceptibility variants for PCOS have been used in Mendelian randomization studies to identify causes and consequences of PCOS.
- Identification of PCOS susceptibility genes will expand our understanding of pathways and processes implicated in the syndrome's etiology, allowing development of new diagnostic and treatment modalities.

The Heritable Basis of PCOS

In recent years the complex genetic architecture of polycystic ovary syndrome (PCOS) has begun to come into focus. Early family aggregation studies focused on the prevalence of PCOS-related traits in the siblings of PCOS cases and provided the first evidence for a genetic basis to the disorder [1–3]. These studies suggested an autosomal-dominant mode of inheritance based on the incidence of PCOS-related traits in the first-degree relatives of probands of 51–66% [4, 5]. Larger studies provided further evidence for an autosomal-dominant model of inheritance, with as many as 50% of mothers or sisters, 25% of aunts, and 20% of grandmothers of 250 PCOS probands having either hirsutism alone or hirsutism with

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_4

oligomenorrhea [6]. Following the initial reports, however, systematic genetic investigations failed to support an autosomal-dominant mode of inheritance; rather, PCOS appears to be inherited as a common complex disorder, with multiple susceptibility loci. Twin studies that used a large cohort of more than 3000 Danish twins identified a small number of self-reported PCOS cases ($n = 92$), with an estimate of the monozygotic twin correlation for PCOS of 0.72 and a dizygotic correlation of 0.39 [7]. The identification of such a large proportion of variance in risk for PCOS in monozygotic twins provided strong evidence that there is a significant genetic component to the disease.

Candidate Gene Approaches Revealed an Incomplete Understanding of PCOS Biology

More than 100 candidate genes were studied as potential causal risk genes for PCOS; however, only the region surrounding the gene encoding the insulin receptor, *INSR*, was replicated in subsequent large, well-powered genome-wide association studies (GWAS) [8]. The initial studies of the region combined linkage and association analyses to identify the microsatellite marker D19S884, located in intron 55 of the fibrillin-3 gene (*FBN3*), which is 1.3 cM distal to *INSR*, the candidate gene targeted with this variant [9]. It remains unclear whether the causal gene at this locus is *FBN3*, or in fact *INSR*. *FBN3* was known to be expressed in the pituitary, but its role there is unknown. Contemporary epigenomic datasets from the ENCODE project [10] provide strong evidence to suggest this microsatellite is within an active gene regulatory element, but its target remains unknown. Histone modification data indicates likely promoter and/or enhancer activity across the region spanning the microsatellite, with clear cell type-specific modification of histones H3k4me3, H3K27ac, and H3K4me1 in conjunction with open chromatin identified using DNase hypersensitivity site analysis. There is currently no transcriptional isoform of the *FBN3* gene with a promoter position overlapping this microsatellite and active regulatory region, but it is plausible that an isoform with corresponding promoter and transcriptional start site may exist in a cell type not yet comprehensively assayed as part of the ENCODE project. The close proximity of this marker to the *INSR* gene made it a popular target in candidate gene studies. Seven individual studies identified an association between single nucleotide polymorphisms (SNPs) across the *INSR* locus and PCOS risk [11–19]. Many of these studies included a small number polymorphisms, and modest sample sizes, as did three additional studies that were not able to replicate a significant association between PCOS risk and variants at the *INSR* locus [11, 14, 20].

Additional candidate gene studies focused on genes with known roles in obesity [21–24], type 2 diabetes [25–30], hormone metabolism, and synthesis and ovarian biology [31–35] did not yield any robust loci for PCOS. These studies were largely hampered by small sample sizes and small numbers of variants that provided incomplete tagging across the locus, focusing on coding regions which we now know are unlikely to harbor causal variants for complex traits [36].

GWAS Studies in PCOS

High-throughput genotyping platforms have enabled GWAS and facilitated rapid advancement in the understanding of the complex genetic architecture of many common traits. The first GWAS in PCOS reported in 2011 identified three risk loci: at 2p16.3 (*LHCGR*), 2p21 (*THADA*), and 9q33.3 (*DENND1A*) in Chinese PCOS cases and healthy controls [37]. This three-stage study used a modestly sized discovery cohort of 744 PCOS cases and 895 controls in the GWAS, with replication of suggestive risk loci in a two-stage approach in two cohorts: cohort I, 2840 PCOS cases and 5012 controls; cohort II, 498 PCOS cases and 780 controls [37]. A second study, also performed in Chinese PCOS cases and controls, identified an additional eight risk loci: 2p16.3, 9q22.32, 11q22.1, 12q13.2, 12q14.3, 16q12.1, 19p13.3, and 20q13.2 [8]. This study identified a second, independent risk signal at the 2p16.3 locus, implicating both *LHCGR* and *FSHR* as potential causal genes in the region. *LHCGR* and *FSHR* encode the luteinizing hormone/choriogonadotropin receptor and the follicle-stimulating hormone receptor, which play important roles in hormone signaling in the gonads, making them very plausible susceptibility genes for PCOS. The 2p16.3 region had been the focus of candidate gene studies that profiled only coding variants, without success [20, 38, 39], highlighting the importance of haplotype tagging approaches that include extensive coverage of non-coding variants at gene regions to enable risk locus discovery. The *INSR* locus at 19p13.3 was discussed above. Additional signals identified in the two Chinese GWAS (*THADA* and *HMGA2* associated with type 2 diabetes [40], *RAB5B/SUOX* associated with type 1 diabetes [41]) are near genes from insulin and glucose metabolism pathways, supporting the importance of insulin resistance and metabolic disturbance in PCOS [42]. Two subsequent GWAS performed in Korean cases and controls did not identify any genome-wide significant loci, likely due to small sample size [43, 44].

The first two GWAS for PCOS performed in European-origin populations were published in 2015 [45, 46]. These analyses provided replication of loci reported by Chen and Shi [8, 37] and identified novel loci not previously identified as risk loci for PCOS (Table 4.1). In an initial study that used discovery and replication cohorts of European descent from North America that included a total of 3000 PCOS cases and more than 5000 controls, two novel risk loci were identified: 8p23.1 (*GATA4/NEIL2*) and 11p14.1 (*FSHB*) [45]. The potential causal gene at 8p23.1 is not immediately apparent. Due to linkage disequilibrium (LD) across the region, the association interval spans almost 30 kb. The lead SNP resides between *GATA4* and *NEIL2*, and SNPs in LD with this variant intersect known regulatory regions that connect to the promoters of *C8orf49*, *NEIL2*, and *FDFT1*. NEIL2 is a transcription factor that is ubiquitously expressed [47, 48] and targets the promoter of more than 240 genes [47, 48], many of which are themselves transcription factors and are important in pathways that include the regulation of development that are dysfunctional in cancer (e.g., HOX family of genes) [49] and in hormone signaling (e.g., FST, which inhibits FSH release). Both *C8orf49* and *GATA4* are highly expressed in the ovary [47] and present possible causal genes at this locus. The association signal identified by Hayes et al. [45] at 11p14.1 intersects with the coding region for

Table 4.1 Loci associated with PCOS in genome-wide association studies

Locus	Nearest gene	First GWAS report	GWAS replication
2p21	*THADA*	Chen 2011	Shi 2012, Day 2015, Meta 2018
2p16.3 (A)	*LHCGR*	Chen 2011	Shi 2012
2p16.3 (B)	*FSHR*	Shi 2012	
2q34	*ERBB4*	Day 2015	Meta 2018, Zhang 2020
5q31.1	*IRF1/RAD50*	Day 2015	Meta 2018
6q25.3	*FNDC1/SOD2*	Zhang 2020	
8p23.1	*GATA4/NEIL2*	Hayes 2015	Meta 2018
9p24.1	*PLGRKT*	Meta 2018	
9q33.3	*DENND1A*	Chen 2011	Shi 2012, Meta 2018
9p24.1	*C9orf3*	Hayes 2015	Meta 2018
11p14.1	*FSHB*	Hayes 2015	Day 2015, Meta 2018
11q22.1	*YAP1*	Shi 2012	Day 2015, Meta 2018
11q23.2	*ZBTB16*	Meta 2018	
12q13.2	*RAB5B/ SUOX*	Shi 2012	Meta 2018
12q14.3	*HMGA2*	Shi 2012	
16q21.1	*TOX3*	Shi 2012	Meta 2018
19p13.3	*INSR*	Shi 2012	
20q13.2	*SUMO1P1*	Shi 2012	
12q21.2	*KRR1*	Day 2015	Meta 2018
20q11.21	*MAPRE1*	Meta 2018	

Chen 2011 refers to [37], Shi 2012 refers to [8], Hayes 2015 refers to [45], Day 2015 refers to [46], Meta 2018 refers to [52], Zhang 2020 refers to [55]

FSHB, the gene encoding follicle-stimulating hormone beta subunit, which is a strong candidate as the causal gene at this locus. Genome-wide significant association signals were reported across a 300 kb interval at this locus, and the lead SNP is located >20 kb upstream of the *FSHB* gene within a highly conserved 450 bp region upstream of the coding region for *FSHB*. In vitro studies have since shown this region binds the transcription factor steroidogenic factor 1 (SF1) and enhances the transcription of *FSHB* in an allele-specific manner, supporting the hypothesis that the risk allele at rs11031006 upregulates *FSHB* expression [50]. In this GWAS of European cohorts, more than half of the loci discovered in GWAS of Chinese cohorts exhibited nominal ($P < 0.05$) association with PCOS.

A second GWAS performed in PCOS cases and controls of European descent was published in 2015, by Day et al. [46]. In this study the discovery analysis was performed in a cohort of more than 5000 self-reported PCOS cases and 82,000 healthy controls from the 23andme research resource, with replication performed in 2000 clinically identified cases and nearly 100,000 controls. This analysis successfully replicated genome-wide significant signals at 2p21 (*THADA*) and 11q22.1 (*YAP*), initially reported as PCOS risk loci in Chinese populations [8, 37] and 11p14.1 (*FSHB*), previously reported as a risk locus in European PCOS cases [45]. In this analysis there was directional consistency in effect on PCOS risk at 10 of the initially reported 11 signals identified in Chinese PCOS cohorts; however, only 6 were nominally ($P < 0.05$) associated, and due to consistently smaller effect sizes, none were genome-wide significant in the discovery GWAS. The effects of different LD structures between Han Chinese and European populations resulted in three of

these loci (2p21 (*THADA*), 9q33.3 (*DENND1A*), and 11q22.1 (*YAP1*)) having different lead SNPs, only one of which (rs11225154; *YAP1*) is in LD with the lead SNP reported in Chinese PCOS cases [46]. Three novel loci were identified in this GWAS at 2q34 (*ERBB4*), 5q31 (*IRF1/RAD50*), and 12q21.2 (*KRR1*) as PCOS risk regions at genome-wide significance. Three members of the EGFR gene family (*ERBB4*, *ERBB3*, and *ERBB2*) were identified as risk loci at, or close to, genome-wide significance in this analysis. Recent studies identified a role for Erbb4 in the ovary, where it regulates anti-Müllerian hormone (AMH) level and folliculogenesis [51]. The risk association signal detected at 5q31 is within a complex, gene dense region. The index SNP lies within intron 3 of *C5orf55* and intron 4 of *IRF1* as well as within the reading frame for an uncharacterized protein-coding transcript AC116366.3. Nearby genes also include the transporter *SLC22A5*, an anti-sense RNA to the nearby gene *IRF1*, *IRF1-AS1*, the B cell growth factor IL5, and the double strand break repair gene *RAD50*. It is difficult to identify a candidate causal transcript at this locus given its complexity and what is known about the function of the genes in this region. To further identify potential biological mechanisms by which identified risk variants may impact PCOS biology, a quantitative analysis of the six genome-wide significant loci identified by Day et al. 2015 revealed an association between these six PCOS risk alleles and AMH levels in girls [46], suggesting that PCOS risk alleles from across the genome act through endocrine and reproductive pathways.

An international collaborative consortium assembled the largest GWAS of PCOS to date in order to identify risk loci in PCOS cases of European descent [52]. This analysis included more than 10,000 cases and 100,000 controls from seven cohorts (effective sample size 18,000), including a large proportion of previously analyzed cases [45, 46]. Imputation was conducted using the 1000 Genomes database, yielding over ten million SNPs for the GWAS. Fourteen risk loci were identified in this consortium effort. Three loci initially reported in GWAS studies of Chinese PCOS cases were replicated at genome-wide significance: 2p21 (*THADA*), 9q33.3 (*DENND1A*), and 16q21.1 (*TOX3*). The two risk loci, located at 8p23.1 (*GATA4/NEIL2*) and 11p14.1 (*FSHB*), reported by Hayes et al. [45] were confirmed in this large meta-analysis, as were the three risk loci at 2q34 (*ERBB4*), 5q31.1 (*IRF1/RAD50*), and 12q21.2 (*KRR1*) reported by Day et al. [46]. Three novel loci were identified in this collaborative meta-analysis at 9p24.1 (*PLGRKT*), 11q23.2 (*ZBTB16*), and 20q11.21 (*MAPRE1*). An additional novel genome-wide significant locus was identified on the X chromosome at the *ARSD* locus but was excluded from the formal results of the analysis due to low imputation quality, low minor allele frequency, and heterogeneity of effect across the three cohorts that had SNP data available for the X chromosome [52]. Additional analyses of this region in a larger sample size are needed to resolve the potential role of this locus in PCOS risk. Given that this GWAS included PCOS cases identified by self-report and two different clinical diagnostic criteria, heterogeneity analysis was performed to identify loci that demonstrated a difference in effect by these strata. The analysis identified heterogeneity at a single locus, 8p23.1 (*GATA4/NEIL2*), where the effect size associated with the risk allele was significantly less in self-reported PCOS cases and significantly greater in PCOS cases diagnosed using the NIH criteria [52]. For the

remaining 13 loci, the magnitude of association with PCOS was similar regardless of mode of diagnosis. This lack of heterogeneity across PCOS cases identified using these different criteria, along with the consistent replication of PCOS risk loci across individual studies, underscores a conserved shared genetic architecture for this phenotype.

Day et al. 2018 combined the PCOS GWAS data with results from GWAS for other traits to carry out genetic correlation analyses [52]. Such analyses suggest shared etiology but do not indicate directionality or causality. This investigation found genetic correlation between PCOS and body mass index (the most correlated trait), childhood obesity, fasting insulin, type 2 diabetes, high-density lipoprotein cholesterol, triglyceride levels, age of menarche, coronary artery disease, and depression. No genetic correlation was observed between PCOS and age of menopause or male pattern balding.

As the use of research biobanks has grown over recent years, the ability for case identification via electronic medical records has facilitated the analysis of population-based cohorts recruited through large medical care systems. Two such systems are the Geisinger MyCode Community Health Initiative that has recruited more than 250,000 research participants throughout the care system in Pennsylvania [53] and the collaborative eMERGE (electronic MEdical Records and GEnomics) network that combines biobanks or studies with clinical data derived from medical records from across many sites [54]. Two such programs performed a GWAS in close to 3000 PCOS cases that met two of the following: (a) diagnosis of PCOS or polycystic ovaries; (b) hyperandrogenism or its related signs, or hyperandrogenemia; and (c) oligomenorrhea, amenorrhea, or infertility (i.e., Rotterdam diagnosis criteria) and 53,000 controls that did not meet any of the three criteria [55]. A small validation cohort of 253 cases and 2161 controls was available from the Vanderbilt BioVu study. This analysis identified three genome-wide significant signals (at 6q25.3, 2q34, and 3q25.1). The locus at 6q25.3 had not been detected in prior studies. The index SNP at this locus is more than 200 kb from the nearest genes (*FNDC1* and *SOD2*) and does not overlap known regulatory elements from ENCODE or 3D chromatin interactions reported by GeneHancer. It is not immediately apparent what the causal gene is at this locus. The previously reported risk signal at 2q34 (*ERBB4*) was identified in this study at a suggestive level of significance, and additionally a novel independent risk variant was identified at this locus at genome-wide significance. A third locus at 3q25.1 (*WWTR1*) was reported as nearing genome-wide significance; this locus has not been previously reported as a risk locus for PCOS [55]. It should be noted that 17% of the total cohort in this study was listed as African American, although the numbers of cases and controls were not provided. A lookup of the three reported risk loci identified in this study was performed in an analysis of only African American participants, and only the novel risk SNP identified at 2q34 (*ERBB4*) passed quality control metrics. Despite having a higher minor allele frequency in African American populations, this SNP was only nominally associated with PCOS risk ($P > 0.01$) [55]. Genome-wide association studies in populations of other ethnicities have not been performed. Our lack of understanding of the shared or differing genetic architecture of PCOS in populations that are

not of Chinese or European ancestry represents a significant deficit in our understanding. A major focus of ongoing research should prioritize the recruitment and profiling of PCOS cases and controls of other ancestries (e.g., Hispanic, African) to address this lack of knowledge.

To better identify the biological pathways through which susceptibility loci act to increase risk of PCOS, association of these loci with phenotypic traits related to PCOS has been performed in several studies, including the recent meta-analysis. Significant associations between known risk loci and polycystic ovarian morphology, ovulatory dysfunction, and hyperandrogenism were all identified [52]. GWAS analyses within PCOS cases also found that the allele associated with increased risk of PCOS at the *FSHB* locus was also associated with increased circulating LH level, decreased FSH level, and increased ratio of LH to FSH [45, 46]. Taken together these analyses further support the role for much of the genetic basis for PCOS to act through disrupting hormone pathways.

Polygenic Risk Scores for Disease Risk Prediction in PCOS

Polygenic risk scores (PRS) have been under active development in recent years, leveraging the increasing pace of discovery of the polygenic genetic architecture of many complex traits and the increasing sample sizes that are becoming available for testing and validation of such scores. The development of methods used to generate such scores is an active area, with empirical and Bayesian methods currently being applied. The long-term goal of PRS application in the population is to allow the early detection of risk for disease prevention strategies to be deployed [56]. This strategy is underway in cardiovascular traits, where the polygenic genetic risk estimated by GWAS equals the known monogenic risk and clinical risk factors [57]. A polygenic risk score for PCOS was developed based on the meta-analysis performed on clinically diagnosed cases included in the collaborative meta-analysis [52] and applied to a cohort of more than 120,000 individuals for whom electronic health records were available through the eMERGE network [58]. The best performing PRS in this analysis demonstrated a prediction accuracy of PCOS cases of 0.55 with an area under the curve (AUC) of 0.715 in eMERGE participants of European ancestry. When combined with information available based on PCOS component phenotypes, the PRS plus phenotype model performed with an accuracy of 0.873 and an AUC of 0.87, indicating that the PRS model built from this analysis is able to predict PCOS phenotype in individuals of European ancestry [58]. This genetic PRS model was also used to perform a phenome-wide association study (PheWAS), where the genetic risk score of an individual is used to identify anthropometric and clinical traits that are enriched in individuals of high genetic risk. This analysis can identify cross phenotype associations that may be the result of pleiotropy – whereby risk alleles impact multiple traits or phenotypes [59]. A significant PheWAS relationship was identified between the PCOS PRS and traits related to endocrine and metabolic traits (obesity, lipid dysfunction, type 2 diabetes), neurological traits (sleep apnea), circulatory system (hypertension), and digestive traits (esophageal

disease) [58]. Many of these associations remained significant after the analysis was repeated without any PCOS cases included in the cohort, suggesting that there are likely undiagnosed PCOS cases within the eMERGE network.

Mendelian Randomization Using GWAS Signals

Even before causal genes are identified at risk loci, GWAS information can be used to dissect the biology of disease. A major example is that robust loci identified by GWAS can be used to interrogate causality between an exposure and an outcome using Mendelian randomization (MR). In this approach, SNPs associated with the exposure are used as instrument variables to estimate the genetically driven effect of the exposure on the outcome, yielding causal effect estimates. Reports of PCOS GWAS included MR analyses that suggested increased body mass index (BMI), age at menopause, decreased sex hormone-binding globulin (SHBG), fasting insulin, male pattern balding, and depression were causal factors for PCOS [46, 52]. The relationship between BMI and PCOS has been extensively investigated using MR, with results finding that while obesity appears to be causal for PCOS, PCOS does not cause obesity [60, 61]. MR studies found that testosterone levels, but not AMH levels, are causal for PCOS [62, 63].

A series of MR studies examined PCOS as the exposure against various outcomes, using PCOS SNPs from the largest GWAS for PCOS [52] as instrument variables. PCOS was found not to have a genetic causal effect on type 2 diabetes, coronary heart disease, or stroke [64]. Given that prior MR studies had demonstrated causal effects of BMI, higher testosterone, and lower SHBG on diabetes and/or cardiovascular disease, the authors concluded that these features commonly present in PCOS, rather than PCOS in and of itself, explain the association between PCOS and cardiometabolic disease. Genetically predicted PCOS was associated with increased risk of breast cancer overall and estrogen receptor-positive breast cancer; no effect on estrogen receptor-negative breast cancer was observed [65]. Consistent results were observed in a study that examined several subtypes of breast cancer [66]. MR studies found a protective effect of PCOS against invasive ovarian cancer and endometrioid ovarian cancer [67, 68]. These MR studies yielded key insights on causes and consequences of PCOS, avoiding confounding variables that affect epidemiological association studies.

Identifying Causal Genes at PCOS Risk Loci

Colocalization analysis of disease and intermediate cellular phenotypes (e.g., gene expression and protein level across different relevant tissues) is performed by measuring the probability that the two traits share a causal variant [69]. A recent analysis applied this approach and successfully identified seven proteins with strong evidence of colocalization [70]. The FSH protein was clearly implicated at the 11p14.1 locus where the significant correlation between genotype at risk-associated

SNPs and circulating FSH level presents a clear colocalization of the same causal SNPs acting on both PCOS and FSH level. This approach was unable to resolve a single likely causal transcript at the 12q13.2 locus but implicated *SUOX*, *ERBB3*, *IKZF4*, *RPS26*, and *GDF11* as potential causal genes. A single likely causal gene, *ZFP36L2*, was identified at 2q21 (*THADA* locus), and *C9orf3* was implicated at 9p24.1. Colocalization analysis at 8p23.1 identified both *C8orf49* and *NEIL2* as potential causal transcripts [70].

Conclusion and Future Directions

Advances in genomic technology have led to rapid progress in our understanding of the genetic architecture of PCOS. Though PCOS is clinically heterogeneous, GWAS have found little genetic heterogeneity across PCOS diagnostic criteria. Twenty loci across the genome have been identified at genome-wide significance in Chinese and/or European cohorts (Table 4.1). The causal gene at many of these loci is unknown; however, genomic analysis and in vitro studies have provided some suggestion of the likely causal gene at specific loci. These results indicate that disruption of hormone signaling pathways, particularly related to the synthesis and signaling of FSH and the signaling of the LH receptor, are key to the pathogenesis of PCOS. As with many complex traits, much of the heritability for PCOS has yet to be identified. Identifying additional risk alleles will contribute to improved PRS accuracy and sensitivity and may identify further biological pathways to be targeted for the treatment of PCOS symptoms. Increasing sample sizes will be required for the discovery of additional risk alleles, and the continued efforts of the International PCOS Consortium (iPCOS) are focused on including increasing numbers of PCOS cases and controls for ongoing meta-analysis for risk allele discovery. A second focus of the iPCOS consortium is to foster the inclusion of PCOS cases and controls of Hispanic and African ancestry, so that we may begin to understand the shared and differing genetic architecture of PCOS between these populations and those already studied. The current move of genomic technologies beyond array-based genotyping into population-level whole genome sequencing will provide opportunities to discover additional types of risk variants (e.g., structural variants) and variants with rare and very rare risk allele frequencies, allowing a deeper understanding of the complex genetic underpinnings of PCOS.

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The Hypothalamic-Pituitary Axis in PCOS

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Key Points

- The neuroendocrine abnormalities in PCOS involve dysfunction at both the hypothalamic and the pituitary levels.
- In PCOS there is hypersecretion of GnRH, characterized by a fast GnRH pulse frequency and an increase in the overall amount of GnRH secreted.
- Kisspeptin, GABA, AMH, hyperinsulinemia, and decreased ghrelin may all contribute to hypersecretion of GnRH in PCOS.
- There is also evidence that in women with PCOS, androgens and possibly insulin contribute to a relative insensitivity to progesterone-induced slowing of GnRH pulse frequency.
- Women with PCOS have an abnormal pituitary response to GnRH with increased LH but not FSH secretion leading to an increased LH to FSH ratio.
- Faster GnRH pulse frequency favors increased secretion of LH over that of FSH, while continuous low levels of estradiol inhibit pituitary secretion of FSH to a greater degree than LH.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_5

- LH and the LH/FSH ratio are inversely related to BMI in women with PCOS, an effect that is mediated at the pituitary, possibly through the direct inhibitory effects of leptin and/or insulin on the gonadotrope.
- Abnormalities in gonadotropin stimulation of the ovary play a key role in the ovarian dysfunction of PCOS and are also perpetuated by abnormal ovarian feedback on the hypothalamus and pituitary gland.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most complex of endocrine disorders. Both genetic and non-genetic factors play a part in the etiology and clinical presentation of this disorder that affects between 7% and 10% of reproductive aged women [1]. The criteria for diagnosis of PCOS have evolved over time, but the key pathophysiologic features include ovarian hyperandrogenism which is almost always accompanied by abnormal ovarian morphology on ultrasound. Patients with these features also have irregular cycles and oligoovulation or anovulation. Increased luteinizing hormone (LH) secretion together with an increase in the LH to follicle-stimulating hormone (FSH) ratio contribute to abnormal ovarian hormonal dynamics. The excess LH results in overproduction of theca cell androgenic substrates that accumulate in the absence of an increase in FSH, which typically converts the androgens to estradiol within the ovary [2]. Both theca cell hyperplasia and an abnormal steroidogenic profile within the polycystic ovary augment hyperandrogenemia as does hyperinsulinemia which is common in PCOS and exacerbated by obesity.

Ovarian hyperandrogenism inhibits the normal progression of follicle development whereby repeated early follicle development is followed by regression, manifesting as anovulation or oligoovulation. The low levels of estradiol that result from early follicle development combined with peripheral conversion of androgens to estrone, a relatively weak estrogen, provide near-constant negative estrogen feedback that compromises the dynamic changes in FSH that are required for normal follicle recruitment and development. Thus, abnormalities in gonadotropin stimulation of the ovary play a key role in the ovarian dysfunction of PCOS and are also perpetuated by abnormal ovarian feedback on the hypothalamus and pituitary gland. This chapter briefly reviews the hypothalamic and pituitary dynamics in normal women with ovulatory cycles as a critical background for understanding the neuroendocrine abnormalities in PCOS and the potential mechanisms which underlie these abnormalities.

Normal Female Reproduction

Neuroendocrine Function

The neuroendocrine regulation of the menstrual cycle involves a complex integrated network of feedback mechanisms between the hypothalamus and pituitary gland and the ovaries. The hypothalamic-pituitary-ovarian (HPO) system is comprised of

the gonadotropin-releasing hormone (GnRH) producing neurons of the hypothalamus, the pituitary gonadotropes that secrete LH and FSH, and the ovary that responds to FSH and LH with follicular development and ovulation and with secretion of the ovarian steroids (estradiol and progesterone) and peptides (inhibin A and inhibin B). The ovarian steroids and peptides, in turn, modulate the hypothalamic and pituitary components of the HPO axis (Fig 5.1a); for more detailed reviews, see [3, 4].

Studies in which frequent blood sampling with measurement of LH pulses as a marker of the frequency of GnRH pulses and the use of pharmacological probes, such as GnRH antagonists, have been used to evaluate the physiology of GnRH secretion in women with normal menstrual cycles [5] and in women with PCOS [6]. In normal women, the frequency of pulsatile GnRH secretion is dynamically regulated across the menstrual cycle; importantly, this dynamic regulation of GnRH pulse frequency is key to maintaining the repetitive cycles of follicle development that are required for normal reproductive function in women (Fig 5.1b). The transition from the end of one cycle to the beginning of the next is marked by an increased frequency of LH (and by proxy, GnRH) pulses from the luteal phase frequency of one pulse every 4 h to one pulse every 90 min in the early follicular phase. During the mid-follicular phase, LH pulse frequency increases further to one pulse every hour, and this frequency is maintained through the mid-cycle gonadotropin surge. After the mid-cycle surge and ovulation, the LH pulse frequency slows to one pulse every 90 min, followed by a further decline to one pulse every 4 h during the late luteal phase. The luteal phase decrease in LH pulse frequency is secondary to rising progesterone levels in the presence of estrogen [5]. Although gonadal steroid levels fluctuate less because of their prolonged half-life due to binding to circulating sex hormone-binding globulin (SHBG), progesterone concentrations can vary dramatically in the mid and late luteal phases (from 2.3 to 40.1 ng/mL) in response to the relatively infrequent LH pulses [5].

GnRH pulse frequency plays a critical role in the differential regulation of pituitary LH and FSH synthesis and secretion. In pituitary cell cultures, slow frequencies of pulsatile GnRH stimulation favor the synthesis of FSH over LH, while faster frequencies favor synthesis and secretion of LH. At a slow GnRH pulse frequency, the concentration of GnRH receptors (GnRHR) on the pituitary gonadotrope cell membranes is relatively low; there is activation of a single signal transduction pathway that stimulates the expression of the common gonadotropin α-subunit and the LH and FSH β-subunits. However, with faster GnRH pulse frequencies, the concentration of GnRHR on the gonadotrope cell membranes increases, resulting in greater activation of this signal transduction pathway in addition to the stimulation of a second signal transduction pathway that specifically inhibits FSH gene expression [7]. The effect of GnRH pulse frequency has been shown in men and women with GnRH deficiency in whom the frequency of pulsatile GnRH administration can be controlled. At slow frequencies of GnRH administration, FSH secretion predominates over that of LH, while at faster frequencies, LH secretion increases dramatically with little effect on FSH [4]. An important caveat is that the effect of GnRH pulse frequency on LH and FSH secretion is masked in the presence of inhibitory levels of gonadal steroids [8, 9]. Thus, slower GnRH pulse frequencies, as seen in

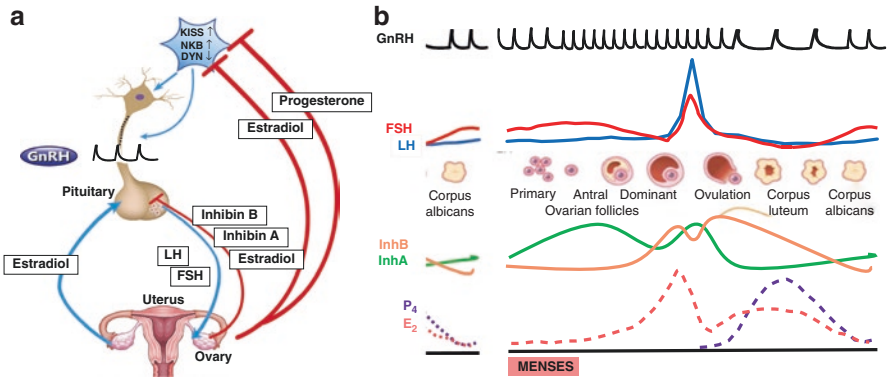

Fig. 5.1 (**a**) Normal reproductive function in women requires the integration of signals from the hypothalamus, pituitary, and ovary with the uterus as a critical end organ. (**b**) Dynamic negative feedback loops from the ovary to the hypothalamus and pituitary control: (i) the increase in FSH that is required for recruitment of a new cohort of follicles with the transition from one cycle to the next, due to release from the negative feedback of declining ovarian steroid and peptide hormones with regression of the corpus luteum and (ii) suppression of FSH by estradiol and the inhibins secreted from the new cohort of follicles that prevents the maturation of multiple follicles. In normal cycles, positive feedback of estradiol at the pituitary induces the pre-ovulatory surge

the luteal phase of the normal menstrual cycle in women, favor synthesis of FSH, while faster GnRH pulse frequencies, as seen in the transition to the early follicular phase, favor the synthesis and secretion of LH . During the luteal-follicular transition, the inhibitory effect of estradiol and inhibin A on FSH secretion decreases dramatically with regression of the corpus luteum. With the increase in GnRH secretion that accompanies release from the progesterone negative feedback (Fig 5.1b), FSH levels rise initiating a new cycle of follicle development, ovulation, and luteal phase function.

Gonadal Feedback on the Neuroendocrine System

FSH levels increase up to threefold during the luteal-follicular transition [10, 11]. This luteal-follicular rise in FSH is essential for initiation of folliculogenesis (Fig 5.1b). With the recruitment and early development of a new cohort of ovarian follicles, estradiol and inhibin B levels begin to rise. Inhibin B selectively inhibits FSH secretion from the pituitary in response to GnRH, while estradiol inhibits the FSH response to GnRH to a greater extent than LH [12] as well as inhibiting the overall secretion of GnRH without affecting GnRH pulse frequency. This mid-follicular phase decrease in FSH ensures that in the majority of cycles only a single follicle emerges as dominant and reaches maturity.

While the initial increase in estradiol inhibits LH and FSH secretion, the exponential rise in estradiol that results from growth of the dominant follicle exerts a positive feedback effect at the pituitary, triggering the mid-cycle LH surge. In animal models, the exponential rise in estradiol increases GnRH secretion in addition

to pituitary responsiveness to GnRH [13, 14], while in women the overwhelming effect is mediated through an increased pituitary response to GnRH. Ovulation typically occurs within 36 h after the mid-cycle LH surge.

LH levels decrease rapidly after the pre-ovulatory gonadotropin surge. There is a minor increase in progesterone secretion as granulosa cells in the mature pre-ovulatory ovarian follicle acquire LH receptors. However, the major increase in progesterone occurs with the luteinization of the theca-granulosa cells, induced by the LH surge, and formation of the corpus luteum. Progesterone reaches peak circulating concentrations in the mid-luteal phase. In addition to progesterone, the corpus luteum secretes estradiol and inhibin A. In the presence of estradiol, progesterone slows the frequency of pulsatile GnRH, and therefore LH, secretion, while estradiol and inhibin A preferentially inhibit pituitary secretion of FSH [4]. In the absence of conception, the pre-programmed demise of the corpus luteum sets in motion the hormonal changes that result in an increase in FSH and the beginning of another cycle of follicle recruitment, selection of the dominant follicle, ovulation, and development of the corpus luteum.

Neuroendocrine Abnormalities in PCOS

Neuroendocrine abnormalities play a significant role in the pathogenesis of the abnormal follicle development and oligo-anovulation that characterize reproductive function in the majority of women with PCOS. In 94% of women with PCOS who have irregular cycles, LH levels are elevated, while FSH levels are normal or slightly low, resulting in an increased LH/FSH ratio compared to normal women in the early follicular phase [6]. However, recent ovulation or the use of a progestin can temporarily reverse this abnormality.

Hypersecretion of LH

The frequency of LH pulses is solely dependent on the frequency of GnRH stimulation of the gonadotropes, and thus LH pulse frequency can be used as an indicator of pulsatile hypothalamic GnRH secretion, as indicated above. In women with PCOS, LH pulses occur every 50–60 min (Fig. 5.2) [12], similar to the pulse frequency in women after menopause, but significantly more often than the 90 min pulse frequency in normal women in the early follicular phase. This increase in pulse frequency is accompanied by a concomitant increase in the overall amount of GnRH secreted, assessed using incomplete receptor blockade with a submaximal dose of a competitive GnRH receptor antagonist. Taken together, there is hypersecretion of GnRH in women with PCOS compared to normal women. Interestingly, the magnitude of the change in overall amount of GnRH secreted is similar in magnitude to the increase in pulse frequency [12], suggesting that the bolus of endogenous GnRH being secreted per pulse in women with PCOS is not increased. The amplitude of spontaneous LH pulses in women with PCOS is also increased. The

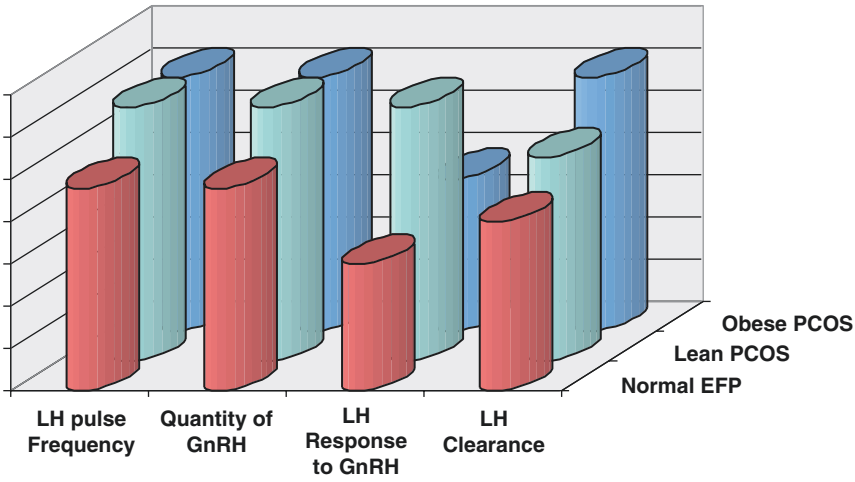

Fig. 5.2 Neuroendocrine features of lean and obese PCOS women relative to normal women in the early follicular phase (EFP) of their menstrual cycle. Hypothalamic function, indicated by LH (GnRH) pulse frequency and overall quantity of GnRH, is increased in PCOS but not affected by obesity, while the pituitary response to GnRH is increased in PCOS but attenuated by obesity. The effect of obesity on pituitary responsiveness and the increased clearance of LH that occurs in obese PCOS both contribute to the relative normalization of both LH levels and the LH/FSH ratio in obese PCOS

lack of an increase in the bolus of GnRH being secreted is consistent with an increase in pituitary sensitivity to GnRH in PCOS and is supported by an increased LH response to a physiologic dose of exogenous GnRH in lean women with PCOS compared to women with GnRH deficiency receiving GnRH to create a normal menstrual cycle [15].

Relative FSH Deficiency

In contrast to LH, FSH levels are normal to low and are relatively adynamic over time in PCOS women compared with regularly menstruating women. There are several factors that may account for this discrepancy between LH and FSH in PCOS. The first is that the synthesis of FSH is favored at slow GnRH pulse frequencies in contrast to the faster GnRH pulse frequency that is seen in women with PCOS. This is important in PCOS because fast frequency GnRH stimulation recruits a second signal GnRH transduction pathway that inhibits FSH gene expression [7]. The second factor relates to the differential influence of GnRH on LH and FSH secretion from the pituitary gonadotrope. While LH is solely dependent on pulsatile GnRH for its synthesis and secretion, about half of FSH control is regulated by the activin/follistatin system within the pituitary with activin stimulating FSH and follistatin controlling this effect by irreversibly binding to activin. Fast frequency GnRH stimulation increases follistatin within the pituitary which would be expected to suppress activin stimulation of FSH. Finally, in PCOS, estradiol levels are

generally in the early to mid-follicular phase range, and estrone, a relatively weak estrogen, is increased due to peripheral conversion of the amplified secretion of ovarian androgens. These low and relatively constant estrogen levels feed back directly at the pituitary, inhibiting FSH to a greater degree than LH.

Importance of the Abnormal Gonadotropin Environment in PCOS

In women with isolated polycystic ovarian morphology but regular ovulatory cycles, gonadotropin dynamics are identical to those in normal ovulatory women [15]. The implications of this observation are twofold. First, this finding indicates that abnormal ovarian morphology is insufficient for the development of the anovulatory cycles that characterize PCOS, even though androgen levels are slightly elevated in these women. Second, it implies that in the setting of polycystic ovarian morphology, an abnormal gonadotropin environment is required for development of the menstrual cycle dysfunction that is seen in the majority of women with PCOS.

The Role of Obesity in Gonadotropin Secretion in PCOS

Studies in normal women and adolescent girls have demonstrated a significant inhibitory effect of obesity on gonadotropin secretion [6, 16]. Obesity is prevalent in PCOS, occurring in approximately 30–60% of patients [17]. Both BMI and percent body fat are negatively correlated with LH levels; thus, while a high LH/FSH ratio is common in PCOS, in obese women with PCOS, this ratio may be relatively normal [6, 18]. In women with PCOS, there is no effect of BMI on hypothalamic GnRH neuronal activity (increased pulse frequency and overall amount of GnRH), indicating that obesity does not appear to exert its effect at the hypothalamus but at the level of the pituitary (Fig. 5.2) [19]. This conclusion is further supported by the finding that obesity attenuates the exaggerated LH response to a physiologic and weight-based dose of GnRH that is evident in lean women with PCOS [15].
LH and FSH are glycoprotein hormones composed of an amino acid backbone conjugated to oligosaccharides. The terminal residues on the oligosaccharide chains create heterogeneous LH and FSH isoforms which can have a significant effect on biological activity and clearance of these hormones. LH isoforms are influenced by gonadal steroids and also by obesity, and in PCOS, the isoforms of LH secreted result in a decrease in the half-life of LH [20]. The increased clearance of LH in obese women with PCOS would further contribute to the inverse relationship between BMI and LH levels in women with PCOS.

Summary

PCOS is thus characterized by hypersecretion of GnRH, with an increase in GnRH pulse frequency and a concomitant increase in the overall amount of GnRH secreted. In addition, there is an exaggerated LH response to GnRH in PCOS,

indicating both hypothalamic and pituitary dysfunction contribute to the abnormal reproductive function in PCOS. The resultant increase in LH levels in the absence of a similar increase in FSH contributes to ovarian hyperandrogenism with estradiol and estrone levels that are both relatively constant and in the range that induces negative feedback to the pituitary [15, 21]. Thus, the discrepancy between the levels of secreted LH and FSH in PCOS is likely due to both the increased frequency of pulsatile GnRH secretion and the relatively constant negative feedback of estrogens at the pituitary that differentially affect LH and FSH. Gonadotropin secretion is normalized after spontaneous or induced ovulation or administration of a progestin, but this effect is not sustained. Gonadotropin secretion is also markedly influenced by obesity in women with PCOS, decreasing LH and the LH/FSH with evidence that the effect of obesity is mediated at the pituitary.

Mechanisms Underlying Neuroendocrine Dysfunction in PCOS

LH and FSH are secreted from the same cell within the pituitary and are stimulated by the same hypothalamic releasing factor, yet they are differentially regulated across the menstrual cycle in normal menstruating women and are also differentially affected in PCOS. As discussed above, there are several mechanisms that account for this differential regulation in normal women that serve as an important point of departure for understanding gonadotropin regulation in PCOS: (1) LH is exclusively under the control of GnRH, and its pulsatile secretion is a direct reflection of the pulsatile secretion of GnRH, while approximately half of FSH synthesis and secretion is controlled by GnRH while the other half is controlled by the activin/follistatin system; (2) LH and FSH are differentially regulated by the frequency of pulsatile GnRH secretion, in part through changes in the concentration of GnRH receptors on gonadotropes, such that faster frequencies of GnRH stimulation increase the secretion of LH over FSH while slow frequencies of GnRH stimulation favor the synthesis of FSH over LH; and (3) FSH is more sensitive to estrogen negative feedback at the pituitary than LH, and the negative feedback of the inhibins on the paracrine effect of activin is FSH-specific. To understand the neuroendocrine regulation in PCOS, it is therefore necessary to consider both neuronal regulation of GnRH secretion into the pituitary portal system and factors that act directly at the pituitary gland.

Dysfunctional Control of GnRH Neuronal Secretion

Kisspeptin, Neurokinin B and Dynorphin (KNDy) and Gamma-Aminobutyric Acidergic (GABAergic) Neurons

There is now overwhelming evidence for the upstream control of GnRH neuronal activity by neuropeptides secreted by the KNDy neurons which are co-located with the GnRH neurons in the median eminence of the hypothalamus. The KNDy neurons secrete the neuropeptides, kisspeptin, neurokinin B, and dynorphin [22–25].

Kisspeptin directly stimulates the GnRH neurons to secrete GnRH. Neurokinin B enhances while dynorphin lessens the stimulatory effects of kisspeptin on the GnRH neurons. Importantly, gonadal feedback to GnRH neurons is mediated through the androgen, progesterone, and estrogen receptors expressed on the KNDy neurons [4].

Given the hypersecretion of GnRH that characterizes PCOS, it has been hypothesized that there is upregulation of kisspeptin and/or neurokinin B secretion in women with PCOS (Fig. 5.3). This hypothesis is supported by the positive correlation of kisspeptin and LH levels in women with PCOS [22] and the further observation that the degree of elevation in kisspeptin in PCOS is associated with increased ovulatory dysfunction [23]. While kisspeptin antagonists are not available,

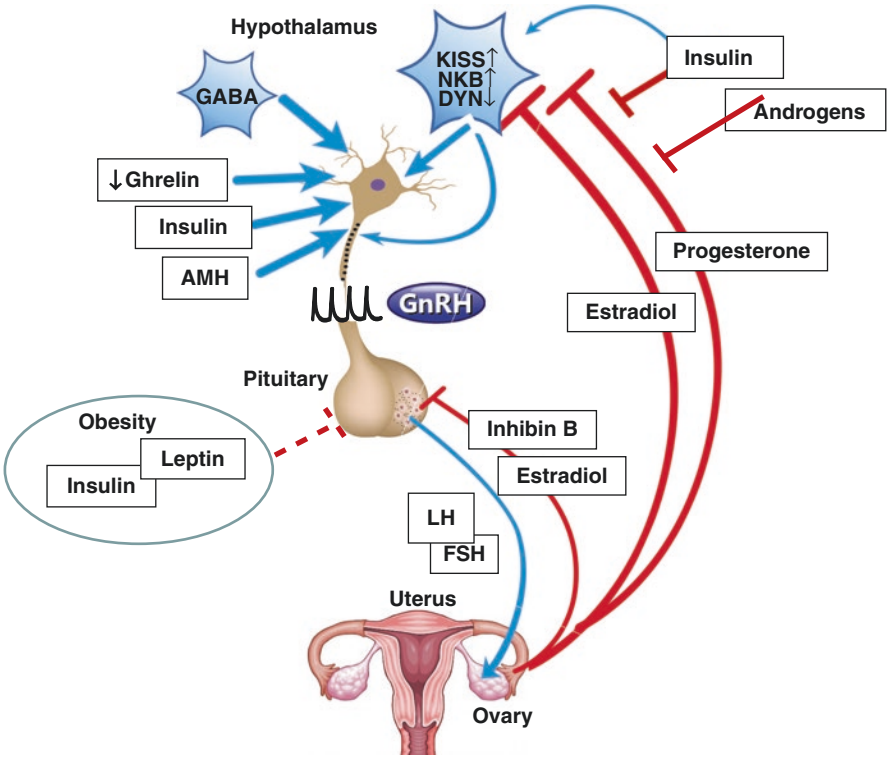

Fig. 5.3 The neuroendocrine abnormalities in PCOS are characterized by an increased frequency of pulsatile GnRH secretion and an increase in the LH response to GnRH, both of which lead to an increased LH/FSH ratio. Kisspeptin, GABA, AMH, hyperinsulinemia, and/or decreased ghrelin may all contribute to hypersecretion of GnRH. There is also evidence that women with PCOS are relatively insensitive to progesterone-induced slowing of GnRH pulse frequency, whether during puberty or following ovulation, and that androgens and possibly insulin play a role in antagonizing the effect of progesterone. Faster GnRH pulse frequency favors increased secretion of LH over that of FSH, while continuous low levels of estradiol inhibit pituitary secretion of FSH to a greater degree than LH. LH and the LH/FSH ratio are inversely related to BMI in women with PCOS, an effect that is mediated at the pituitary, possibly through the direct inhibitory effects of leptin and/or insulin on gonadotropes

administration of a neurokinin B antagonist in women with PCOS resulted in a decrease in LH pulse frequency and mean LH [26], again supporting a role for the KNDy neurons in the GnRH hypersecretion in PCOS.

In the hypothalamus, GABAergic neurons exert a stimulatory effect directly on GnRH neurons that is mediated by dihydrotestosterone and an inhibitory effect that is mediated by progesterone [27]. Hence, in PCOS, where there are lower levels of progesterone and higher levels of androgens, one would predict a net stimulatory effect of the GABAergic neurons on GnRH neurons (Fig. 5.3). Evidence from animal models of PCOS show that activation of GABAergic neurons is associated with overactivity of GnRH neurons leading to increased LH secretion and ovulatory dysfunctions, as is observed in women with PCOS [25]. Higher levels of GABA have been reported in the cerebrospinal fluid in women with PCOS compared to their normal menstruating counterparts, suggesting that GABA may be an important player in the hypothalamic dysfunction that characterizes PCOS [28].

Hyperandrogenemia

A direct hypothalamic role of hyperandrogenemia was initially hypothesized to explain the neuroendocrine abnormalities in PCOS as correction of hyperandrogenemia with the removal of an androgen-producing tumor [29] or with ovarian wedge resection or ovarian drilling in PCOS [30] restored normal ovulatory cycles. However, androgen administration in normal menstruating women, as in men, [9, 31], decreases, rather than increases, LH pulse frequency [32]. These studies indicate that improvement in reproductive function with removal of androgen-secreting tumors or wedge resection is likely due to a decrease in the effects of androgens within the ovary per se. Thus, the observation that androgens slow LH pulse frequency is counter to the hypothesis that increased androgens play a direct role in the increase in GnRH pulse frequency that characterizes PCOS.

Hyperinsulinemia

Insulin resistance and hyperinsulinemia are features of PCOS across the weight spectrum, although the degree of hyperinsulinemia increases with increasing BMI [33, 34]. In studies in male rats, hyperinsulinemia resulted in a 50–60%, dose-dependent increase in LH pulse frequency [35]. In male and female fetal mice, insulin stimulated increased GnRH expression in hypothalamic neurons [35]. Insulin also activated the phosphatidylinositol 3-kinase and Erk1/2 MAP kinase signaling pathways in a rat GnRH neuronal cell line (Gnv-3 cells) known to express insulin receptors, resulting in increased secretion of GnRH [36]. Glycemia did not affect insulin-stimulated LH secretion in either in vivo or in vitro studies. Further studies have shown that Kiss1 neurons express insulin receptor mRNA and insulin administration depolarizes the neurons resulting in increased secretion of kisspeptin [37].

These pre-clinical studies raise the possibility that hyperinsulinemia plays a role in the increased GnRH secretion in women with PCOS (Fig. 5.3). A small, but important, study used a hyperinsulinemic, euglycemic clamp and compared women with PCOS to weight match control women. Hyperinsulinemia was associated with

an increase in LH pulse frequency in control women to a frequency comparable to women with PCOS at baseline but did not further increase pulse frequency in women with PCOS [38]. This finding confirms an effect of insulin on GnRH secretion in normal women, raising the possibility that early or long-term exposure to hyperinsulinemia may contribute to the abnormal GnRH secretion in PCOS.

Progesterone Negative Feedback in PCOS: The Effects of Androgens and Insulin

Progesterone has a dramatic effect on GnRH secretion as evidenced by the marked slowing of LH pulse frequency in the luteal phase of the normal menstrual cycle. The inhibitory effect of progesterone on GnRH pulse frequency is mediated, at least in part, through effects on the KNDy neurons. There is also evidence that the negative feedback of progesterone on GnRH neuronal activity may be mediated through the progesterone receptor membrane component 1 (PgRMC1) expressed on GnRH neurons; the binding of progesterone to this receptor results in the reduction of intracellular calcium, an inhibitory effect that is independent of other factors such as GABAergic and glutamatergic input [39].

In women with PCOS, spontaneous ovulation or the use of progesterone or a progestin transiently slows GnRH pulse frequency and improves the abnormal LH/FSH ratio [6]. However, sensitivity to progesterone-induced slowing of GnRH pulse frequency is reduced in women with PCOS compared to normal women [32]. This reduced sensitivity has been attributed to the hyperandrogenemia and possibly hyperinsulinemia seen in PCOS (Fig. 5.3). In women with PCOS, antiandrogen treatment for 4 weeks restored normal GnRH pulse generator sensitivity to progesterone [40]. Ovulation occurs intermittently in some women with PCOS. The androgen-induced decrease in sensitivity to the progesterone-induced slowing of GnRH pulse frequency that is critical for dynamic secretion of FSH may explain why ovulation is often seen following a course of medroxyprogesterone acetate or discontinuation of oral contraceptives in PCOS but is not followed by the repeated cycles of follicle development, ovulation, and corpus luteum function that characterize normal menstrual cycles.

In adolescent girls, the neuroendocrine transition in early puberty is initially manifest by an increase in nocturnal LH pulse amplitude and mean FSH, driven by reactivation of pulsatile GnRH secretion. These nocturnal changes are accompanied by a small but significant increase in progesterone in the early morning [16, 41]. In the daytime, gonadotropin secretion is suppressed by the early morning increase in progesterone. This diurnal pattern in early puberty is followed by an increase in pulsatile LH secretion throughout the 24-h day in the later stages of puberty [42, 43]. Increased adrenal androgen production associated with adrenarche is thought to be the initial source of androgen exposure impairing sensitivity to gonadal steroid feedback during normal pubertal maturation in normal girls [32]. Girls with premature adrenarche develop an accelerated LH pulse frequency similar to that in adult women with PCOS with a loss of the typical diurnal variation; it is hypothesized that premature adrenarche this abnormality secondarily results in the ovarian hyperandrogenemia and PCOS associated with premature adrenarche [44, 45]. Pubertal

girls with hyperandrogenemia have increased LH pulse frequency compared to non-hyperandrogenic girls across pubertal Tanner stages, suggesting that the abnormalities in the GnRH pulse generator occur prior to menarche [16]. Furthermore, increased testosterone in early adolescence correlates with increased LH pulse frequency, again consistent with the hypothesis that hyperandrogenemia may decrease sensitivity to the progesterone-mediated inhibition of GnRH [32].

The timing of exposure to excess androgens during the course of development may be important in conferring risk for PCOS. Exposure to inappropriate levels of androgens prior to puberty and perhaps even during prenatal life may impact gonadotropin secretion by decreasing the expression of progesterone receptors in the hypothalamus. Animal studies demonstrate that prenatal androgen exposure increases mean LH and LH pulsatility, mechanisms identified as contributory to amenorrhea, hyperandrogenemia, and possibly polycystic ovarian morphology [46–48]. In rhesus monkeys, administration of moderate supraphysiologic doses of testosterone during pubertal development (1–5 years of age) results in LH hypersecretion and increased sensitivity to GnRH [49]. Longitudinal observational studies will be required to confirm these findings in adolescent and early adult girls.

In addition to the potential stimulatory effect of hyperinsulinemia on GnRH pulse frequency described above, there is evidence that insulin may also block progesterone negative feedback on GnRH secretion. However, decreasing insulin with insulin-sensitizing agents decreased gonadotropin levels in some [50, 51], but not all studies [52, 53] in women with PCOS unlike the more consistent findings with anti-androgens.

Anti-Müllerian Hormone (AMH)

While initially thought to be involved only in sex differentiation during embryogenesis, AMH is now known to be synthesized in small ovarian follicles in the ovary up to the small antral stage and serves as a marker of the number of follicles in the ovary and ovarian aging. AMH also plays a functional role within the ovary to restrain the actions of FSH on both recruitment of follicles into the growing follicle pool and aromatization of androgens to estrogens. AMH is elevated in women with PCOS compared to age-matched women without PCOS [17]. AMH and LH levels are correlated in a PCOS mouse model [54]. Anti-Müllerian hormone receptor 2 (AMHR2) is expressed on GnRH neurons in both fetal and adult animal and human tissues [54] and there is growing evidence of the stimulatory effect of AMH on GnRH neuron activity [17, 54–57]. Exogenous central administration of AMH in both in vivo and in vitro studies in mice led to increased GnRH neuronal activity and secretion that was dose-dependent [54]. Taken together with the finding that AMH levels are elevated in women with PCOS, these studies provide evidence for a possible role for AMH in GnRH hypersecretion in PCOS through direct stimulation of GnRH neurons (Fig. 5.3).

Leptin and Ghrelin

Leptin and ghrelin are key hormones that provide metabolic information from the periphery to the hypothalamus to regulate appetite [58, 59] and the reproductive

system [59–61]. Leptin is positively correlated with obesity and is permissive in the activation of pulsatile GnRH secretion at puberty [62]. Leptin levels are higher in women with PCOS than their normal cycling counterparts [63–66]. However, leptin is inversely related to LH in women with PCOS [64–66] and there is no effect of BMI on LH pulse frequency or overall amount of GnRH. Taken together, these data suggest that leptin does not play a major role in the hypersecretion of GnRH in women with PCOS.

Ghrelin is a gut peptide that is inversely related to energy availability. There is ample evidence from studies in rodents supporting an inhibitory role of ghrelin on GnRH [67] and further evidence that ghrelin administration inhibits LH pulse frequency in both men and women, also supporting a hypothalamic site of action in humans [61]. The inverse relationship of ghrelin to BMI in non-PCOS women is exaggerated in obese women with PCOS, possibly relating to the associated metabolic abnormalities [68, 69]. However, the hypersecretion of GnRH in women with PCOS is not affected by obesity and it is therefore unlikely that ghrelin alters GnRH secretion in PCOS.

Summary

The mechanisms underlying the hypersecretion of GnRH in women with PCOS are likely to be both complex and multifactorial. While androgens have an inhibitory rather than stimulatory effect on GnRH secretion, there is evidence that hyperandrogenemia inhibits progesterone-induced slowing of pulsatile GnRH acting through the upstream KNDy neurons. Hyperinsulinemia may stimulate GnRH secretion through kisspeptin, either directly or through inhibiting progesterone-induced slowing of pulsatile GnRH secretion, and may also stimulate GnRH neurons directly. There is also evidence that both GABA and AMH may play a further role in direct stimulation of GnRH neurons in PCOS. It is unlikely that any changes in leptin or ghrelin in PCOS contribute significantly to the hypersecretion of GnRH secretion in this disorder.

Dysfunctional Control of Pituitary Secretion of LH and FSH

GnRH Pulse Frequency

As discussed above, GnRH pulse frequency is one of the physiologic mechanisms that controls the dynamic and differential secretion of LH and FSH from the pituitary that is critical for normal ovulatory cycles. A relatively constant feature in women with PCOS is that GnRH pulse frequency is relatively fast; it varies little over time in the absence of ovulation or a progestin and is associated with an increase in LH relative to FHS. Faster pulse frequencies of GnRH stimulation increase GnRH receptors on gonadotropes. While incompletely understood, the frequency of GnRH stimulation and GnRH receptor density in turn control specific signaling pathways that differentially stimulate and suppress LH and FSH synthesis [7]. An addition mechanism through which GnRH pulse frequency may exacerbate the abnormal secretion of LH and FSH is that fast LH pulse frequencies also increase

the synthesis of follistatin [70], limiting the autocrine-paracrine effect of activin which would otherwise increase the synthesis of FSH, but not LH.

Estrogens and Androgens

Although the majority of women with PCOS do not have the dynamic changes in estradiol associated with normal follicle development, they are not hypoestrogenic. The effective use of estrogen receptor blockers or aromatase inhibitors in some, though not all, women with PCOS underscores the important negative feedback role that estrogens play [71, 72]. In addition to inhibiting the overall amount, but not the frequency of GnRH secretion, there is now evidence for a direct inhibitory effect of early to mid-follicular phase levels of estradiol at the pituitary itself that is greater for FSH than it is for LH [73, 74].

In all ovulation induction treatment modalities including use of exogenous gonadotropins and pulsatile GnRH, the ovulatory response is negatively affected by the severity of hyperandrogenemia, high BMI, and insulin resistance or hyperinsulinemia [75, 76]. However, rather than stimulatory, androgens are inhibitory to LH secretion at the pituitary in addition to their direct inhibitory effects at the hypothalamus [9, 31], suggesting the effect of androgens on fertility in women with PCOS is mediated through the intraovarian effect of androgens rather than through central effects.

Insulin

Hyperinsulinemia is observed in approximately 50–75% of lean and obese women with PCOS and is positively correlated with anovulation and hyperandrogenemia [77]. The insulin receptor localizes to pituitary gonadotropes in animals and humans and to the pituitary plasma membrane in the gonadotropin-derived LβT2 cell line [78]. Insulin stimulates secretion of LH from pituitary cells in culture [79] and in rodents deletions in the insulin receptor substrate, IRS-2, and the insulin receptor (IR) resulted in decreased LH secretion [80, 81]. In contrast, insulin increased GnRH-induced gonadotropin secretion and fertility in pituitary insulin receptor knockout mice with diet-induced obesity [81–83] suggesting that, at least in this model, the stimulatory effect of insulin is at the hypothalamic level, as reviewed above. Early studies found that insulin infusions had no effect on pulsatile LH secretion in women with PCOS [84, 85] or in normal women [86]. More recent studies, however, showed that in normal women, LH is inversely related to insulin and the LH response to GnRH is suppressed at high insulin concentrations; in women with PCOS, hyperinsulinemia resulting from a euglycemic hyperinsulinemic clamp also resulted in decreased rather than increased pituitary sensitivity to GnRH [87]. Thus while some data suggests that insulin stimulates LH secretion from gonadotropes, human data primarily points to insulin-mediated attenuation of gonadotrope responsiveness to GnRH. It is unlikely that this effect in women is due to pituitary insulin resistance as results were similar in normal women and women with PCOS and no relationship has been found between pituitary responsiveness and markers of insulin resistance including HOMA and stimulated insulin secretion [19]. Taken together, any direct pituitary role that insulin may play on LH secretion in PCOS is likely to be inhibitory rather than stimulatory.

Potential Mediators Underlying the Inhibitory Effect of BMI on LH Secretion

Mean LH, LH pulse amplitude, and the LH response to GnRH are markedly decreased in obese compared to lean women with PCOS with no effect of obesity on LH pulse frequency or the overall quantity of GnRH secreted. There are several factors associated with PCOS that must be considered as potential mediators of the inhibitory effect of obesity on pituitary secretion of LH. As discussed above, studies in women suggest that insulin has an inhibitory effect on the pituitary response to GnRH suggesting that the associated hyperinsulinemia may contribute to the attenuation of LH secretion that is evident with obesity.

Leptin receptors are located on pituitary gonadotropes in addition to their presence in the hypothalamus [88]. In vitro studies suggest that low levels of leptin stimulate LH secretion, while high levels are inhibitory [89]. In women with PCOS, there is an inverse relationship between leptin and mean LH and LH pulse amplitude [64, 90]. In addition, short-term caloric restriction in women with PCOS leads to a marked decrease in leptin levels and a concomitant increase in LH pulse amplitude [91]. Taken together, these studies suggest that the inhibitory effects of increasing leptin may underlie the inverse relationship between BMI and LH, acting directly at the pituitary.

Ghrelin is another potential mediator of the effect of obesity on LH secretion, but it is unlikely to be a significant factor in mediating the inhibitory effect of increasing BMI on LH through altering the pituitary responsiveness to GnRH. While ghrelin is inversely related to BMI in normal women and women with PCOS, studies of ghrelin administration to ovariectomized monkeys demonstrated a decrease in LH pulse frequency, but no effect on pulse amplitude [92], suggesting that any effect of ghrelin on LH secretion is mediated at the hypothalamus rather than the pituitary. Importantly, ghrelin is not correlated with the LH to FSH ratio in PCOS [93].

Summary

The persistent increase in GnRH pulse frequency that characterizes anovulatory women with PCOS is likely to play a major role in the increased secretion of LH relative to FSH from the pituitary, augmented by chronically low but persistent estrogen levels that inhibit FSH to a greater extent than LH. It is of interest that the abnormal gonadotropin dynamics in women with PCOS are attenuated with obesity, marked by a decrease in LH and the LH to FSH ratio. It is most likely that this effect is mediated through the associated increase in leptin which has a direct inhibitory effect on gonadotropin secretion and/or obesity-associated hyperinsulinemia.

Genetic Evidence of the Complex Pathophysiology of PCOS

A large number of genes have now been associated with PCOS through genetic studies on increasingly large populations of women with this disorder [94–97]. The identified genes further support the complex and interactive pathophysiology of PCOS as variants have been found in genes known to affect the hypothalamus, the

pituitary, the ovary, and metabolic function. For example, DENN/MADD domain containing 1A (*DENND1A)* is involved in calcium-mediated exocytotic GnRH/LH/FSH secretion in the hypothalamus and pituitary; luteinizing hormone/choriogonadotropin receptor *(LHCGR)* encodes LH receptors; and microtubule-associated protein RP/EB family member 1 *(MAPRE1*) is implicated in ovarian angiogenesis and follicle development. In addition, thyroid adenoma-associated protein *(THADA)* is associated with hyperandrogenemia and insulin resistance; plasminogen receptor (*PLGRKT)* is inhibited by plasminogen activator inhibitor type 1 (PAI-1) which is associated with hyperinsulinemia; and zinc finger and BTB domain containing 16 *(ZBTB16)* is associated with obesity. Chapter 4 in this text provides a more detailed overview of the role of genetics in PCOS.

Conclusions

From a neuroendocrine perspective, PCOS is characterized by both increased GnRH pulse frequency and increased pituitary responsiveness to GnRH resulting in increased secretion of LH relative to FSH. While androgens have an inhibitory rather than stimulatory effect on GnRH secretion, hyperandrogenemia inhibits progesterone-induced slowing of pulsatile GnRH acting through the upstream KNDY neurons. Hyperinsulinemia may have a similar effect. Insulin, GABA, AMH, and ghrelin may also contribute to GnRH hypersecretion through direct effects at the level of the GnRH neurons. The overall stimulatory effect of increased leptin on GnRH is less likely to contribute to GnRH hypersecretion given the lack of relationship between GnRH pulse frequency and leptin. The persistent increase in GnRH pulse frequency is likely to play a major role in the increased pituitary secretion of LH relative to FSH, augmented by chronically low but persistent estrogens which inhibit FSH to a greater extent than LH. The abnormal gonadotropin dynamics in women with PCOS are attenuated by obesity through a direct inhibitory effect of increased leptin and/or insulin on the gonadotrope response to GnRH. Genes associated with PCOS include not only those involved with ovarian function but also with hypothalamic, pituitary, and metabolic function, consistent with the complex and integrated pathophysiology of this disorder.

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Ovarian Dysfunction in Polycystic Ovary Syndrome (PCOS)

6

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Abbreviations

11βHSD	11β-hydroxysteroid dehydrogenase
11βHSD1	Type 1 11βHSD
11βHSD2	Type 2 11βHSD
AF	Antral follicle
AMH	Anti-Müllerian Hormone
AMHRII	AMH-specific type II transmembrane serine/threonine kinase receptor
AR	Androgen receptors
BMP	Bone morphogenetic protein
CYP17A1	Cytochrome P450 17α-hydroxylase
E2	Oestradiol
ECM	Extracellular matrix
FBN 3	Fibrillin-3 (FBN 3)
GLUT4	High-affinity glucose transporter 4
GLUTs	Glucose transport proteins
GnRH	Gonadotropin-releasing hormone
GWAS	Genome-wide association studies
LH	Luteinising hormone

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_6

LHCGR	LH chorionic gonadotropin receptor
mTOR	Mammalian target of rapamycin
PCO	Polycystic ovaries
PCOM	Polycystic ovary morphology
PCOS	Polycystic ovary syndrome
PI3K	Phosphatidylinositol 3-kinase
pSAMD	Phosphorylating proteins SMAD-1/5/8
SNPs	Single nucleotide polymorphisms
TGFβ	Transforming growth factor beta
WSD	Western-style diet (high fat and fructose)

Key Points

- Follicle numbers in polycystic ovaries (PCO) are increased once growth commences: the complement may be altered prenatally.
- Genome-wide association studies have revealed links to genes encoding proteins involved in ovarian function.
- Members of the transforming growth factor beta (TGFβ) family appear to have a role in many aspects of follicular dysfunction in PCOS.
- Overproduction of androgens by the ovarian theca cells of PCO is intrinsic to ovarian dysfunction of PCOS.
- Hyperandrogenaemia and hyperinsulinemia synergise in the polycystic ovary to affect follicle growth and oocyte maturation, contributing to defects in folliculogenesis and anovulation.

Introduction

Polycystic ovaries (PCO) are defined on ultrasound by two key features: the increase in size and density of the ovarian stroma and the large number of subcapsular antral follicles [1]. Due to the technical difficulties in tracking follicles in vivo, it remains unknown precisely how the life cycle of these follicles in PCO differs from that in normal ovaries, but evidence is accumulating to suggest that development goes awry at the earliest stages. Either the resultant PCO may then continue to ovulate with some frequency [2, 3], or a second defect or 'hit', presumably as a result of the combination of a highly androgenic and hyperinsulinemic environment, may cause chronic anovulation. Indeed, polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility [4, 5]. Experiments on follicular function in PCOS by necessity therefore fall into two categories: those investigating the cause of the development of the polycystic appearance per se and those determining why some polycystic ovaries ovulate whereas others do not. Increasingly it appears that the manifestation of morphological changes in PCO has genetic origins, as discussed elsewhere in this book, whereas the subsequent ovulatory status may be a combination of genes and environment. That patients across the spectrum and diagnosed by different criteria share a common genetic makeup was however highlighted in a recent large-scale study [6]. It was always to be hoped that as the genome-wide association studies (GWAS) began to produce results, they would

further illuminate the mechanisms of the development of the polycystic ovarian phenotype and this is gradually proving to be the case. The first such studies were performed in women of Han Chinese ancestry and revealed 11 candidate genes [7, 8]. Collection of adequate samples from women of European ancestry to perform GWAS of sufficient power took a while longer but confirmed associations of some of these susceptibility loci and also identified additional candidate loci as might be expected given the different ancestry of the groups [9, 10]. Together, 4 GWAS [7–10] and PCOS GWAS meta-analyses [6] have identified 22 candidate loci/genes for PCOS. As hoped, there were susceptibility loci close to genes encoding proteins with obvious roles in ovarian function, for example, *FSHR*, *LHCGR* and *INSR*. Other linked loci have more tenuous connections but have gradually revealed themselves to be highly interesting, such as *DENND1A* a member of the connecdenn family of proteins which modulates thecal androgen production [11]. These linkages are discussed in the appropriate sections below.

The search for the cause of anovulation in PCOS initially focussed on intrafollicular polypeptides [12] and has increasingly concentrated on anti-Müllerian hormone (AMH); evidence for this will be discussed in some detail in this chapter and is further examined elsewhere in this textbook. Aetiologically, it appears that the PCO may develop prenatally and animals exposed to increased levels of androgens in utero manifest many aspects of the syndrome [13]. Rather intriguingly, AMH may itself also have a role in utero in this regard [14]. This chapter will address in detail each of the above facets of disordered ovarian function in PCOS.

Development of the Polycystic Ovary

At Which Stage Do Follicle Numbers Increase?

The vast majority of follicles in the ovary are in the 'resting', primordial stage [15]. These gradually activate across the reproductive lifespan, first into the preantral primary and secondary follicles and then, with further growth, into the largely gonadotrophin-dependent antral follicles. Only a very small percentage of the primordial pool will eventually reach the large antral follicle stage, while the rest are destined to undergo atresia; thus, mathematically, even a small change in the atretic proportion would alter the balance in follicle numbers and the ovarian appearance. In order to elucidate the cause of the morphological changes in polycystic ovaries, it is clearly important to initially establish the precise stage at which follicle numbers become disordered: this has been problematic due to the paucity of appropriate ovarian tissue available for examination in women of reproductive age. It was initially reported from counting of full sections of surgically removed ovaries and in ovarian wedge resections performed for infertility management that the number of primordial follicles in PCO was identical to that in normal ovaries but that numbers of all growing and atretic follicles were increased in PCO [16]. Comparative counts in ovarian biopsies from women with anovulatory PCOS later confirmed the latter, in that there was a sixfold increase in the median density of preantral follicles in ovarian biopsy samples from women with PCOS [17]. The majority of these excess growing follicles were in the primary stage, i.e. the earliest stages of activation.

Conversely, there was a comparative decrease in the size of dormant primordial follicle pool in ovarian tissue from women with PCOS suggesting an 'overactivation' mechanism. In another study, follicles were counted in sections of fixed archived ovaries [18]. In contrast, this group observed no difference in the number of primordial follicles but again showed a specific fivefold increase in primary follicle counts in PCO compared to normal ovaries, a phenomenon the authors described as 'stockpiling'. As no differences in atretic follicle counts were noted between normal and PCO, the authors concluded that the increased numbers must be a consequence of follicles spending longer at the primary stage, i.e. that growth was slower. Counting human ovarian follicles is a laborious and complex task, and follicle staging is something of an art that will, in itself, produce variability. When this is coupled with the disparate sources of material used in these studies, i.e. full sections of ovary, wedge resections taken for infertility treatment or small ovarian biopsies, it is not surprising that we do not yet have a definitive conclusion. Cumulatively, however, these publications have produced a clearer picture of the timing of disordered follicle growth in PCO and point to it being at the earliest stage of activation leading to an increase in early growing forms and possible changes in growth rates.

What Might Be Causing These Changes?

The above discussed findings logically lead to an examination of the possible changes in follicle activation and growth that could result in this specific increase in small growing follicle numbers in PCO. This may occur with or without a reduction in primordial follicles, but with no resultant change in the timing of menopause: indeed, such a change is not apparent [19]. Theoretically such stockpiling could only result from a reduction in the rates of atresia, and indeed, cultured ovarian follicles obtained from anovulatory women with PCO did undergo atresia at lower rates than follicles from normal ovaries [20], indicating that follicles may persist to cause the characteristic ovarian appearance. Very little is known however regarding the factors that might be responsible for these reduced rates of preantral follicle atresia. The fact that survival persists in culture suggests that they are intrinsic to the follicle (i.e. connections between the oocyte and surrounding granulosa cells) rather than a function of the endocrine milieu. Discovery of the changes in gene expression as follicles activate and grow even in normal ovaries has been hampered by both challenges in access to the appropriate tissue as mentioned above, coupled with the tiny quantum of genetic material extractable from the preantral follicles [21]; however, the increasing utilisation of ovarian tissue cryopreservation by reproductive age women diagnosed with various cancers as a fertility preservation strategy is creating increasing opportunities of access to ovarian tissue for research. The revolution in techniques for mRNA amplification and single-cell RNA-seq analysis has also made these experiments feasible, has revealed the transcriptome during folliculogenesis [22] and has unmasked salient functional pathways that are significantly overrepresented in both oocytes and granulosa cells during the primordial-primary transition, including insulin, gonadotropin-releasing hormone (GnRH), neurotrophin, mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K) signalling pathways, as well as many more of unknown functions or significance [23].

Role of Androgens

Given the hyperandrogenised environment of the polycystic ovary, it was inevitable that androgens would be implicated as a cause of the reduced follicular atresia of PCO as discussed earlier [17, 18]. It must be borne in mind that follicles only develop a theca layer on reaching the secondary stage and so androgens are not acting in a paracrine fashion within individual follicles at this stage to cause developmental dysfunction. Rather preantral follicles at earlier stages of development than secondary are exposed to a highly androgenic milieu created by adjacent antral follicles. Androgen receptors (AR) are present in multiple cell types within ovary including fibroblast, stroma, immune and endothelial cells [24]. While androgen receptors have not been shown to be present on granulosa cells in primordial human follicles, at least from normal ovaries, there is an increase in the number of AR-positive follicles with progressive follicular development [21] indicating that the androgen signalling mechanism is at least present as the follicles grow. Experimentally, testosterone administration to adult female primates was shown to increase the number of growing preantral and small antral follicles and cause targeted loss of AR signalling in the granulosa cells with increased follicle atresia [25]; a likely explanation for testosterone-mediated follicular atresia is through an effect on FSH receptor acquisition [25–27]. However, ideally these experiments should be conducted in vitro such that confounding effects of the endocrine milieu can be removed. In addition to the paucity of ovarian tissue material, there is one further experimental hurdle for scientists wishing to grow follicles for the requisite experiments, and that is the wholesale activation and subsequent atresia of primordial follicles when ovarian tissue or individual follicles are placed in culture. Indeed, even the very processes of dissecting follicles from the ovarian cortex initiates a cascade of release of factors resulting in activation [28]. In order to overcome these experimental limitations, it has been necessary to grow tissue ‘in ovo’ or to develop culture matrix systems designed to reproduce the physical environment of the ovarian cortex. In our ‘in ovo’ model, follicles in sections of lamb ovary were grown inside chicken eggs where they become revascularised, permitting the majority of follicles to remain healthy and preventing wholesale initiation [29]. Interestingly, exposure of this in ovo tissue to androgens produced a significant reduction in follicle atresia with primary follicles being the class most protected [29]. Other studies have indicated that androgens attenuate follicular atresia by increasing granulosa cell expression of miRNA125b, which suppresses the expression of proapoptotic proteins [30].

Collectively, these results support a role for elevated androgens in, on one hand, stimulating and prolonging the life of early follicles in PCO by rescuing them from atresia but, on the other hand, leading to arrested follicle development in later stages, thereby contributing to the altered morphology of PCO. It might be assumed, therefore, that this ‘stockpile’ of primary follicles forms the basis of the PCO, feeding an increased pool of preantral and subsequently antral follicles to give the PCO its characteristic appearance.

Although these changes can be reproduced experimentally in animals and in tissue in culture, it now appears very likely that follicle development is reprogrammed in the ovary in utero. The clearest support for androgens having a role in this regard comes from models of prenatal androgenisation; prenatal exposure to exogenous

androgens is observed to disrupt subsequent adult ovarian phenotype and reproduce other metabolic aspects of the syndrome [13]. Specifically, in prenatal androgen exposure models in sheep, the ovaries contain multiple antral follicles that appear to have prolonged survival, and this was revealed to begin at the preantral stages [31]. Normally, the circulating maternal androgens get aromatised by the placenta with little ingress to the foetal compartment [32]; however, overwhelming the placental capacity to aromatise or exposure to excess non-aromatisable androgens can modulate the foetal ovarian microenvironment with changes persisting after birth.

Finally, the search for successful methods of culturing preantral follicles for both experimentation and clinical utility has unexpectedly revealed a further reason why follicles may exhibit enhanced survival in PCO, both in vivo and in vitro. Follicles initiate growth in the dense outer cortical layer of the ovary; therefore, it was considered that imitation of the rigid cortical environment as a three-dimensional framework of matrix would allow a more physiological environment for follicular growth [33]. Intriguingly, follicular survival was directly related to the degree of rigidity of the matrix. Anyone who has dissected or operated on a polycystic ovary is aware that the outer tunica and the ovarian cortex are excessively dense compared to normal ovaries, almost certainly a function of a lifetime of exposure of this tissue to raised thecal androgens. Immature follicles cultured in denser and more rigid three-dimensional environments were less able to proceed through folliculogenesis to the antral stage, suggesting that more dense, rigid hydrogels phenocopy the stroma-rich PCOS ovary. In addition, follicles grown in these rigid alginate hydrogels synthesised a greater ratio of androgens to oestrogens [34]. Androgen-induced rigidity of the ovarian tissue in PCO may itself, therefore, be another key to the reduced follicular atresia and the consequent increase in follicle number.

Role of TGFβ Family Members

PCOS has clear genetic origins. A search for determinant genes in a candidate gene screen investigated markers close to the *INSR* gene and provided the first strong evidence for both linkage and association with markers mapping intron 55 of the fibrillin-3 (FBN 3) gene [35]. These findings have been confirmed in two additional independent case-control studies [36, 37], although this locus does not appear in GWAS meta-analysis studies [7–10]. Fibrillin-3 is an extracellular matrix protein which interacts with members of the transforming growth factor beta (TGFβ)-binding protein family and thereby controls the bioactivity of members of the TGFβ family in tissues [38]: many members of this family, one of which is AMH, have recognised roles in ovarian function [39] and in PCOS in particular [40] making this an exciting discovery. When levels of fibrillin-3 expression in the adult ovary were compared, the levels were either very low or comparable in normal and polycystic ovaries [41]. The results were more exciting for the foetal ovary however, and fibrillin-3 was found not only to be highly expressed but also to be localised between nests of developing follicles [42]. Furthermore, later work in the prenatally androgenised primate model of PCOS has strengthened the case for a role of TGFβ modulation and foetal origins of PCOS. The promoter regions of genes expressed in the adipose tissue were examined to identify those that were differentially

epigenetically altered (methylated) between primate control and a model of PCOS; many of the genes identified to be differentially expressed belonged to the TGFβ signalling pathway [43, 44]. This effect could only have been produced prenatally. This offers an intriguing prospect that in PCOS, the altered expression of fibrillin-3, linked to abnormalities in the extracellular matrix and increased stromal deposition via an effect on TGFβ action, alters the population of ovarian follicles at the earliest stages and that these effects may occur prenatally.

There is mounting evidence that disordered secretion of AMH and the action of its receptors is implicated in the morphological changes seen in PCO, although the data are conflicting and at times even confusing. This paracrine factor that was first identified for its effects on male foetal differentiation is also expressed by the ovarian granulosa cells in human ovaries from 36 weeks gestation onwards, with levels peaking in the small antral follicles [45]. AMH is generally considered to be an inhibitor of follicle growth initiation, as first demonstrated by the AMH knockout mouse model [46] in which there was rapid initiation of growth of the primordial pool, eventually leading to an early cessation of fertility. Serum AMH levels are elevated in women with PCOS [47], and it was envisioned that overexpression of AMH might therefore hold back follicle development in the ovary leading to the reported stockpiling of small follicles. AMH also enhanced follicle survival in non-human primates [48, 49] and in human ovarian cortical strips [50]; these and similar experimental results however are confounded by intrinsic AMH production. This was tackled in an elegant series of experiments in Macaque ovaries in which the endogenous expression was removed by employing adenoviral vector-mediated shRNA/siRNA silencing of the *AMH* gene and then culturing of follicles in vitro with or without AMH supplementation. AMH also proved to be a survival factor in these experiments, and conversely, in the AMH-depleted environment, there was reduced survival of preantral follicles giving support to the hypothesis that AMH is causative of the follicle stockpiling seen in PCO [51].

In one of the few studies involving human ovaries, AMH was actually expressed in a *lower* percentage of preantral follicles in PCO than in normal ovaries [50], suggesting a mechanism that permitted accelerated follicular initiation, as seen in PCOS. Interestingly, we were unable to find many preantral follicles that actually expressed the AMH receptor in normal human ovaries [21] although we were not able to study follicles from PCO. Perhaps AMH has differing roles at each follicle stage in PCO, with those primordial follicles not expressing AMH receptor escaping the inhibitory effect and initiating growth, whereas those follicles that do express receptors are later protected from atresia by subsequent exposure to high levels of AMH in the ovarian milieu. Clearly this requires more investigation, and it is hoped that improved methods of ovarian follicle culture will assist in providing clarity in this confusing area.

Intriguingly, elevated serum levels of AMH in women with PCOS were shown to persist during pregnancy [14], introducing the possibility that in pregnancies in women with PCOS, the foetus is exposed to higher levels of AMH during development. Whether this maternal elevation is reflected in foetal levels is however unclear as in one study in newborn offspring of women with PCOS the levels of AMH were elevated [52], whereas in another study they were not [53]. In a landmark study, the offspring of mice with raised maternal AMH developed PCOS-like GnRH pulse

patterns and masculinisation of the sexually dimorphic regions of the brain [54]. It appears possible therefore that AMH is capable of foetal neural reprogramming, either through a direct effect on the maternal or foetal HPO axis or indirectly by reducing placental aromatase and increasing bioavailable androgen to the developing foetus [14, 52, 54]. It remains to be determined whether this also includes effects on the ovarian follicle population.

Overall, it appears that members of the TGFβ family are closely linked to the abnormal growth and survival of preantral follicles in PCO. In addition to their obvious roles in the adult ovary, evidence increasingly points to a role for these factors in determining the polycystic ovarian morphology prenatally. This will be an exciting area of research to follow in the future.

Theca and Granulosa Cell Dysfunction in PCOS

Hyperandrogenism is one of the defining features of PCOS; the major source of excess androgen in PCOS is the ovaries [5, 55]. Within the ovary, the theca cell layer converts cholesterol to androgens through a succession of intermediary steps, the primary ovarian androgenic output being androstenedione. Initially the excessive production of androgens was thought to be a consequence of the increased mass of androgen-producing theca cells in polycystic ovaries, but in fact each theca cell is hyperandrogenic. Evidence of exaggerated steroidogenesis in theca cells of PCOS came from culture of isolated cells in the 1990s, which showed in decreasing order an overproduction of androstenedione, progesterone, 17α(alpha)-hydroxyprogesterone and dehydroepiandrosterone [56, 57]. These findings led to the concept of an *intrinsic defect* in PCO and directed the search for 'PCO genes' towards the steroid synthesis pathway [57, 58],

A great deal of progress has now been made in this regard thanks to efforts to overcome the shortage of primary human theca cells, by developing a PCOS theca cell line that even in long-term culture retained its hyperandrogenic phenotype [59]. Increased expression of genes encoding the key steroidogenic enzymes, cholesterol side chain cleavage, CYP11A1 and CYP17A1 and increased stability of their mRNAs have been described in this PCOS theca cell line compared to cells from normal ovaries [59–61]. The mechanisms of this increased expression and stability have now also been revealed in more detail, greatly assisted by the ability to harvest sufficient cellular material to perform microarray analyses [62].

As mentioned in the introduction, GWAS have revealed a number of candidate genes with or without obvious links to ovarian function, but there is consistent linkage to one gene in particular that has provided insight into disordered androgen production in PCOS theca [62, 63]. Differentially expressed in neoplastic versus normal cells domain containing 1A, (*DENND1A*) is a member of the connecdenn family of proteins that are found within endosomes, the organelles responsible for intracellular sorting and recycling [64]; it is highly expressed in the theca cells [11]. The *DENND1A* gene yields two principal transcripts via alternative splicing: DENND1A variant 1 (*DENND1A.V1*) and *DENND1A.V2* [65]. Whereas variant one expression was decreased, variant 2 and its mRNA were overexpressed in the

PCOS theca cell line [65]. That variant two was directly involved in thecal androgen production was then neatly demonstrated by converting normal cells to a PCOS phenotype of increased *CYP17A1* and *CYP11A1* gene transcription and androgen biosynthesis by forced expression of *DENND1A.V2* in normal cells. In contrast, knockdown of DENND1A.V2 expression, or ablation of DENND1A.V2 function in PCOS theca cells utilising DENND1A.V2-specific antibodies, converts the cells to a normal phenotype [66]. Unfortunately, subsequent sequence analysis of the *DENND1A* gene of patients with PCOS did not identify alterations that pointed to a possible cause of PCOS; however small sample size was a major limitation of this study [11], and additional efforts are required to elucidate the precise role of connecdenns in the ovary and the change in function conferred by the mutation to fully implicate *DENND1A* in the hyperandrogenism of PCOS.

Finally, it should be noted that in addition to an intrinsic theca cell defect, there are a number of other factors acting in concert to influence androgen production in PCO. The first of these is the luteinising hormone (LH), which is often raised in PCOS [67, 68], as are the expression levels of LH receptors in both theca and granulosa cells [69]: the LH/hCG receptor gene *LHCGR* has been linked to PCOS in a number of GWAS. However, none of the isoforms are found consistently or appear to cause hyper-responsiveness, and thus the significance of these findings is unclear [63].

Hyperinsulinemia and insulin resistance are emerging as possibly the most important mechanisms driving ovarian production of androgens in PCOS. Uniquely, in the ovarian tissue, insulin not only stimulates glucose uptake and metabolism but also has gonadotrophic effects on steroid production in both granulosa and theca compartments [70]. As with other cells in PCOS, ovarian cells display resistance to insulin-stimulated glucose uptake, whereas the stimulatory effects of insulin on steroidogenesis remain sensitive [71, 72]. As circulating insulin rises to compensate for cellular resistance, the drive to this latter pathway increases and amplifies the intrinsic steroidogenic defect of PCO. The possibility of a link with insulin action was further raised by the finding in GWAS of a susceptibility locus for PCOS on the insulin receptor gene *INSR*, although the mutations found have not proven to be of any interest in terms of altered activity [73].

The link between androgens and insulin was further reinforced in a non-human primate model of exposure to chronically raised androgens pre- and post-pubertally and subsequent addition of a Western-style diet (high fat and fructose, WSD). Female Macaques on a WSD and chronic testosterone exposure had increased body weight and percentage body fat and decreased insulin sensitivity compared to those on normal diet. Interestingly, ultrasound imaging and immunohistochemistry also showed increased numbers of small antral follicles in each ovary [74]. Insulin also increases *CYP17* expression and enzymatic activity in human theca cells, further reinforcing this synergism [75].

It is of course possible, in addition to changes within genes of interest, that the function of genes is altered by differential expression of microRNAs. MicroRNAs are involved in post-transcriptional regulation of gene expression. It was first demonstrated in 2013 that a large variety of miRNAs are present in follicular fluid and that the profile differed between fluid collected during oocyte aspiration from

normal and polycystic ovaries [76, 77]. This was confirmed a year later, albeit with some differences in the results which may be explained by the differing populations studied [78]. A number of groups have subsequently pursued this methodology to reveal further miRNAs that may be differentially expressed and to determine a possible role for them in the disordered ovarian function of PCOS [79]. Of particular interest was the identification of miR-130b-3p which was predicted to target *DENND1A*, the gene shown to be linked to disordered theca function. The expression of miR-130b-3p was reduced in PCOS theca cells compared to normal, and this correlated with increased DENND1A.V2, the DENND1A transcript previously shown to be overexpressed in PCOS theca [80]. In addition, the level of miR-130b-3p also correlated with cytochrome P450 17α-hydroxylase (CYP17A1) mRNA and androgen biosynthesis [80]. It is exciting to see thecal cell function gradually dissected at the molecular level to reveal the disorders in both genetic and post-translational function in PCOS. Many more miRNAs have been revealed to be of interest in these studies, and this will be a fertile ground for research for some years to come.

Anovulation

Ovulatory Versus Anovulatory PCO

Once the PCO has gained its stockpile of preantral follicles, it is clear that a second (or more) defect then determines whether the ovary will become anovulatory or not. The clinical significance of ultrasound-detected PCO 'morphology', in the absence of other symptoms of the syndrome, is still unclear. High-resolution transvaginal ultrasound scanning has shown that PCO appearance of the ovary is very common: up to 68% of young Danish women in one series [81]. Indeed, such findings led to a recommendation from the Androgen Excess and PCOS Society Taskforce to change the ultrasound diagnostic criteria to a minimum of 25 antral follicles per ovary [82]. Ovulatory frequency can clearly be changed by the gain or loss of adiposity [83, 84], indicating that women who appear to have the polycystic ovary morphology (PCOM) alone are part of a spectrum and may move along the spectrum in either direction (Fig. 6.1). They can however be differentiated from women with normal ovaries in that they have higher circulating androgens and AMH and lower FSH levels [85] and, even when ovulating apparently regularly, lower luteal phase serum progesterone levels [3, 85]. Even those women deemed to be anovulatory were found to attain a significant number of ovulatory cycles when closely tracked by ultrasound [85]. The ovulatory status of the ovary in PCOS is therefore also part of a spectrum, and establishing a clear differentiation between anovulatory and ovulatory is difficult when making comparisons.

PCOS remains the most common cause of anovulatory infertility [86], and within the anovulatory ovary, follicle growth ceases once the follicles reach 5–8 mm in diameter [4]: in fact, with contemporary ultrasound the mean diameter at which follicles ceased growth was found to be 7.2 mm [85]. Numerous hypotheses have been proposed as to the cause of anovulation in PCOS. The common finding of androgen

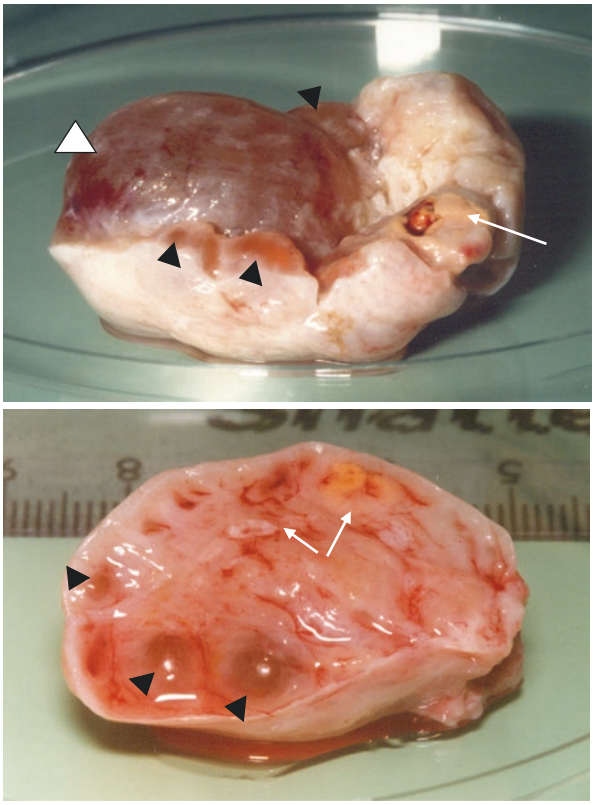

Fig. 6.1 Photomicrographs of human polycystic ovaries. *Top panel* – Ovulatory polycystic ovary dissected to show a large preovulatory follicle (white arrowhead), surrounded by smaller antral follicles of various sizes (black arrowhead) and corpus luteum from the previous cycle (arrow). *Bottom panel* – Anovulatory polycystic ovary dissected to show small antral follicles (black arrowheads) arranged around the periphery. In the centre can be seen the remnants of a white corpus albicans adjacent to a yellow corpus luteum (arrows). (*Copyright – Pictures courtesy of Professor H.D. Mason*)

overproduction by theca of PCOS ovaries led to this being suggested as a causative mechanism for anovulation; the validity of this assumption however is rendered questionable given that theca cells of PCO from *ovulatory* women also exhibit excess androgen production [5].

Role of Gonadotrophins

Serum LH is raised in anovulatory women with PCOS, and granulosa cells from small antral follicles from polycystic ovaries were found to gain LH receptors prematurely [87]. Normally follicle growth ceases shortly after LH receptor acquisition [88], suggesting that these follicles undergo *premature* luteinisation leading to early growth cessation [87]. LH receptor mRNA is overexpressed in granulosa cells from polycystic ovaries compared to normal ovaries [69], adding weight to this hypothesis, although lack of access to suitable tissue for experimentation has prevented further investigation of this hypothesis.

As discussed earlier, increasing utilisation of elective ovarian tissue cryopreservation by reproductive age women has allowed opportunities of access to non-luteinised granulosa cells from normal as well as polycystic ovaries. Only the cortical tissue containing preantral follicles is cryopreserved, and fluid and enriched

populations of granulosa and theca cells can be extracted from antral follicles in the same pieces [89]. Collection of granulosa cells from normal and polycystic ovaries revealed that whereas there were no differences in the levels of LHCGR (LH chorionic gonadotropin receptor) expression, overall, 20% of follicles from PCOS had expression up to 20-fold higher than that in normal ovaries [90]. This would certainly lend weight to the premature luteinisation theory, and determining the aetiology of this follicle population will be important in the future.

One further area of investigation that may help to shed light on the question of premature luteinisation is that of telomere length. The length of telomeres in granulosa cells reduces with successive divisions as might be expected. Intriguingly, granulosa lutein cells harvested from patients with PCOS at the time of oocyte retrieval were found to have longer telomeres than those collected from normal ovaries [91], indicating reduced cell divisions or a higher activity of telomerase. In a second study, however, the opposite was found to be the case [92].

Selection and maturation of the follicle from its small antral and antral phases are principally dependent on FSH-stimulated aromatase production [4, 88]. Follicles tracked by ultrasound ceased growth at an average of 7 mm in the anovulatory PCO and regressed approximately 4 days later [85], strongly suggesting a defect in FSH signalling. FSH levels are slightly lower in women with PCOS and do not show the intercycle rise and fall that recruits the dominant follicle in the normal menstrual cycle [4, 88]; however, once exposed to FSH, these granulosa cells express increased binding and are readily responsive both in vitro and in vivo [93–95]. Indeed, these findings first pointed to the likely presence of an inhibitor of aromatase within the PCO follicle [12]. In the comparison of cells from ovarian tissue removed for cryopreservation, FSHR expression was lower in PCOS than controls, but despite this the levels of aromatase gene expression (*CYP191A*) were higher in PCOS [90], supporting the earlier findings. Similar to LH, the FSH receptor gene also displayed linkage in GWAS, but the variants encoded by the major and minor FSHR variants seen did not alter FSH binding or function when transfected into model cell systems [96, 97] indicating that other, possibly locally produced factors are at play.

Insulin Resistance

Insulin resistance, mainly linked to adiposity, is a common finding in PCOS [98] and as previously discussed plays a significant role in excess androgen production [70]. Women with PCOS have intrinsic insulin resistance, and in the same way that obesity increases the extrinsic insulin resistance in the general population, it also does this in women with PCOS [98]. Even lean women with PCOS have level of insulin resistance, as determined by the gold standard euglycaemic-hyperinsulinaemic clamp, that is 75% higher than matched women with normal ovaries [99]. From the first finding of an inverse correlation between the annual number of menstrual cycles and insulin sensitivity [100], there have been numerous studies showing an association between anovulation and insulin resistance [reviewed in 101]. Incubation of granulosa cells with insulin induced early LH receptor acquisition and

'premature luteinisation' [87, 102] implicating hyperinsulinaemia as a cause of anovulation via LH action. This hypothesis has not been tested subsequently mainly again due to the immense difficulty in obtaining the requisite unluteinised cells for experimentation. When overweight insulin-resistant patients with PCOS lose weight and their insulin sensitivity parameters consequently improve, there is often a resumption of ovulatory cycles [83, 84, 103] further reinforcing the link between ovulatory dysfunction and insulin signalling. Interestingly there was a PCOS susceptibility locus close to the gene encoding LHCGR in the GWAS, but no obvious functional significance has been determined [reviewed in 63].

The use of exogenous gonadotrophins during fertility treatment in women with PCOS can often override the factors attenuating spontaneous follicle growth; however, this often occurs at the cost of lower quality eggs, reduced numbers of embryos and lower implantation rates compared to normal [104]. Proliferation of granulosa cells is dependent on increased glucose metabolism driven by insulin-mediated glucose uptake via the insulin-sensitive, high-affinity glucose transporter 4 (GLUT4) [105]. Interestingly this pathway also converges with the FSH signalling pathway at several critical points such as Akt or MAPK [106] indicating possible effects of hyperinsulinemia on FSH-mediated activity.

Anti-Müllerian Hormone

In addition to its role in preantral follicle abnormalities in PCO, the discovery that AMH circulates at significantly higher levels in women with anovulatory PCOS led to the hypothesis that AMH may be implicated in anovulation [107, 108]. In the ovary, AMH production peaks in small- to medium-sized antral follicles and declines around the time of follicle selection for dominance [108–111] (Fig. 6.2-normal). The decline is necessary because AMH inhibits FSH- and LH-induced aromatase expression in granulosa cells and further reduces the activity of the ovary-specific aromatase promoter II, resulting in a significant reduction in oestradiol production [107, 110], all of which would prevent follicle growth and progression. Interestingly, Pellatt et al. also demonstrated that AMH inhibits FSH-stimulated FSH receptor mRNA expression [107]. These data add to our understanding of the relevance of AMH in disturbed folliculogenesis in PCOS as AMH production was shown to be 75 times higher per granulosa cell in anovulatory PCO compared to normal ovaries [110], a level which would be sufficient to inhibit follicle growth (Fig. 6.2-PCOS). The cause of this high AMH production by PCO however remains to be elucidated, but again the highly androgenic environment in these follicles was suspected.

In our experiments to investigate this possibility, though the effects were modest, testosterone and 5α-dihydrotestosterone at doses equivalent to normoandrogenemic levels did inhibit AMH production by granulosa cells; however, doses equating to the hyperandrogenemic levels seen in PCOS appeared to favour persistence of AMH expression [111]. The fact that the presence of flutamide, an androgen receptor antagonist, made no discernible difference to testosterone's actions indicates that

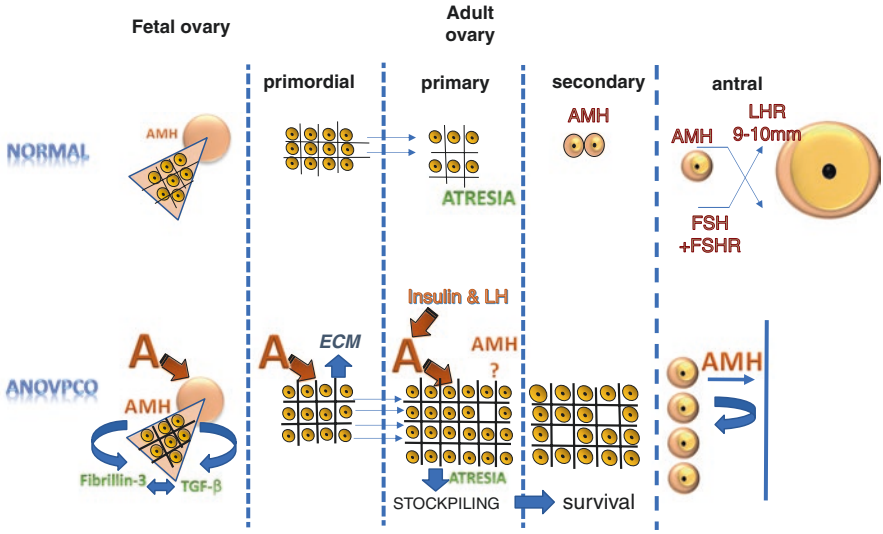

Fig. 6.2 Comparison of the follicle lifespan in a normal or anovulatory polycystic ovary. During foetal life, follicles develop in the extracellular matrix (ECM), which is thickened in response to excess androgens (A) in PCOS. Members of the TGFβ superfamily interact with fibrillin-3 which is localised between developing follicles and is highly expressed in the foetal ovary. ECM rigidity increases developing follicle survival and androgen production and may result in the stockpiling of follicles in PCOS. Raised levels of insulin and LH further drive thecal androgen production. At the antral stage, follicles in PCO overexpress AMH, and levels do not fall as in the normal ovary, thereby inhibiting FSHR acquisition, preventing the selection of a dominant follicle and later acquisition of granulosa cell LH receptors, resulting in anovulation

the reduction in AMH expression seen with testosterone could be indirectly through its conversion to oestradiol. There is a growing body of evidence to support the contention that oestradiol represses the expression of AMH [111–113], and this would be physiologically sound as it would establish an inhibitory 'loop' mode of action. Androgens therefore do not appear to be responsible for the increased AMH per se, but in PCOS they may interfere in the mechanisms requiring the fall in AMH that is critical for follicle growth.

It might be anticipated that AMH promoter activity is altered in cells from women with PCOS, although little is known in this regard. To date no association of single nucleotide polymorphisms (SNPs) of AMH or AMHR2 has been found to be linked to an increased risk of PCOS [114], though this does not preclude a role for functional variants in the AMH signalling pathway being involved.

The majority of research on the role of AMH in the ovary has focussed on AMH production or action or on *AMHR* expression with little attention being given to the AMH intracellular signalling pathway. AMH signalling occurs upon it's binding to the AMH-specific type II transmembrane serine/threonine kinase receptor (AMHRII), which then forms a complex with and activates a type-1 receptor, phosphorylating proteins SMAD-1/5/8 (pSAMD) and leading to the formation of a tetrameric complex consisting of two AMHRIIs and two Type-I receptors [115]. The pSMADs-1/5/8 form a complex with the common SMAD-4 and translocate to the

nucleus to regulate target gene expression via interaction with other factors. Inhibitory SMADs negatively regulate intracellular SMAD signalling: SMAD-6 specifically inhibits activation of BMP pathways by competing with pSMAD-1/5/8 for binding to co-SMAD-4, whereas SMAD-7 inhibits activation by binding to the type I receptor [116].

In normal granulosa luteal cells, AMH increased pSMAD-1/5/8 and SMAD-4 protein levels but, strikingly, significantly *decreased* levels in the equivalent cells from women with PCOS [111]. Furthermore, we found that in cells from polycystic ovaries, AMH *increased* the levels of the inhibitory SMAD-6 and SMAD-7, an effect not seen in normal cells. The outcome would therefore be a reduction in AMH signalling in PCOS which may partially negate the effects of the high levels, a phenomenon we named 'AMH resistance' [111]. We speculate that in vivo these high levels of AMH may cause uncoupling or desensitisation of the AMH signalling pathway leading to the dysregulated follicle growth seen in PCOS (Fig. 6.3).

In the Müllerian duct, the classical role of AMH is to cause cellular apoptosis [117], and we have questioned why levels of AMH seen in PCOS that are equivalent to those in the male [118] do not therefore appear to have the same effects on other tissue expressing receptors, such as follicular cells. Treatment of a granulosa cell line with increasing doses of AMH did not induce apoptosis as measured by caspase activity [111]. This lack of an apoptotic effect of AMH in vivo explains the ability to 'rescue' stalled follicles with superovulation regimes. We speculate that the lack of apoptotic effect on follicular cells in comparison to the Müllerian ducts may be due to an indirect action by AMH on the epithelial cells in the latter occurring after initial interaction with mesenchymal cells [111, 117], an action not possible in the ovary.

Role of Glucocorticoids

For a number of years, there has also been speculation that glucocorticoids have a role in the pathophysiology of PCOS. Within target cells, the actions of the physiological glucocorticoid, cortisol, are modulated by 11β-hydroxysteroid dehydrogenase (11βHSD) enzymes which catalyse interconversion of cortisol with its inert 11-ketosteroid metabolite, cortisone [119, 120]. To date, two 11βHSD isoenzymes have been cloned: type 1 11βHSD (11βHSD1), a relatively low-affinity, bidirectional enzyme which, in most tissues, acts predominantly as an 11-ketosteroid reductase (11KSR) to regenerate cortisol [119, 120], and type 2 11βHSD (11βHSD2), an 11β-dehydrogenase enzyme that inactivates cortisol [120]. Several authors had reported that in PCOS patients, there is a decreased urinary ratio of cortisol:cortisone [121–123], but it was not known whether this was reflected within the ovary. Within human granulosa-lutein cells, 11βHSD1 appears to be the sole isoenzyme expressed, with no detectable expression of 11βHSD2 mRNA or protein [124]. The high levels of intrafollicular androgens were hypothesised to cause dysregulated intra-ovarian 11βHSD1 and consequently affect cortisol-cortisone metabolism in the polycystic ovary. Intrafollicular cortisol to cortisone ratios were indeed elevated in the follicular

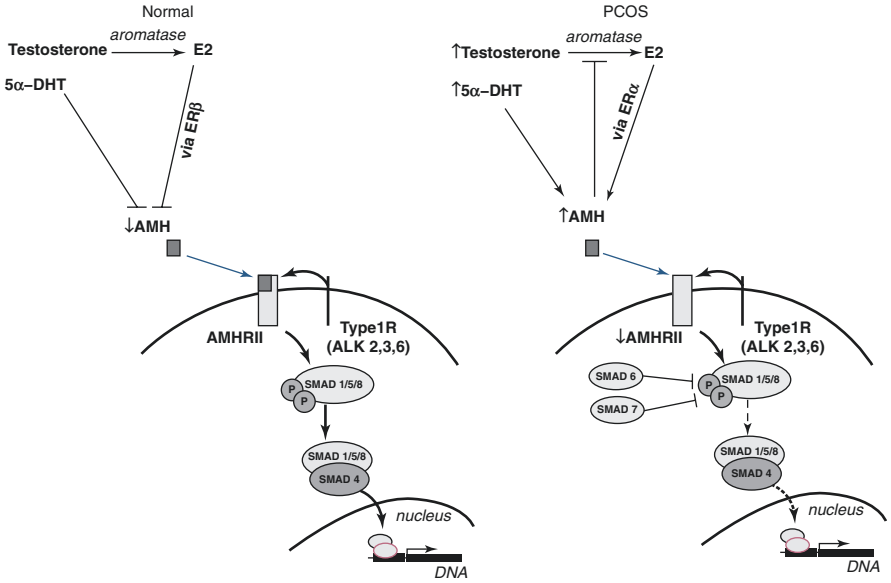

Fig. 6.3 Proposed model of AMH regulation and signalling in the normal ovary and in women with PCOS. *Normal ovary* – It is necessary to achieve a timely reduction in AMH to allow for antral follicle (AF) growth and selection of the dominant follicle. Testosterone can contribute to this via conversion to oestradiol (E2) and action through ERβ. The non-aromatisable 5α-DHT attenuates AMH production directly. AMH binds and signals exclusively through its type IIR, and this interaction regulates its actions. The recruitment of the common type IR opens up interaction of the highly restricted type IIR with other shared bone morphogenetic protein (BMP) and transforming growth factor-β (TGFβ) signalling pathways, to allow for the measured growth of follicles. *PCOS ovary* – In cells from women with PCOS, hyperandrogenaemia prevents the attenuation in AMH directly (5α-DHT) or indirectly via testosterone's conversion to E2 and action through the increased expression of ERα. Prolonged exposure to elevated AMH also reduces aromatase expression which contributes to stalled AF growth. In addition, the normal signalling events downstream of AMH binding to AMHRII are perturbed in PCOS by high levels of AMH that increase protein levels of the inhibitory SMADs, which has implications for progression of follicles. There is also a reduction in the expression of AMHRII, which could contribute to the dysregulated signalling events and follicle growth. (Reprinted from Dilaver et al. (2019) [111] – *Original illustration created by authors for article. Still awaiting reprint permission from OUP and Human Reproduction Journal*)

fluid aspirated from size-matched small antral follicles from anovulatory PCOs compared to ovulatory PCO and normal ovaries [125]. In addition, in granulosa-lutein cells recovered from patients with PCOS, 11βHSD1 activity was lower compared to cells from women with all other causes of infertility [125]. The basis of this inhibition appeared to be local testosterone action as it inhibited cortisol activation in a concentration-dependent manner in these cells in culture [125]. Physiologically, in the bovine and porcine ovary at least, progressively increasing cortisol activation is a marker of healthy antral follicle growth [126, 127], so although the precise impact of inactivation in follicles from anovulatory PCOS requires further investigation, it may also contribute to the arrested folliculogenesis.

It is clear that the mechanism of anovulation in PCOS remains something of an enigma with many possible factors being involved. It is impossible to determine in many cases which of these may represent a primary defect and which simply reflect the dysfunctional growth occurring in these follicles. It is to be hoped that discovery of the primary defects may assist in the identification of new forms of strategies to restore ovulation in anovulatory women with PCOS.

Energy Balance

From the primordial resting phase through to ovulation, follicles increase in size dramatically [85]; there is clearly thus a need for an adequate energy supply to fuel the multiplying granulosa cells and the maturing oocyte within the growing follicle. The dividing granulosa cells utilise glucose as the energy source, primarily via the glycolytic pathway. This allows for energy production in the form of ATP and metabolites (pyruvate and lactate), which are then secreted into the follicular fluid [128–131] or passed directly to the oocyte via gap junctions [132]. The concentration of glucose in the follicular fluid is comparable to plasma levels, at about 3.3 mM in humans [133]. Follicular fluid glucose concentrations are positively correlated with ovarian follicle size [134].

The application of metabolomics has facilitated the search for metabolic markers of PCOS, with the most common matrices investigated being plasma/serum, urine and follicular fluid due to the scarcity of ovarian tissue [135]. A vast range of metabolites have been identified as being altered in women with PCOS, highlighting the complexity of this endocrinopathy; those in the tricarboxylic acid cycle and glucose metabolism appear to be the major pathways that are deranged. This further implicates dysregulated insulin signalling and metabolism in PCOS, but the challenge however remains with the high level of inter-individual variability in these types of studies.

The high capacity of the follicle to metabolise glucose is characterised by the presence of glucose transport proteins (GLUTs; gene nomenclature SLC2A), which act as shuttles to move glucose across the cell membranes by forming an aqueous pore through which glucose can move [136]. In insulin-sensitive tissues, the translocation of the insulin-regulated GLUT4 from the intracellular pool to the cell surface accounts for most of the insulin-stimulated increase in glucose uptake [136]. Given that other insulin target tissues display insulin resistance in patients with PCOS [98], the possibility of the ovary also being resistant to insulin-stimulated glucose uptake in PCOS was investigated. Insulin-stimulated glucose uptake and metabolism pathway in granulosa cells from women with PCOS were noted to be impaired, as evidenced by an attenuated insulin-mediated lactate production response in cultured granulosa cells [71, 72, 137]. The observed impairment of insulin-mediated lactate accumulation is likely to represent both attenuated glucose uptake and a reduction in glycolytic activity in cells from anovulatory women with PCOS [72]; these phenomena occurring in vivo may account for impaired growth and proliferation of granulosa cells due to compromised energy availability.

While impairments in glucose metabolism are well described in granulosa cells from women with PCOS, as discussed earlier, this defect appears to be confined to the insulin-mediated pathway, since there was no difference in LH-stimulated lactate production in luteinised granulosa cells collected from women with normal ovaries and ovulatory and anovulatory PCOS undergoing in vitro fertilisation [72]. In addition, insulin-mediated steroidogenesis remained unaltered in the insulin-resistant PCO, indicating a divergence in the pathway such that insulin-mediated glucose uptake is resistant whereas steroidogenesis remains sensitive to insulin stimulation [71, 72, 137]. At a molecular level, this defect of insulin action in women with PCOS appears to be secondary to a post-receptor binding defect in insulin signalling, with reduced tyrosine phosphorylation of the insulin receptor and insulin receptor substrate proteins 1 and 2 [98]. Interestingly, we showed that metformin, an insulin-sensitising agent commonly utilised in the management of PCOS, was capable of directly activating IRS-1 and IRS-2 in granulosa cells, leading to an increased insulin-stimulated translocation of GLUT-4 to the plasma membrane [105]. Metformin thus has the potential to increase glucose uptake and metabolism in the insulin-resistant PCO and facilitate follicle growth through direct ovarian effects. Indeed, a recent Cochrane review on metformin concluded that ovulation and pregnancy rates were higher in women with PCOS taking metformin [138], though whether metformin can improve the developmental competence of PCOS oocytes remains to be established.

As stated earlier, pyruvate is the preferred substrate for the human oocyte, and abnormalities in pyruvate consumption have been implicated in abnormal meiosis in oocytes from PCO. Conversely, and in keeping with earlier discussion, prior exposure to metformin significantly decreased pyruvate consumption by in vitro matured oocytes from women with PCOS [139]. A study investigating carbohydrate metabolism and meiotic status in immature oocytes donated by women with PCOS and control patients and matured in vitro showed a high pyruvate uptake in the oocytes from PCOS and that this was associated with oocyte aneuploidy [140].

Collectively, existing data indicate that the distinct phenotype of PCO is accompanied by defects in steroidogenic pathway as well as impairments in glucose uptake and metabolism which are likely to have adverse effects on both oocyte development and follicle growth.

Oocyte of PCO

Given the disordered follicular development characteristic of PCOS, and the fact that oocyte energy metabolism may be abnormal, it is pertinent to further question the health of the oocytes contained within the follicles of polycystic ovaries. Evidence from in vitro fertilisation suggests that despite the higher oocyte yield that is common in women with PCOS, the implantation rate per oocyte is lower in this population compared to patients without PCOS [140]. Interestingly in vitro maturation of metaphase 1 oocytes in high glucose concentrations (such as would be seen with hyperinsulinemia or gestational diabetes) resulted in decreased rates of first polar body extrusion and altered DNA methylation levels [141]. The oocyte is

exposed to high intrafollicular levels of androgens and abnormal levels of other factors, including insulin, throughout its lifespan in PCO. The impact of endocrine and metabolic influences on gene expression in donated oocytes from PCOS was addressed by a comparison with oocytes from normal ovaries using microarray analysis [142]. These donated oocytes by women with PCOS were morphologically indistinguishable from those obtained from controls but displayed consistently different gene expression profiles. Of interest was the fact that the majority of differentially expressed genes were upregulated in oocytes from women with PCOS. Genes involved in the mitotic cell cycle and maternal effect genes (i.e. genes expressing proteins that are required around activation of the zygotic genome, i.e. at the time of resumption of mitosis) were amongst those altered. Many of the proximal promoters of the altered genes contained putative nuclear receptor binding sites including for androgen receptor. While difficulty in access to oocytes from clinically well-characterised patient populations for similar studies remains a limitation, future endeavours are clearly needed to fully assess the implications of an abnormal follicular environment on the developing oocyte in order not only to gain a better understanding of the reproductive capacity in PCOS but also to appreciate implications therein for the progeny of this population.

Summary

Collectively, data suggest that genetic and epigenetic modifications in the perinatal ovary lay a platform for abnormalities in the development of ovarian follicles, which persist into adult life. The cause of the failure of follicle selection for dominance in the majority of women with PCOS manifesting ovulatory disturbances appears to be multifactorial. Abnormalities in theca cell steroidogenesis of PCO are well characterised, are probably genetic in origin and result in an overproduction of androgens. This androgen excess which is responsible for the many manifestations of PCOS is in turn exacerbated by the interplay between cellular insulin and LH signalling pathways, and this abnormal ovarian milieu of paracrine factors, particularly AMH, is strongly implicated in the disturbed folliculogenesis of PCOS. The hyperandrogenaemia and hyperinsulinemia (as a consequence of prevalent insulin resistance) not only contribute to the premature arrest of follicle growth but also impact on normal oocyte maturation. Despite the strides in our understanding, the polycystic ovary remains an enigma, and the field for research on the many aspects of the ovarian dysfunction of PCOS will likely remain open and fertile for many years to come.

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The Role of the Adrenal Glands in the Hyperandrogenism Associated with the Polycystic Ovarian Syndrome

7

Ruth G. Freeman

Key Points

- PCOS in the adult is a complicated multietiologic disorder whose origin is difficult to determine.
- As many as 30–50% of PCOS patients have excessive androgens originating from the adrenal glands.
- Androgen excess from any source at any phase of life can be a precursor to PCOS.
- Most also have androgen excess of ovarian origin.
- Early onset of adrenal androgen increase, particularly DHEA and DHEA-S seen in children with premature adrenarche or precocious puberty, often precedes the development of PCOS in late adolescence.

Adrenal Androgens and PCOS

Polycystic ovarian syndrome (PCOS) is a multietiologic disorder of young premenopausal women [1]. It is a syndrome of oligomenorrhea and hyperandrogenism associated with either clinical findings (hirsutism and/or acne) or elevated blood androgen levels. In recent years the diagnosis by Rotterdam criteria adds women with only oligomenorrhea and enlarged polycystic ovaries to the overall group of PCOS [2]. This increases the number of women who have this heterogeneous disorder and makes finding an etiology more difficult. In this discussion only PCOS with hyperandrogenism will be considered (the NIH criteria, discussed in greater detail in Chaps. 1 and 3 of this book). The source of the androgens in PCOS is still controversial. The effect of androgens on ovarian function has been reviewed by Walters [3]. Basically, increased androgens, endogenous, or exogenous, at any phase of life,

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_7

fetal, childhood, adolescence, or adulthood, can alter ovarian function. Even exogenous administration of androgens can result in a PCOS-like picture with anovulation, and oligomenorrhea as well as hirsutism [3]. Walters concludes that no specific androgen receptor-mediated action has been proven as the mechanism for the development of PCOS. In fact this author suggests that androgen receptor activation in the brain altering GnRH or LH production is likely to play a role. Different sources of androgen excess may be operative in different individuals.

There is no consensus on the role of the adrenal in the pathophysiology of PCOS. An extensive review by Futterweit was published in a monograph by Springer Verlag in 1984 [4]. Some studies have shown that the androgens in this disorder may be entirely of ovarian origin, whereas others provide evidence that it is largely from the adrenal, and still others provide evidence that both the ovaries and the adrenals contribute to the hyperandrogenism of PCOS. Zeng et al. in a 2020 review regarding the role of hyperandrogenemia, insulin resistance, and obesity were unable to decide which of the glands capable of making androgens play a causative role in the development of PCOS [5].

The steroid-producing tissues of both the adrenals and the ovaries have a common embryonic origin, the early mesonephric region. In some individuals, adrenal rest tissue can be identified within the ovary, further obscuring the source of elevated androgens. This has been described in patients diagnosed with congenital adrenal hyperplasia (CAH). Adrenal tissue responsive to ACTH is present in their gonads. This occurs more commonly in the testis than in the ovary [6–8] making it uncertain whether the ovarian output of a steroid is due to adrenal rest or native ovarian tissue. Androgen production by the ovary may in fact cause alterations in steroidogenesis in the adrenal [9, 10]. The reverse may also be true, as elevated exogenous androgens during or prior to puberty can cause increased ovarian steroidogenesis via a positive feedback at the hypothalamo-pituitary level with consequent LH elevation [11]. In the discussion that follows, the available data will be reviewed with a systematic focus on adrenal function during childhood and adolescence in those who later develop PCOS, then in the adult with this syndrome, and finally in the peri- and postmenopausal women who were diagnosed with PCOS in reproductive years of life.

The Role of the Adrenal in Childhood and Subsequent Development of PCOS

Some children who have classical CAH with excessive androgen production by the adrenals during fetal life develop PCOS after adolescence. In a long-term follow-up of CAH patients, Mnif et al. [12] found that 6 of 15 women also had PCOS.

Does exposure to androgens in the fetus or neonate result in an alteration in the postpubertal ovarian hormone production? In animal studies, administration of testosterone to a newborn mouse or rat results in persistent estrus and polycystic ovaries [13]. Female monkeys exposed to androgens just prior and during puberty developed increased LH pulsatility with circulating levels similar to those observed in PCOS

[11]. Similar findings were seen in adolescent girls given testosterone acutely [14]. Thus, exposure of the fetus or the pubertal girl to androgens, regardless of the source, may result in altered functioning of the hypothalamic-pituitary-ovarian axis consistent with PCOS [15]. There have been suggestions that the fetus of a mother who has PCOS gets exposed to increased androgen levels via the placenta. It is also possibly seen in situations where the placenta does not have adequate aromatase activity to prevent androgens from mother to get to the fetus [3]. Recently, with increasing ability to measure AMH (anti-Müllerian hormone), Sova et al. [16] reported that mothers who have PCOS have higher AMH levels during pregnancy and this may result in increased AMH in the female fetus [17]. Tata et al. [18] studied mice who were given AMH during pregnancy which resulted in increased testosterone in the mothers and masculinization of the female fetus, eventually resulting in a PCOS-like picture in these mice in adulthood. The role of AMH, whether in fetal life or later after puberty, is still unclear and needs further investigation.

Premature adrenarche, which is known to be associated with elevated dehydroepiandrosterone (DHEA and DHEA-S) levels, is frequently seen in children who will subsequently develop PCOS [19]. The onset of puberty in normal girls starts with breast development (thelarche) followed by the growth of pubic hair (pubarche). It has long been recognized that girls experiencing premature adrenarche (or pubarche) manifested by early onset of pubic and axillary hair are likely to go on to develop PCOS. A detailed review of the effects of premature adrenarche was published by Idkowiak et al. in 2011 [20] and recently by Voutilainen and Jaaskeoainen [21]. Elevation in serum DHEA occurs at the time of the onset of pubic hair growth [22]. It may be due to an alteration in the function of CYP17A1 (P450c17α), an enzyme that has dual actions of (1) 17 hydroxylation of pregnenolone and progesterone (Fig. 7.1) and (2) removal of C20 and C21 (lyase activity) from the resultant intermediary products 17-OH pregnenolone and 17-OH progesterone to yield DHEA and androstenedione. Activation of CYP17A1 in the zona reticularis of the adrenal glands is a physiological event resulting in a rise in DHEA during normal puberty [23]. Activity of CYP17A1 may be enhanced in girls experiencing premature adrenarche, resulting in excess DHEA and androstenedione. An extensive

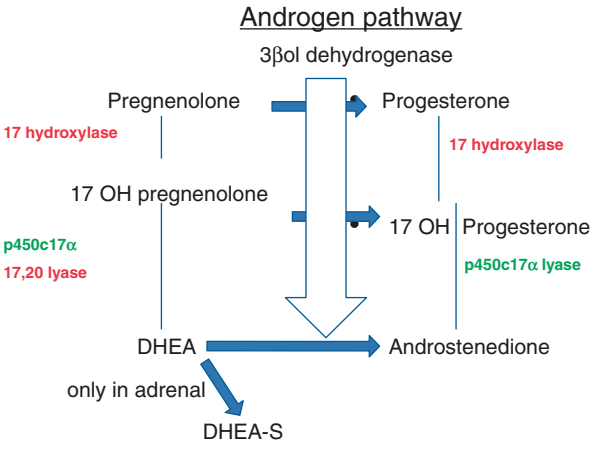

Fig. 7.1 Androgen production pathways in the adrenal and ovary. Sulfation of DHEA (dehydroepiandrosterone) resulting in production of DHEA-S is limited to the adrenal gland

review of this paradigm in adults who started out with early pubarche (adrenarche) has been reported by Ibanez et al. [22]. Prior to puberty, lyase activity is very low. (Note: This is true of adrenal and ovary.) Zeng [5] has proposed that the early development of the zona reticularis produces increased amounts of DHEA and DHEAS resulting in increased production of AMH in pre-antral follicles leading to irregular functioning of ovarian cyclic activity.

This can be further exacerbated if the individual also has insulin resistance. In this setting there is decreased SHBG further increasing free testosterone, which is secondarily derived from the precursor DHEA. It is still not clear whether hyperinsulinemia causes hyperandrogenism or vice versa, as reviewed by Moghetti and Tossi [24]. In clinical studies, Nestler has shown that reducing insulin either by metformin or a glitazone reduces the ovarian androgens and leads to resumption of menstrual cyclicity [25].

Oberfield et al. reviewed the pubertal increase in the size of the zona reticularis of the adrenal and increasing function of the P450c17α activity producing increased DHEA in normal puberty, as well as the progression from premature adrenarche to PCOS [26]. Girls who have precocious puberty commonly develop PCOS with early abnormal hair growth and irregular menses [27]. Alteration of the lyase pathway may be part of the pathophysiology of increasing DHEA production in the adrenal and/or increasing conversion of 17-OH progesterone to androstenedione in the ovary of women with PCOS. The same enzyme is active in the adrenals and ovaries. Its control is likely due to other proteins, some of which decrease 11 hydroxylation in the adrenal, thereby increasing the amount of androstenedione due to the increased levels of the precursor 17-hydroxyprogesterone (see Fig. 7.1). A study of the daughters of women who had PCOS found higher than normal levels of DHEA-S and greater increase in ACTH-stimulated DHEA-S in 30% of the PCOS daughters than in a control group of normal girls [28]. Thus, an alteration in the production of adrenal androgens in children may, by a diverse array of mechanisms, result in the disordered menstrual cyclicity that is characteristic of PCOS.

A review of the interaction of androgens, AMH, and estrogens by Dewaily et al. [29] suggests that androgens, from any source adrenal, ovarian, or exogenous, either increase FSH or make the granulosa cells more sensitive to FSH resulting in large numbers of pre-antral follicles. The pre-antral follicles produce increased amounts of AMH which inhibits FSH production and reduces the further maturation of many of the pre-antral follicles thereby interfering with dynamics of follicular growth and ovulation. The elevated estradiol produced by the multiple follicles results in increased LH production by the pituitary. The elevated level of LH increases the androgen production by the theca cells in the ovary.

While abnormalities of adrenal steroidogenesis may contribute to the reproductive axis dysfunction that is characteristic of PCOS, of interest is the observation that excess lyase activity of P450c17α function may be associated with insulin resistance [30] or with an increase in IGF1. These latter data provide a mechanism that links functional differences in adrenal steroidogenic pathway with the metabolic picture of PCOS. The frequent occurrence of insulin resistance in adults who started out with early pubarche (adrenarche) has been detailed by Ibanez et al. [22].

In summary, existing data suggest that abnormal production of androgens by the adrenals early in life (in the fetus or prepubertal child) may be a precursor of the PCOS phenotype.

Adults with PCOS and the Role of Adrenal Androgens

PCOS in the adult is a complicated disorder whose origin is difficult to determine. Overall it seems that between 30 and 50% of women meeting criteria for PCOS exhibit excess androgens of both adrenal and ovarian origin. Determining the source of androgen excess is important from the perspective of ruling out differential diagnoses (such as an adrenal or ovarian tumor, or late-onset CAH); it may also allow for more specific treatment strategies.

Clinical presentation of PCOS may be somewhat insidious early in its presentation; the condition is usually manifest early in the reproductive years, usually within 5–10 years following menarche. For some, menstrual cycles may be erratic from the time of menarche, whereas for most others, menses may be predictable ovulatory cycles in the early years, with progressive deterioration in menstrual cyclicity and onset of signs of androgen excess, such as body odor related to apocrine gland activity, acne, and excess facial and body hair growth. By the time the young woman seeks medical attention, the source of the androgens may be difficult to ascertain.

Adrenal androgens, after conversion to estrogens in the peripheral body fat (especially in obese individuals) and/or liver, increase the pituitary secretion of LH, which in turn causes increased ovarian androgen production. Therefore, there are likely some women with PCOS in whom the process started with an increase in adrenal androgens that secondarily affected ovarian steroid production. Conversely, the reverse may also be true, as ovarian androgens have been shown, in vitro, to alter the adrenal production of androgens by blocking the 21 hydroxylation of 17-hydroxyprogesterone and 11-deoxycortisol [31] thus channeling the steroidogenic precursors towards androgen synthesis. The effect of estrogen in altering the production of adrenal androgens has been well summarized by Gonzalez et al. [9].

Catheterization of the adrenal and ovarian veins has been used to identify the source of excess androgen production. The studies by Kirschner et al. [32] and Wajchenberg [33] suggest that the androgens are largely from the ovary, whereas the studies by Abraham et al. provide evidence for excessive adrenal production of androgens [4]. Elevated DHEA and particularly DHEA-S have been suggested as evidence that the adrenals are the source of the abnormally high androgens in women with PCOS. Kumar et al. examined 182 patients who had PCOS and found that 30% of black and 20% of white subjects had excessive amounts of DHEA-S, suggesting an adrenal source of androgen excess [34]. The age-associated decline in DHEA-S was seen both in normal women and in those who had PCOS. As these PCOS women age with decreased production of DHEA-S, they may resume normal menstrual cycles. Whereas in normal women most of the DHEA is produced by the adrenal, the ovary is capable of secreting DHEA as well. DHEA-S, however, is thought to be produced only by the zona reticularis of the adrenals, suggesting an

adrenal source of androgen excess for those PCOS patients who have elevated DHEA-S levels. A concomitant ovarian contribution to androgen excess may exist in this population as well.

Of interest is a recent finding by Al Nofal et al. [35] in a study of obese PCOS adolescents compared to thin PCOS adolescents suggesting that those who had a higher adrenal androgen (DHEA-S) output as measured by DHEA-S/FT (free testosterone) were less likely to have abnormal metabolic syndrome parameters including insulin resistance and elevated triglycerides.

Stimulation and suppression tests are commonly used to differentiate the source of excess androgens; the ACTH and GnRH stimulation tests evaluate for adrenal and ovarian source of steroid hormone excess, respectively; similarly, suppression of androgen levels following a trial of exogenous glucocorticoid (usually dexamethasone) suggests an adrenal source but doesn't prove it. Successful suppression of androgens with GnRH antagonist or following long-term use of a GnRH agonist favors an ovarian source of androgen excess.

Trial of a GnRH agonist, reducing gonadotropins to very low levels, resulted in reduced production of ovarian hormones and has clearly shown that at least some PCOS subjects only have ovarian hyperandrogenism with negligible adrenal contribution. Chang et al. in 1983 suppressed the ovarian function with long-term GnRH agonist use, reducing ovarian estradiol, estrone, and androstenedione production to levels that were comparable to castrated controls [36]. In their subjects, DHEA but not cortisol was elevated and remained unaltered by the GnRH agonist treatment, suggesting that the DHEA was essentially of adrenal origin. These authors concluded that in their PCOS patients, both adrenal and ovarian sources contributed to androgen excess. It is important to appreciate that in the short term, administration of GnRH agonist results in a flare effect, with an acute rise in pituitary release of both LH and FSH, with a resultant transient increase in ovarian androgens. When administered over a longer period of time (at least 2–3 weeks), however, suppression of pituitary gonadotropins is achieved with use of GnRH agonist secondary to a downregulation of GnRH receptors on the gonadotrophs, with consequent ovarian suppression. A complicated study by White et al. using GnRH agonists first and then followed by ACTH stimulation testing could not show increased adrenal androgen production when ovarian function was blocked by GnRH administration [37], thus adding to the confusion of the source of androgens in this disorder.

Although adrenal suppression with potent glucocorticoids (usually dexamethasone) can show reduction of adrenal androgens, they may also alter the ovarian production of hormones and may, in fact, reduce LH secretion, which in turn would lower ovarian androgen production. In a complicated study of 60 PCOS patients and 39 normal control patients, Rosenfield et al. reported that lack of suppression of testosterone by dexamethasone provided evidence of an ovarian source of the androgens in the PCOS group [38]. These authors further proposed that greater response of DHEA after ACTH administration, which was found in roughly 30% of the PCOS subjects, additionally suggested an adrenal source of excess androgens. ACTH stimulation tests have shown increased response with higher 17-hydroxyprogesterone levels in about 20–30% of PCOS patients. Azziz et al.

separated women with PCOS into those with elevated DHEA-S and a second group with normal or minimally elevated DHEA-S levels and found that the high DHEA-S group had an excessive response to ACTH [39]. These authors also measured ACTH response to CRH (corticotrophin-releasing hormone) stimulation and found that to be similar in both groups. In patients with demonstrable suppression of androgen levels following exogenous glucocorticoid exposure, ACTH stimulation test can help identify women with PCOS from those with a diagnosis of late-onset CAH. It is important to appreciate an overlapping phenotype between these two diagnostic entities, and some of these women may, in fact, have both PCOS and CAH, similar to the classical CAH patients who develop PCOS during adolescence. Azziz et al. [39] studied the ACTH response in PCOS subjects using a complicated system of increasing low doses of ACTH followed by the usual 250 μg dose and demonstrated an increased output of androstenedione, which was greater than the cortisol production, which in turn was greater than the output of DHEA. The response was higher in PCOS patients than in normal controls, suggesting an increased adrenal source of androstenedione in these subjects; androstenedione is normally produced equally by the adrenal and the ovary.

In a recent study, Rosenfield et al. showed that about 27% of the PCOS patients had excessively high response of DHEA to ACTH stimulation [38]. This was found in a group of PCOS patients who when given GnRH agonist (GnRHag) acutely to stimulate the ovary did not have the expected rise in 17-hydroxyprogesterone or androstenedione. The authors proposed heterogeneity within PCOS with *typical* and *atypical* PCOS phenotypes. Some women with PCOS in both the groups had less suppression of testosterone with dexamethasone, suggesting an ovarian (often as well as an adrenal) source of hyperandrogenism. In fact, they identified a group of women with BMI >40 who had a PCOS-like picture but responded normally to dexamethasone suppression, ACTH stimulation, and GnRHag. However, not all of the very obese subjects had this finding; some of the typical PCOS group also had >40 BMI. Sahin et al., in contrast, found that the initial increase in 17-hydroxyprogesterone was greater in PCOS patients than in normal controls. After pituitary suppression with GnRHag, the ACTH response in PCOS patients was the same as in controls, suggesting an ovarian rather than adrenal source for androgens [40]. Carmina et al. have suggested that ovarian disturbance could affect the adrenal response to stimulation; these investigators provided evidence that only those subjects who at baseline have elevated 11-hydroxyandrostenedione (11 hydroxylation occurs exclusively in the adrenal tissue and not at all in the ovaries) had increased adrenal androgen production in the first place [10]. These authors suppressed the ovaries with 3 and 6 months of GnRH followed by ACTH stimulation and found that in some subjects this altered the DHEA response to ACTH stimulation.

Futterweit's monograph *Polycystic Ovarian Syndrome* [4] offers an extensive review of the classic stimulation and suppression studies up through 1984 utilized for evaluating adrenal gland function in PCOS. More recently, studies have suggested that there may be many different alterations in the enzymes affecting steroidogenesis pathways in both the adrenal and the ovary. Pathways leading to the production of androstenedione and testosterone are shared, and both tissues require

the cytochrome P450c17α activity for hydroxylation of the steroid molecule at the 17 position; the same enzyme is required for cleavage (lyase) of the 20 and 21 carbon side chains (see Fig. 7.1). Thus, excessive lyase activity of P450c17α in both the ovary and the adrenal can contribute to excessive production of both adrenal and ovarian androgens.

There has been much speculation of the role of insulin and androgen production particularly in the ovary. Do increased insulin levels raise androgen production, or is it vice versa and increased androgens cause insulin resistance? Nestler et al. in 1997 reported that correction of high insulin levels in lean PCOS subjects reduced the abnormality of ovarian P450c17α; the authors utilized GnRH stimulation to assess the ovarian component [30]. Moghetti and Tosi recently reviewed the subject of insulin resistance in PCOS patients summarizing the available literature through 2021 [24]. However, in clinical practice, many PCOS patients who can be shown to have increased insulin levels respond to medication, metformin, or glitazones by resuming normal cycles and lowering androgen levels. Obesity may increase insulin resistance thereby resulting in increased ovarian androgen production. If these women can reduce their weight, some of them may return to normal menstrual cycles and fertility. It is likely that they have a mild form of PCOS which gets worse with increased weight. Many of the hyperinsulinemic PCOS women go on to develop type 2 diabetes. However not all women who have elevated levels of insulin and diabetes have a history of PCOS. Therefore answers to other pieces of this puzzle still need to be found.

Vassiliadi et al. demonstrated increased 5α-reductase activity in both obese and non-obese PCOS patients as compared to normal women of comparable BMI [41]. In fact, cortisol production in these PCOS patients was shown to be higher than the controls; however, circulating cortisol levels were in the low normal range. This suggested an exaggerated corticosteroid degradation in patients with PCOS, suggesting that the increased adrenal androgens were a side product of the compensatory increase in cortisol production. A cause-effect relationship to the observed phenomenon, however, could not be elucidated given the cross-sectional study design. Gambineri et al. suggested that increased clearance of cortisol due to 5β-reductase activity resulted in increased adrenal androgen production due to elevated ACTH levels in 90 subjects with PCOS by demonstrating that these women had higher responses to ACTH than did the 45 women without the enzyme abnormality [42]. They concluded that increased inactivation of cortisol resulted in increased ACTH secretion (not proven) and a more robust response in adrenal 17-hydroxyprogesterone to ACTH stimulation. Direct measurement of ACTH by Chang et al. [43] in PCOS patients, however, failed to show any increase in circulating ACTH levels in PCOS patients. The ratio of cortisol:ACTH was the same as in normal women, but DHEA-S:ACTH was much higher in PCOS women than the normal control; this latter observation implies that in women with PCOS, for every unit of cortisol production there was excessive output of androgens.

Glintborg et al. reviewed data on 650 PCOS patients and found that those who smoked (260 subjects) had exaggerated 17-hydroxyprogesterone responses to ACTH stimulation [44]. This suggested increased adrenal responsiveness with

increased androgen production in smokers who had PCOS. Smoking has been shown to increase the degradation of estrogens, possibly resulting in higher production of androgens as a side product of increased or decreased estradiol production (no evidence of increased estrogen production has been demonstrated) [45].

Adrenal Androgens in Perimenopausal and Postmenopausal Women with PCOS

There is little information regarding the PCOS patient's adrenal androgen production in the postmenopausal period. Whereas adrenal androgen production decreases in aging normal control perimenopausal women in response to ACTH stimulation, PCOS women had continued high response of 17-hydroxyprogesterone into perimenopausal ages [46]. In fact, this was also true of six postmenopausal women in the same study. Markopoulos et al. reported that 20 postmenopausal women who had a history of PCOS continued to have higher DHEA-S and androstenedione levels and had a greater response to ACTH stimulation than in the normal women [47]. In these women, the response of ACTH to CRH was the same as in normal women, suggesting increased sensitivity of the adrenals to normal levels of ACTH. Schmidt et al. examined 25 women well past menopause who had previously undergone ovarian wedge resection as a management strategy for PCOS. Increased degree of hirsutism and higher free testosterone levels were observed in this population when compared to control women [48], although DHEA-S levels were comparable to the controls. No stimulation studies were conducted, however, thus limiting interpretability of these findings.

Conclusion

Despite numerous studies regarding the source of the hyperandrogenemia observed in most PCOS patients, the basic underlying cause is not as yet clear. Approximately 50% of PCOS women can be shown, at some time, to have elevated adrenal androgens (DHEA-S or DHEA) but also have increased ovarian androgen production. Another 20–30% appear to have largely increased adrenal androgens. Long-term GnRH analogue administration, which preferentially suppresses ovarian function, can demonstrate persistent high levels of androgens produced by the adrenal.

In utero fetal exposure to excess androgens, for example, from an adrenal steroidogenic enzyme abnormality or maternal hyperandrogenemia resulting from an androgen secreting tumor or luteoma of pregnancy, or even hyperandrogenemia of PCOS can predispose the exposed progeny to the development of post pubertal PCOS (about 50% may be normal).

The child who has premature adrenarche with elevated adrenal androgens (specifically DHEA-S and DHEA) is most likely to go on to develop PCOS. Children Children who have CAH may develop PCOS, but again, not all of them do.

Adults who have been given androgens may also develop a PCOS picture. In the adult increased obesity may bring out PCOS, which is probably a mild form of this disorder. If they succeed in losing weight, normal ovarian function returns. Insulin resistance may increase androgen production, but this is largely in the ovary. Medication to improve insulin sensitivity may return function to normal. Whether elevated androgens "cause" insulin resistance is still not clear.

Overall it is likely that a number of different abnormalities may cause this phenotypically similar syndrome. Whether the abnormality is of genetic or environmental etiology remains to be proven as does the absolute role of adrenal androgens.

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Insulin Resistance and Lipotoxicity in PCOS: Causes and Consequences

8

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Key Points

- Most of women with PCOS are insulin resistant and hyperinsulinemic.
- Insulin resistance seems to be an aggravating factor for hyperandrogenism.
- Abnormalities in fatty acid metabolism are common in women with PCOS.
- Lipotoxicity is known to cause insulin resistance but may also directly induce hyperandrogenism.
- Lipotoxicity treatment improves insulin sensitivity and lowers androgen levels.
- Lipotoxicity is an appealing candidate for PCOS pathogenesis since it could explain both the insulin resistance and hyperandrogenism of PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy mainly characterized by androgen excess and a major cause of anovulatory infertility in women. Therapeutic approaches often succeed in normalizing androgen production and/or restoring ovulatory cycles in women with PCOS. However, the exact etiology of the syndrome remains unknown. Several studies have established links between PCOS and various metabolic anomalies that are more prevalent in women with PCOS. Indeed, metabolic syndrome (30–46%), dyslipidemia (33%), glucose intolerance (30%), fatty liver disease (50%), and type 2 diabetes risks (32%) are all increased in women with PCOS compared to women with similar BMI without

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_8

PCOS [1–10]. Other known consequences of the insulin resistance syndrome are also commonly encountered in women with PCOS, including pro-coagulant [11–13], pro-inflammatory states [14–16] and endothelial dysfunction [17–20]. Importantly, all of these conditions, which are associated with severe health consequences, share a common underlying feature: insulin resistance [21–23]. Accordingly, recognizing these associated metabolic anomalies will help manage not only the clinical manifestations of the syndrome but also the long-term cardiometabolic risks of these women.

The diagnostic criteria established by the NIH conference in 1990, the Rotterdam conference in 2003, and the Androgen Excess and PCOS Society (AEPCOS) in 2006 use two criteria for the diagnosis of PCOS: (1) evidence of hyperandrogenism and (2) existence of ovarian dysfunction, manifested by oligo-anovulation or polycystic ovaries [5]. However, in the clinic, the wide phenotypic heterogeneity that is observed among women with PCOS makes it difficult to target and characterize the underlying causes. For example, a majority of women with PCOS present with overweight or obesity and display insulin resistance and compensatory insulinemia [24, 25]. Paradoxically, because a minority of women with typical PCOS display no sign of insulin disturbance [26, 27], insulin resistance does not seem to be necessary to develop the syndrome and should, therefore, not be part of the diagnostic criteria per se. However, it is currently unknown whether insulin resistance is a causal factor, an important contributor to the disorder, or rather just a not so innocent bystander. Consequently, determining the role of insulin resistance in the development of PCOS is an important challenge that needs further investigation.

Reminders Concerning Insulin Resistance

Definitions of Insulin Resistance

Physiologically, insulin exerts several effects on insulin-sensitive tissues such as the liver, skeletal muscles, and adipose tissue, promoting glucose promoting glucose uptake, glucose oxidation, glycogen storage and inhibiting hepatic gluconeogenesis and lipolysis from adipose tissue, all of which are known as its “metabolic” effects. However, insulin also exerts important “mitogenic” effects on cell proliferation and differentiation.

Glucose homeostasis relies on a delicate balance between insulin sensitivity and secretion. Insulin resistance occurs when insulin sensitivity is impaired in such a way that insulin can no longer exert its metabolic effects on insulin-sensitive tissues. When insulin insensitivity hampers glucose uptake in target tissues, insulin secretion is usually increased, resulting in compensatory hyperinsulinemia for as long as the pancreatic beta cells can compensate. Several techniques are used to assess insulin sensitivity, the euglycemic-hyperinsulinemic clamp (HEC) being the gold standard method [28]. However, it is important to distinguish the metabolic effects of insulin from its other effects, such as mitogenic effects, which will be discussed later.

Typically, in insulin resistance, the metabolic effects of insulin are preferentially disturbed, whereas the non-metabolic effects of insulin, such as its mitogenic effects, may remain intact.

Evolution of Insulin Resistance Knowledge in PCOS

The very first association between insulin resistance and pathophysiology of hyperandrogenism was actually reported in 1920 by Emile Charles Achard and Joseph Thiers. These investigators described the Achard-Thiers syndrome, in which highly virilized women developed type 2 diabetes. The characterization of PCOS by Stein and Leventhal occurred in 1935 [29]. Almost a century later, a large body of data affirms a relationship between endocrine and metabolic aberrations of PCOS with insulin resistance; despite the consistency across studies, however, a cause and effect relationship to the observed associations remains unclear, and the existence of intrinsic anomalies leading to insulin resistance in PCOS is still open for debate.

In 1980, Burghen and colleagues observed higher levels of insulin in patients with PCOS and obesity compared to healthy control women of similar BMI. They also observed significant positive correlations between circulating androgen and insulin levels [30]. Others also revealed new and significant positive correlations between testosterone or androstenedione with insulin levels, either under fasting state or during an oral glucose tolerance test (OGTT) [26]. However, Dunaif and colleagues were the first to assess insulin sensitivity in a small group of women with PCOS, both with and without obesity, using the HEC technique [31]. They showed lower insulin sensitivity in women with PCOS (n = 29) compared to BMI comparable healthy women (n = 18), suggesting that lower insulin sensitivity in PCOS could be independent of obesity and, thus, intrinsic to the syndrome. Results were confirmed later by the same group [32] and others [33–36], using different techniques to assess insulin sensitivity. More recently, also using the gold standard HEC method, a study showed that 95% of overweight/obese women with PCOS displayed insulin resistance, defined by insulin sensitivity less than the 25th centile of lean women, in comparison to 62% of overweight/obese women without PCOS. Also, 75% of normal-weight women with PCOS displayed increased insulin resistance [37]. Furthermore, insulin resistance was shown to appear early in the syndrome as early pubertal girls and adolescents with PCOS were shown to be more insulin resistant than aged-matched and BMI-matched adolescents without PCOS. This insulin resistance was present in normal-weight and obese girls with PCOS [33, 34, 38]. Moreover, peripubertal girls with a first-degree relative with PCOS display insulin resistance, beta-cell dysfunction, and resistance to insulin-mediated inhibition of lipolysis compared to age-matched control girls [39, 40]. Also, first-degree relatives of women with PCOS, men, women, and adolescents who did not display signs and symptoms of PCOS also show similar insulin resistance, hyperinsulinemia, and increased lipid anomalies, independent of adiposity levels [41, 42].

In general, it is accepted that the majority of women with PCOS will display a higher level of insulin resistance and compensatory hyperinsulinemia than a similar control woman, independently of adiposity. However, data are not entirely consistent in this regard, and some studies have shown that insulin resistance is not entirely intrinsic to the syndrome. In a study [43] based on a phenotypically heterogeneous PCOS group including 49 women with PCOS and 42 control women with similar BMI ranging from approximately 18–38 kg/m^2, lower insulin sensitivity was observed in women presenting with elevated BMI (>28 kg/m^2) but not in lean subjects with PCOS. In addition, the lower insulin sensitivity observed in some lean women with PCOS disappeared upon correction for truncal-abdominal subcutaneous fat distribution. According to this study, not all normal-weight women with PCOS would be insulin-resistant, but those with insulin resistance would display increased abdominal fat distribution, as already described in the general population. This suggests that weight and adipose tissue distribution are important determinants for the development of insulin resistance in women with PCOS. Interestingly, a mean loss of 12.4 kg, at the end of a 14-month diet, increased insulin sensitivity in 13 women with PCOS and obesity as compared to those seen in control women [44], thus suggesting once again a key role for weight in insulin resistance associated with PCOS. Increased BMI is associated to a large proportion to the development of metabolic syndrome features, but the evidence also shows that some women with PCOS and without obesity are not necessarily insulin-resistant.

The apparent discrepancies observed among studies regarding insulin sensitivity in women with PCOS versus those without PCOS are probably explained by the heterogeneity of PCOS phenotypes across studied populations and the potential impact of genetic background on insulin resistance in women with PCOS. Phenotypes of PCOS may vary according to the definition used, namely, the NIH, Rotterdam, or AEPCOS diagnostic criteria. Women with the phenotypic manifestations according to the 1990 NIH criteria are those with increased risk for type 2 diabetes, glucose impaired tolerance, and other metabolic syndrome components [22]. The heterogeneity of the disease is also affected by the complexity of the pathophysiology, with various source of hyperandrogenism (adrenal and/or ovarian) that may be identified within women with hyperandrogenism [45]. Obesity is a factor that enhances the metabolic phenotype of PCOS, with increasing metabolic syndrome traits and insulin resistance in these women. A high percentage of women with PCOS may display obesity (estimated to 50–80% of women with PCOS in the United States), but this percentage may represent an overestimation because of a referral bias in PCOS clinical studies [46, 47]. Our knowledge of insulin resistance in PCOS may then be largely influenced by the phenotypes that are clinically identified and included in clinical studies and may not represent the large array of manifestations in all women with PCOS.

Cumulating evidence shows that PCOS carries an inheritable susceptibility including genome-wide association studies (GWAS) [48–53], monozygotic twin study [54], familial clustering for PCOS [55], and, more recently, evidence that prenatal fetal exposure and epigenetic modifications may impact fetal metabolic pathways in women with PCOS [55, 56]. GWAS showed various genetic variants associated with PCOS, with impact either for metabolic function, androgen

biosynthesis, or gonadotropin physiology. The variety of affected pathways and various genetic susceptibilities argue for a heterogeneous syndrome that may display various levels of metabolic dysfunction.

Taken together, data from the literature suggest that insulin resistance and hyperinsulinemia are contributing factors that potentially exacerbate or even uncover PCOS in genetically or epigenetically predisposed women. This may explain why the majority of women with PCOS are more insulin-resistant than similar women without PCOS. Despite several lines of evidence of the contribution of insulin resistance to the pathophysiology of PCOS, the exact mechanisms involved remain poorly understood.

Insulin and Hyperandrogenemia in PCOS

The reduced action of insulin on metabolic regulation that is observed in the setting of insulin resistance is the result of a series of alterations affecting insulin signaling in insulin-sensitive tissues such as the liver, skeletal muscles, and adipose tissue. Importantly, various tissues may be differentially affected by insulin resistance, as well as various metabolic pathways. Compensatory hyperinsulinemia and/or alterations in the insulin signaling pathway leading to metabolic insulin resistance are suggested to directly affect the androgen biosynthesis pathway, thus contributing to the PCOS phenotype (Fig. 8.1). The next section describes how insulin influences androgen production as well as possible mechanisms involved in PCOS hyperandrogenemia.

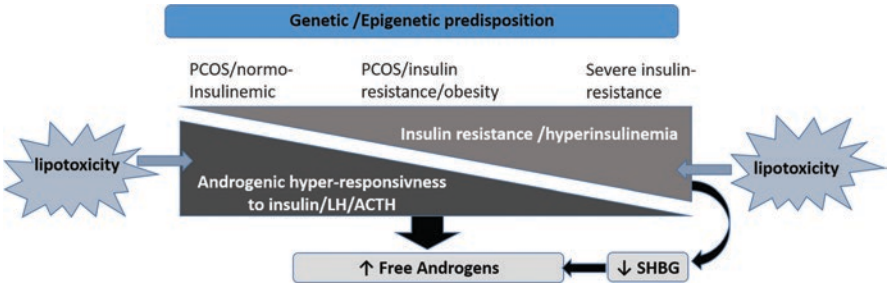

Fig. 8.1 Effects of insulin resistance and compensatory hyperinsulinemia on the development of hyperandrogenism in PCOS. Hyperandrogenism results from the interaction between lipotoxicity and hypersecretion of androgen by ovaries/adrenals in response insulinemia (and other physiological stimuli, such as LH and ACTH). PCOS is represented by a spectrum of phenotypes. On one end of the spectrum, PCOS may manifest because of a high genetic or epigenetic predisposition to the effects of lipotoxicity on androgen-secreting tissue despite low degree or absence of insulin resistance and hyperinsulinism. On the opposite end of the spectrum, women with high insulin resistance and hyperinsulinism may have hyperandrogenic manifestations despite a relatively low genetic/epigenetic predisposition. Insulin resistance and hyperinsulinemia are responsible for the increase in free androgen plasmatic levels, both directly, through the stimulation of ovarian and adrenal androgen synthesis, and indirectly, by decreasing hepatic production of SHBG. Obesity, promoting lipotoxicity and insulin resistance, exacerbates hyperandrogenism and clinical manifestations

In Vivo Observations

Elevated androgens may have an impact on insulin sensitivity [22], on the other hand, insulin resistance may have a central role on increased androgen production in women with PCOS. Several in vivo studies have attempted to dissect the role of insulin on androgen production, either in women with and without PCOS. Different approaches aimed at reducing insulin levels in hyperinsulinemic women with PCOS were first used to study the direct implication of insulin on androgen hypersecretion in PCOS. In 1989, Nestler and colleagues observed that in hyperinsulinemic women with PCOS and obesity, a short-term decrease in fasting and glucose-stimulated insulin levels, using diazoxide for 10 days, significantly reduced both free and total testosterone levels [57]. Similarly, a randomized controlled trial (RCT) showed that acarbose, which slows intestinal glucose absorption and thus postprandial insulinemia, significantly decreased the free androgen index in 30 hyperinsulinemic women with PCOS and obesity, compared to placebo [58]. In agreement with these data, a meta-analysis showed that acarbose decreased testosterone in comparison to placebo in a few other randomized controlled studies, without impact on BMI [59].

Regarding weight loss interventions, a recent 6-month intervention with a GLP-1 receptor agonist in 36 women with PCOS showed a decrease in insulin resistance measured by HEC, along with decreased free androgen index and inflammatory circulating markers [60]. Importantly, insulin sensitization in overweight women with PCOS, either with metformin, with GLP-1 receptor agonist, or with thiazolidinedione (TZDs), alone or in combination, improves hyperandrogenism [5, 61]. Weight loss interventions such as bariatric surgery [62] or lifestyle interventions [63] were also shown to improve hyperandrogenism in women with PCOS.

Similar benefits of insulin lowering therapies such as diazoxide, and insulin sensitizers such as metformin and the TZD rosiglitazone, have also been observed in normal-weight normoinsulinemic women with PCOS, even in women with normal insulin sensitivity [5, 27, 64]. In an RCT showing that metformin and rosiglitazone decrease androgen levels in such normal-weight women with PCOS and normal insulin sensitivity, it was interesting to note that metformin lowered insulin levels, an effect not seen with rosiglitazone [27]. Thus, lowering of androgens with metformin could be attributed to a decline in circulating insulin, similar to that seen with diazoxide, whereas lowered hyperandrogenemia with rosiglitazone may be due to restoration of the normal androgenic response to insulin.

An interesting study was conducted in nine young women with PCOS and normal weight or obesity and used a GnRH agonist stimulation followed by an insulin infusion for 17 h, as compared to a saline infusion, under adrenal steroidogenesis suppression with dexamethasone. This study reported that circulating progesterone and androstenedione levels were significantly increased 17 h post GnRH agonist stimulation under insulin infusion, as well as testosterone levels after 24 h [65]. Since gonadotropic hormone levels (LH/FSH) did not differ between infusion conditions, these results suggest that the hyperinsulinemic condition potentiated ovarian androgenic response to gonadotropic hormones. However, studies in women without PCOS have showed that lowering insulin does not decrease basal androgen production and that the induction of transitory hyperinsulinemia during an HEC

procedure does not increase androgen production [66, 67]. Taken together, these observations support that hyperinsulinemia in itself does not induce PCOS in a woman without predisposition to PCOS but does contribute to hyperandrogenemia in both insulin-resistant and insulin-sensitive women with PCOS.

Insulin also indirectly contributes to hyperandrogenemia by lowering sex hormone-binding globulin (SHBG) levels. This carrier protein binds testosterone in the plasma, reducing levels of its free circulating bioactive form. Plasma SHBG levels have been shown to be lower in women with PCOS [68, 69], causing an increase in the bioavailable testosterone. In hyperinsulinemic women with PCOS and obesity, the direct reduction in insulin levels with diazoxide for 10 days [68] or with acarbose for 6 months [58] increases SHBG levels. These results suggest that the low SHBG levels found in women with PCOS are directly caused by the compensatory hyperinsulinemia associated with insulin resistance. Such reduction in SHBG levels is not limited to PCOS but is also commonly observed in pre-diabetic states or established type 2 diabetes, in both men and women [70–73]. Moreover, SHBG is produced by the liver, and low levels of SHBG have been associated with fatty liver disease in PCOS [74, 75] and in individuals without PCOS [76]. It has been suggested that SHBG production is impacted by increased de novo lipogenesis, a consequence of hepatic insulin resistance and hyperinsulinemia, which could also contribute to increased androgen concentration found in women with PCOS and fatty liver disease, the latter being more prevalent than in women without PCOS of similar BMI [76, 77].

Another important characteristic of PCOS is increased ovarian and adrenal responsiveness to LH or ACTH, respectively [78–80]. Moreover, it has been shown that suppression of LH secretion with a GnRH long-acting analog in women with PCOS does not correct ovarian hypersensitivity to hCG stimulation [81]. However, treatments improving insulin resistance in lean and obese women with PCOS have been shown to curb the exaggerated androgenic response to ACTH [82, 83] and LH [84, 85], suggesting that such hyperresponsiveness is probably due to factor(s) related to insulin sensitization rather than to LH, ACTH, or ovarian steroids per se.

In Vitro Observations

Several defects in insulin action, which are mostly located at the level of the insulin signaling pathway, have been observed in vitro in different PCOS cell types or tissues. Differential defects in different pathways and tissues in women with PCOS explain the coexistence of insulin resistance in many tissues with insulin-dependent androgen overproduction in androgen-producing tissues.

Insulin Signaling in Physiological Conditions

Under physiological conditions, the interaction of insulin with its receptor results in receptor dimerization and autophosphorylation by its own tyrosine kinase activity (Fig. 8.2). This enables recruitment of the first proteins involved in signal

transduction, mainly the insulin receptor substrate protein family, or IRS. Thereafter, the numerous actions of insulin are mediated via two main pathways, with cross-talks between them. The first is responsible for the "metabolic" effects of insulin and regulate energy balance. The "mitogenic" effects of insulin on cell proliferation and survival are mediated via the second pathway, which is aptly called the *mitogenic pathway*.

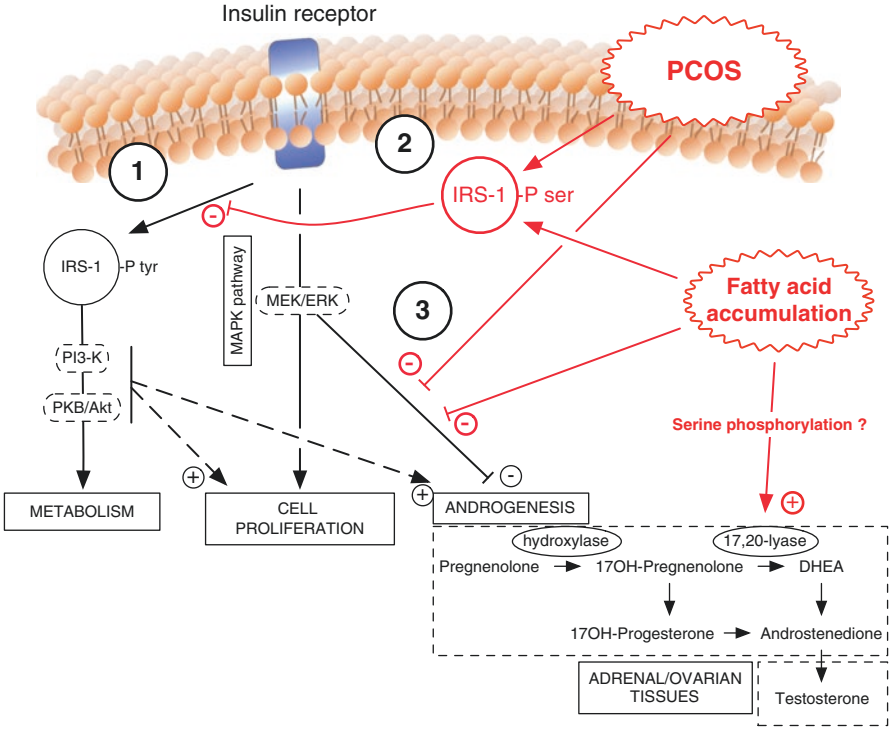

Fig. 8.2 Cellular consequences of lipotoxicity in PCOS. A summary of theoretical mechanisms leading to the establishment of insulin resistance and androgen overproduction in insulin-sensitive tissues and androgen-secreting cells in women with PCOS. (1) Insulin binding to its receptor activates IRS-1 protein by tyrosine phosphorylation, which in turn activates the PI3-kinase and PKB/Akt proteins that mainly mediate metabolic effects of insulin and could also affect cell proliferation and androgenesis. (2) Serine phosphorylation of IRS-1 observed in PCOS and insulin resistance states impairs metabolic effects of insulin by preventing its tyrosine phosphorylation. This phenomenon could potentially be caused by fatty acid accumulation. (3) The constitutive inhibitory effect that the mitogenic pathway of insulin (MEK/ERK) exerts on androgenesis could be removed by fatty acid accumulation through the inhibition of MEK/ERK; such inhibition of MEK/ERK activity is observed in PCOS and could lead to increased androgenesis. In addition, fatty acids could directly increase the 17,20-lyase activity of the P450c17 through the serine phosphorylation mechanism by unknown serine/threonine kinase(s). (Adapted with permission from Baillargeon [138]. (Endocrine Updates; vol 27))

Metabolic Pathway Anomalies

Insulin metabolic pathway alterations in PCOS have been studied in classically defined insulin-sensitive tissues (i.e., tissues that are sensitive to its main metabolic actions: myocytes, adipocytes and hepatocytes), showing impaired glucose utilization in these tissues [32, 86, 87].

Impaired metabolic pathway defects involve insulin receptor and post-receptor alterations. Indeed, studies found that insulin-stimulated autophosphorylation of the insulin receptor β (beta)-subunit was decreased in myocytes, as well as fibroblasts from women with PCOS [88, 89]. In these tissues, serine phosphorylation of the insulin receptor is associated with a decrease in tyrosine phosphorylation and impaired activation of insulin receptor. In addition, treatment with a non-specific serine kinase inhibitor or a protein kinase A-specific inhibitor, respectively, totally or partially restored insulin-stimulated tyrosine phosphorylation of the receptor. Treatment of the cells with Gö6983, a pan-protein kinase C inhibitor, had no effect. Thus, the drop in signal transduction after insulin stimulation in PCOS cells could be the result of altered basal serine phosphorylation of the insulin receptor.

The mechanisms of serine phosphorylation of insulin receptor and its substrates have been abundantly studied in research related to type 2 diabetes [90–92]. Among the main proteins recruited by the activated insulin receptor are the IRS proteins. Following recruitment, these proteins are usually phosphorylated on tyrosine residues by the insulin receptor itself. Once again, serine phosphorylation of IRS-1 and 2, instead of tyrosine phosphorylation, impairs transmission of the insulin signal and may thus be responsible for insulin resistance in PCOS. This phenomenon has been observed in muscle fibers from women with PCOS [93] displaying an increase in IRS-1 serine phosphorylation compared to muscle fibers from normal women. These results support the notion that excessive serine phosphorylation of the insulin receptor and/or IRS by a yet unidentified serine kinase may be involved in the metabolic insulin resistance of PCOS.

Furthermore, post-receptor defects impairing insulin signaling have also been suggested in the setting of PCOS. One of the terminal effects of metabolic insulin action is the translocation of GLUT4 glucose transporters to the cell membrane in order to allow glucose uptake in insulin-sensitive tissues. Post-receptor defects in the insulin signaling cascade have also been suggested to lead to reduced GLUT4 translocation and/or expression and, thus, deficient glucose uptake, which is typical of metabolic insulin resistance. Reduced GLUT4 expression has been directly detected in adipocytes from women with PCOS in several studies [94]. As compared to control adipocytes, Ciaraldi et al. [87] detected in PCOS adipocytes a drop of maximal insulin effect under a sub-maximal dose of insulin (0.17 nM), but not at a supra-physiological dose of insulin (8.5 nM), which could be indicative of the presence of adipocyte insulin resistance under low doses of insulin in these women. Other results of this study suggested that the signaling defects responsible for the loss of metabolic effects of insulin in adipocytes or muscle cells of women with PCOS may not be explained by the level of Akt activation nor by differences in the expression of several other metabolic insulin signaling proteins, although the activation state of these proteins was not assessed.

Muscle insulin resistance was also associated with mitochondrial dysfunction that may be intrinsic to the syndrome. Indeed, myocyte mitochondrial gene expression implicated in oxidative metabolism has been shown to be reduced in muscle of women with PCOS [95]. In vivo impaired mitochondrial function was associated with peripheral insulin resistance in adolescents with PCOS [33] suggesting a contribution of impaired energy metabolism to peripheral insulin resistance in PCOS.

Mitogenic Pathway Anomalies

Besides its actions on glucose regulation, insulin also regulates cell proliferation and differentiation via the insulin mitogenic pathway. Following insulin stimulation, a cascade of phosphorylation mediators leads to the activation of several transcription factors, which in turn regulate the expression of genes involved in cell proliferation and differentiation [96]. Interestingly, defects in the metabolic pathway do not necessarily affect the mitogenic pathway and vice versa [97], since each possesses a respective set of exclusive signaling actors. Differential alterations between metabolic and mitogenic pathways have been characterized experimentally in different cell models [90, 98, 99]. For example, Books et al. [100] found that PCOS skin fibroblasts displayed metabolic insulin resistance compared to cells from healthy controls, but mitogenic functions per se seemed unaltered. This was evidenced by the absence of significant difference in thymidine incorporation, a marker of proliferation, between PCOS and control fibroblasts under insulin or IGF-1 stimulation.

Existence of potential crosstalk and interaction between both insulin signaling pathways has been observed in vitro in muscle cells from women with PCOS [101]. For example, inhibition of MEK1/2 activation, one of the main mediators of the insulin mitogenic pathway, may result in a decrease of IRS-1 serine phosphorylation and an increase of its interaction with Akt, which is important for the metabolic actions of insulin. These results highlight the fact that, in women with PCOS, the presence of defects affecting insulin's mitogenic pathway may also affect metabolic signaling, at least in specific tissues such as skeletal muscle.

Insulin-signaling defects have also been reported in steroidogenic organs in PCOS. In vitro studies have been performed in bovine or porcine ovarian thecal cells as well as in ovarian tissue from women with PCOS. It is worth noting that different insulin-signaling defects have been reported for the ovarian tissue, some being the opposite of those observed in muscle or adipose tissue. Moreover, defects in the mitogenic insulin-signaling pathway directly impact androgen biosynthesis in the ovarian tissue. Indeed, Nelson-Degrave et al. found that cultured PCOS ovarian thecal cells displayed a significant decrease in the activation of the mitogenic pathway mediator MEK/ERK, compared to the same type of cells from healthy women [102]. They showed in vitro that the loss of MEK/ERK activity could explain, at least in part, increased androgen production in PCOS thecal cell under insulin stimulation. Indeed, hyperactivation of MEK/ERK pathway inhibited androgen production, and conversely, inhibition of this pathway significantly stimulated androgen synthesis. These results suggest that, under physiological

conditions, the MEK/ERK pathway in the ovary could exert constitutive inhibition of androgen production and that lifting of this inhibition due to insulin-signaling defects associated with PCOS could contribute to the development of ovarian hyperandrogenism.

Altogether, in vitro studies suggest that women with PCOS are characterized by defects in both the metabolic and mitogenic insulin-signaling pathways that can explain, respectively, the metabolic insulin resistance and ovarian hyperandrogenism that are typical of PCOS. The mechanisms of such defects are still unknown, and it is unclear whether there is a link between the excessive serine phosphorylation of the insulin receptor and IRS and the constitutive inhibition of the MEK/ERK signal, for example. Interestingly, one mechanism that could possibly explains both the cellular increase in serine phosphorylation state and decrease in the MEK/ERK signal is lipotoxicity, which is explained in the next section.

Lipotoxicity in PCOS

Evidence of Lipid Metabolism Dysfunction in PCOS

Insulin resistance induced by lipid ectopic deposition and/or mitochondrial dysfunction, impacting insulin signaling and glucose utilization, is well described in type 2 diabetes and other metabolic conditions [92]. Indeed, adipocyte dysfunction and abnormal fatty acid metabolism are important defects widely investigated for their implication in muscle and liver insulin resistance as well as beta-cell failure [92, 103]. Under dysmetabolic conditions, adipocyte dysfunction can lead to impaired free fatty acid storage, including dietary free fatty acid spillover and excess adipose tissue lipolysis, leading to excess free fatty acids in circulation. The deleterious effects of fatty acids in overexposed tissues are gathered under the term of "lipotoxicity." Thus, the lipotoxicity theory is important to characterize the perturbations that could ultimately lead to the development of type 2 diabetes [103].

In addition to lipotoxicity, adipocyte dysfunction is also associated with adipose tissue inflammation, low-grade systemic inflammation, increased secretion of adipokines implicated in insulin resistance development (leptin, resistin, visfatin, etc.), and reduced production of adiponectin, an adipokine known to improve insulin sensitivity [103–106]. The role of inflammation in PCOS is discussed elsewhere in this book and is not the focus of this chapter.

Lipotoxicity has not yet been widely studied in PCOS research, despite the well-established link between PCOS and a risk for several metabolic complications, including type 2 diabetes [2–4, 107]. Some studies have shown an increased levels of circulating non-esterified fatty acid (NEFA) and higher postprandial triglycerides in lean and obese women as well as adolescents with PCOS [108–111]. Also, serum untargeted metabolomics studies have shown increased serum long-chain fatty acids and lipid metabolites in women with PCOS in comparison to women without PCOS [112]. In adolescents with PCOS and obesity, long-chain NEFA (C14:0, C16:1, and C18:1) under HEC conditions were higher in girls with PCOS compared

to BMI-matched controls, suggesting impaired insulin-mediated inhibition of lipolysis or clearance of NEFA. Indeed, C16:1 and C18:1 were strongly associated with insulin-mediated glucose disposal during the HEC, which classically defines metabolic insulin resistance [113]. In this study, free androgen index related negatively to short-, medium-, and long-chain acylcarnitines, markers of incomplete long-chain fatty acid oxidation that can lead to the lipotoxic effects of NEFA [113].

In adolescents with obesity, suppression of the rate of appearance of NEFA under hyperinsulinemic conditions was lower in girls with PCOS than BMI comparable adolescent controls, suggesting impaired adipose tissue insulin sensitivity and excess lipolysis [108]. However, in normal-weight adolescents with PCOS, this was not the case, suggesting that obesity may exacerbate lipotoxicity, early in the disease [34]. Furthermore, in peripubertal girls at high risk for PCOS, namely, with a first-degree relative with PCOS, insulin suppressed to a lesser extent NEFA concentration during an intravenous glucose tolerance test than age-matched controls [39]. This was associated with insulin resistance that was also higher in this group, independent of BMI.

Adipose Tissue Depot in Women with PCOS

Subcutaneous adipose tissue tends to have a protective storage function, whereas visceral adipose tissue has a more dysfunctional profile that is associated with increased lipolysis, fatty acid spillover, and inflammatory profile. Several studies assessed the impact of PCOS on body fat distribution among women with obesity, overweight, and/or normal weight. The recent literature does not allow to conclude if women with PCOS are more prone to increased visceral fat than similar women without PCOS, due to important discrepancies in the conclusions of these studies [114]. However, a recent systematic review showed that women with androgen excess (mostly women with PCOS) display an association between androgen levels (free and total) and increased abdominal and/or visceral adipose tissue accumulation [114]. Increased adipocyte cell size, decreased lipoprotein lipase activity, and plasma adiponectin were shown in normal-weight, overweight, and obese women with PCOS [115]. Subcutaneous adipose tissue dysfunction, with limited expansibility to the expense of increased visceral fat, and adipose tissue insulin resistance may be some characteristics explaining increased lipotoxicity in women with PCOS, contributing to lean organs dysfunction and insulin resistance.

Impact of Lipotoxicity on Androgenesis

Lipotoxicity could contribute to the insulin resistance of PCOS, as demonstrated in obesity [103] and other conditions characterized by insulin resistance, such as type 2 diabetes, Cushing's syndrome, and inherited or acquired states of lipodystrophy [103, 116–118]. However, a more interesting question is whether lipotoxicity could contribute directly to PCOS hyperandrogenism; if the latter was indeed true, this

would suggest that lipotoxicity may be a key factor in PCOS pathogenesis. To begin to answer this question, the effects of overexposure to NEFA on androgenesis have been investigated in vivo [119, 120]. Mai and colleagues first studied the effects of an intralipid/heparin (IH) infusion on androgen production in eight healthy young men [119]. The infusion of IH induced a transitory increase in whole-body circulating NEFA levels because heparin stimulates endothelial LPL activity and hydrolysis of the triglycerides from the intralipid emulsion in all tissues. The authors observed an increase in the levels of the adrenal androgens DHEA and androstenedione during a 6-h IH infusion as compared to saline infusion. In addition, they detected this increase before any increase in circulating insulin levels was apparent, suggesting that hyperandrogenic effect was independent of NEFA-induced insulin resistance and/or compensatory hyperinsulinemia [119]. Moreover, neither ACTH, LH, nor FSH levels significantly changed during the infusion, suggesting an increase in the androgen responsiveness to physiological stimuli. Two years later, the same team performed a similar experimentation on 12 healthy young women and confirmed their earlier findings. In addition, they observed an increase in the production in both adrenal and gonadal androgens, as well as estrogens, in women following IH infusion [120]. These results are the first to suggest that overexposure to circulating NEFA directly increases androgen production in healthy women. We also found that, in follicular fluid of women undergoing in vitro fertilization, follicular testosterone concentration was associated with follicular lipids and lipid metabolites (long-chain acylcarnitines), suggesting that ovarian androgen production is related to ovarian lipotoxicity [121].

Using bovine adrenal fasciculata/reticularis cells as an in vitro androgen-producing cell model, we observed similar results [122]. We reported significant effects of a 48-h exposure of these cells to palmitate, a saturated fatty acid, on ACTH- or forskolin-induced androgen production. Indeed, bovine adrenal fasciculata/reticularis cells treated with palmitate (100 μM) produced 37% and 68% more DHEA, the main adrenal androgen, under ACTH or forskolin stimulation, respectively, when compared to untreated cells. Moreover, under forskolin stimulation we observed significant decrease of the ERK1/2 phosphorylation after palmitate exposure. This result is consistent with the loss of MEK/ERK phosphorylation reported in ovarian thecal cells from women with PCOS by Nelson-Degrave et al. [102] and the stimulation of androgen production that they observed in vitro after experimental MEK/ERK inhibition [102]. These findings thus suggest that palmitate could impair MEK/ERK pathway under forskolin-stimulated condition in adrenal androgen-producing cells and, therefore, induce androgen production. However, complementary results are necessary, and specific inhibitors of the MEK/ERK pathway should be used to confirm whether fatty acids could induce such a defect in vitro.

In PCOS, several anomalies directly affecting the key enzymes involved in steroidogenesis and more specifically in androgen production have been described. Expression of P450c17, the key enzyme responsible for DHEA and androstenedione synthesis, and the stability of its transcripts were increased in ovarian thecal cells from women with PCOS [123, 124]. In addition, it has been shown recently that

upstream enzymes in the steroidogenic pathway, such as P450 cholesterol side-chain cleavage, could probably be also affected in a similar way [125]. The P450c17, which possesses two distinct activities (17α-hydroxylase and 17,20-lyase), may be regulated through serine phosphorylation mechanisms [126–128], although this has not been clearly characterized yet. Indeed, evidence suggests that a serine phosphorylation of this enzyme can increase its 17,20-lyase activity over its 17α-hydroxylase activity, thus increasing androgen synthesis relatively to glucocorticoids. We previously mentioned the existence of abnormal serine phosphorylation at different steps of the metabolic insulin-signaling pathway that was associated with metabolic insulin resistance in women with PCOS. All these findings thus suggest dysregulated serine kinase activity in PCOS that could affect both the metabolic insulin-signaling pathway and the P450c17/17,20-lyase activity. Moreover, a potential mechanism for lipotoxicity is the activation of serine kinase cascades after intracellular accumulation of reactive lipids, such as diacylglycerol (DAG), long-chain acyl-coenzyme A, acylcarnitines, and ceramides [92, 103]. These lipotoxic mediators accumulate in NEFA-exposed cells when their ability to esterify or beta-oxidize NEFA is exceeded [103]. In muscle or liver, it has been shown that the insulin resistance induced by increased serine phosphorylation of IRS-1/2 was likely due to activation of a serine kinase, such as novel PKC isoforms, after intracellular accumulation of DAG, acyl-coA, and ceramides [92, 129–131]. Therefore, both PCOS insulin resistance and hyperandrogenesis may be due to an increase in the serine phosphorylation state of insulin-sensitive and androgenic cells following accumulation of these reactive lipids.

Directs and Indirect Effects of Thiazolidinediones on Androgenesis

Many studies have demonstrated that peroxisome proliferator-activated receptor gamma (PPARγ) agonists, the thiazolidinediones (TZDs), can normalize insulinemia and androgen levels in women with PCOS [83, 132]; these data underscore the implication of NEFAs in the development of PCOS. TZDs, namely, rosiglitazone or pioglitazone, activate PPARγ receptors that will increase the expression of key genes for fatty acid storage in the adipose tissue and beta-oxidation in non-adipose tissues (see Fig. 8.3). Therefore, TZDs can improve pan-corporal fatty acid metabolism, decrease ectopic fat deposition, and improve insulin resistance in insulin-sensitive tissues [133, 134], presumably by reducing lipotoxicity. Interestingly, TZDs have been shown to significantly improve androgen levels in women with PCOS [135, 136]. However, it is unclear whether the effects on androgens are due to insulin lowering following improvement of insulin resistance or directly to improved beta-oxidation of NEFAs in androgen-secreting cells and, thus, reduction in lipotoxic effects. Our finding that 6-month treatment with a TZD significantly reduced androgen levels in lean normoinsulinemic women with PCOS, without affecting insulin levels, greatly supports a direct mechanism independent of insulin stimulation [27]. Furthermore, an in vitro study suggested that pioglitazone

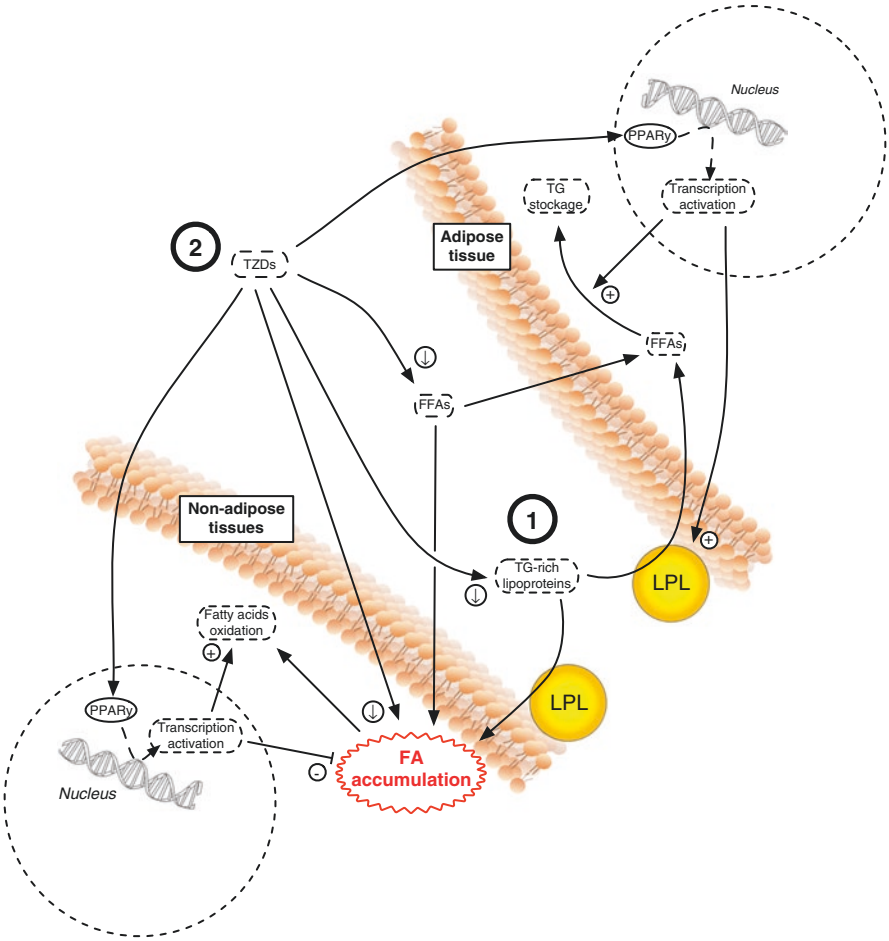

Fig. 8.3 General effects of thiazolidinediones on lipid metabolism in adipose and non-adipose tissues. (1) TG-rich lipoproteins release FFAs under the action of the LPL; this release is followed by the entry of fatty acids that will be stored as TG in adipose tissue or will be accumulated and oxidized in non-adipose tissues. (2) TZDs bind PPARγ, which will in turn activate the transcription of several genes. In adipose tissue, it stimulates the LPL activity and enhances the entry and the storage of FFAs. In non-adipose tissues, it enhances fatty acid oxidation and inhibits their accumulation. Through these mechanisms, TZDs decrease lipid accumulation in non-adipose tissues and promote fat redistribution in adipose tissue. TZDs thiazolidinediones, FFAs free fatty acids, FF fatty acid/s, LPL lipoprotein lipase, TG triglyceride, PPARγ peroxisome proliferator-activated receptor gamma

could directly correct the increased expression of P450c17 that occurs in NCI-H295R cells after inhibition of the MEK/ERK pathway [137]. Once again, these data corroborate the results observed by Nelson-Degrave et al. [102] regarding a potential implication of constitutively decreased activation of the MEK/ERK pathway in PCOS hyperandrogenemia, which may be due to lipotoxicity and released by the anti-lipotoxic effects of TZDs.

Summary

It is difficult to determine whether insulin resistance is the main cause, the consequence, or a bystander of hyperandrogenism in PCOS. Since insulin resistance and hyperinsulinemia do not seem to be necessary for the development of the syndrome, they are more likely contributing factors rather than being the essential trigger. However, depending on individual characteristics (phenotype and genotype/epigenome), the contributions of insulin resistance to the development of PCOS may strongly vary among individual women. On the other hand, current evidence in the literature suggests that lipotoxicity in insulin-sensitive tissues is a key mechanism in the establishment of insulin resistance. Moreover, studies have shown that overexposure of tissues to NEFA could also increase androgen production. Together, these data support the hypothesis that impaired NEFA metabolism in women with PCOS could explain both the insulin resistance and the hyperandrogenism that characterize the syndrome. An increased susceptibility of androgen-secreting cells to altered NEFA metabolism may exist in women destined to develop PCOS. This underpinning may also predispose most, but not all, women with PCOS to the same susceptibility in their insulin-sensitive tissues. The lipotoxicity paradigm can thus explain the metabolic and endocrine aberrations of PCOS. The degree of tissue susceptibility to lipotoxicity however could greatly differ among women with PCOS and explain the large variety of phenotypes in PCOS, including why insulin resistance may be absent in some women with otherwise typical features of PCOS.

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Obesity, Dysmetabolic and Proinflammatory Milieu of Polycystic Ovary Syndrome

9

James J. Morong and Frank González

Key Points

- Beyond its energy storing capacity, adipose tissue acts as an endocrine and immunological organ.
- Epigenetic silencing of genes relevant to metabolism during fetal development is triggered by inflammation in response to a high-fat maternal diet setting the stage for postnatal accumulation of adiposity.
- Accumulation of adiposity causes dysfunction of the adipose tissue compartment that leads to chronic low-grade inflammation, metabolic derangement, and endocrine imbalance.
- Obesity exacerbates the signs and symptoms of polycystic ovary syndrome (PCOS) by worsening pre-existing chronic low-grade inflammation and insulin resistance.
- The amount of fat accumulation determines the extent to which adiposity promotes insulin resistance and hyperandrogenism in PCOS.
- Obesity increases the risk of developing type 2 diabetes mellitus, dyslipidemia, and hypertension in PCOS.
- Nutrient-induced inflammation that is independent of adiposity can induce molecular alterations in PCOS that may be the underpinning of insulin resistance, β-cell dysfunction, atherogenesis, and ovarian dysfunction in this disorder.

Introduction

Obesity has grown in pandemic proportions in industrialized countries throughout the world likely due to inexpensive calorie dense food, community structure, and technology that reduces physical activity and favors inexpensive nonphysical

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_9

entertainment [1]. More recent evidence suggests that epigenetic programming of obesity begins in the fetus exposed to the abnormal metabolic environment of obese mothers [2]. Beyond its energy storing capacity, adipose tissue acts as an endocrine and immunological organ and contains several cell types that contribute to its various functions. Evidence suggests that insulin resistance is the link between obesity and metabolic derangement leading to an increased risk of developing type 2 diabetes mellitus (T2DM), dyslipidemia, and hypertension [3]. Obesity can initiate or exacerbate the signs and symptoms of polycystic ovary syndrome (PCOS) by worsening metabolic abnormalities present in PCOS such as chronic low-grade inflammation and insulin resistance. Lessons learned from the proinflammatory state of obesity have proven to be useful in understanding key pathogenic mechanisms in PCOS that are independent of adiposity.

Definition of Obesity and Body Fat Distribution

Obesity is defined as excess adipose tissue in the body. In the clinical setting, obesity is defined as an excess in body mass index (BMI) (weight in pounds/(height in inches)^2 × 703 or weight in kilograms/(height in meters)^2) [4, 5]. Overweight and obese BMI ranges have been established (Table 9.1) [5]. The interpretation of the application of BMI has been widely scrutinized [6]. The cutoff points for identifying health risks are arbitrary. Moreover, lower BMI thresholds have been suggested when health risks are correlated with more accurate measures of body fat [7]. It has also been suggested to establish separate ethnic-based BMI designations [8].

Although a BMI ≥30 kg/m^2 is generally associated with excess adipose tissue, BMI alone cannot differentiate between lean and fat mass stores, the proportions of which are affected by age, gender, athleticism, and menopausal status [4, 5]. Obesity, as defined by BMI, tends to be more representative of increased fat mass in women than in men. Sarcopenic obesity from age-related muscle loss is more profound in women than men [7]. Thus, greater BMI in the average sedentary women tends to be more reflective of greater adiposity when compared with men [9].

The BMI should be considered a starting point for the assessment of obesity. Indirect measures of body fat include waist, thigh, and hip circumferences, calculated waist-to-hip ratio (WHR), and waist-to-thigh (WTR) ratio [4]. Waist

Table 9.1 Classification of body mass index[a]

Classification		Body mass index, kg/m^2
Underweight		<18.5
Normal weight		18.5–24.9
Class 1	Overweight	25–29.9
Class 2	Obesity	30–34.9
Class 3		35–39.9
Class 4		≥40

[a]Modified from Ref. [5]

circumference (WC) is measured at the level of the iliac crest after a normal expiration, while hip circumference (HC) is measured at the widest point around the buttocks. Mid thigh circumference (TC) is measured with the individual positioned with slightly flexed knees and both feet flat on the floor [10].

In humans, adipose tissue is present in subcutaneous and visceral locations throughout the body. WC reflects both subcutaneous and intra-abdominal/visceral adiposity and correlates most strongly with BMI. Conversely, HC and TC reflect mostly subcutaneous fat and muscle [11]. Although elevated WHR and WTR suggest increased visceral adipose, these may alternatively indicate decreased muscle or subcutaneous fat [11, 12]. With aging, adiposity becomes more centrally localized within the abdomen independently of weight [4]. Since central obesity is associated with higher morbidity, it has been recommended to measure WC and/or WHR besides BMI in the clinical setting [11].

Neck circumference is emerging as a new anthropometric index for obesity because it is a more stable measurement than the aforementioned measures of body fat. Moreover, measurement of WC in particular requires identification of anatomic landmarks that are not always obvious in obese individuals [13]. Neck circumference is measured at the inferior margin of the laryngeal prominence, and closely correlates with BMI, as well as with obesity-related cardiovascular risks and visceral fat content [13].

Direct measures of body composition provide reliable estimates of total and regional body fat. Although techniques such as bioelectrical impedance, dual energy X-ray absorptiometry, computerized tomography, and magnetic resonance imaging have all been utilized for measurement of body composition, there currently is no universally recommended approach to assess body composition in either clinical or research settings. The choice of using either clinical or direct methods to examine body composition is typically based on the patient population and specific characteristics desired for interpretation [14]. Gender-specific thresholds for classifying obesity using direct measures of body composition suggest ≥25% body fat for men and ≥30% for women [5].

Prevalence of Obesity

The most recent data from the National Health and Nutrition Examination Survey 2017–2018 found that 42.8% of adults in the United States were obese, which has increased from prior years. Men and women had similar prevalence of obesity, and no significant increase was observed with age for either gender [15].

Adipose Tissue Anatomy

The adipocyte is the primary cell type of the adipose tissue. Adipocytes comprise 50% of the adipose tissue contents and are traditionally characterized as brown or white [16]. Brown and white adipocytes are of different embryonic origins [16].

More recent evidence suggests a third category of "beige" adipocytes possessing characteristics of both white and brown cells may be commonly found in adults [16]. Aggregates of adipocytes are held in a framework by tissue collagen (Fig. 9.1) [17]. The non-adipocyte components of the adipose tissue are known as the *stromal-vascular fraction* and consist of mononuclear cells (MNC), resident MNC-derived macrophages, pre-adipocytes, fibroblasts, mesenchymal stem cells, and blood vessels [18]. Although the brown adipocytes are more richly perfused, each white adipocyte is still in close proximity to a capillary [17, 19]. The blood supply facilitates the delivery of metabolic substrates to and removal of metabolic end products from the adipose reservoirs [20].

White adipocytes are by far the predominant type of adipocyte in both genders and appear as spherical unilocular vacuoles. The cytoplasm of the white adipocytes is mostly composed of lipid and contains small mitochondria that are pushed to the periphery. These cells produce the hormone leptin, which

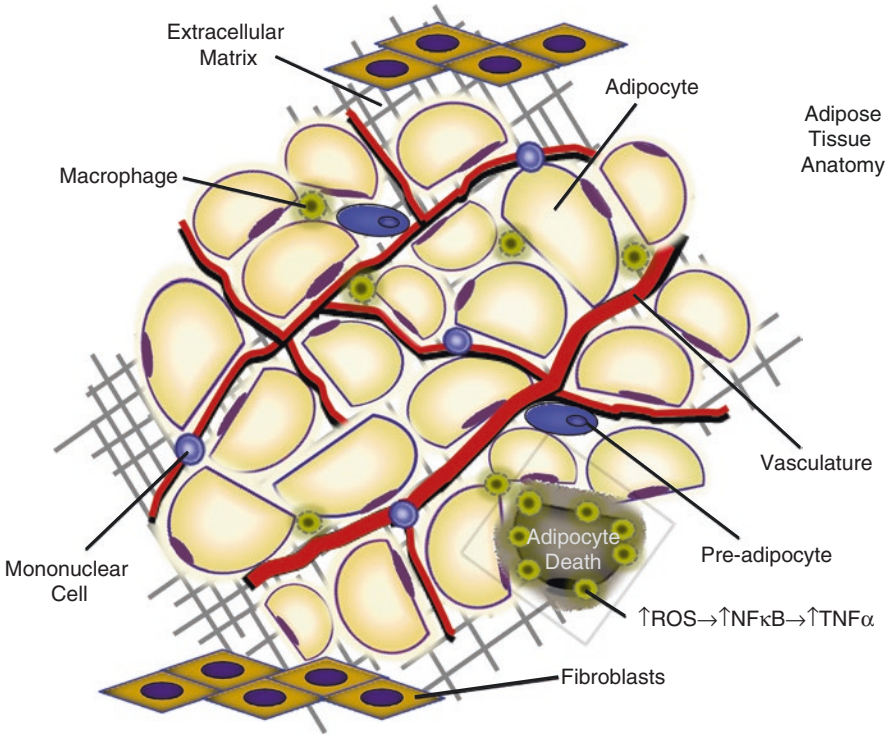

Fig. 9.1 Adipose tissue holds aggregates of adipocytes in an extracellular collagen matrix [12]. The non-adipocyte components are contained in the stromal-vascular compartment and consist of mononuclear cells (MNC), MNC-derived macrophages, pre-adipocytes, fibroblasts, and blood vessels [14]. Adipocyte death induced by hypoxia in the expanded adipose tissue of the obese leads to migration of circulating MNC into the adipose stromal-vascular compartment [118, 119]. Phagocytic activity by MNC-derived macrophages induces oxidative stress and inflammation [121, 122]

influences food intake and promotes release of stored energy during fasting in the form of free fatty acids (FFA) [13]. White adipocytes can enlarge in size (hypertrophy) by increasing the lipid content within their cytoplasm or undergo cell division to increase in quantity (hyperplasia). Hypertrophy increases with overall fat mass and transitions to hyperplasia with further lipid accumulation once the cytoplasm reaches a critical volume [13, 17] as a result of aging or chronic overfeeding. Brown adipocytes, in contrast, contain numerous cytoplasmic vacuoles; the mitochondria are large and are innervated by adrenergic nerves that are activated by low temperatures to generate heat [16]. These cells are primarily found in the intrascapular region of infants but can also be present in adults chronically exposed to extreme cold [16].

Adipose Tissue Metabolism

Dietary Fat Absorption

Dietary fat absorption (Fig. 9.2) involves three steps as follows: (1) enterocyte uptake, (2) intracellular processing, and (3) delivery to the mesenteric lymph. Ingested dietary fats consist mostly of triglycerides. Pancreatic triacylglycerol lipase hydrolyzes triglycerides into FFA and glycerol in the lumen of the small intestine [21]. The FFA and glycerol cross from the lumen into the enterocytes by simple diffusion and are shuttled into the endoplasmic reticulum with the aid of fatty acid binding proteins. The translocated FFA and glycerol are reformed into triglycerides and get packaged with dietary or endogenously synthesized cholesterol into prechylomicrons within the enterocytes. Recent evidence suggests that FFA in the enterocytes serve many purposes including re-esterification into triacylglycerol, phospholipids, or cholesterol esters that will be secreted as lipoproteins and used in cell membrane synthesis or cytoplasmic lipid resynthesis. FFA may also be used for fatty acid oxidation or cellular signaling [22]. Final processing of the prechylomicrons occurs in the Golgi apparatus of the enterocytes to form chylomicrons [23]. Transport vesicles carry mature chylomicrons to the basolateral

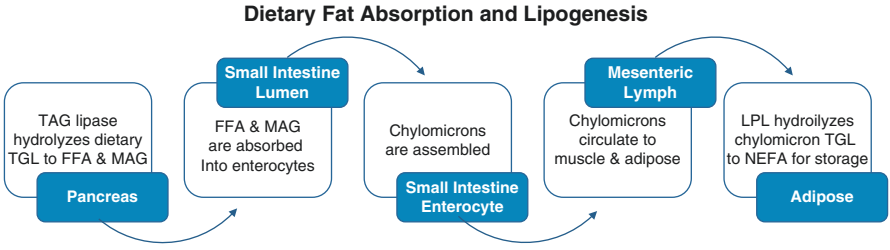

Fig. 9.2 The process of dietary fat absorption and lipogenesis occurs with the aid of enzymes in the gut and circulation that break down or reform fat molecules into various sizes for transport and eventual storage into adipose tissue. TAG triacylglycerol, FFA free fatty acids, MAG monoacylglycerides, LPL lipoprotein lipase, NEFA nonesterified fatty acids [21, 23]

membrane for exocytosis into the mesenteric lymph, and the chylomicrons eventually enter the circulation for transport to key target organs such as muscles and the adipose tissue [21, 23].

Energy Storage and Utilization

Chylomicrons and very low density lipoproteins (VLDLs) are too large to pass through the adipose tissue capillaries. The triglycerides they contain get released into the capillary lumen and hydrolyzed by adipocyte-derived lipoprotein lipase into nonesterified fatty acids (NEFA) (Fig. 9.2). NEFA subsequently enter the adipocytes by passive diffusion, where they are esterified to reform triglycerides [24].

Utilization of fatty acids as a source of energy is dependent on glucose availability. NEFA are important sources of energy during times of energy deprivation, particularly during fasting or physical exercise. Adipose tissue metabolism has opposing regulation by insulin and catecholamines. Insulin promotes fat storage in a process called lipogenesis, whereas catecholamines promote fat breakdown in a process called lipolysis [24, 25]. Lipogenesis consists of both the de novo formation of FFA from carbohydrate stores in the liver and the formation of triglycerides from NEFA in the adipose tissue [26]. Insulin stimulates the uptake of glucose into adipocytes where it is converted to glycerol; the resulting glycerol combines with NEFA to form triglycerides, which then accumulate in the adipose tissue [24].

Lipolysis consists of the breakdown of the triglycerides into NEFA and glycerol and is regulated by catecholamine-induced hormone-sensitive lipase and adipose triglyceride lipase/desnutrin [24]. Other proteins such as perilipin either cover or uncover the core of the triglycerides, controlling their exposure to these lipases [24, 25].

Adipose Tissue as an Endocrine and Immunological Organ

Steroid Hormone Peripheral Conversion: Role of Adipose Tissue

Adipose tissue is capable of metabolizing steroids, but is not a site for de novo synthesis of steroid hormones [27, 28]. White adipocytes within adipose tissue express 17β-hydroxysteroid dehydrogenase, which converts androstenedione to testosterone, a more potent androgen, and estrone to estradiol, a more potent estrogen (Fig. 9.3). Cytochrome-P450-dependent aromatase is also expressed in white adipocytes and is responsible for aromatization of androgens to estrogens within the fat stores (see Fig. 9.3) [26, 29]. Thus, the expanded adipose tissue in obesity increases the peripheral conversion of androgens to estrogens, thereby promoting a hyperestrogenic state.

White adipocytes express the enzyme 11β-hydroxysteroid dehydrogenase type 1 which converts the less potent circulating glucocorticoids such as cortisone and 11-dehydrocorticosterone to the more potent cortisol and corticosterone,

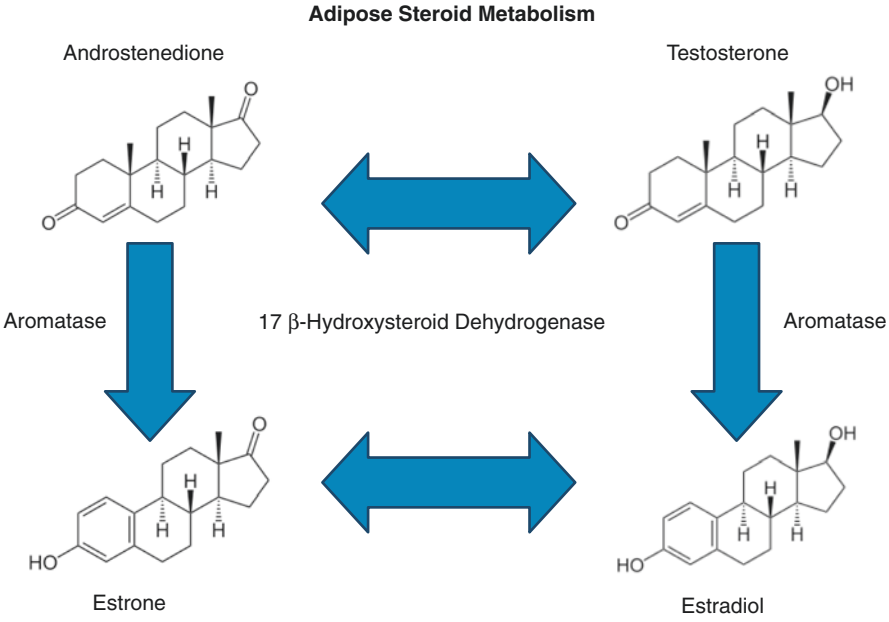

Fig. 9.3 Adipose tissue is an endocrine organ. Androstenedione and testosterone are interconverted by 17β-hydroxysteroid dehydrogenase activity and are respectively converted to estrone and estradiol by aromatase activity [26, 29]

respectively [30]. Local glucocorticoid activity within the adipose tissue is involved in maturing pre-adipocytes from the stromal-vascular fraction to adipocytes [30]. However, overexpression of 11β-hydroxysteroid dehydrogenase type 1 in obesity leads to glucocorticoid excess within the adipose tissue. The subsequent increase in local glucocorticoid activity promotes abdominal fat accumulation, particularly in the visceral compartment similar to what is observed in Cushing's syndrome via mechanisms that are incompletely understood [30].

Adipokine Function

Adipose tissue produces many proteins called adipokines that aid in adipose tissue metabolism as well as in immunomodulation for host defenses [18]. The substantial listing of adipokines includes proinflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin (IL-6), also known as chemokines, as well as leptin and adiponectin [18]. Resident macrophages in the adipose tissue can generate reactive oxygen species (ROS) and produce TNFα and IL-6 to a greater extent in visceral fat compared with subcutaneous fat. TNFα in particular, through paracrine effects on the adipocytes, enhances lipolysis that causes the release of FFA. Resident MNC-derived macrophages and adipocytes express toll-like receptor-4 (TLR-4) that can bind either FFA or lipopolysaccharide (LPS), both of which stimulate

chemokine production from either of these cells to perpetuate local and systemic inflammatory responses. Interestingly enough, pre-adipocytes can transform into macrophage-like cells that are also involved in processes that determine the innate immunity [31].

Leptin is primarily secreted by subcutaneous adipose tissue and acts as a hormone to enhance satiety at the level of the hypothalamus, and thus plays a critical role for regulation of body weight [32]. Leptin also acts locally in the white adipose tissue to regulate energy expenditure by stimulating triglyceride depletion without increasing FFA release. This favors the use of FFA instead of glucose as a fuel source [32–34]. Although leptin-deficient states are associated with hyperphagia and insulin resistance, obesity itself induces a state of leptin resistance despite high circulating leptin levels [18]. These leptin elevations promote inflammation characterized by increased chemokine production from circulating mononuclear cells (MNC) and resident MNC-derived macrophages of the adipose tissue. In contrast, adiponectin, another adipokine, inhibits inflammation by preventing macrophage differentiation into foam cells and decreases LPS-mediated TNFα secretion from macrophages. Adiponectin also improves insulin resistance by improving skeletal muscle glucose uptake and decreasing hepatic gluconeogenesis [18].

Development of Obesity

The development of obesity in pandemic proportions is a multifactorial phenomenon with the foundations of an obesogenic environment laid in the early 1980s [1]. The modern technologically advanced urban lifestyle is more sedentary now than before, which has reduced opportunities for energy expenditure [35]. Fewer occupations require physical labor, transportation is readily available so fewer people walk, and entertainment such as television and computers has decreased leisure activities that require physical effort [1, 35–37]. This has been compounded by a widespread availability of processed and sugar-rich foods. Excess macronutrient intake exceeding expenditure forms a basis for a positive energy balance that ultimately sets the stage for progressive weight gain [1, 38].

Specific dietary components play a role in the maintenance of a healthy weight and body composition versus promotion of weight gain and adipose tissue accumulation. Dietary protein content is more satiating than the dietary carbohydrates. Fat contains the most energy per gram, but dietary fat is the least satiating [38, 39]. Body can easily oxidize excess protein and carbohydrates, whereas it tends to store excess consumed fats [38, 40, 41]. The unfortunate overemphasis on a “low fat” diet in the late 1980s led to replacement of fat with sugar and carbohydrates that compensated for taste and calories in the process [1]. However, excessive intake of carbohydrates that are high on the glycemic index scale due to their rapid metabolism that causes blood sugar fluctuations serves as a stimulus for excessive insulin secretion. Since insulin is an anabolic hormone, these types of carbohydrates get easily stored as fat [34]. Conversely, high-fat diet has also been associated with overeating due to its poor effect on satiety culminating in weight gain [35, 38, 42].

The satiety cascade relies on key peripheral peptides such as ghrelin and leptin [38]. Ghrelin is a hormone secreted by gastric mucosal cells that increases appetite and stimulates food intake [40, 42]. In fact, ghrelin administration has been shown to increase appetite scores and caloric intake compared with placebo [34, 41]. Leptin inhibits ghrelin secretion in the brain as well as from the gastric mucosa [31, 33, 43]. Fasting and weight loss suppress leptin secretion [44]. A high-fat diet has a variable effect on leptin secretion but can clearly suppress the secretion of ghrelin [39]. The mechanical stretch on the stomach caused by a large meal initiates a secretion cascade of a variety of satiety peptides and hormones within the gastrointestinal tract such as cholecystokinin, glucagon-like peptide-1, gastric inhibitory peptide, and peptide YY ultimately culminating in decreased ghrelin secretion [39]. However, physiological ghrelin suppression in response to a meal is attenuated in obese individuals causing a continued hunger sensation [42]. Furthermore, leptin resistance in the presence of high body fat mass increases leptin secretion and maintains adiposity in a status quo [39]. While leptin administration causes profound weight loss in leptin-deficient and severely leptin-insufficient individuals, this approach only leads to modest weight loss in individuals with obesity because of their leptin resistance [45].

Genetic factors also play a role in the development of obesity since there is 40–70% heritability [46]. Genome-wide association studies have identified several single nucleotide polymorphisms (SNP) that likely contribute to obesity and obese traits. Data do not support the concept of there being a single obesogenic gene; it appears that there are SNPs related to individual traits in the obesity syndrome such as body fat, birthweight, WHR, visceral adiposity, and extreme obesity. At the center of these is FTO, a SNP variant that is present in nearly all obesity phenotypes across multiple ancestries [47], and affects leptin, its receptor, and post-receptor signaling [43, 44, 47]. These genetic factors as well as other imprinted genetic syndromes, copy number variants, and susceptible genetic loci increase the susceptibility to gain weight by promoting a positive energy balance [1, 44–46].

Growing evidence suggests that prenatal epigenetic phenomena significantly contribute to the development of obesity of the modern era [2]. Maternal diet high in fat content results in a lipid-rich fetal environment [48]. In fact, lipid excess triggers inflammation that causes epigenetic modifications such as DNA methylation and histone deacetylation to silence genes that are of metabolic relevance and that are normally expressed during fetal development [49]. These phenomena permanently alter fetal organ structures, cell numbers, and metabolism, and the effects can be transmitted across generations [2, 49]. Microarray variations serving as epigenetic biomarkers are now being identified to predict metabolic disorders later in life [50]. Several genes have been shown to be epigenetically regulated, including the genes for leptin, suppressor of cytokine signaling-3 (SOCS-3), and glucose transporters [51, 52]. Consequently, maternal obesity perpetuates a paradigm of worsening obesity in the offspring [2, 36, 53, 54], a paradigm that can explain the prevalent and growing pandemic of adiposity.

Medical Disorders Associated with Obesity

Obesity is associated with a higher risk of developing impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, cardiovascular disease (CVD), and obstructive sleep apnea (OSA) [55]. In fact, increased adipose tissue alone has been uniformly documented to increase CVD-related mortality after controlling for BMI [56]. The prevalence of OSA also increases as the neck circumference and percentage of total body and visceral fat increase [57, 58].

Weight gain and physical inactivity collectively lead to insulin resistance in the skeletal muscle, liver, and the adipose tissue [59]. This in turn causes a compensatory increase in pancreatic insulin secretion and to eventual pancreatic β-cell failure in predisposed individuals. In fact, a family history of T2DM is a genetic marker for the development of glucose intolerance and T2DM [60].

The incidence of dyslipidemia is increased in obesity. Very low density lipoprotein (VLDL) transports triglycerides to peripheral tissues and is formed by a lipoprotein synthesis cascade that proceeds from apolipoprotein B (apoB) to intermediate density lipoprotein (IDL) to low density lipoprotein (LDL) to VLDL [61]. All of these lipoproteins along with triglycerides tend to be elevated in obesity due to delayed hepatic clearance as a result of increased hepatic secretion of VLDL apoB [62]. The more atherogenic smaller particle size LDL in particular is more prevalent in individuals with obesity compared with their lean counterparts [3]. High density lipoprotein cholesterol (HDL-C) transports cholesterol from peripheral tissues back to the liver either directly or through reverse cholesterol transport [61]. HDL-C tends to be low in obesity as a result of increased hepatic uptake [62].

Obesity contributes to hypertension through several mechanisms. Increases in circulating leptin, insulin, and FFA activate both the sympathetic nervous system and the renin-angiotensin-aldosterone system [63]. In the latter instance, increases in plasma aldosterone levels cause abnormal sodium retention which raises the arterial blood pressure [3]. Obesity is also associated with vascular inflammatory changes that lead to endothelial dysfunction, decreased nitric oxide responsiveness, increased endothelin-1, and renal dysfunction, all of which contribute to systemic hypertension [63, 64].

Obesity is one of the criteria for metabolic syndrome, a condition associated with a significantly higher risk of coronary artery disease and all-cause mortality even in the absence of cardiovascular symptomatology. In an effort to accurately identify women with metabolic syndrome worldwide, the International Diabetes Federation has refined the previous North American-based definition as the presence of an elevated waist circumference >80 cm or a BMI ≥30 kg/m^2, plus any two of the following four additional clinical criteria: elevated triglycerides >150 mg/dL, low HDL cholesterol <50 mg/dL or previous treatment for dyslipidemia, elevated blood pressure ≥130 mmHg systolic or >85 mmHg diastolic or previously used antihypertensive regimen, and elevated fasting glucose >100 mg/dL or previously diagnosed T2DM [65, 66].

Metabolic Dysfunction in PCOS: Prevalence, Pattern, and Health Risks

Insulin resistance affects 50–70% of women with PCOS and is an underlying risk factor for developing metabolic dysfunction [67, 68]. When present in normal weight women with the disorder, the degree of insulin resistance can be similar to that of obese individuals without PCOS [69, 70]. Thus, metabolic abnormalities in PCOS can be independent of obesity. There are much higher rates of glucose intolerance, T2DM, dyslipidemia, hypertension, and subclinical atherosclerosis in PCOS compared with the general female population [66]. In a study by Legro et al., the prevalence of impaired glucose tolerance (IGT) and T2DM among rural women with PCOS was 31% and 8% overall, respectively, and 10% and 1.5%, respectively, in the absence of obesity [71]. Additionally, there are ethnic differences in the prevalence of metabolic disorders within PCOS, with a higher risk of IGT in women from Asia and the Americas compared with European women [72]. Nonobese women with PCOS have higher LDL-C and higher total cholesterol compared with nonobese women without the disorder after adjusting for alcohol intake, smoking, and exercise [71]. Hypertension is also more likely to occur in women with PCOS even after accounting for age, BMI category, diabetes, and dyslipidemia [73, 74]. Thus, it is not surprising that there is a high prevalence of the metabolic syndrome in PCOS [75], with a twofold higher prevalence in women with PCOS diagnosed by NIH criteria compared with women in the general population when matched for age and BMI (43–47% versus 23%) [76, 77].

Impact of Metabolic Dysfunction on Ovarian Dysfunction in PCOS

Although the cause of ovarian dysfunction in PCOS is poorly understood, insulin resistance is also thought to play a role in this abnormality. Theca cell androgen production capacity is increased in the polycystic ovary [78]. Ovarian androgen hypersecretion occurs in women with PCOS in response to hCG administration regardless of weight class [79, 80]. The compensatory hyperinsulinemia of insulin resistance is considered to be a promoter of the hyperandrogenism and chronic oligo- or anovulation in PCOS, particularly when there is concomitant obesity. Hyperinsulinemia is thought to impede ovulation in the disorder by altering the synthesis and pulsatile pattern of luteinizing hormone (LH) and FSH secretion and by potentiating the secretion and bioavailability of androgens [81, 82]. While insulin may serve as a co-gonadotropin that amplifies LH-mediated androgen synthesis [83], exposure to insulin alone only increases androgen production from cultured theca cells of women with PCOS in the presence of supraphysiological insulin concentrations [84]. In contrast, physiological insulin infusion alone does not increase circulating androgens in PCOS [70]. Given that as many as 30–50% of women with PCOS are of normal weight and do not have insulin resistance [85], it is likely that another modulator is involved in driving the ovarian dysfunction in PCOS.

Impact of Obesity on PCOS

Perpetuation of Signs and Symptoms of the Disorder

PCOS with Superimposed Obesity

The prevalence of obesity in PCOS is greater (30–75%, pooled estimate 61%) compared with the general population (~25%), although estimates vary widely [72, 74, 75]. Obese individuals regardless of whether they have PCOS are more insulin resistant than normal weight women with PCOS [86]. The resultant compensatory hyperinsulinemia is evident in both the fasting and postprandial states. In contrast, normal weight women with PCOS exhibit a milder form of insulin resistance that only results in postprandial hyperinsulinemia [86].

As opposed to the lesser degree of hyperinsulinemia observed in normal weight women with PCOS, the more profound hyperinsulinemia present with obesity per se inhibits hepatic sex hormone binding globulin (SHBG) synthesis, thereby increasing androgen bioavailability to heighten the hyperandrogenic effect in obese women with PCOS [87]. The superimposed obesity in PCOS is also associated with hyperactivity of the hypothalamic-pituitary-adrenal axis, leading to increased adrenal androgens and worsening of the hyperandrogenic state [88]. In contrast, obese ovulatory women have a relative hypogonadotropic hypogonadism characterized by decreased LH pulse amplitude that dampens LH-mediated ovarian androgen production, thus minimizing the androgenic effects of low SHBG [89, 90]. In obese women with PCOS, further weight gain towards the morbidly obese range can eventually lead to superimposed hypogonadotropic hypogonadism that presents with normal or low normal androgen levels in the face of secondary amenorrhea and persistent stigmata of hyperandrogenism [91].

Obese women with PCOS have lower SHBG levels than normal weight women with PCOS. This translates into greater elevations in circulating free testosterone, and as a consequence, there is a greater degree of hirsutism and menstrual disturbance in the obese compared with the normal weight population of women with PCOS [87, 92]. Treatment of anovulatory infertility in obese women with PCOS often requires higher doses of fertility medication, and subsequent ovulation and birth rates are lower compared with those of normal weight women with PCOS [93]. Conversely, significant improvements in ovulatory responses can be seen with weight loss alone.

It has been proposed that PCOS itself may predispose to weight gain. Although resting energy expenditure is normal in PCOS, studies conflict as to whether thermogenesis is decreased in the postprandial state [94, 95]. Disordered hunger and satiety signals have also been reported in PCOS and may be due to ghrelin dysregulation [96, 97]. More importantly, the superimposed obesity, once entrenched, promotes further weight gain. This is because the more profound hyperinsulinemia of obesity exerts insulin's anabolic action to a greater extent, thereby leading to a more rapid adipose deposition [34]. Thus, PCOS per se does not lead to the development of obesity.

Obesity-Induced PCOS

It is possible that obesity promotes the development of PCOS in predisposed individuals rather than the converse. The signs and symptoms of PCOS typically become evident around menarche in normal weight women with the disorder [98]. In contrast, some obese women with PCOS encountered in clinical practice develop these signs and symptoms later in life following progressive weight gain to the obese range. This observation is supported by the restoration of menstrual cyclicity, normalization of hyperandrogenemia, and resolution of acne in these individuals once sufficient weight loss has been achieved with lifestyle modification or following bariatric surgery [99, 100]. However, hirsutism is a more permanent malady and usually persists after weight loss, even though the progression is halted [101].

A predisposing factor is needed for obesity to induce PCOS because the disorder does not develop in all obese women. Although this predisposing factor remains unclear, the presence of polycystic ovaries is a prime candidate. Polycystic ovaries are quite common in premenopausal women, especially at an early age, with a prevalence of 24–32% [102, 103]. In contrast, polycystic ovaries are present in 87–92% of women with PCOS [104, 105]. In a small, longitudinal study over 8 years, asymptomatic women with polycystic ovaries did not develop PCOS if they remained normal in weight [106]. Polycystic ovarian morphology typically resolves with age even if modest weight gain below the obese range occurs in the interim [106]. Clinically relevant endocrine and metabolic abnormalities are lacking in this population [103]. Nevertheless, ovarian and metabolic dynamic testing has uncovered subclinical abnormalities characterized by androgen secretion and insulin sensitivity that are intermediate between women with PCOS and normal ovulatory women [107, 108]. It is possible that obesity-related inflammation and insulin resistance has an adverse impact on ovarian function should polycystic ovaries be present at the time the critical weight limit is superseded and that PCOS develops in the process [109]. For confirmation, long-term longitudinal studies are needed that prospectively evaluate the effects of weight gain on asymptomatic normal weight women with polycystic ovaries.

Perpetuation of Medical Risks of Metabolic Dysfunction in PCOS

The risk of medical illness attributable to metabolic dysfunction increases in PCOS in the presence of obesity. There is a preponderance of an android body habitus in PCOS even in normal weight women with the disorder [110]. Consequently, most obese women with PCOS exhibit fat accumulation in the abdomen, particularly within the visceral compartment. Abdominal obesity is strongly correlated with the development of insulin resistance [111]. Visceral adipose tissue in particular is highly sensitive to lipolytic stimulation by androgens that facilitates increased portal FFA availability [112]. This in turn leads to fat accumulation in the liver, reduced hepatic insulin clearance, and the induction of hepatic insulin resistance [113].

The greater degree of insulin resistance that occurs in PCOS with the additional presence of obesity further increases the prevalence of IGT and T2DM and worsens

the fasting lipid profile [70, 89, 114]. Moreover, obese women with PCOS tend to exhibit higher triglycerides and decreased HDL-C levels [115, 116]. Obesity-related increases in circulating endothelin-1 and activation of both the sympathetic nervous system and the renin-angiotensin-aldosterone system also contribute to worsening hypertension in PCOS [74, 75]. The increased severity of all of these metabolic abnormalities when obesity is present in PCOS can lead to the development of metabolic syndrome that increases the risk of incurring a cardiovascular event. Not surprisingly, the majority of women with PCOS who meet the criteria for metabolic syndrome are obese. In fact, abdominal obesity is the most common feature of metabolic syndrome in the disorder [78, 117].

OSA occurs more often in obese women with PCOS compared with obese women who lack the syndrome [60]. In fact, the prevalence of OSA in obese women with PCOS approaches the higher prevalence known to occur in men [60]. Hyperandrogenemia and low progesterone related to chronic ovulatory dysfunction are implicated in the pathogenesis of OSA in PCOS; androgens are recognized to decrease upper airway stability and ventilatory drive, and progesterone stimulates respiration to lower upper respiratory resistance [118, 119]. OSA is particularly associated with insulin resistance and appears to be an obesity-related phenomenon given the very low prevalence of this sleep disorder in nonobese women with PCOS [120–123]. OSA is discussed in greater detail elsewhere in this textbook.

Metabolic Inflammation in PCOS

Obesity-Related Inflammation

It is well established that obesity is a prooxidant, proinflammatory state. Hypoxia-induced adipocyte death in the expanded adipose tissue of the obese leads to migration of peripheral blood MNC into the adipose stromal-vascular compartment (Fig. 9.1) [124, 125]. These MNC subsequently undergo morphological alteration to become resident macrophages. Phagocytic activity by MNC-derived macrophages induces membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [126]. Oxidation of NADPH by the NADPH oxidase generates superoxide, a ROS that induces oxidative stress [127]. This phenomenon activates the transcription factor known as nuclear factor κB (NFκB), the cardinal signal of inflammation, by promoting its dissociation from the inhibitory protein, inhibitory κB (IκB) [128]. NFκB is freed to undergo nuclear translocation and subsequent DNA binding to promote transcription of a variety of inflammatory mediators that upregulate the aforementioned molecular events and directly promote insulin resistance and atherogenesis.

TNFα and IL-6 are proinflammatory cytokines produced in excess inflamed adipose tissue following upregulation of the NFκB inflammation pathway. These cytokines primarily originate from MNC-derived macrophages, but also serve to stimulate TNFα and IL-6 production in adipocytes through paracrine interaction [129]. While TNFα is a known mediator of insulin resistance, the impact of IL-6 on

insulin resistance is variable [130–132]. Moreover, IL-6 is clearly involved in the promotion of atherogenesis [133].

TNFα mediates insulin resistance in obesity-related diabetic syndromes by upregulating SOCS-3 that can bind tyrosine 960 of the insulin receptor [134] and by activating the JNK1 MAP kinase pathway that increases serine phosphorylation of insulin receptor substrate-1 (IRS-1) [130, 135]. Both of these mechanisms prevent IRS-1 from binding to the insulin receptor in insulin-sensitive tissues [130, 135]. In the process, insulin signaling is truncated, leading to decreased expression of GLUT4, the insulin-sensitive glucose transport protein, and a subsequent decrease in glucose transport that prompts a compensatory hyperinsulinemia [136, 137]. On the other hand, IL-6 mediates atherogenesis in consort with other proatherogenic inflammatory mediators produced in response to NFκB activation such as soluble intercellular cell adhesion molecule-1 (sICAM-1) and monocyte chemotactic protein-1 (MCP-1). IL-6 stimulates synthesis of the acute phase reactant known as C-reactive protein (CRP) to some extent in adipose tissue, but primarily in the liver through its unique endocrine effect [138]. Whereas sICAM-1 causes attachment of MNC to the vascular endothelium, MCP-1 facilitates migration of MNC into the vascular interstitium. CRP subsequently promotes the uptake of lipids into MNC-derived foamy macrophages within the atherosclerotic plaques [139, 140]. Thus, the proinflammatory environment of the expanded adipose tissue compartment in obesity is a major driver of metabolic aberration and clinically relevant consequences.

Chronic Low-Grade Inflammation in PCOS

There is a genetic basis for the chronic low-grade inflammation observed in PCOS. Several proinflammatory genotypes including those that encode TNFα and the type 2 TNF receptor as well as IL-6 and its signal transducer are associated with PCOS [141–144].

Chronic low-grade inflammation has been implicated as the underpinning of insulin resistance in PCOS. The insulin receptor in the disorder is genetically and functionally normal. Insulin resistance in PCOS unrelated to obesity is caused by a post-receptor defect in insulin signaling, with increases in SOC-3 expression and serine phosphorylation of IRS-1 implicated as mechanisms for decreased insulin-stimulated IRS-1 activation and decreased GLUT4 mobilization [81, 145, 146]. The discovery that circulating levels of TNFα are elevated in normal weight women with PCOS not only provided the initial clue that PCOS is a proinflammatory state [147], but also raises the possibility that TNFα is a mediator of insulin resistance in the disorder. Moreover, the ability of TNFα to drive the aforementioned mechanisms makes it an ideal candidate for initiating the molecular events that truncate insulin signaling in PCOS (Fig. 9.4) [130, 134–136].

A number of studies addressing the status of chronic low-grade inflammation in PCOS have focused on the measurement of circulating CRP using high-sensitivity assays. In fact, a meta-analysis revealed that CRP is the most reliable circulating marker of chronic low-grade inflammation in PCOS [148]. In the general

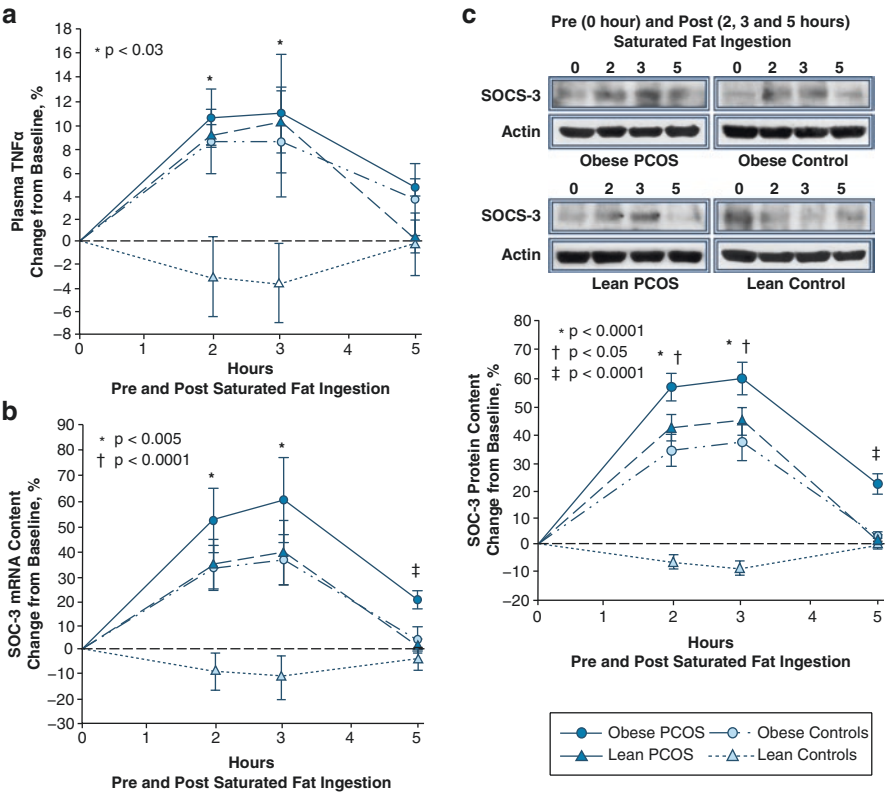

Fig. 9.4 Change from baseline (%) in (**a**) plasma tumor necrosis factor α (TNFα) levels along with mononuclear cell (MNC)-derived (**b**) suppressor of cytokine signaling-3 (SOCS-3) mRNA content and (**c**) SOCS-3 protein content from blood samples collected while fasting and 2, 3, and 5 hours after saturated fat ingestion. Representative Western blots (**c**) from the four study groups showing the change in quantity of SOCS-3 and actin in MNC homogenates in samples collected before and after saturated fat ingestion. *Response in lean and obese women with PCOS and obese control subjects was significantly greater compared with lean control subjects at 2 and 3 hours post-saturated fat ingestion. †Response in obese women with PCOS was significantly greater compared with obese control subjects at 2 and 3 hours post-saturated fat ingestion. ‡Residual response in obese women with PCOS was significantly greater compared with lean women with PCOS and both control groups at 5 hours post-saturated fat ingestion. (Adapted from González et al. [79], with permission. Copyright Oxford University Press and the Endocrine Society, 2019)

population, CRP levels >3 mg/L are equally predictive of a cardiovascular event compared with the ATP III criteria for metabolic syndrome [149]. However, the CRP elevation in normal weight women with PCOS (<3.0 mg/L) is still much lower in magnitude compared with what is observed in the obese (>3.0 mg/L) regardless of PCOS status [150, 151]. Thus, the milder CRP elevations observed in normal weight women with PCOS are obscured in the presence of obesity but are nonetheless indicative of intermediate atherosclerotic cardiovascular risk [152]. Furthermore,

circulating inflammation markers such as CRP are but one reflection of systemic inflammation in conjunction with circulating MNC as outlined in the next section.

Nutrient-Induced Inflammation in PCOS

Chronic low-grade inflammation is manifested by persistent activity of circulating MNC [153]. In fact, circulating MNC has been the object of intense investigation in efforts to increase the understanding of the mechanisms responsible for metabolic abnormalities in PCOS. The discovery that nutrients such as glucose and saturated fat can trigger an inflammatory response in MNC has shed light on the molecular events that may promote insulin resistance and cardiovascular risk in PCOS.

Upregulation of the NFκB Inflammation Pathway

Mitochondrial respiration within MNC is the result of glycolysis and β-oxidation of lipids, which are driven by the ingestion of glucose and saturated fat, respectively [154]. Some glucose is diverted to the hexose monophosphate shunt to generate NADPH, while some saturated fat in the form of FFA is converted to diacylglycerol [155, 156]. Saturated fat ingestion also upregulates the gene expression of $p47^{phox}$, the key cytosol component of NADPH oxidase [157]. Diacylglycerol promotes phosphorylation of $p47^{phox}$ to initiate its translocation to the cell membrane to form a functional enzyme complex [158]. Increased NADPH activity leads to ROS-induced oxidative stress and NFκB activation [126, 159]. This in turn promotes TNFα and IL-6 gene transcription in a fashion similar to what occurs within excess adipose tissue in obesity [159].

In PCOS, ingestion of glucose and saturated fat induces a prooxidant inflammatory response as evidenced by increases in MNC-derived ROS generation, $p47^{phox}$ mRNA and protein content, NFκB activation, TNFα mRNA content, and SOCS-3 mRNA and protein content, in addition to increases in circulating TNFα, all of which are independent of obesity [152, 160–163]. Compared with normal ovulatory women, MNC secretion of TNFα and IL-6 is also altered in PCOS by glucose and saturated fat ingestion in vivo and by physiological exposure to these nutrients in vitro [164–168]. Furthermore, these markers of oxidative stress and inflammation are associated with glucose- and lipid-stimulated measures of insulin sensitivity and/or fasting measures of insulin resistance in normal weight and obese women with and without PCOS combined [152, 160–168]. Thus, nutrient-induced inflammation in PCOS culminates in proinflammatory signaling known to be involved in the development of insulin resistance and atherogenesis.

Upregulation of Atherothrombotic Inflammation Pathways

In metabolically aberrant conditions such as advancing age and obesity, glucose ingestion activates activator protein-1 (AP-1) and early growth response-1 (EGR-1), two additional proinflammatory transcription factors involved in the initiation of a

cardiovascular event [169, 170]. AP-1 regulates the transcription of a family of matrix metalloproteinases (MMP) [171]. MMP-2 and MMP-9 in particular are involved in extracellular matrix remodeling within the blood vessel wall [172, 173]. EGR-1 regulates the transcription of tissue factor (TF), the receptor for coagulation factor VII that induces thrombin generation to promote fibrin formation and platelet activation [174]. MMP-2, MMP-9, and TF are produced by MNC-derived foamy macrophages and activated vascular smooth muscle cells within atherosclerotic plaque [175]. Excessive extracellular matrix breakdown by MMP-2 and MMP-9 culminates in atherosclerotic plaque rupture, thereby exposing TF to the circulating blood. This in turn triggers thrombosis and culminates in blood vessel occlusion [176, 177].

Saturated fat ingestion also promotes atherogenesis and insulin resistance in metabolically aberrant conditions through classic inflammation signaling involving the toll-like receptor (TLR) system and its ligands in MNC-derived macrophages [178]. Oxidative stress in response to saturated fat ingestion damages vital cellular proteins, but also induces a concomitant rise in a variety of circulating heat shock proteins (HSP) such as HSP-70 that bind and stabilize these proteins [178–180]. In the process, however, HSP-70 binds TLR-2 [177] that culminates in AP-1 activation to upregulate the transcription of MMP-2 and MMP-9 [181]. Furthermore, lipopolysaccharide (LPS) from gut-related bacteria enters the circulation with saturated fat ingestion and binds to TLR-4, after delivery by LPS binding protein (LBP) previously intercalated to the cell membrane [182–184], thereby activating NFκB to upregulate the transcription of TNFα, IL-6, sICAM-1, and MCP-1 [128, 159].

In PCOS, circulating levels of MMP-2, MMP-9, TF, HSP-70, LPS, and MCP-1 are elevated [185–190]; and ingestion of glucose or saturated fat activates AP-1 and increases circulating HSP-70 and the MNC-derived protein content of TLR-2 and MMP-2 independent of obesity [190, 191]. In contrast, ingestion of glucose or saturated fat increases circulating LPS and sICAM-1, the protein content of EGR-1 and TF, and the mRNA and protein content of TLR-4 only in the obese regardless of whether they have PCOS [81, 186, 190]. The lipid-stimulated LPS and TLR-4 responses in particular are greater in obese women with PCOS compared with obese ovulatory women [81]. Interestingly, circulating lipopolysaccharide binding protein (LBP) is elevated in normal weight women with PCOS but similar to normal ovulatory women in overweight and obese women with PCOS [192]. Thus, the lack of LPS and TLR-4 response to saturated fat in normal weight women with PCOS may be due to the LBP levels in these individuals which can sequester LPS within circulating LBP-LPS complexes, thereby barring LPS access to membrane-bound LBP for binding to TLR-4 [184]. Atherothrombotic inflammation markers are associated with abdominal adiposity and basal and hCG-stimulated androgen secretion [81, 186, 187, 189–191]. *These findings are striking because they show that both PCOS and obesity separately contribute to an atherothrombotic state at an early age* [81, 186, 190, 191]. Furthermore, excess abdominal adiposity and hyperandrogenism may be specific risk factors for atherosclerotic plaque rupture and vascular thrombosis. Additional studies are needed to clarify whether these findings translate into actual cardiovascular events.

Link to β-Cell Dysfunction

In PCOS, the risk of developing T2DM is five- to tenfold higher compared with the general population [193]. Susceptible individuals with prolonged or worsening insulin resistance develop pancreatic β-cell dysfunction which eventually leads to a rise in circulating glucose and ultimately culminates in β-cell failure and T2DM [194]. Obese women with PCOS who have a first-degree relative with T2DM can exhibit β-cell dysfunction that increases the risk for developing T2DM later in life [195]. Infiltrates of MNC-derived macrophage within pancreatic islets of primates and humans with T2DM are capable of disrupting pancreatic insulin secretion [196, 197]. Whereas glucose-induced oxidative stress subjects β-cells to lipid peroxidation and DNA damage, TNFα secreted from macrophages activates NFκB within β-cells, leading to endoplasmic reticulum stress and eventual β-cell apoptosis [198, 199]. Hyperglycemia-induced oxidative stress augments the disruptive process by generating a local inflammatory response that has further deleterious effect on β-cell function and contributes to the development of T2DM.

In PCOS, β-cell dysfunction is linked to oxidative stress and inflammation. Within the β-cell, glucose ingestion triggers the release of a readily available pool of insulin (1st phase) that is followed by de novo insulin synthesis to manage postprandial glucose fluctuations (2nd phase) [200]. Even in the face of normal glucose tolerance, insulin resistant normal weight women with PCOS exhibit mildly decreased 1st phase β-cell function, whereas obese women with PCOS exhibit decreases in both 1st and 2nd phase β-cell function [201]. In PCOS, 1st phase β-cell function is negatively correlated with MNC-derived ROS generation, $p47^{phox}$ protein content, and TNFα secretion in response to glucose ingestion, whereas both 1st and 2nd phase β-cell functions are negatively correlated with glucose-stimulated NFκB activation from MNC and circulating levels of CRP and thiobarbituric acid-reactive substances, a recognized measure of lipid peroxidation [201, 202]. Thus, nutrient-induced oxidative stress and inflammation are implicated in the development of β-cell dysfunction in PCOS even prior to the onset of overt hyperglycemia, with a worsening of β-cell dysfunction when obesity is also present.

Proinflammatory Impact of Excess Abdominal Fat

When obesity is present in PCOS, the inflammatory load of excess adipose tissue contributes to the promotion of insulin resistance and atherogenesis. Excess abdominal fat is present in PCOS across all weight classes, with a prevalence of 30% in normal weight women with the disorder [203]. However, molecular inflammation marker expression in the adipose tissue is in proportion to the degree of adiposity in PCOS and is not uniquely greater when compared with women who do not have PCOS [204–206].

While abdominal adiposity is more common in PCOS, it is not the primary source of inflammation in normal weight women with PCOS. Moreover, ingestion of glucose and saturated fat induces a prooxidant inflammatory response in normal weight

women with PCOS who lack abdominal adiposity when compared with normal weight ovulatory women who lack abdominal adiposity as evidenced by increases in MNC-derived ROS generation, $p47^{phox}$ mRNA and protein content, NFκB activation, and TNFα mRNA content [207–210]. These individuals are insulin resistant in the face of normal circulating TNFα, IL-6, and CRP levels. In contrast, markers of oxidative stress and inflammation trend higher, and circulating TNFα, IL-6, and CRP levels are elevated in normal weight women with PCOS who have abdominal adiposity compared with those who lack abdominal adiposity [166, 208]. Thus, nutrient-stimulated oxidative stress and inflammation from MNC are independent of excess abdominal fat in normal weight women with PCOS. Nevertheless, abdominal adiposity is the likely source of basal elevations in circulating TNFα, IL-6, and CRP in normal weight women with PCOS. Most importantly, when compared with inflamed adipose tissue, circulating MNC play a separate and discrete role in the development of chronic low-grade inflammation in PCOS.

In PCOS, the amount of fat accumulation may determine the extent to which adiposity promotes insulin resistance and hyperandrogenism. In obesity, excess abdominal fat promotes insulin resistance and is negatively correlated with measures of insulin sensitivity in studies that include obese individuals, regardless of whether they have PCOS [81, 160–163, 211]. On the other hand, abdominal adiposity and insulin resistance are not correlated in normal weight women with PCOS [207–210]. This latter finding suggests that in the absence of increased body weight in PCOS, the degree of inflammation generated in excess abdominal fat is insufficient to promote insulin resistance. In studies that include obese women with PCOS, abdominal adiposity is also positively associated with circulating androgens [212]. This finding has contributed to the dogma that insulin resistance is the primary driver of hyperandrogenism in PCOS [213]. In contrast, circulating testosterone is negatively correlated with abdominal adiposity in normal weight women with PCOS and is elevated to a greater extent in normal weight women with PCOS who lack abdominal adiposity compared with those who have abdominal adiposity [207–210]. Increases in visceral fat lipolysis due to upregulation of protein kinase A hormone-sensitive lipase activity have been reported in normal weight women with PCOS [214]. Testosterone is a known stimulator of lipolysis and may contribute to this defect when present at higher levels to limit abdominal fat accumulation in normal weight women with PCOS [112]. Thus, the smaller inflammatory load of abdominal adiposity may have limited metabolic impact when present in normal weight women with PCOS; and the degree of hyperandrogenemia before weight gain may regulate abdominal fat accumulation in PCOS.

Link Between Inflammation and Hyperandrogenism

Circulating and molecular markers of oxidative stress and inflammation are highly correlated with basal and hCG-stimulated androgens [81, 160–168, 186–188, 207–210]. This finding suggests that in PCOS, hyperandrogenemia can pre-activate MNC to account for the inflammation that is induced by nutrients, or conversely

that nutrient-stimulated inflammation can promote excess ovarian androgen production. Data exists that is in keeping with both mechanisms [215–222].

Hyperandrogenism as an Inducer of Inflammation

In PCOS, there is increased MNC-derived ROS generation, NFκB activation, and TNFα secretion in the fasting state to indicate that MNC are pre-activated [215, 216, 220]. This can explain the increased MNC sensitivity to glucose ingestion in the disorder. In contrast, MNC of normal weight ovulatory women are not sensitive to hyperglycemia and do not exhibit an inflammatory response to nutrients [81, 160–163, 165–168, 207–210]. Acute oral androgen administration to increase circulating androgen levels in normal weight ovulatory women to the range observed in PCOS increases ROS generation, $p47^{phox}$ protein content, activated NFκB, TNFα mRNA content, and TNFα secretion from MNC in the fasting state and in response to glucose ingestion [216–220]. This prooxidant, proinflammatory response occurs in MNC in an androgen receptor-dependent fashion [219]. Thus, hyperandrogenemia to the level present in PCOS can activate MNC and increase MNC sensitivity to glucose ingestion.

Prenatal androgenization can induce the PCOS phenotype in animals [223]. Placental enzyme alterations in pregnant women with PCOS favor production of androgens that may reach the fetus [224]. These findings support the concept that PCOS has an epigenetic origin. Given that hyperandrogenemia can alter immune function in a fashion that is known to promote metabolic aberration [216–220], the question is raised whether fetal exposure to excess androgens culminates in epigenetic immune alteration. Thus, it is possible that hyperandrogenism, a hallmark feature of PCOS, is the progenitor of nutrient-induced inflammation.

Hyperandrogenism as a Suppressor of Inflammation when Obesity Is Present

Circulating CRP is a useful measurement of inflammatory load since its alteration reflects exacerbation or amelioration of inflammation in clinical practice [225–227]. In obese women with PCOS, CRP levels increase along with an increase in IL-6 levels and body weight and a decrease in FFA levels in response to chronic gonadotropin-releasing hormone (GnRH) agonist-induced androgen suppression [228]. The ability of elevated circulating androgens to promote lipolysis may be responsible for these alterations. Once again, testosterone is known to stimulate catecholamine-induced hormone-sensitive lipase activity, which in turn limits adipose tissue expansion [112, 229]. Conversely, decreased lipolysis following androgen suppression can lead to adipose tissue expansion to account for the observed weight gain and is corroborated by the fall in FFA levels. Indeed, a progressive increase in subcutaneous adipocyte size that is associated with worsening insulin resistance has been documented in PCOS when hormone-sensitive lipase activity is

low [230]. Furthermore, visceral fat accumulation has clearly been demonstrated in obese women with PCOS in response to chronic GnRH agonist-induced androgen suppression [231]. The rise in IL-6 in this group during GnRH agonist treatment is most likely the result of increased IL-6 production from the excess inflamed adipose tissue, which in turn can stimulate hepatic CRP synthesis to account for the rise in CRP. Thus, hyperandrogenism exerts an anti-inflammatory effect in obese women with PCOS.

No Effect of Hyperandrogenism on Inflammation when Obesity Is Absent

Normal weight women with PCOS respond quite differently to chronic GnRH agonist-induced androgen suppression. In this group, CRP levels along with IL-6 and FFA levels remain unaltered, and body weight does not change significantly during treatment [228]. This is most likely because circulating androgens have a limited effect on subcutaneous lipolysis in normal weight women with PCOS. Moreover, the subcutaneous adipose tissue of these individuals is catecholamine resistant, thereby precluding adequate androgen induction of hormone-sensitive lipase activity [232]. These findings are important because they show that once the PCOS phenotype is established, hyperandrogenism does not promote inflammation in PCOS.

Inflammation as an Inducer of Hyperandrogenism

There is a growing body of literature showing that oxidative stress and inflammation can directly stimulate hyperandrogenism in PCOS. MNC-derived macrophages increase within the ovary in response to saturated fat ingestion [233]. CYP17, the gene encoding the androgen-producing enzyme P450c17 within the ovarian theca cells, is upregulated by prooxidant and proinflammatory stimuli including interleukin-1β and LPS [234, 235], and inhibited by antioxidants such as resveratrol and statins [236]. TNFα secreted from macrophages can promote serine phosphorylation via the MAP kinase p38α pathway which increases the 17,20-lyase activity of P450c17 [237–239]. TNFα also activates the MAP kinase ERK 1/2 pathway known to promote cellular proliferation [236]. Indeed, theca cell proliferation from rat and human polycystic ovaries is stimulated by TNFα and suppressed by statins and resveratrol [240–242]. In corroboration, antioxidant treatment using resveratrol lowers circulating androgens in women with PCOS [243], and chronic suppression of lipid-stimulated oxidative stress and inflammation from MNC with salicylate therapy reduces basal and hCG-stimulated ovarian androgen secretion and induces ovulation in lean insulin-sensitive women with PCOS (Fig. 9.5) [222]. These findings suggest that trafficking of MNC into the polycystic ovary in response to nutrient ingestion can incite oxidative stress and a local inflammatory response that increases the proliferation and steroidogenic activity of theca cells to directly stimulate ovarian androgen production even in the absence of insulin resistance and adiposity.

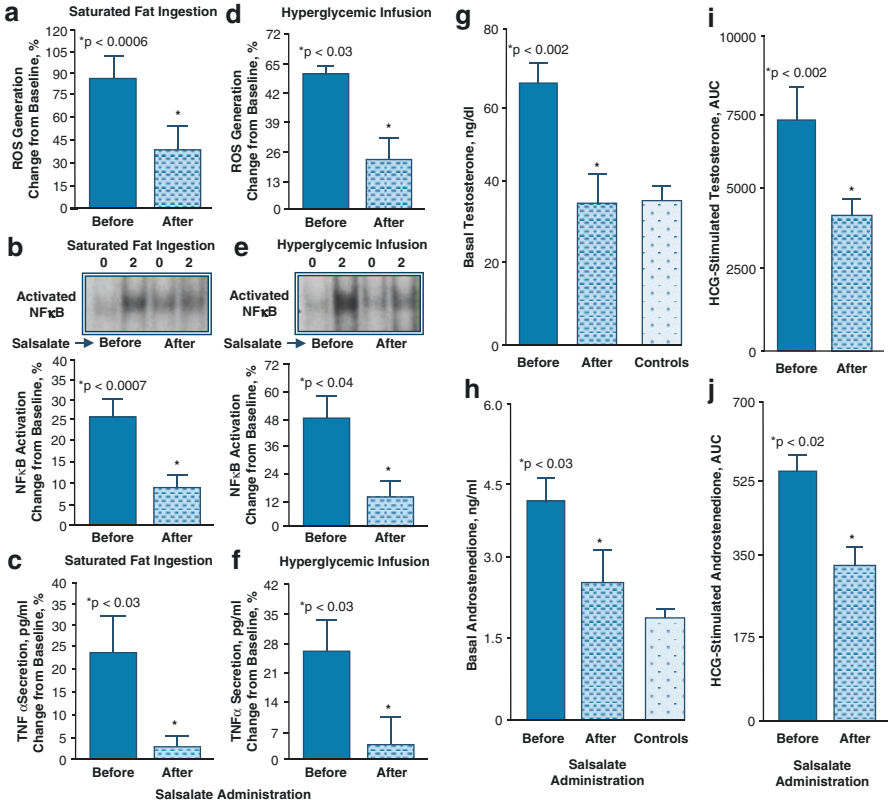

Fig. 9.5 Change from baseline (%) in mononuclear cell (MNC)-derived markers of oxidative stress and inflammation in women with PCOS before and after salsalate treatment in response to saturated fat ingestion, (**a**) reactive oxygen species (ROS) generation, (**b**) activated nuclear factor κB (NFκB), and (**c**) tumor necrosis factor α (TNFα) secretion, and in response to a hyperglycemic infusion, (**d**) ROS generation, (**e**) activated NFκB, and (**f**) TNFα secretion. Representative electrophoretic mobility shift assay bands in B and E showing the change in quantity of NFκB in nuclear extracts from MNC in samples collected at 0 and 2 hours after the respective prooxidant, proinflammatory triggers. *Pre-treatment was significantly different compared with post-treatment; (**a**) $P < 0.0006$, (**b**) $P < 0.0007$, (**c**), (**d**), and (**f**) $P < 0.03$, (**e**) $P < 0.04$. Basal levels of (**g**) testosterone and (**h**) androstenedione in women with PCOS before and after salsalate treatment and in control ovulatory women at baseline. Area under the curve (AUC) for human chorionic gonadotropin (HCG)-stimulated (**i**) testosterone and (**j**) androstenedione secretion in women with PCOS before and after salsalate treatment. *Significantly decreased after treatment, (**a**) and (**c**) $P < 0.002$, (**b**) $P < 0.03$, and (**d**) $P < 0.02$. (Adapted from González et al. [216], with permission. Copyright the American Physiological Society, 2020)

Summary

Adipose tissue serves multiple functions involved in normal metabolism and maintenance of normal endocrine and immune status [25, 29, 31]. Accumulation of adiposity to the point of obesity causes dysfunction of the adipose tissue compartment [124, 125]. This ultimately leads to immune alteration characterized by

inflammation, which causes metabolic derangement and endocrine imbalance [29, 129, 130]. The ovarian dysfunction of PCOS appears to be intimately related to and even driven by the metabolic aberrations discussed in this chapter [82, 213, 223]. Thus, it is not surprising that the signs and symptoms of the endocrinopathy manifested by hyperandrogenism and chronic anovulation, much less the risk of medical illness, are made worse when obesity is superimposed on PCOS [92].

The metabolic pathophysiology of obesity and PCOS in many ways runs in parallel, except that the adipose tissue-related inflammatory load of obesity is greater compared with the nutrient-triggered inflammation originating from circulating MNC in PCOS alone [154, 191]. Inflammation triggered by nutrients has emerged as a key contributor to the pathogenesis of PCOS. In this new paradigm, nutrient-induced oxidative stress and inflammation from MNC are responsible for molecular alterations that may be the underpinning of insulin resistance, atherogenesis, pancreatic β-cell dysfunction, and ovarian dysfunction in the disorder [81, 160–163, 186, 190, 201, 202]. It is now clear that inflammation in PCOS is independent of excess adipose tissue [166, 207–210]. However, the superimposed inflammatory load from obesity is manifested clinically in PCOS by even greater metabolic aberration [81, 160, 190]. While it is possible that epigenetic immune alteration induced by hyperandrogenism is the progenitor of inflammation in PCOS [217, 223, 224], hyperandrogenism does not drive inflammation upon establishment of the PCOS phenotype [228]. Aside from promoting metabolic aberration, inflammation in PCOS is capable of directly stimulating excess ovarian androgen production [222].

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Part III

Management of Polycystic Ovary Syndrome

Managing PCOS in the Adolescent

10

Tania S. Burgert and Emily Paprocki

Key Points

- Combined oral contraceptive pills, metformin, and anti-androgens are current strategies commonly utilized for symptom control and risk reduction in adolescents with PCOS; long-term data in this population are lacking.
- Existing data underscore a need for a long-term follow-up of adolescents with PCOS to quantify long-term risks relating to the diagnosis and to gain a better understanding of benefits of insulin-sensitizing strategies that are increasingly being employed in this population.
- Given the lack of evidence for superiority of one treatment over the other as well as the different mechanisms of action for each drug, pharmacotherapy in adolescents is individualized and should take into consideration the adolescent's values, priorities, and preferences.

Studies are being done to evaluate effectiveness of medications alone or in combination to ameliorate symptomatology of PCOS. A meta-analysis has compared the effectiveness of metformin versus combined oral contraceptive use in adolescents with PCOS in terms of improving menstrual cyclicity, clinical hyperandrogenism, and metabolic profile. Four randomized controlled trials met inclusion/exclusion criteria leaving a sample size of 170 adolescents. There was no statistically significant difference between metformin and COCP in regard to effect on hirsutism, triglycerides, high density lipoprotein (HDL), or total testosterone levels. Improved menstrual regularity favored COCP use compared to metformin. Improvement in dysglycemia as assessed by oral glucose tolerance test [OGTT]), and body mass index (BMI, as well as low density lipoprotein (LDL) favored metformin use [1].

More recently, a Cochrane Review was done to assess the effectiveness and safety of metformin versus COCP (alone or in combination) in improving clinical,

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_10

hormonal, and metabolic features of PCOS. The majority of included studies were in adult women, although 5 adolescent studies were included for a total of 206 adolescent patients. Unfortunately, meaningful conclusions were limited due to low quality of studies included. The study did suggest that in adult women, either metformin alone or COCP alone may be less effective in improving excessive facial and body hair compared to the combination of the COCP with metformin. Well-designed and well-conducted randomized controlled trials are needed to better evaluate metformin versus COCP and combinations of these interventions, especially in adolescents with PCOS [2].

Patient-Centered Approach to Treatment

Case 1 A 17-year-old female presented with oligomenorrhea and hirsutism. Menarche was at the age of 11 years and cycles were every 3–6 months. She was sexually active with her boyfriend. She had no history of migraines or blood clots/bleeding disorders. Physical exam showed an evidence of hirsutism with a Ferriman Galleway (FG) score of 21; severe acne on the face, chest, and back; no acanthosis nigricans; and BMI normal at 23 kg/m^2.

Clinical Course Diagnosis of PCOS was given based on hyperandrogenism and oligomenorrhea (see Table 10.1). Other etiologies of hyperandrogenism and oligomenorrhea were excluded by laboratory evaluation showing normal 17-OHP, DHEAS, prolactin, and TSH. Treatment with COCP was chosen to improve hirsutism and acne, regulate menstrual cycles, and provide contraception. She was started on 0.03 mg ethinyl estradiol (EE)/3 mg drospirenone containing COCP which was chosen for its anti-androgenic effects. At 6-month follow-up, she had significant improvement in acne and normalization of menstrual cycles. At 12-month follow-up, she was satisfied with her improvement in hirsutism and was no longer requiring frequent shaving.

Clinical Lesson Treatment with COCP should be considered for its beneficial effect in lowering testosterone levels, regulating menstrual cycles, and providing contraception in adolescents who are sexually active.

Table 10.1 Case 1 laboratory evaluation

Testosterone	88 (<40–50 ng/dL)
Free testosterone	1.45 (<1.09 ng/dL)
Luteinizing hormone	10.2 (0–2.7 mIU/mL)
Follicle stimulating hormone	2.5 (1.9–20 mIU/mL)
HbA1c	5.5 (<5.7%)
Oral glucose tolerance test	
0 min serum glucose	90 (<100 mg/dL)
120 min serum glucose	115 (<140 mg/dL)
0 min insulin	11 (<14 uIU/mL)
120 min insulin	48 (<50 uIU/mL)
25-OH vitamin D	18 (30–80 ng/mL)

Hormonal Contraceptive Approach

Combined oral contraceptive pills (COCPs) containing both an estrogen and a progestin component not only regularize the menstrual cycle but also alleviate clinical features such as acne and hirsutism. Specifically, the estrogen component of the pill increases hepatic production of sex hormone binding globulin (SHBG), which leads to a reduction in circulating free and bioavailable androgens. The progestin component of COCPs ensures endometrial decidualization and thus protects against proliferative endometrial pathologies, including endometrial hyperplasia. Therefore, the potential risk for endometrial cancer resulting from prolonged periods of anovulation is reduced. By negative-feedback effect on pituitary LH and thereby lowering LH-driven ovarian androgen production, progestins can also contribute to improving hyperandrogenemia associated with COCP use. Fourth-generation progestins such as drospirenone have an added anti-androgenic effect and therefore have been deemed more effective in improving PCOS symptoms [3]. In adolescents with PCOS, switching from a third-generation progestin to a less androgenic fourth-generation progestin improved adiposity [4]. However, concerns regarding somewhat higher thromboembolic risk with fourth-generation progestins compared to older third-generation formulations have led to more cautious use in general and especially in a pro-inflammatory/pro-thrombotic disease such as PCOS [5, 6]. The current 2018 international evidence-based guidelines on PCOS do not favor one progestin over the other when it comes to COCP use in PCOS.

Another consideration in prescribing COCPs in overweight adolescents with PCOS is the negative effect on lipid profiles and insulin resistance [7, 8]. The higher the estrogen dose and the more androgenic the progestin, the more unfavorable effects can be observed on lipid and glucose metabolism. A study comparing a combination product of 30 μg EE with either a third- or fourth-generation progestin found that both led to similar increase in HDL and triglyceride profile in lean women with PCOS. However, fourth-generation progestin combination kept the LDL levels stable, therefore increasing the HDL/LDL ratio, leading to a beneficial constellation in terms of vascular disease [9]. Another study in adult nonobese women with PCOS demonstrated that low-dose EE (30 μg EE) did not affect gluco-insulinemic homeostasis [10]. Also, estrogen-containing COCPs are contraindicated in adolescents with factor V Leiden mutations, greatly enhancing thromboembolic risk. Sometimes family moral/religious or stigma concerns about oral contraceptives also prompt younger patients to seek alternative treatment to their PCOS. In such cases, uterine protection can also be obtained by inducing periodic withdrawal bleeds by a short course of progesterone every 2–3 months.

Case 2 A 15-year-old female presented with oligomenorrhea. Menarche was at the age of 12 years and cycles were every 4–5 months beginning the third year postmenarche. She had a history of homozygous factor V Leiden deficiency and irritable bowel syndrome. She was not sexually active. Physical exam showed a FG score of 4, moderate acne on the face and back, no acanthosis nigricans, and BMI normal at 24.8 kg/m^2.

Clinical Course Diagnosis of PCOS was given based on hyperandrogenism and oligomenorrhea (see Table 10.2). Other etiologies of hyperandrogenism and oligomenorrhea were excluded by laboratory evaluation showing normal 17-OHP, DHEAS, prolactin, and TSH. Due to her history of factor V Leiden deficiency, COCP was contraindicated. Progesterone-only formulations were discussed including etonogestrel implant, progesterone-only pill, progesterone-containing IUD, and intermittent medroxyprogesterone use. She was given a 10-day medroxyprogesterone course of 10 mg daily which led to withdrawal bleeding. She was started on metformin using extended-release formulation with the goal of minimizing any gastrointestinal discomfort due to history of irritable bowel syndrome. Extended-release metformin was started at 500 mg daily for 2 weeks and increased to 1000 mg daily thereafter. This led to normal monthly menstrual cycles beginning at 2.5 months after starting treatment. She lost 2.5 kg and had improvement in acne. She experienced no side effects and began a daily multivitamin which included vitamin B_{12}.

Clinical Lesson Metformin can normalize menstrual cycles in nonobese adolescents.

Metformin

Insulin resistance has been documented in clamp studies in 75% of lean women and 95% of overweight women with PCOS, which supports the use of metformin in this population [11]. Given the perpetuating effect of insulin on androgens, metformin was the first antidiabetic drug to be examined in the context of treating the PCOS condition in adult women [12]. Metformin is an insulin sensitizer with its main action in the liver. Metformin is not FDA approved as a treatment for PCOS, although it is widely used off-label, is cost-effective, and has a good safety profile for long-term use. Although metformin is used off-label in adolescents with PCOS,

Table 10.2 Case 2 laboratory evaluation

Testosterone	64 (<40–50 ng/dL)
Free testosterone	0.65 (<1.09 ng/dL)
Luteinizing hormone	12.2 (0–2.7 mIU/mL)
Follicle stimulating hormone	3.5 (1.9–20 mIU/mL)
HbA1c	5.4 (<5.7%)
Oral glucose tolerance test	
0 min serum glucose	95 (<100 mg/dL)
120 min serum glucose	95 (<140 mg/dL)
0 min insulin	15 (<14 uIU/mL)
120 min insulin	68 (<50 uIU/mL)
25-OH vitamin D	22 (30–80 ng/mL)

a study assessing metformin prescription patterns in the USA showed PCOS is one of the more commonly used indications for metformin treatment. Specifically, in girls aged 15–19 years, PCOS is the most common diagnosis leading to metformin prescription [13].

Many studies have confirmed the benefits of metformin for restoring cycle regularity, fertility, and favorable metabolic profile for adult women. In 2001, the first studies emerged examining the effect of metformin in adolescent PCOS. For a group of 18 nonobese girls with PCOS and a history of premature adrenarche, Ibanez et al. found that 14 (78%) had restored menstrual cycle regularity by 6 months of metformin therapy [14]. Six months of metformin restored normal menstrual cycles in 10/11 obese subjects [15] and improved both ovarian and adrenal hyperandrogenism in another group of obese subjects [16]. A double-blind, placebo-controlled study in obese girls with PCOS found that metformin/lifestyle versus placebo/lifestyle was more effective in reducing testosterone levels and menstrual cycle regularity than lifestyle alone [17]. Albeit there may be a prescribing bias of metformin to obese adolescents with PCOS, it is equally effective in lean adolescents in restoring menstrual regularity [18]. Metformin in combination with oral contraceptives and anti-androgens has been extensively studied by Ibanez and Zegher for girls of the Catalan region of Spain. Intriguing are their data on starting metformin at the presentation of premature adrenarche, a common antecedent to the later manifestation of PCOS [19]. In terms of body composition and metabolism, the authors show that earlier treatment appears to be more effective than later treatment [20] and may even augment final height by delaying early menarche [21].

Metformin is a good choice for the lean and overweight young adolescents who are not in need of contraception and who do not suffer from severe acne and hirsutism that would require more immediate symptomatic therapy with COCPs. Metformin has an excellent safety profile in patients with healthy kidney and liver function and can be used in patients with concern of thromboembolic risk. Menstrual regularity in both lean and obese adolescents will likely be restored after 6 months of therapy. Should endometrial buildup from prolonged amenorrhea be a concern, then a short-course progesterone-induced withdrawal bleed may be indicated before starting metformin.

If the dose is slowly and incrementally increased to 1500–2000 mg daily, divided twice or thrice daily over a course of several weeks and taken with food, gastrointestinal side effects such as diarrhea can be avoided in most patients [22, 23]. Additionally, the use of extended-release metformin should be considered due to the benefit of once daily dosing and the potential decrease in gastrointestinal side effects which may lead to improved adherence. Long-term metformin use may decrease levels in B_{12} [24]; therefore, a supplemental daily multivitamin that contains B_{12} can prevent this long-term use effect. There is an increased risk of lactic acidosis with metformin use, although this is rare in pediatrics and typically only a concern with other compounding risk factors, such as prolonged fasting.

Combining COCPs and Metformin

Ibanez and Zegher have examined varying drug combinations in adolescents with PCOS [25]. From their data, we know that metformin has additive beneficial effects, especially in improving body composition and dyslipidemia. For obese adolescents, a double-blind, placebo-controlled study confirmed the beneficial effect of additive therapy [26]. They first compared single-agent therapy (COCP vs. lifestyle vs. metformin) over a 6-month time period. Subsequently, they examined combinations of lifestyle plus COCP with either metformin or placebo. In this study, lifestyle alone was highly effective in reducing FAI with an increased SHBG. COCPs improved androgen profile but had negative metabolic effects with increased C-reactive protein and LDL cholesterol. Interestingly, adding metformin to COCP and lifestyle improved total testosterone, HDL, and waist circumference. It therefore appears that adding metformin to COCPs ameliorates some of the negative metabolic effects of OCPs. In adult women, a study done comparing thrombin generation with COCP and COCP plus metformin showed that metformin ameliorated the thrombin generating effect of COCP [27].

An important consideration when treating adolescent PCOS is that COCPs are often desired therapy for their contraceptive benefit. Monotherapy with metformin, on the other hand, can enhance fertility by way of restoring normal ovulatory cycles and therefore may increase the risk of teen pregnancy. Hence, combining both therapeutics may sometimes be the best choice, especially for sexually active, overweight adolescents with cardiometabolic risk.

Case 3 A 16-year-old female presented with primary amenorrhea and hirsutism. Thelarche was at the age of 12 years and adrenarche at the age of 10 years. She had a negative past medical history. She had no history of migraines or blood clots/bleeding disorders. She was not sexually active. Family history included father who had a history of a blood clot and was identified as an alcoholic who smoked four packs of cigarettes per day. There was no other family history of blood clots. Physical exam showed a FG score of 36; deep voice; no clitoromegaly; moderate acne on the face, chest, and back; mild acanthosis nigricans; and a BMI of 34 kg/m^2.

Clinical Course Diagnosis of PCOS was made based on amenorrhea and biochemical/clinical hyperandrogenism (see Table 10.3). Other etiologies of hyperandrogenism and amenorrhea were excluded by laboratory evaluation showing normal 17-OHP, DHEAS, prolactin, and TSH. She had a normal 46, XX karyotype and pelvic US. She was given a medroxyprogesterone course which led to withdrawal bleeding. She was started on COCP, 0.035 mg EE/0.25 mg ethinyl estradiol/0.25 mg norgestimate. The combination of higher estrogen and less androgenic progestin was chosen for potential beneficial effect on hirsutism. One month after beginning COCP, she was hospitalized for a pulmonary embolism. Hypercoagulability workup was negative. COCP was discontinued and treatment switched to the combination of metformin and spironolactone. She was started on metformin immediate release 500 mg daily for 2 weeks and then increased to 500 mg twice a day. Spironolactone

Table 10.3 Case 3 laboratory evaluation

Testosterone	76 (<40–50 ng/dL)
Free testosterone	2.4 (<1.09 ng/dL)
Luteinizing hormone	21.5 (0–2.7 mIU/mL)
Follicle stimulating hormone	7.5 (1.9–20 mIU/mL)
HbA1c	5.0 (<5.7%)
Oral glucose tolerance test	
0 min serum glucose	97 (<100 mg/dL)
120 min serum glucose	135 (<140 mg/dL)
0 min insulin	21 (<14 uIU/mL)
120 min insulin	125 (<50 uIU/mL)
25-OH vitamin D	15 (30–80 ng/mL)

was started at 50 mg daily and then increased to 100 mg daily. The potential teratogenic effect of spironolactone was discussed and agreement made to discontinue medication if she became sexually active. By 12-month follow-up visit, FG score improved to 18, BMI decreased to 26 kg/m^2, and menstrual cycles were regulated.

Clinical Lesson Thromboembolic risk is heightened in PCOS. Necessity of COCP should be carefully evaluated and alternative options considered including the combination of anti-androgens and metformin.

Anti-androgens

Hyperandrogenemia is not only closely tied to the pathogenesis of the condition but also responsible for the metabolic complications. Hyperandrogenemic obese adolescents have a greater risk of metabolic syndrome and an inflammatory state than non-hyperandrogenemic, similarly obese counterparts [28]. Therefore, anti-androgen therapy may be indicated not only to benefit cosmetic manifestations such as hirsutism and acne but also as a metabolic management strategy.

Flutamide, a nonsteroidal drug with a potent androgen receptor blocker, has been very effective in both cosmetic and metabolic effects when added to oral contraceptives and metformin in a PCOS treatment regimen [4, 25, 29]. However, few pediatric investigators have studied flutamide due to the widespread concern about its hepatotoxic effect. It appears, though, that this effect is dose dependent and not idiosyncratic. In low dose (62.5 mg/day), flutamide did not affect the liver transaminases, as demonstrated by an epi-analysis of 12 prospective studies, each with at least 20 patients treated for at least 12 months [30, 31]. While Ibanez and Zegher have published most of the data about the safety and efficacy of flutamide in combination therapy for adolescent PCOS, an independent observational study confirmed low-dose flutamide safety for other young women with hirsutism [32].

Spironolactone, used for decades as a potassium-sparing diuretic, was found to have anti-androgenic effect in the 1970s [33]. Its anti-androgenic effect is carried out through a dual mechanism of androgen receptor blockade, and via spironolactone's inhibition of 5-alpha reductase, thus reducing the conversion of testosterone

Table 10.4 Efficacy of commonly utilized therapeutic options for managing PCOS-related problems in adolescents

Treatment	Menstrual dysfunction	Hyperandrogenism	Metabolic benefit
Insulin sensitizer Metformin	±	±	+
Combined oral contraceptive pill (COCP)	+	+	–
Cyclic progesterone	+	–	–
COCP+ metformin	+	+	+
Anti-androgen	±	+	±

to its biologically more potent metabolite, dihydrotestosterone. Studies in adult women have shown higher doses of 100 mg daily to be safe and effective in improving hirsutism scores over time. Spironolactone, while more commonly used in the USA to help with hirsutism, has the unnerving side effect of irregularizing menses. Therefore, it is often used in combination with OCPs in patients with PCOS. In young women at risk for pregnancy, both anti-androgens must only be used with secure contraceptives due to the potential effect of undervirilization of a male conceptus.

Table 10.4 identifies the efficacy of the commonly utilized therapeutic regimens against the common symptoms of PCOS.

Lifestyle

Multicomponent lifestyle intervention including diet, exercise, and behavioral strategies should be recommended in all adolescents with PCOS in order to reduce BMI, central obesity, and insulin resistance. Intensive lifestyle modification, even with only a modest change in BMI, has shown to improve menstrual cycle regularity and normalize serum androgens in adolescents with PCOS [26, 34]. In these lifestyle studies, adolescents received intense nutrition education and psychological support and participated in group exercise classes at least once a week. However, even when enrolled in an intensive lifestyle program for a year, only 26/59 obese girls with PCOS lost >0.2 SDS in BMI [34]. Maintaining lifestyle changes outside of clinical trials is even more challenging and, therefore, pharmacotherapy often becomes necessary. There are a range of reduced energy diets with different macronutrient contents that have been effective in women and adolescents with PCOS, but no single diet has been proven to be more beneficial. At least 60 minutes of moderate to vigorous intense physical activity per day is recommended for prevention of weight gain and maintenance of health in adolescents [11]. A recent study investigated the effects of a supervised aerobic exercise intervention on health-related quality of life in women with PCOS. Quality of life was significantly improved in treatment group versus control including the domains of physical functioning, general health, and mental health [35]. A recent meta-analysis including 11 studies (412 patients) confirmed that lifestyle changes through reduced calorie intake and regular physical

activity can improve clinical, metabolic, and hormonal parameters in adolescent girls with PCOS. Lifestyle changes for 3–12 months decreased LH levels and free androgen index (FAI), although not all hormonal parameters were significantly improved. Specifically, exercise was significantly associated with improved menstrual regulation and FG score. However, this study did not show that lifestyle changes were significantly associated with improvements in glucose metabolism [36].

Additional Therapies

Inositol is another treatment option that has been studied and utilized in PCOS particularly in the past two decades. The rationale underlying the use of inositol as a therapeutic regimen in PCOS is due to its function as an insulin-sensitizing agent and beneficial effect on metabolism. There are nine stereoisomeric forms of inositol; focus has been given to myo-inositol (MI) and D-chiro-inositol (DCI) in the treatment of PCOS. Both MI and DCI function as insulin second messengers and mediate different actions of insulin. At the ovarian level, it has been shown that an MI-based second messenger is involved in both glucose uptake and FSH signaling, whereas a DCI-based second messenger is devoted to insulin-mediated androgen production [37–39].

In women with PCOS, a deficiency of insulin second messengers in tissues, or altered metabolism of inositols, could play a role in inducing insulin resistance [40]. The first controlled clinical trial of inositols versus placebo in PCOS was published in 1999. In that study, DCI was given orally once a day for 6–8 weeks to 44 obese PCOS women. They showed improved insulin sensitivity and decreased circulating free testosterone levels, whereas there was no effect of placebo. DCI administration also resulted in ovulation in 19 of 22 women (86%), whereas only 6 of 22 women (27%) ovulated in the placebo group [41]. A 40:1 MI/DCI combination is well supported by many preclinical and clinical studies. Most recently, a 2019 clinical trial compared the efficacy of seven different ratios between MI and DCI in PCOS therapy. The 40:1 MI/DCI ratio was the best for PCOS therapy directed at restoring ovulation and normalizing progesterone, LH, SHBG, estradiol, and testosterone. With higher doses of DCI, there was decreased efficacy in PCOS treatment [42].

There is a recent increase in studies looking at the effect of inositols in adolescents with PCOS as well [43, 44]. A prospective study of 61 adolescent girls aged 13–19 years with PCOS evaluated MI and OCP (drospirenone/ethinyl estradiol) alone and in combination. They showed MI is an effective method to prevent and correct metabolic disorders in teenagers affected by PCOS. A significant reduction in weight, BMI, glucose, C-peptide, insulin, HOMA-IR, free testosterone, and LH were detected. With the combination of MI and OCP, the anti-androgenic effects are enhanced, the negative impact of OCPs on weight gain is balanced, and the metabolic profile is improved [45].

Vitamin D deficiency and vitamin D insufficiency are common in adolescents with PCOS. In a recent study, 25-OH vitamin D levels were shown to be significantly lower in a group of 59 adolescents with PCOS (diagnosis based on meeting

Rotterdam Criteria) compared to 38 adolescents without PCOS (Rotterdam criteria for PCOS not met). This was confirmed in an adjusted analysis for season during which level was collected, age, BMI, ethnicity, race, and insurance status [46]. In adult women with PCOS, treatment of vitamin D deficiency has been shown to normalize menstrual cycles and improve insulin resistance [47, 48]. Vitamin D deficiency may be a mitigatable factor in improving symptomatology of PCOS in adolescents.

Summary

Treatment of PCOS in adolescence centers around symptomatic relief and prevention of comorbidities. Combined oral contraceptives, metformin, and anti-androgens are current strategies commonly used along with lifestyle recommendations. Initial medication approach should be tailored to each patient based on their symptoms, personal preferences, and comorbidities.

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Managing the PCOS-Related Symptoms of Hirsutism, Acne, and Female Pattern Hair Loss

11

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Key Points

- Hirsutism, acne, and female pattern hair loss are commonly encountered symptoms of hyperandrogenism of PCOS.
- Symptoms of hyperandrogenism are significant contributors to decreased quality of life and poor self-image in women with PCOS.
- Familial and genetic underpinnings must be considered when quantifying facial and bodily hair as excessive.
- Assessment of hyperandrogenemia with laboratory tests is an important component in the workup, but elevations in values are not necessary to make a clinical diagnosis.
- Updated guidelines from the Endocrine Society in 2018 suggest testing for elevated androgen levels in all women with an abnormal hirsutism score.
- PCOS-related hyperandrogenism must be differentiated from features of virilization.
- Provided there are no contraindications, combined hormonal contraceptives remain the first-line approach in managing symptoms of hyperandrogenism.
- Addition of antiandrogens to combined hormonal contraceptive regimen can offer additive benefit against symptoms of hyperandrogenism.
- Teratogenic potential of antiandrogens must be taken into consideration when considering antiandrogen therapy for managing symptoms of hyperandrogenism in reproductive-age women.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_11

Clinical Vignette

T.K. is a 26-year-old gravida 0, para 0 of South Asian descent who presents to the gynecologist with a long-standing history of irregular menses since menarche at the age of 13; she also complains progressively bothersome unwanted facial hair and persistent mild acne. She reports being on combined oral contraceptive pills (OCPs) on and off for the past few years, which she had discontinued a few months prior to her visit. On inquiry, the progestin constituent in her OCP regimen was levonorgestrel. She notes excessive hair on the chin and upper lip for which she has utilized bleaching, plucking, and chemical depilation with some success. Her acne is on the cheeks, and there is no evidence of inflammatory pustules or papules. She feels self-conscious with her excessive facial hair growth, reports a low self-image, and desires management. She is otherwise healthy and is currently not taking any medications. Physical exam reveals a BMI of 26 kg/m^2, normal blood pressure, and excessive hair noted on the chin, upper lip, and lower abdomen with a Ferriman-Gallwey score (FGS) of 9; there is normal scalp hair growth, and there is no evidence of cutaneous striae, acanthosis nigricans, or goiter. Laboratory evaluation indicates a normal TSH, prolactin, normal DHEA-S and 17-OHP, and normal total testosterone with mildly elevated free testosterone. A pelvic ultrasound confirmed an endometrial thickness of 8 millimeters and polycystic appearing ovarian morphology. A diagnosis of PCOS was made. Implications of the diagnosis and management options for addressing bothersome hyperandrogenism were discussed.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine conditions affecting 5–13% of reproductive-age women, based on US National Institutes of Health (NIH), Rotterdam, and the Androgen Excess and PCOS Society (AE-PCOS Society) estimates [1, 2]. The Centre for Research Excellence in Polycystic Ovary Syndrome (CREPCOS) research in partnership with the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) has developed and updated international evidence-based guidelines in 2018 to reflect clinical recommendations [3]. A diagnosis of PCOS can be made based on the presence of at least two out of the following criteria: (1) androgen excess manifested by either clinical features of hyperandrogenism (such as hirsutism, acne, or androgenic alopecia) or evidence of hyperandrogenemia, (2) ovulatory dysfunction (oligo-ovulation or anovulation), and (3) polycystic appearance on ultrasound examination (transvaginal ultrasound with a follicle number per ovary of >20 and/or an ovarian volume ≥10 ml). Technology advancements in transvaginal ultrasound transducers underlie the increase in the antral follicle count threshold for calling out an ovary as polycystic compared to the originally specified count criterion of 12 [3].

Clinical Hyperandrogenism

Hirsutism

Hirsutism is defined as the increase in *terminal hair* growth in the androgen-dependent areas of the body, such as the chin, upper lip, chest, abdomen, back, and anterior thighs. It can be identified in approximately 5–10% of reproductive-age women, representing over four million women in the United States alone [4–6]. Hirsutism is usually a clinical sign of an underlying androgen excess, although familial and idiopathic variants are also well recognized. The most common cause of hirsutism is polycystic ovary syndrome (PCOS). Of patients with PCOS, 57–82% report being bothered by excess facial and body hair. Other less common causes of hirsutism in the setting of androgen excess are late-onset congenital adrenal hyperplasia (CAH), insulin-resistant acanthosis nigricans syndrome, androgen-secreting tumors, and iatrogenic exposures. Hirsutism when present in the setting of normal ovulatory function and androgen levels, as can be seen in 10–15% of women, is deemed idiopathic. Genetic predisposition in familial variants is also well described [6, 7].

The pathophysiology of hirsutism in PCOS is a result of the sensitivity of the hair follicle to the hormonal milieu of androgens as well as local growth factors.

Hair can be categorized as either *vellus* (fine, soft, and not pigmented) or *terminal* (long, coarse, pigmented, greater than 5mm in length if left untreated). Hair growth is comprised of three phases: the growth phase (anagen) lasting 3–4 months, the involutional phase (catagen) lasting 2–3 weeks, and the resting phase (telogen) that can last up to 3–4 months [8]. Androgen bioactivity is regulated in the hair follicle primarily by the activity of enzyme 5α-reductase, which converts free testosterone to the more potent dihydrotestosterone (DHT). The type 2 isoenzyme of 5α-reductase is primarily found in the scalp hair follicles. Type 2 5α-reductase activity is increased with elevated circulating testosterone and contributes to a dysregulation of hair follicle growth. Thus, hyperandrogenemia encourages the transition from *vellus* to *terminal* hair in androgen-sensitive skin sites [9]. The expression of 5α-reductase is also stimulated by several other factors, including insulin and insulin-like growth factor [10]. This is pertinent to the understanding of a shared underlying pathophysiology between PCOS and hirsutism. Given the involvement of other factors, hirsutism is not solely dependent on androgen excess, as evidenced by normal androgen levels that can be present in hirsute PCOS patients [11]. Androgens additionally increase hair follicle size, hair fiber diameter, and the proportion of time that the *terminal* hair spends in the anagen phase [12].

A male pattern distribution of *terminal* hair in androgen-sensitive sites in women characterizes hirsutism [6]. However, it is important to note ethnic and genetic differences in hair follicle density, as well as in 5α-reductase activity. These variations allow for a differential expression of cutaneous hair growth profiles and thus severity of hirsutism across populations; 60–80% of Caucasian American women experience hirsutism with PCOS but only 20% of Japanese PCOS patients experience it [13].

Evaluation of Hirsutism

History

A detailed history should include the ages of therlarche, adrenarche, and menarche. The menstrual and reproductive history can elucidate potential ovulatory dysfunction. The timing and progression of hirsutism can help discern PCOS from other androgen excess etiologies. A rapid onset of symptoms or progression in the severity of hirsutism, in addition to other signs of virilization (deepening of the voice or clitoromegaly), makes a chronic disorder such as PCOS less likely and an androgen-secreting tumor more likely. Taking a thorough medication history by addressing the use of anabolic steroids, danazol, or testosterone can identify iatrogenic causes as a result of pharmacologic agents. Significant family history for menstrual irregularities, hirsutism, PCOS, obesity, infertility, diabetes mellitus, or enzymatic deficiency should be obtained [6, 14, 15]. The clinician should ascertain the degree of emotional distress caused by the excess hair, along with a record of previous therapies, which will be helpful to guide future management.

Physical Exam

On physical examination, indices of body mass and blood pressure and signs of insulin resistance, such as obesity, central fat distribution, skin tags, and acanthosis nigricans, should be evaluated in addition to seeking signs of hyperandrogenism [15].

Severity of hirsutism should be objectively assessed; the Ferriman-Gallwey screen, created in 1961, represents one of a few tools that are available to the healthcare providers to quantify the severity and pattern of hair excess. Since its initial description, this scoring system has been modified to retain 9 of the original 11 anatomical sites at which hair growth is quantified (upper lip, chin and neck, upper chest, upper abdomen, lower abdomen, thighs, upper back, lower back, and upper arms); based on pictorial assessment, hair growth severity at each of the 9 sites is a score from a range of 0 (absence of *terminal* hairs) to 4 (extensive *terminal* hair growth), and the scores in each area are summed for a total hair growth score. This scoring system is depicted in Fig. 11.1.

A modified Ferrimen-Gallwey Score (FGS) of >8 is commonly considered to reflect objective hirsutism [14, 16, 17]. There are, however, some limitations of using rigid cutoff scores of FGS that merit consideration. The screen was derived from a cohort of Caucasian women and thus does not reflect the ethnic differences in expression of hair growth [3]. For Mediterranean, Hispanic, and Middle Eastern women, FGS ≥9 to 10 is considered abnormal, and for Southeast Asian women, a score ≥2 has been deemed to reflect excess hair [3, 18, 19]. A simplified FGS of

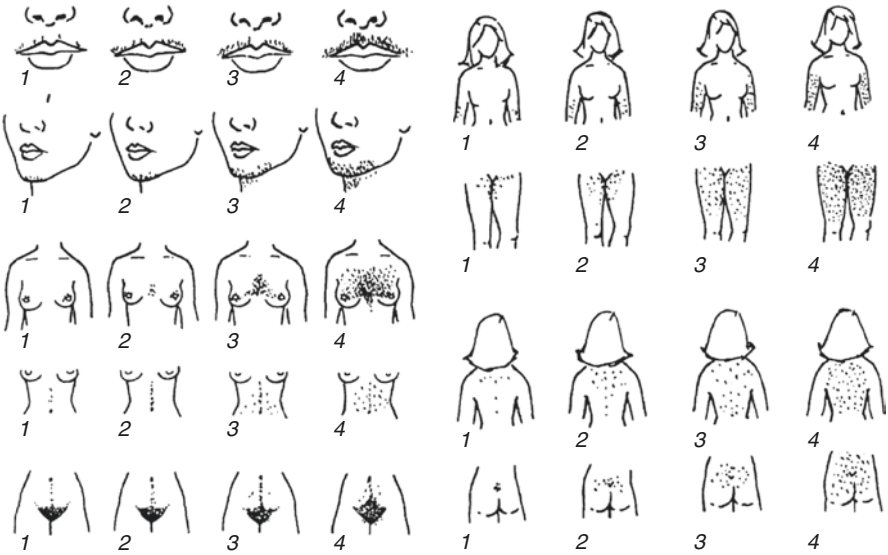

Fig. 11.1 Visual method of scoring hair growth in women, modified from the system originally reported by Ferriman and Gallwey in 1961 [16]. Each of the nine body areas depicted is scored from 0 (absence of terminal hairs) to 4 (extensive terminal hair growth), and the scores in each area are summed for a total hair growth score. (Reprinted with permission from: Hatch et al. [17])

four sites with a cutoff of ≥3 was validated in a Chinese cohort PCOS population with a sensitivity and specificity of 97.6% and 96.4%, respectively [20]. Updated 2018 international guidelines now recommend using ≥4–6 as the cutoff for hirsutism, depending on ethnicity [3]. Another limitation of the hirsutism scoring system is that women often use cosmetic measures before seeking consultation, and therefore FGS at the time of initial assessment may not accurately reflect the severity and distribution of hair excess. Additionally, the inter-observer measure of evaluating hirsutism is generally poor, with too much variation for clinical use [21]. Lastly, the clinical relevance of the score may be limited since low scores can still be a cause of significant distress for many women [22].

On physical exam, one must also distinguish hirsutism from *hypertrichosis*, which is increased hair growth in the androgen-independent areas. Hypertrichosis is commonly seen with systemic disorders such as hypothyroidism, anorexia nervosa, or malnutrition [23].

Biochemical Evaluation of Androgen Excess

Updated guidelines from the Endocrine Society in 2018 suggest testing for elevated androgen levels in all women with an abnormal hirsutism score, which are now in agreement with AE-PCOS Society [21]. Hyperprolactinemia should be considered

in the list of differential diagnoses for PCOS [24]. It is important to note, however, that a robust correlation between the quantity of hair growth and circulating androgen levels is not always present as although androgen excess underlies most cases of hirsutism, there is, however, only a modest correlation between the quantity of hair growth and circulating levels [3, 25].

When diagnosing hyperandrogenism, both clinical and biochemical evidence of hyperandrogenism must be evaluated. Clinical signs of androgen excess in the PCOS population can manifest as hirsutism, acne vulgaris, and female pattern hair loss. These manifestations can negatively impact quality of life; women report lower body satisfaction, negative body image, and depression [26–28]. While a majority of women with PCOS can present with clinical hyperandrogenism, often there is a discordance between clinical phenotype and the hormonal milieu [25].

In females, androgens originate from three primary sources: (1) the ovarian theca, (2) the adrenal cortex, and (3) within end organs by peripheral conversion. The major androgens include testosterone, dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), and androstenedione. In healthy women, testosterone is largely bound by sex hormone-binding globulin (SHBG) and albumin, leaving only approximately 1% freely circulating as bioactive free testosterone [29]. PCOS is considered a diagnosis of exclusion, and it is imperative that other potential etiologies of androgen excess are considered as part of a thorough biochemical evaluation, as has been discussed extensively elsewhere in this textbook. Etiologies that should be considered in the list of differential diagnoses include idiopathic hirsutism, androgen-secreting ovarian or adrenal neoplasm, late-onset CAH, Cushing's syndrome, and iatrogenic exposures.

Based on a systematic review, free testosterone can detect biochemical hyperandrogenism in 50–60% of women with PCOS, although it is dependent on the accuracy of the currently available assays [3]. Total testosterone alone can identify 20–30% of PCOS women with biochemical hyperandrogenism [3]. Most women with PCOS have serum total testosterone concentrations less than 150 ng/dL and often in the normal range (2–45 ng/dL). Serum total testosterone levels above 150 ng/dL warrant consideration of a testosterone-secreting ovarian neoplasm, and persistent elevation in this range should guide further evaluation for neoplasm using pelvic imaging [30].

Dehydroepiandrosterone sulfate (DHEA-S) is an adrenal androgen precursor that should be assessed in women manifesting signs of hyperandrogenism. While DHEA-S may have limited value in the diagnosis of PCOS, significant elevations in serum levels greater than 700 μg/dL should raise suspicion for an adrenal tumor as a source of androgen excess, a finding that merits further evaluation using abdominal imaging. Late-onset CAH should be considered as a differential diagnosis, particularly in certain ethnic populations, such as women of Ashkenazi Jewish decent [14]. A morning value of 17-hydroxyprogesterone

(17-OHP) greater than 200 ng/dL in the early follicular phase of the menstrual cycle is suggestive of CAH and must be further pursued by undertaking an adrenocorticotropic hormone (ACTH) stimulation test. If there is an exaggerated response to ACTH, a 17-OHP value greater than 1500 ng/dL, a diagnosis of CAH is made [31]. Additionally, androstenedione can be elevated in 21-hydroxylase-deficient CAH. Cushing's syndrome should be considered and screened for in the setting of hyperandrogenism if a patient manifests evidence of centripetal obesity, hypertension, diabetes and/or purple cutaneous striae. A 24-hour urine collection for estimation of urinary free cortisol (UFC) is a sensitive screening test for Cushing's syndrome. A UFC level more than three times the upper limit of normal should prompt further investigations. A separate chapter in this textbook offers a more detailed discussion on the relevance of adrenal function and dysfunction in the context of PCOS diagnosis.

It is important to note that reliable assessment of biochemical hyperandrogenism in women on hormonal contraception is compromised, due to stimulant effects of the estrogenic component of the OCP regimen on hepatic production of the sex hormone-binding globulin (with resulting decrease in circulating free androgens), and dampening effects of both estrogen and progestin components of OCP regimen on the pituitary and resultant suppression of gonadotrophin-dependent androgen production by the ovarian theca. Thus, drug withdrawal is recommended before undertaking biochemical evaluation of hyperandrogenemia; recommendation for nonhormonal contraceptive strategies must be underscored during this time for all at risk for an unplanned pregnancy [3]. This biochemical hyperandrogenism evaluation is applicable for the evaluation of androgen excess in all suspected to have PCOS.

Treatment of Hirsutism

The 2018 Endocrine Society guidelines recommend treatment in any woman with "patient-important" hirsutism, defined as "unwanted sexual hair growth of any degree that causes sufficient distress for women to seek additional treatment" [21]. Setting of realistic goals is critical for ensuring treatment-related satisfaction. Attainable treatment goals are targeted at excess hair becoming less coarse, growing more slowly, and requiring less frequent use of depilatory methods over time. With the discussion of treatment modalities, it is imperative to discuss fertility goals, as most pharmacotherapies are contraindicated in women seeking pregnancy. A trial of at least 6 months is recommended for observable effects, due to the duration of the hair growth cycle, provided the intervention/s is/are well tolerated.

Management approach to and treatment options for hirsutism are outlined in Table 11.1 [10].

Table 11.1 Management approach to hirsutism

Evaluation	History
	Chronicity, progression, and rapidity
	Menstrual
	Medications-anabolic steroids, danazol, testosterone
	Examination
	Severity
	Signs of virilization
	Stigmata
	Striae
	Acanthosis nigricans/skin tags
	Thyromegaly/ocular features
	Common tests
	Total (and free) testosterone
	DHEA-S
	17-OH progesterone
	24-h UFC (if suspecting cortisol excess)
	Pelvic ultrasound (if indicated)
	Pharmacotherapy
	OCPs
	CPA
	Anti-androgens
	Spironolactone
	Flutamide
	Finasteride
	GnRH agonist
	GnRH antagonist
	Glucocorticoids
	Eflornithine hydrochloride cream
	Insulin-sensitizing agents
	Cosmetic
	Shaving
	Waxing
	Epilation
	Depilation
	Laser and light therapies
	Electrolysis

DHEA-S dehydroepiandrosterone sulfate, *17-OH progesterone* 17-hydroxyprogesterone, *UFC* urinary free cortisol, *OCP* combined oral contraceptive, *CPA* cyproterone acetate (not available in the United States), *GnRH agonist* gonadotropin-releasing hormone agonist
Modified with permission from: McAvey and Lieman [10]

Pharmacotherapy for Managing Hirsutism

Systemic Treatments for Hirsutism

Combined Hormonal Contraceptives

Oral contraceptive pills (OCPs) are the first-line therapy for managing the hyperandrogenism of PCOS [21]. While this indication is an off-label use of OCPs, the evidence supports its use for hyperandrogenism and treatment of hirsutism. The additional benefits of menstrual regularity, contraception, and decreased

endometrial cancer risk must be weighed against the individualized risks of OCP use [21].

The mechanisms in which OCPs function is multifactorial. The estrogen and progestin components of the pill collectively inhibit pituitary gonadotropin secretion through feedback suppression; it is the suppression of luteinizing hormone (LH) that results in mitigation of androgen production by the LH-sensitive ovarian theca [32]. The estrogenic component of OCPs stimulates the hepatic production of SHBG, thereby increasing androgen binding in serum and reducing serum free androgen concentrations [33]. The combination of these effects can decrease serum free testosterone concentrations by approximately 50% in hyperandrogenic women treated with an OCP [34]. Another suggested mechanism is a decrease in adrenal androgen production [35]. Subjective improvement in hirsutism with OCP use has been reported in the range from 60% to 100% [36, 37]. Dewis et al. noted an objective 25% improvement in hair growth [37], while Cullberg et al. recorded longer intervals for needing depilatory measures [38]. A network meta-analysis of 43 randomized controlled trials demonstrated significant reduction in pooled-weighted mean of FGS with OCP we compared to placebo [39].

There are no universal recommendations on types or dose of progestins or estrogens in OCP formulations. Nevertheless, certain progestins are known to have inherent androgenic potential (e.g., levonorgestrel) and others to have antiandrogenic properties (such as drospirenone and cyproterone acetate [CPA]). While evidence of superiority of one over the other among formulations is limited, for the most part, all combination OCP regimens have relatively similar efficacy on symptoms of androgen excess. However, when choosing an OCP regimen, consideration should be given to the progestin component, given the varying androgenicity potential of the available progestin; OCPs containing progestins of low androgenicity (e.g., desogestrel and norgestimate) or antiandrogenic progestins such as drospirenone or CPA may be preferentially considered for women with features of hyperandrogenism. DSP is structurally similar to spironolactone [40, 41], and CPA is a 17-hydroxyprogesterone derivative that competitively inhibits the action of DHT at its receptor. Given that an increase in both venous and arterial thromboembolic events has been suggested in users of DSP compared to levonorgestrel-containing OCP regimens, judicious caution must be exercised and general population-based guidelines and individualized profiles must be kept in perspective when deciding on the choice of OCP regimen [42].

A double-blind randomized controlled trial compared the effects of CPA alone to an OCP containing desogestrel or DSP. Results indicated that CPA significantly increased SHBG and decreased the free androgen index, in addition to significantly decreasing the modified FGS [43].

Antiandrogens

Antiandrogens are commonly employed, often in conjunction with OCPs, as a second-line strategy for the management of bothersome hyperandrogenism. Addition of an antiandrogen to an OCP regimen is recommended when the initial response after 6 months to 1 year of OCP monotherapy has been inadequate. Antiandrogens can also be used as a monotherapy in those for whom use of OCPs is contraindicated (such as those with inherited thrombophilia).

Finasteride is an inhibitor of 5α-reductase type 2, an enzyme that converts testosterone to the more potent androgen DHT. In a literature review, it has been shown to reduce the overall hair diameter and number in androgen-sensitive areas [44]. A systematic review and meta-analysis demonstrated a significant reduction in hirsutism with finasteride compared with placebo [45].

Spironolactone, an aldosterone antagonist, exhibits dose-dependent competitive inhibition of the androgen receptor, in addition to inhibition of 5α-reductase activity [46]. The drug is generally well tolerated, but it may also cause increased diuresis, postural hypotension, or dizziness. A 2009 Cochrane meta-analysis and 2018 network meta-analysis both confirm spironolactone showed a significant reduction in hirsutism compared to placebo [39, 46]. A rare adverse effect of spironolactone is hyperkalemia, and therefore it should not be used in patients with renal impairment.

Flutamide is an androgen receptor antagonist that is used off-label in the treatment of hirsutism. There are a few retrospective and nonrandomized studies that indicate flutamide is superior to placebo in the treatment [47, 48]. The major limitation with the use of flutamide is the adverse event of hepatic toxicity. Complications can range from elevated transaminases found in a retrospective study to liver failure requiring transplantation and death noted in a case series [21, 49]. Given the potential for hepatotoxicity with flutamide, alternative safer regimens such as finasteride and spironolactone are preferred over flutamide for the management of hirsutism.

In the network meta-analysis of randomized controlled trial mentioned earlier, spironolactone, finasteride, and flutamide, each showed a significant reduction in hirsutism scores compared to placebo; furthermore, the addition of antiandrogens to OCPs was shown to be more effective in reducing FGS compared to OCPs alone [39]. Despite evidence of efficacy, utilization of antiandrogen regimen for the management of hirsutism represents an off-label use of these agents and the choice of antiandrogen depends upon its side effect profile and potential toxicity, availability, and cost. Given their teratogenic potential (i.e., potential for adversely affecting the development of external genitalia in an inadvertently exposed male fetus), antiandrogens must not be used as single agents by reproductive-age women who are deemed at risk for unplanned pregnancy; a concomitant contraceptive strategy must be instituted for women who are deemed at risk for conception [3, 21, 24].

Antiandrogenic potential of DSP and CPA was discussed earlier; both are available as combined hormonal contraceptives as well as monotherapy regimens; CPA-based products however are not approved for use in the United States.

GnRH Analogues

Suppression of the hypothalamic-pituitary-ovarian axis with the use of gonadotropin-releasing hormone (GnRH) agonists has been shown to be effective in the treatment of hirsutism. GnRH agonist therapy in women with hyperandrogenism has reported significant decreases in LH, testosterone, and androstenedione [50, 51]. Clinical assessment has shown a decrease in FGS and subjective reports of hair growth [52, 53]. Although GnRH agonist treatment is more effective than placebo for hirsutism, it may be considered as third-line therapy. GnRH agonist therapy is expensive and requires injections, and estrogen add-back therapy is necessary when considering

long-term use in premenopausal women to prevent bone loss. Therefore, the use of GnRH agonists should be reserved for women with severe forms of hyperandrogenemia who fail treatment with OCPs and antiandrogens [21, 24].

The approval and availability of an oral GnRH antagonist formulation (elagolix) has been a meaningful addition to the spectrum of available therapeutics. It acts as a direct competitive inhibitor of the endogenous GnRH to suppress the hypothalamic-pituitary-ovarian axis. It offers advantages over GnRH agonists given its convenience of oral administration and has a potentially lesser suppressive effects on bone turnover compared to the GnRH agonist formulations, and its efficacy and safety in the PCOS population is currently being investigated in a phase 2, multicenter, double-blind (sponsor-unblinded), randomized, placebo-controlled study [54].

Glucocorticoids

Glucocorticoids are effectively used to manage symptoms of hyperandrogenism in cases where the predominant source of androgen excess is adrenal, such as in late-onset CAH, due to 21-hydroxylase deficiency. Exogenous glucocorticoids suppress hypothalamic corticotropin-releasing hormone production and thus pituitary corticotropin and adrenal androgen production. Mitigation of hyperandrogenemia, improvement in acne, and resumption of menstrual cyclicity are well described with glucocorticoid use in women with late-onset CAH; effectiveness in reducing severity of hirsutism is less evident. Despite evidence of efficacy, given the significant risk for adverse effects with chronic use of systemic glucocorticoid which includes weight gain, adverse effects on metabolism, on blood pressure indices, on mood, on the skeleton and skin, and a risk for adrenocortical atrophy with long-term use, current recommendations caution against utilizing glucocorticoid therapy for routine treatment of hirsutism, the exception being those women with late-onset CAH who fail to respond to or cannot tolerate OCP or antiandrogen therapies [10, 21, 24].

Insulin-Sensitizing Agents

As discussed earlier in this chapter, and covered extensively elsewhere, hyperinsulinemia and insulin resistance are stimuli for ovarian androgen synthesis. Thus, pharmacotherapy targeting the reduction of insulin levels can attenuate hyperandrogenemia, and indeed, improvements in androgen profiles are well described following use of insulin-sensitizing agents including metformin and thiazolidinediones in women with PCOS, albeit inconsistently. However, when evaluated specifically for benefit against hirsutism, a meta-analysis of six randomized controlled trials (RCTs) found no statistically significant difference between metformin and placebo, regardless of BMI [3]. Similarly, an updated systematic review of nine trials in a pooled analysis showed metformin was no more effective than placebo for lowering hirsutism scores [21]. Randomized treatment with other insulin sensitizers, such as rosiglitazone, similarly had no significant effect on hirsutism [21]. Current understanding is that insulin sensitizers provide limited or no important benefit against hirsutism

and current recommendations therefore do not support using insulin-lowering drugs for the sole indication of treatment of hirsutism.

Topical Treatment for Hirsutism

Eflornithine Hydrochloride

Eflornithine hydrochloride is an inhibitor of the enzyme *ornithine decarboxylase* which catalyzes the rate-limiting step for follicular polyamine synthesis. A topical formulation of eflornithine hydrochloride 13.9% (Vaniqa) is available in the United States as an FDA-approved product for the management of unwanted facial hair. Noticeable results occur in 6–8 weeks; however, since it acts to inhibit hair growth and is not depilatory, discontinuation of treatment will cause hair growth to return to pretreatment levels as early as 8 weeks following discontinuation. In one study, eflornithine hydrochloride cream was superior to placebo, 58% as compared to 34%, in reducing hair growth [55]. Furthermore, combined eflornithine and laser therapy demonstrated a more rapid response when compared with laser treatments alone [56]. Common adverse effects include itching and dry skin. Unfortunately, this formulation is rarely covered by insurance plans, thus limiting widespread use.

Cosmetic Strategies for Managing Hirsutism

Temporary Measures

Temporary cosmetic hair removal methods include shaving, plucking, epilation, waxing, or depilation. These methods may be sufficient when used in mild or localized cases or can be used in combination with pharmacologic interventions. Shaving is the mechanical process of removing hair just below the surface of the skin. Skin irritation, risk of folliculitis keratosis, and discoloration are potential adverse events, and this technique may require daily maintenance [24]. Plucking, epilation, and waxing are relatively safe and inexpensive methods to remove hair by its root. The effects can last for 6–8 weeks, although these may be uncomfortable and time consuming to endure. Chemical depilation dissolves the hair via thioglycolates, which disrupt disulfide bonds in the hair. The dissolved hair can be wiped away. Contact dermatitis and even hyperpigmentation can result from chemical methods [21, 57], and results are transient, with effects often lasting no more than about 2 weeks.

Although not an actual method of hair removal, bleaching with the use of a product that contains hydrogen peroxide is a relatively common method for masking the presence of undesired hair; side effects include irritation and possible skin discoloration.

Permanent Measures

Photoepilation therapies such as laser may be used to reduce hair density via selective photothermolysis, a process that directs energy and sufficient heat into the target area of hair to destroy the hair bulb; the surrounding areas remain relatively untouched. This

process usually results in a reduction in hair growth; new hairs in the treated area are lighter and thinner in nature, although multiple treatment sessions are usually required before any benefit may become apparent, and one is unlikely to achieve complete resolution of excess hair. Photoepilation uses pulses of light of wavelength that is absorbed by the melanin in the hair shaft and follicle and causes selective photothermolysis of the pigmented terminal hair. This method requires multiple sessions for increased efficacy, given that hairs at a given site are at different stages in the growth cycle. Terminal hairs that regrow following photoepilation are lighter and thinner in texture. Photoepilation has traditionally worked best on light-skinned individuals with dark hair, but the development of the long-pulsed Nd:YAG (neodymium:yttrium-aluminum-garnet) laser has demonstrated efficacy in dark-skinned individuals with a low rate of pigment changes. A systematic review of 11 RCTs reported a 50% reduction in hair growth lasting up to 6 months [58]. Additionally, a meta-analysis of 24 studies found significant hair reduction at 6 months after the last treatment [59]. A RCT that focused on the PCOS population compared five laser treatments versus five sham laser treatments and found a significantly greater decline in the severity of facial hair and improved quality of life with laser treatment [60]. Although an effective strategy, the adverse effect of paradoxical hypertrichosis following laser hair ablation has been reported in the literature, particularly in women of Mediterranean and Middle Eastern descent [21]. Patients should be counseled regarding setting realistic expectations as complete hair removal is rarely achieved, whereas responding individuals can expect an average of 80% reduction in excess hair after 6 months of the final treatment [61]. Furthermore, periodic maintenance treatments are often required to maintaining sustaining benefit.

Electrolysis, a therapy that is performed by inserting a needle into the hair follicle and applying an electrical current that creates heat, is technically the only permanent method of hair removal. The therapeutic efficacy is based on permanent destruction of the rapidly dividing cells of the matrix and the follicle itself. Electrolysis can be effective in reducing hair counts and creating a permanent removal of hair in 75–85% of patients. The procedure is generally limited to small treatment areas given that electrolysis can be uncomfortable, often requiring use of local anesthetics to improve tolerance. Furthermore, inflammatory pigment changes and residual scarring can occur. The advantage of electrolysis over photoepilation is that it does not depend on hair pigmentation. A prospective study compared electrolysis and photoepilation with a split face treatment and found that 96% of the patients preferred photoepilation to electrolysis treatment, with higher efficacy and satisfaction [62].

Acne

Acne vulgaris, characterized by inflammation of the pilosebaceous unit, is a prevalent cutaneous manifestation of PCOS. The prevalence of acne in women with PCOS has been estimated to be 10–34%, with as high as 50% prevalence reported in adolescent girls [63]. In a study of 52 women aged 18–35 years with mild acne versus 59 age-matched controls, PCOS was significantly more common in patients

with acne than in age-matched controls [64]. Pathogenesis of acne entails the formation of comedones due to increased sebum accumulation, which allows bacterial colonization and subsequent inflammation. Physiology of the pilosebaceous unit is influenced by several factors, including hormones such as androgens and insulin, as well as local factors. Androgens stimulate the growth and secretory function of sebaceous glands. The increased sebum production is thought to provide a growth medium for the bacterium *Cutibacterium acnes* (*C. acnes*) and leads to a predisposition to formation of comedones [65]. It is the immune response to *C. acnes* that can lead to more severe manifestations of acne by developing inflammatory papules and pustules. Androgen-sensitive sites for acne include the face, neck, mid-chest, and upper back [66, 67]. Comedonal acne can affect up to 85% of adolescents, and in adult females it can range from 7% to 50% [68]. The presence of acne is common and multifactorial, including genetic factors, diet, skin trauma, and even stress [69]. When examining acne specifically in the PCOS population, a retrospective cross-sectional study found the prevalence of acne among women without PCOS was 40.4%, but 61.2% in women with PCOS. This study additionally showed that increased serum androgen levels were not associated with acne in the PCOS population [70]. An older study corroborated these results and found only 25% of women with moderate acne had an elevated plasma free testosterone level [71]. These data suggest that acne is a complex multifactorial entity with diverse pathophysiological underpinnings other than androgen excess. However, the subset of patients with persistent moderate-to-severe inflammatory acne vulgaris should warrant an endocrinological workup, given a higher likelihood of hyperandrogenemia and an underlying systemic disorder such as PCOS [72].

Evaluation of Acne

A thorough history and focused clinical examination should precede biochemical evaluation for evidence of hyperandrogenemia. Despite existence of a number of scoring systems such as the Leeds technique, acne lesion counting, or global acne grading system (GAGS), there is no accepted universal standard for assessing and quantifying acne [73]. Physical exam should focus on examining the affected sites and detailing the nature of lesions (comedones, papules, pustules, nodules) as well as existence and severity of inflammatory response and coexisting cutaneous stigmata including hirsutism, acanthosis nigricans, skin tags, and striae.

Acne Treatments

The goals of therapy are to reduce the amount of sebum production, clear the existing comedones, and reduce the amount of bacterial colonization and inflammation (Table 11.2).

Table 11.2 Management approach to acne

Evaluation	History
	Rapidity of progression in symptoms
	Menstrual
	Medical
	Medication
	Examination
	Site
	Severity
	Inflammation
	Additional stigmata
	Common tests
	Total (and free) testosterone
	DHEA-S
	17-OH progesterone
	Systemic pharmacotherapy
	OCPs
	CPA
	Oral antibiotics
	Oral retinoids
	Anti-androgens
	Spironolactone
	Flutamide
	Finasteride
	Topical agents
	Benzoyl peroxide
	Retinoid cream
	Antibiotic cream
	Cosmetic therapy
	Laser/light therapy

DHEA-S dehydroepiandrosterone sulfate, *17-OH progesterone* 17-hydroxyprogesterone, *OCP* combined oral contraceptive, *CPA* cyproterone acetate (not available in the United States)
Modified with permission from: McAvey and Lieman [10]

Systemic Treatments for Acne

Combined Hormonal Contraceptives

Similar to hirsutism management, OCPs are an acceptable first-line approach to the management of acne. Hormonal therapy is often considered to treat acne in the settings of hyperandrogenemia, late-onset acne (>25 years of age), jawline acne distribution, acne with menstrual flare, and acne that is resistant to conventional therapies. OCPs limit the exposure of the pilosebaceous units to the androgens, thereby decreasing sebum production. There are currently four FDA-approved combination OCPs for the treatment of acne that contain low androgenicity or antiandrogenic progestins: Ortho Tri-Cyclen (norgestimate/ethinyl estradiol), Estrostep (norethindrone acetate/ethinyl estradiol), YAZ (drospirenone/ethinyl estradiol), and Beyaz (drospirenone/ethinyl estradiol/levomefolate) [74, 75]. The ethinyl estradiol in OCPs works to increase production of SHBG and suppress ovarian androgen

production by decreasing pituitary LH, thereby reducing the level of biologically available circulating free androgen.

With the presence of an estrogenic component, several OCPs exhibit some degree of efficacy in the reduction of acne lesion count by 3–6 months of use [76]. A pooled analysis of two placebo-controlled randomized trials found that patients treated with a drospirenone-containing OCP were more likely to attain clear skin than those receiving placebo, and a similar superior therapeutic efficacy was seen when compared against a norgestimate-containing OCP in another study [77, 78]. A subsequent Cochrane review, however, evaluated 31 trials and demonstrated no consistent differences in acne reduction among different formulations of combined OCPs, thereby signifying non-inferiority [75].

Oral Antibiotics

Oral antibiotics improve inflammatory acne by inhibiting the growth of *C. acnes* within the pilosebaceous unit and decreasing inflammation. Oral antibiotics are typically used for moderate-to-severe acne, acne on the chest and back, and with inflammatory lesions when topical agents have not been efficacious. Systemic antibiotics produce more rapid clinical improvement than topical preparations but may induce side effects such as vaginal candidiasis or gastrointestinal distress. Tetracyclines are the most commonly prescribed oral antibiotic for acne. A systematic review of 13 studies has demonstrated the effectiveness of various tetracyclines to treat acne [79]. Doxycycline is FDA approved for the adjunctive treatment of severe acne and has even been found to be effective at subantimicrobial dosing as low as 20 mg [80]. Sarecycline is a novel tetracycline designed with a narrower spectrum of antibiotic activity to target acne. It is also less likely to cause *C. acnes* bacterial resistance or gastrointestinal disturbances [79]. Patients should be counseled about the potential for teratogenicity with discoloration of developing permanent teeth with long-term treatment. The results of a meta-analysis comparing the efficacy of OCP therapy versus oral antibiotic therapy show that antibiotics may produce a more rapid response to treat acne within 3 months, but OCPs and antibiotics were equivalent in reducing acne lesions at 6 months of use [81].

Retinoids

Isotretinoins are vitamin A derivatives that bind to retinoic acid receptors within the keratinocytes and cause a decrease in sebum, inhibition of bacterial proliferation of *C. acnes*, inhibition of comedone development, and reduce inflammation. It is considered the most efficacious strategy against moderate-to-severe inflammatory acne; however, the severity of adverse effects limits its use [82]. This medication is contraindicated in pregnancy; it can cause severe congenital malformations and spontaneous abortions with exposure in the first trimester. Thus, patients must be counseled on the monitoring and contraception that are required to prescribe isotretinoin therapy. Additional adverse effects include photophobia, headache, depressed mood, dry skin, elevated liver enzymes, and decreased appetite [83]. Treatment duration usually lasts for a minimum of 6 months. In one study of PCOS patients with severe cystic acne with a contraindication to use OCPs, oral isotretinoins improved acne scores and had a decrease in FGS and a decrease in free testosterone levels [84].

Antiandrogens

Antiandrogens discussed under hirsutism management are also effective in reducing acne, primarily due to effects at reducing sebum production. The side effect profile of flutamide limits its use, since several other safer treatment modalities can be employed to treat acne. Spironolactone is the most commonly prescribed antiandrogen for the treatment of acne [76]. Data do not consistently demonstrate greater efficacy with the use of spironolactone over OCPs; however, the off-label use of spironolactone is often reserved for those patients who fail prior treatment or have contraindications to OCPs. A 2009 Cochrane review found insufficient evidence to confirm the efficacy of spironolactone in women with acne, although several published small studies have reported treatment benefit [46]. One prospective study reported that 85% of patients with acne had significant improvement in facial lesions with spironolactone treatment [85]. In a 2020 retrospective study of patients with acne treated with spironolactone, 66% of patients had ≥90% improvement and 19% of patients had 51–89% improvement [86].

Topical Treatments for Acne

Topical treatments are the first-line therapy for mild acne vulgaris and can be used in conjunction with other treatments in more severe cases. They can be prescribed or are available over the counter and are comprised of several classes of pharmacotherapy including antibacterial, retinoid and antiandrogen formulations. They work to decrease comedone production and bacterial colonization. The choice of topical therapy can be determined by affected site, severity of disease, or patient preference [15].

Benzoyl peroxide is found in various formulations over the counter and has potent antibacterial action. Low dose of benzoyl peroxide (2.5%) was more effective than placebo and as effective as the higher doses in treating acne [87]. Exposure to higher concentrations can lead to significant skin irritation.

The topical retinoids prevent the formation of the microcomedone as well as decrease bacterial colonization but usually do not decrease the amount of sebum production [88]. The topical retinoids include all-trans-retinoic acid (tretinoin), adapalene, and isotretinoin (tazarotene). Dry flaky skin, irritation, and photosensitivity are common side effects of topical retinoids and use of sunblock protection is advised. Given the adverse effects, it is recommended to start with the lowest-strength preparation and then increase the potency as needed and as tolerated. Randomized controlled trials of topical retinoids compared to placebo have shown greater reduction in acne lesion count when used alone, as well as improved efficacy in the setting of combination therapy with clindamycin/benzoyl peroxide [89, 90]. Topical retinoids should be avoided in pregnancy.

Topical antibacterial agents such as clindamycin, tetracycline, and erythromycin can be used as monotherapy or in combination with a topical retinoid or benzoyl peroxide. A meta-analysis found that the combination of benzoyl peroxide and clindamycin in gel form was more effective and resulted in more rapid improvement than benzoyl peroxide alone for the treatment of acne [91].

Clascoterone is a new topical cream that was approved in the United States by the FDA in 2020 for the treatment of acne. It competitively inhibits binding of DHT to the androgen receptor. Data from two phase 3 randomized trials show an increased

number of patients with treatment success compared to placebo after 12 weeks of intervention [92]. Data regarding long-term use, however, is lacking in the reproductive-age population of women with PCOS.

Cosmetic Strategies for Managing Acne

Laser and light therapies have become popular cosmetic means to treat acne. Exposure to red and blue visible light therapy is thought to produce free oxygen radicals and bacterial death. Small RCTs found that it was not more effective than topical clindamycin, but there are conflicting data on if it is more efficacious than benzoyl peroxide [93, 94].

Laser therapy is thought to inflict thermal damage to sebaceous glands and decrease sebum production. These light-based therapies can have varying results and require multiple sessions. Side effects may include skin discoloration, lingering pain, scarring, burns, or blisters [95].

Female Pattern Hair Loss

Also known as androgenic alopecia, female pattern hair loss (FPHL) is a hereditary condition, and considered a sequel to disruption of proper androgen signaling [96]. Recognized as the most common type of hair loss in women [97], FPHL is characterized by decreased proliferation of follicle epithelia and progressive miniaturization of the terminal hairs on the scalp. It is the most common type of hair loss that occurs in women [97]. The pattern of hair loss has a classic distribution that preferentially affects central, frontal, and parietal scalp areas. FPHL can have a significant impact on the psychological well-being and quality of life [98].

Hyperandrogenemia, advancing age, menopause, and genetic predisposition have all been suggested as relevant to the pathophysiology of FPHL. Hyperandrogenism can be associated with FPHL but is not necessary for the diagnosis. It is for this reason the AE-PCOS Society prefers the term FPHL over female androgenic alopecia [99].

The prevalence of hyperandrogenemia in the FPHL population varies widely in the literature. In a sample of over one thousand white women, a rate of FPHL was noted to be 3% for women in their 20s, 17% for women in their 30s, and 23% for those in their 50s, suggesting its presence increases with age [96]. Ethnic differences exist and women of Asian descent have a lower prevalence overall, although still showing a similar trend with increasing age. In a recent meta-analysis assessing FPHL in PCOS patients, the prevalence was 20–30% higher than in the general population [99].

The process underlying FPHL involves a progressive miniaturization of the terminal hair follicles and subsequent reduction of the number of scalp hairs. Typically, hair growth is cyclical and comprised of three distinct phases—the growth phase (anagen), the involutional phase (catagen), and the resting phase (telogen). Normally, the majority of hair follicles are in the anagen phase, and as hair is shed from the telogen phase, the same numbers of follicles enter the anagen phase [100]. The loss of hair in FPHL results from a shortening of the anagen phase; the resulting effect

is of shorter and thinner hair shaft. Varying degrees of hair thinning are commonly encountered, primarily at the vertex and frontal areas of the scalp [101]. The process of miniaturization does not permanently destroy the hair follicle but results in an overall reduction in hair density [99]. The hair cycle is influenced by several factors including genetic, hormonal, and inflammatory processes. Enhanced androgen action in the scalp may occur due to an increase in local DHT production. Levels of 5α-reductase mRNA were found to be increased in hair follicles in women in FPHL [102]. DHT binds to androgen receptors in hair follicles and facilitates the gradual transformation of terminal hair follicles to miniaturized hair follicles. The pattern of the hair loss typically seen in FPHL reflects regional differences in the sensitivity of scalp follicles to androgens [103, 104]. The role of androgens in the pathogenesis of FPHL is however complex, given that there was laboratory evidence for hyperandrogenism in only 39% of women [105]. Thus, it is important to note that FPHL may be present in the absence of any detectable signs of androgen excess.

Evaluation of Hair Loss

The diagnosis of FPHL is clinical. History alone can offer meaningful information about timing of onset, chronicity, rate of progression, and coexisting features to suggest virilization. Meaningful information can be gleaned from the menstrual, medical, and family histories (including history of FPHL in female members of the family) and review of list of medications and exposures.

On physical exam, two typical patterns of scalp hair thinning can be seen in FPHL: central loss (with widening of central hair parting and sparing of the frontal hairline) or as frontal accentuation. One may also note the hair has a variety of diameters and lengths in the affected areas and there is an absence of diffuse shedding [99]. The Ludwig scale is one of the most commonly used grading systems that describes and classifies the distinctive features of androgenic alopecia into three grades of severity referred to as Ludwig grades I, II, and III [106].

Although no biochemical tests provide a definitive diagnosis of FPHL, they can aid in identifying an underlying hyperandrogenic state or differentiate FPHL from other etiologies of hair loss. Measurements of blood iron studies, vitamin D, zinc, thyroid profile, and prolactin may additionally be useful to rule out and treat other conditions that may affect hair regrowth in FPHL [99].

Potential differential diagnosis must be considered in the evaluation of hair loss. The diagnosis of FPHL is sometimes difficult to make, as often women use varying methods to hide the hair loss. In addition, there are multiple hair and scalp disorders that may present with clinical features that resemble FPHL, making the diagnosis not always clear. Hair loss can be categorized as either scarring or non-scarring alopecia [70]. Scarring alopecias are conditions that lead to the irreversible cessation of hair cycling and permanent hair loss, while in non-scarring alopecias, the hair follicle is not permanently damaged and, often, spontaneous or treatment-induced regrowth is a possibility. *Telogen effluvium* is a disorder that may be most difficult to distinguish from androgenic alopecia because patients present with acute or chronic non-inflammatory, diffuse hair loss that often follows a significant event, such as childbirth, a major illness, or severe trauma. Hair

loss occurs in all areas of the scalp, although the hair loss may be most evident in the temporal area [70]. Another diffuse form of hair loss includes *alopecia areata*, a disorder that is characterized by a global reduction in pigmented hair density, often detected in circumscribed areas. In addition, these patients may have nail abnormalities and patchy hair loss on other body sites, findings that are not typically associated with androgenic alopecia [70]. Although a scalp biopsy is usually not needed to diagnose androgenic alopecia, it may be helpful in cases when the clinical evaluation does not provide a definitive diagnosis, as in distinguishing FPHL from telogen effluvium or diffuse alopecia areata and, furthermore, in differentiating scarring and non-scarring alopecia [71]. Scarring alopecias such as central centrifugal cicatricial alopecia or frontal fibrosing alopecia may be best distinguished with a dermatoscopic examination and a scalp biopsy. Making the diagnosis may involve multidisciplinary care [99].

Treatment of Female Pattern Hair Loss

Management of FPHL involves systemic pharmacotherapies and local cosmetic therapies, as outlined in Table 11.3.

Table 11.3 Management approach to female pattern hair loss

Evaluation	History
	Onset and rapidity of progression in symptoms
	Menstrual
	Medical/stress
	Medication
	Common tests
	Total (and free) testosterone
	DHEA-S
	17-OH progesterone
	FSH/LH/estradiol
	TSH
	Blood iron studies
	Vitamin D and zinc
	Prolactin
	Scalp biopsy
	Systemic pharmacotherapy
	Minoxidil
	Anti-androgens
	Spironolactone
	Finasteride
	Dutasteride
	Local/cosmetic
	Laser and light therapies
	Surgery (hair transplant)

DHEA-S dehydroepiandrosterone sulfate, *17-OH progesterone* 17-hydroxyprogesterone, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *TSH* thyroid-stimulating hormone
Modified with permission from: McAvey and Lieman [10]

Systemic Treatments for Female Pattern Hair Loss

Oral Antiandrogens

Antiandrogens can be employed for the treatment of FPHL. In a single-center retrospective study, 74.3% of patients receiving spironolactone reported stabilization or improvement of their disease [107]. In an observational study, daily use of spironolactone or CPA was associated with stabilization of hair loss or even hair regrowth [108].

Finasteride (Propecia) is a type 2 *5α-reductase* inhibitor and is also FDA approved for the treatment of androgenic alopecia. Data on the efficacy of finasteride for female hair loss are conflicting. A randomized controlled trial showed that finasteride did not show a statistically significant reduction in Ludwig scores [109]. This finding was corroborated by the 2016 Cochrane meta-analysis which found that finasteride was no more effective than placebo when patients self-rated their hair regrowth. Smaller observational studies, however, suggest finasteride may be effective, particularly in postmenopausal patients [107]. As discussed prior with antiandrogens, there is potential for teratogenicity and contraception should be discussed with patients of childbearing age.

Dutasteride is an inhibitor of both type 1 and type 2 isoforms of *5α-reductase* and can be used off-label for the treatment of FPHL. In two RCTs, dutasteride was found to be superior to placebo and finasteride, making it perhaps a more favorable treatment modality [110].

Topical Treatment for Female Pattern Hair Loss

Minoxidil (Rogaine) is an FDA-approved topical agent for the treatment of alopecia and is considered as first-line treatment. It is thought to promote hair growth by increasing the duration of anagen phase, shortening the telogen phase, and enlarging miniaturized follicles via a mechanism involving nitric oxide [96]. The efficacy of topical minoxidil was reviewed in a 2016 Cochrane systematic review and meta-analysis. Pooled data from six studies found that treated women reported a statistically significant moderate increase in hair regrowth when compared with placebo. Four studies within this Cochrane meta-analysis compared minoxidil (2%) versus minoxidil (5%) and none found a significant difference in efficacy with two different concentrations [111]. Potential adverse effects of minoxidil include scalp pruritus, flaking, and facial hypertrichosis. Topical minoxidil is recommended as a long-term treatment. Optimal results can take up to 12 months [99].

Cosmetic Strategies for Managing Hair Loss

Low-level laser light therapy (LLLT) uses red or near-infrared laser light to promote tissue repair and regeneration, modulate inflammation, and stimulate hair growth [99]. A randomized double-blind clinical trial comparing 26 weeks of LLLT therapy to sham therapy found a statistically significant increase in terminal hair density with the laser as compared to sham-treated subjects, although it may be of modest benefit [112]. Adverse events with use may include pruritis, headache, erythema, and mild paresthesia.

Surgical management of FPHL involves hair transplantation of follicular units and is often utilized when pharmacotherapy fails. In this process, terminal hair

follicles are harvested from unaffected areas of the scalp and transplanted into areas of low hair density. It is important to counsel patients that they can continue to lose hair in susceptible areas where transplantation did not occur, so concomitant medical therapy is recommended. Limitations of hair transplantation include potential for transplant failure and high cost and time [99].

Return to the Clinical Vignette

T.K., a 26-year-old mildly overweight but otherwise healthy female who meets criteria for PCOS (irregular menses, hirsutism, mild persistent acne whose workup identified US evidence of polycystic ovaries and mild elevation in circulating free testosterone levels) and whose principal bother centers on symptoms of hyperandrogenism.

T.K. was started on a trial of OCPs containing ethinyl estradiol and drospirenone. The choice of drospirenone-containing OCP regimen was based on her primary complaint, on her past lack of responsiveness to non-drospirenone-containing regimens, and an individualized assessment of her being at low risk for venous thromboembolism. She was additionally counseled to consider concomitant use of a topical benzoyl peroxide face wash for facial acne.

At 6-month follow-up visit, T.K. endorsed significant improvement in facial acne but acknowledged dissatisfaction with responsivity of excess facial and bodily hair to the current regimen. At this time, discussion for multidrug therapy was introduced, and her fertility plans and goals were assessed. T.K. did not foresee fertility planning in the foreseeable future and expressed an interest in trial of a multiregimen approach. Addition of an antiandrogen formulation to current hormonal contraceptive regimen was deemed as an optimal next step. Risks, benefits, and alternatives of initiating antiandrogen therapy were reviewed, and the importance of concomitant use of a reliable contraceptive when taking antiandrogens was underscored. A basic metabolic panel revealed normal potassium level and kidney function. Spironolactone was started at a dose of 25 mg daily with plans for gradual dose escalation over weeks to a goal dose of 50 mg twice daily. Cosmetic depilatory strategies were encouraged, and T.K. was advised regarding a realistic timeline of 3–6 months for any benefit to become apparent. Periodic biochemical surveillance was recommended to ensure timely detection of any electrolyte imbalance resulting from long-term use of spironolactone. At 4-month follow-up visit following initiation of combination regimen, T.K. endorsed satisfaction with the cutaneous benefit achieved. She is continuing with OCP and spironolactone combination regimen that has effectively harnessed her symptoms of acne and hirsutism in the setting of PCOS diagnosis.

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Role of Insulin-Sensitizing Drugs in PCOS Management

12

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Key Points

- Insulin resistance is recognized as contributory to the clinical picture of PCOS.
- Metformin is the insulin-sensitizing drug of potential benefit for women with PCOS who are planning to conceive.
- Letrozole has gradually replaced clomiphene citrate as the first-line agent for ovulation induction in PCOS-related subfertility.
- Metformin-alone therapy may benefit the nonobese, while metformin combination therapy with clomiphene citrate may benefit obese women with PCOS who are attempting natural conception.
- Metformin co-treatment during IVF/ICSI cycles does not improve any of the reproductive endpoints in women with PCOS, except for a reduction in the risk of ovarian hyperstimulation syndrome.
- The use of metformin during pregnancy to prevent spontaneous miscarriages in PCOS is not supported by existing data.
- Insulin-sensitizing drugs offer no advantage over established anti-androgenic drugs for ameliorating the symptoms of androgen excess.
- There is no established role for insulin-sensitizing drugs in the long-term prevention of type 2 diabetes mellitus and coronary artery disease in PCOS.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_12

Polycystic Ovary Syndrome and Insulin Resistance

Nearly nine decades following its original description, polycystic ovary syndrome (PCOS) remains a poorly understood entity. Various pathophysiologic mechanisms have been proposed, including intrinsic ovarian, adrenal, and hypothalamic-pituitary pathways, but none was shown to provide a complete explanation for the development of this condition. Much interest has accrued lately over the metabolic aspects of the syndrome, rendering insulin resistance with compensatory hyperinsulinemia the mechanism most widely studied today [1–3].

Increased insulin resistance is observed in 20–30% of nonobese and 70–80% of obese women with the syndrome [4–6]. Affected women tend to be more insulin resistant than weight-matched women in the general population [7]. Although obesity is a recognized risk factor for decreased insulin sensitivity [8], at least one aspect of the insulin resistance hypothesis in the context of PCOS is thought to be largely obesity independent [7]. Hyperinsulinemia is believed to stimulate ovarian androgen production by direct action on the theca cells and by potentiating LH effects [9]. The local buildup in follicular androgenic signal has been shown to promote premature follicular atresia [10], primary and pre-antral follicle arrest, and impaired follicle dominance [11], leading to anovulation [12]. High insulin state further potentiates adrenal androgen secretion by enhancing adrenal response to ACTH [13]. Insulin has further been implicated in impairing progesterone inhibition of the gonadotropin-releasing hormone pulse generator [14–16] and suppression of hepatic synthesis of sex hormone-binding globulins (SHBG).

More than 50% of women with PCOS are obese [17]. Since abdominal obesity, expressed by increased waist-to-hip ratio, is considered an independent factor for reduced fecundity [18–20] and increased menstrual disorders [4, 21], behavioral therapy aimed at weight reduction has been proposed as a first-line management strategy for improving fertility in the overweight and obese women with PCOS [22]. The effectiveness of this approach, however, is highly dependent on personal drive in the context of well-structured lifestyle modification programs [23, 24]. The main limitation to such programs, however, lies in the difficulty of safeguarding benefits over time. For this reason, much attention has been diverted lately toward finding an alternative pharmacologic approach to the management of PCOS.

Insulin-Sensitizing Drugs: Background

The management of PCOS has traditionally focused on the presenting complaint(s), i.e., infertility treatment through ovulation-induction strategies, hormonal manipulation for the management of menstrual irregularities, and anti-androgenic therapy for dealing with features of hyperandrogenism (hirsutism and acne). An interest in the role of insulin-sensitizing drugs (ISDs) as a means of reducing compensatory hyperinsulinemia in the hope of improving metabolic and reproductive functions in women with PCOS has grown measurably over the past decade [25, 26].

Metformin

Metformin is an FDA-approved biguanide for the management of type 2 diabetes mellitus (T2DM). Although its mechanism of action remains obscure, metformin was shown to activate adenosine monophosphate-activated protein kinase (AMPK) pathway, inhibiting hepatic production of glucose, reducing oxidation of fatty acids, and increasing peripheral tissue uptake of glucose [27–29]. Metformin is believed to lower fasting serum insulin levels in insulin-resistant states without inducing hypoglycemia [30–32] and helps reduce insulin requirements in insulin-dependent and non-insulin-dependent diabetes. These effects, however, are not universal particularly in the PCOS population. While inducing a significant drop in fasting insulin levels in nonobese women (BMI <30 kg/m^2) with PCOS (mean difference (MD) −5.65 mIU/L; 95% CI −10.25 to −1.06; 4 trials; 85 women), metformin treatment [26] failed to show a similar effect in the obese PCOS population (BMI ≥30 kg/m^2) (MD −2.72 mIU/L; 95% CI −6.50 to 1.05; 12 trials; 488 women).

An additional mechanism by which metformin mitigates the abnormal folliculogenesis associated with PCOS is through its postulated effect on AMH. Elevated AMH levels in PCOS are associated with slowing of the initial "FSH-sensitive" follicular growth and a lower apoptosis rate of granulosa cells from pre-antral follicles resulting in a "stock piling" effect and a subsequent excessive number of growing follicles [33, 34]. In addition, they are associated with inhibition of ovarian aromatase expression and reduced LH receptor expression culminating in follicular arrest [33, 35, 36]. The body of evidence about the effect of metformin on AMH levels is rather limited to a few observational studies with small sample size. While some studies support an association between metformin and lower AMH levels [35, 37–40], others failed to show any similar effects [41–44]. A secondary analysis of an RCT showed a statistically significant decrease in AMH levels by 9.3 pmol/L in obese women after 6 months of metformin treatment [45]. Changes in AMH levels are expected to occur with a 3–6-month delay due to the protracted turnover of the developing follicular cohorts [45].

Despite the lack of consensus on the optimal dosing regimen for metformin, the most commonly used dose is 1500–2000 mg daily (either 500 TID or 850 BID) for 6 months. A study of 108 PCOS patients revealed that compared to overweight patients, obese patients with a BMI of 31.15 (± 0.40) did not demonstrate normalization of the HOMA index with 1500 mg daily dosing and required 2500 mg daily to achieve this target [46].

Bothersome side effects of metformin may be experienced in about 30% of patients and not uncommonly can be a reason for treatment discontinuation. These are predominantly gastrointestinal in nature, consisting of bloating, abdominal discomfort, nausea, vomiting, and diarrhea [47]. A Cochrane systematic review [26] revealed a fourfold increase in the incidence of gastrointestinal disturbances when metformin was used compared with placebo (OR 4.27; 95% CI 2.4–7.59; 5 trials; 318 women). Symptoms are usually dose dependent and can be mitigated by a gradual increment in the daily drug dose from 500 to 2000 mg over a period of 4 weeks.

The gastrointestinal side effects and frequent daily dosing are the two major factors hindering compliance to metformin use. Both of these issues can be tackled by

the more advanced and equally effective formulation, extended-release metformin (XR) [48, 49]. Various studies have demonstrated a lower incidence of gastrointestinal symptoms after XR metformin compared to the immediate-release formulation, leading to higher compliance rates [49, 50]. Slower absorption of metformin accounts for improved tolerability [51, 52]. In fact, a pharmacokinetic study revealed that the maximum drug concentration after the XR formulation was attained by 7 hours vs. 2–3 hours following the immediate-release one [53]. Hence, once daily dosing with dinner results in drug levels peaking during sleep [52].

A rare but serious complication of metformin therapy is lactic acidosis, which has been reported in high-risk conditions such as renal insufficiency, liver disease, and congestive heart failure, with fatality rates approaching 50% [29, 30]. A clinically insignificant decrease in vitamin B_{12} absorption has also been reported in association with metformin use [29].

Metformin remains the preferred ISD for utilization in the management of PCOS due to the vast experience reported in the literature on its use and potential for metabolic benefit [30]. It is a Class B drug with no proven teratogenic risks in animals and no reported fetotoxic effects in humans [12, 30, 47].

Thiazolidinediones

Insulin-sensitizing effects of thiazolidinediones (TZDs) are mediated through nuclear gamma-peroxisome proliferator activator receptor (PPAR) binding, inducing gene transcription and activation [30]. TZDs improve insulin signaling, enhance glucose uptake in adipose tissue and muscle, and confer multiple effects on lipid metabolism and inflammatory pathways [30]. Randomized clinical trials nevertheless failed to demonstrate any measurable reduction in fasting serum insulin levels in women with PCOS following either rosiglitazone [54, 55] or pioglitazone therapy [56]. While TZDs exhibit a greater potency at increasing peripheral glucose uptake, metformin was found to be more effective in decreasing hepatic glucose production [47].

Enthusiasm for TZD use in PCOS management is dampened not just by a predisposition toward weight gain seen with this class of ISDs [47, 56–59] but also by safety concerns as TZD use has been associated with increased risk for cardiovascular events, namely, coronary artery disease, myocardial infarction, and nonfatal heart failure [60, 61]. More recently, an increased risk of bladder cancer has raised additional concern [62], further constraining their use in healthy individuals. Fluid retention leading to edema and dilutional anemia has also been reported in association with TZD use [30, 47, 61]. Concern over the incidence of hepatoxicity as a result of troglitazone use led to its discontinuation and removal from the market [29, 30]. Moreover, rosiglitazone and pioglitazone are classified category C drugs with proven teratogenicity in animals, further limiting their use in reproductive age women [61].

Inositol

Over the past 30 years, light has been shed on a new class of insulin sensitizers: the inositol stereoisomers. The inositol family, which is chemically known as hexahydroxycyclohexane, consists of nine stereoisomers [63]. The most abundant

form is myo-inositol (MI), followed by D-chiro-inositol (DCI) [64]. The conversion of MI to DCI occurs via an epimerase-mediated reaction with different tissue-specific MI/DCI ratios that modulate various metabolic processes [65, 66]. The MI/DCI ratio averages at 40:1 in plasma as opposed to being around 100:1 in follicular fluid [64, 65].

After transformation to inositol phosphoglycans via phosphorylation, both MI and DCI presume the role of secondary messengers in numerous metabolic pathways, the most intriguing of which is glucose metabolism [65]. The modulatory effects of MI on glucose transport and use, as well as that of DCI in glycogen synthesis, culminate in the attainment of insulin-sensitizing activity [64]. As a matter of fact, one of the proposed mechanisms of insulin resistance in DM and PCOS is a reduction in insulin-regulated epimerase activity leading to decreased levels of DCI with higher MCI/DCI ratios [67]. This was portrayed by two recent meta-analyses which showed a significant decrease in fasting insulin (SMD = −1.021 μU/mL; 95% CI −1.791 to −0.251; P = 0.009; 9 RCTs; 496 women) and HOMA index (SMD = −0.585; 95% CI −1.145 to −0.025; P = 0.041; 9 RCTs; 496 women) after MI supplementation (alone and/or in combination with 40:1 DCI) [68, 69].

Apart from being a secondary messenger in insulin signaling pathways, MI has a pivotal role in FSH signaling. Furthermore, MI is also involved in regulating intracellular Ca2+ levels which are crucial in the final calcium-dependent steps of oocyte development and maturation as well as meiotic progression [65]. In fact, one of the main causes of FSH signaling impairment in PCOS was attributed to what has been referred to as "the ovarian DCI paradox" [70]. This paradox is based on the fact that ovaries maintain normal insulin sensitivity despite systemic insulin resistance. Therefore, higher insulin levels are associated with increased epimerase activity leading to high DCI and depleted MI levels in the ovary, with altered FSH signaling and diminished oocyte quality [64, 65, 70]. Intuitively, re-establishing the physiologic balance between MI and DCI levels appears to be crucial for the restoration of normal ovarian function.

Based on the current body of evidence, the optimal therapeutic regimen has been suggested to be a 2 g combination of MI and DCI in a 40:1 ratio twice daily, for at least 3 or 6 months [65]. The safety profile of MI has been confirmed both during and outside of pregnancy [65]. One of the issues with inositol therapy, however, is inositol resistance, whereby no improvement in hormonal or metabolic endpoints may be seen in 30–40% of PCOS patients [65]. This resistance was suspected to be due to decreased absorption. Although evidence on that issue is still limited to only very small trials [71, 72], alpha-lactalbumin (α-LA) appears to be a promising molecule for enhancement of MI absorption [65].

Clinical Use of ISDs in PCOS Management

The scope of metformin use in the treatment of PCOS has changed critically over the last decade. Earlier studies conveyed a highly favorable and promising profile expressed by marked improvement of hyperinsulinemia, reduction of hyperandrogenemia [9], restoration of ovulatory function [73], increase in pregnancy rates [74,

75], and decrease in first-trimester spontaneous abortions in women. Most of these studies, however, were either observational or randomized, involving very small numbers of participants [12]. The largest randomized controlled trials were published only in 2006 and 2007 by Moll et al. [76] and Legro et al. [77] and included 225 and 626 women, respectively.

Effects of ISDs on Reproductive Endpoints in Women with PCOS-Related Subfertility

After having been considered the first-line treatment for women with anovulatory PCOS for numerous years, clomiphene citrate has been replaced by the most recent and effective addition to the PCOS subfertility frontline armamentarium: letrozole [78]. High-quality evidence including the 2014 RCT by Legro et al., which evaluated 750 women, demonstrated that letrozole was associated with higher cumulative live births (103 of 374 (27.5%) vs. 72 of 376 (19.1%), $P = 0.007$) and higher ovulation rates (834 of 1352 treatment cycles (61.7%) vs. 688 of 1425 treatment cycles (48.3%), $P < 0.05$), when compared to clomiphene citrate. No significant differences were however noted for pregnancy loss (31.8% vs. 29.1%, respectively) and twin pregnancy rates (3.4% vs. 7.4%, respectively) [79]. The 2018 Cochrane review update (total of 42 RCTs and 7935 women), which demonstrated improvement in the overall quality of evidence to moderate/high, further confirmed higher live birth rates and clinical pregnancy rates with letrozole after timed intercourse compared to clomiphene with/without adjuncts (OR 1.68; 95% CI 1.42–1.99; $P < 0.05$) and (OR 1.56; 95% CI 1.37–1.78; $P < 0.05$), respectively [80]. No between-group differences were detected for miscarriage and multiple pregnancy rates [80].

While the superiority of CC in terms of higher ovulation and pregnancy rates compared to placebo has been well established [22, 81–83], much debate still exists about the role of metformin for improving fertility outcomes in women with PCOS [77, 82–88].

Metformin use in the context of PCOS was found by several studies to be associated with improved menstrual cyclicity [31, 89, 90], as more regular menstrual patterns were observed compared with placebo (OR 1.72; 95% CI 1.14–2.61; 7 trials; 427 women) [26]. Compared to OCPs, however, metformin therapy was demonstrated to be less beneficial by two RCTs (OR 0.08; 95% CI 0.01–0.45; 2 trials; 35 women) [91, 92].

Legro et al. evaluated the rate of ovulation as a secondary outcome in a randomized controlled design in 626 infertile women with PCOS over a period of 6 months [77]. Women receiving metformin alone had significantly lower ovulation rates compared with combination CC and metformin therapy (absolute difference 31.4%; 95% CI 24.7–38.0). Increased ovulation rates over placebo were also reported with metformin therapy (OR 1.81; 95% CI 1.13–2.93; 16 trials; 1208 cycles) [26]. For one woman to achieve ovulation, the number needed to treat using metformin was estimated to be 4.0 [87]. When compared to CC therapy, metformin was found to be similar in inducing ovulation in the nonobese women (BMI <30 kg/m^2) with PCOS (OR 0.87; 95% CI 0.60–1.26; 2 trials; 497 cycles), but much inferior in the obese PCOS population (OR 0.43; 95% CI 0.36–0.51; 2 trials; 2044 cycles) [26].

Combining metformin with CC therapy offered an added benefit to ovulation rates compared to CC alone treatment (OR 1.74; 95% CI 1.50–2.001; 8 trials; 3265 cycles) [26, 93]. It should be noted that the literature is still lacking head-to-head comparisons between letrozole and metformin monotherapy.

While improvements in ovarian and reproductive function were also demonstrated when rosiglitazone was used in women affected with PCOS, yielding a significant increase in menstrual regularization and ovulation rates compared to placebo [56, 58, 94, 95], the earlier specified concerns relating to TZDs limit their use in the reproductive age women seeking fertility.

While BMI status was previously thought to be a discriminatory factor pertaining to the effect of metformin vs. placebo on clinical pregnancy rate [26], the more recent 2017 Cochrane review [96] showed that subgroup analysis by BMI status did not show a difference between the two arms specifically. Clinical pregnancy rates were higher in the metformin arm without BMI stratification (OR 1.93; 95% CI 1.42–2.64; 9 studies; 1027 women). When metformin was combined with CC treatment, higher clinical pregnancy rates were noted compared to clomiphene citrate alone (OR 1.59; 95% CI 1.27–1.99; 16 studies; 1529 women) with no evidence of a difference after subgroup analysis for BMI status between strata. When metformin was compared to CC therapy, on the other hand, BMI status was, in fact, a discriminatory factor. Metformin alone achieved better clinical pregnancy outcomes in non-obese women (OR 1.56; 95% CI 1.05–2.33; 5 studies; 490 women) and lower outcomes in the obese (OR 0.34; 95% CI 0.21–0.55; 2 studies; 500 women).

Looking for live birth rates as a reproductive endpoint, the use of metformin alone (OR 0.71; 95% CI 0.49–1.01; 5 studies; 741 women) or in combination with CC (OR 1.21; 95% CI 0.92–1.59; 9 studies; 1079 women) failed to show any significant benefits compared with CC alone therapy [96]. Two systematic reviews of RCTs further found insufficient evidence to establish a beneficial effect of metformin on live births in PCOS women treated with CC [82, 83, 93].

Metformin did not confer any protective effect against spontaneous miscarriages for pregnancies achieved during the course of treatment when compared to placebo (OR 1.08; 95% CI 0.50–2.35; 4 studies; 748 women) and to CC therapy (OR 0.71; 95% CI 0.49–1.01; 5 studies; 741 women) [96]. These findings are supported by other systematic reviews [82, 83, 93]. Paradoxically, combined therapy with metformin and CC was associated with a higher risk of miscarriage compared to CC alone according to one review (OR 1.59; 95% CI 1.03–2.46; 9 studies; 1096 women) although the evidence was of low quality [96].

In more recent years, some clinical trials have suggested the prevalence rate of spontaneous miscarriages in infertile women with PCOS to be comparable to the general population [76, 77, 97–99]. According to some investigators, this may explain the lack of measurable improvement in this reproductive endpoint following metformin therapy. Previous studies which indicated a two- to fivefold increased risk [85] and 58.3% baseline prevalence [100] suffer serious shortcomings, such as small sample sizes and biased PCOS phenotypes. None of the available clinical trials reported on cost-effectiveness analyses of available drug therapies for the management of PCOS-related clinical concerns.

Priming with metformin before initiating CC treatment has been used to improve cycle outcomes, although the optimal duration of pre-treatment remains poorly understood. Although several studies suggested 4–12-week pre-treatment periods with metformin [74], two studies demonstrated that an ultrashort 2-week pre-treatment regimen may also be associated with improved ovulation and pregnancy outcomes [101, 102]. Unfortunately, there are no well-designed RCTs to determine the most appropriate duration of ISD pre-treatment for the management of subfertile women with PCOS [103].

As for the effects of inositol on improving ovulation and pregnancy rates in anovulatory PCOS patients, very limited evidence currently exists to infer any valuable associations [65].

Effects of ISDs on Reproductive Endpoints in Women with CC-Resistant PCOS

About 20% of women with PCOS are found to be resistant to CC effect on follicular growth and development, requiring consideration for alternate means of intervention [104]. Several studies have been conducted to assess the effectiveness of using ISDs in women with CC resistance [9, 12, 104–107]. A meta-analysis of RCTs [26] recently demonstrated a significant improvement in ovulation rates in these women when metformin was used as a co-treatment (OR 4.86; 95% CI 2.43–9.74; 5 trials; 179 cycles). Pioglitazone was also found to improve pregnancy rates in women with CC-resistant PCOS [108]. Short-term treatment with rosiglitazone was shown to be effective in inducing ovulation in CC-resistant women with the obese phenotype of the syndrome [109]. These findings seem biologically reasonable because they imply that the amelioration in the metabolic milieu by ISDs improves the likelihood of CC-resistant women to respond to ovulation-induction agents.

Another method of inducing ovulation in women with PCOS is “laparoscopic ovarian drilling” (LOD), which has been shown to be effective in CC-resistant women in meeting reproductive endpoints; a particular advantage of LOD lies in reducing multiple pregnancy risk that is well recognized with the use of medical strategies for achieving ovulation induction [110–112]. LOD nevertheless is an invasive procedure that has been associated with postsurgical pelvic adhesions and reduced ovarian reserve [110], generating serious concerns regarding its use as a primary ovulation-induction tool in women with PCOS. Results on menstrual cycle regularity, ovulation, and pregnancy rates were comparable when metformin/CC combination treatment was used compared with LOD [111, 112]. The findings of a recent Cochrane systematic review [113] in which 26 RCTs involving women with CC-resistant PCOS were analyzed demonstrated a significant improvement in live births when the combination treatment was used compared to LOD (OR 0.44; 95% CI 0.24–0.82; 2 trials; 159 women). There were no differences in miscarriages (OR 1.43; 95% CI 0.70–2.91; 3 trials; 441 women) and multiple pregnancies (OR 0.1; 95% CI 0.01–1.94; 3 trials; 441 women) between both groups. A cost analysis further showed that metformin/CC combination treatment was significantly less expensive than LOD when used over a 6-month period (50 EUR versus 1050 EUR; $P < 0.05$) [110].

Although LOD was shown to be successful in improving fertility outcomes in women with CC-resistant PCOS, metformin/CC combination treatment appears to offer an equally efficacious approach with the added advantages of being less invasive and less costly.

An entire chapter in this text addresses a surgical approach to achieving ovulation in further depth.

Effects of ISDs on Reproductive Endpoints in Women with PCOS Undergoing in Vitro Fertilization and Intracytoplasmic Sperm Injection Cycles

Experience with in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles shows that women with PCOS may experience longer treatment durations, yield a larger number of oocytes, express higher serum estradiol levels, and are at a particularly increased risk for ovarian hyperstimulation syndrome (OHSS) compared to those with other etiologies for infertility; lower conception rates following embryo transfer have also been suggested for women with PCOS undergoing IVF [114].

In women with PCOS undergoing assisted reproductive technologies (ART), the use of ISD pre-treatment, such as metformin, has been evaluated by several studies [5]. The plausible working hypothesis was that suppression of hyperinsulinemia and hyperandrogenism may prove beneficial in improving ovarian response, enhance pregnancy endpoints, and reduce adverse effects [5].

A recent updated Cochrane systematic review [115] analyzing data from 13 RCTs (1132 women) found no evidence that metformin co-treatment with IVF/ICSI cycles reduced either the mean gonadotropin dose (MD 0.24; 95% CI −0.23, 0.70; 8 trials; 868 cycles) or the number of stimulation days (MD −0.17 days; 95% CI −0.71 to 0.37; 9 studies; 796 women) compared to placebo regardless of the type of ovarian stimulation protocol used. In addition, metformin use failed to reduce significantly the number of oocytes retrieved (MD 0.03; 95% CI −1.42 to 1.48; 11 RCTs; 890 women). The clinical results for peak serum estradiol levels remain inconclusive as three of six studies found that metformin pre-treatment was associated with lower estradiol levels (MD −2.43; 95% CI −4.59, −0.26), whereas three other studies failed to support these findings (studies were not pooled due to high heterogeneity, $I^2 = 89\%$).

Following stratification by the type of ovarian stimulation protocol used, the updated Cochrane review [115] confirmed that metformin treatment did not improve clinical pregnancies in short GnRH antagonist cycles (RR 1.38; 95% CI 0.21–9.14; 2 trials; 177 cycles). Metformin nonetheless appeared to improve pregnancy outcome in long GnRH agonist cycles (RR 1.32; 95% CI 1.08–1.63; 10 trials; 915 cycles), with quality of evidence ranging from very low to low. Metformin did not improve miscarriage rates (RR 0.86; 95% CI 0.56–1.32; 8 trials; 821 cycles) in either protocols. While no benefits were found for live births in long agonist cycles (RR 1.30; 95% CI 0.94–1.79; 6 trials; 651 cycles), one study reported a decrease in short antagonist treatment cycles (RR 0.48; 95% CI 0.29–0.79; 153 cycles) [115].

While metformin use during IVF/ICSI treatments did not positively impact fertility-related endpoints including cycle outcome, a significant reduction in the

risk of developing OHSS however was demonstrated with metformin therapy (RR 0.46; 95% CI 0.29–0.72; 11 trials; 1091 cycles) [115]. Reasons for this protective effect are unclear but are seemingly independent of the total number of oocytes retrieved and/or peak estradiol serum levels achieved.

Despite very limited evidence, numerous studies have shown that MI treatment during IVF is associated with decreased consumption of recombinant FSH administration, higher total number of retrieved oocytes, improved oocyte quality, and less immature oocytes [64].

Effects of ISDs on Endocrine and Clinical Endpoints in Women with Symptomatic PCOS-Related Androgen Excess

The androgen excess state appears to play a central role in determining the PCOS phenotype, as affected women often present with the manifestation spectrum of acne and hirsutism [116]. The oral contraceptive pill (OCP) has traditionally constituted the main pharmacologic management approach to symptoms of hyperandrogenism in women with PCOS. Since about 15–20% of women on OCPs suffer significant side effects [117], alternative pharmacologic therapies have been sought. Given the established role of hyperinsulinemia in the development of hyperandrogenism, it seemed plausible to explore the role of ISDs in improving the clinical symptoms of androgen excess in the affected women [116, 118].

Several clinical trials evaluated the effects of metformin on the endocrine milieu of PCOS. Compared with placebo, the use of metformin was found to be significantly more effective in reducing total serum testosterone levels (MD of −0.49 nmol/L; 95% CI −0.59 to −0.39; 15 RCTs; 863 women), with a subgroup analysis by BMI showing a stronger effect in the nonobese group (MD −0.71 versus −0.29 nmol/L) [96]. It did not however reveal any improvement in serum sex hormone-binding globulin (SHBG) levels (MD 0.49; 95% CI −1.82 to 2.81; 15 RCTs; 823 women), even with subgroup and sensitivity analyses [96].

Compared with OCP use however, metformin alone was significantly less effective in reducing total serum testosterone levels (MD 0.39 nmol/L; 95% CI 0.32, 0.47; 17 trials; 818 women) and the free androgen index (MD 3.95%; 95% CI 3.32, 4.58; 10 trials; 433 women) regardless of BMI [119]. The addition of metformin to OCPs in women with BMI ≤25 did not yield any additional benefit for total serum testosterone levels (MD 0.09; 95% CI −0.08 to 0.26; 2 RCTs) or FAI (MD 0.35; 95% CI −1.09 to 1.79; 1 RCT). In those with a BMI between 25 and 30, however, the combined treatment was associated with an increase in testosterone levels (MD 0.79; 95% CI 0.57–1.00; 3 RCTs; 152 women) and an improvement in FAI (MD 3.80; 95% CI 2.91–4.69; 2 RCTs; n = 9) [119].

The repercussions of inositol as an ISD on the overall hyperandrogenic state associated with PCOS have been tackled in numerous studies despite the limited quality of evidence. From a pathophysiologic point of view, DCI is capable of increasing testosterone levels both by acting as an insulin mediator in theca cells and by inhibiting aromatase in granulosa cells [65]. A meta-analysis demonstrated that inositol supplementation resulted in a slight trend toward a decrease of testosterone concentration (SMD = −0.49; 95% CI −1.072 to 0.092; $P = 0.099$), with no

differences in androstenedione levels. Studies that included MI administration for at least 24 weeks showed a significant increase in serum SHBG (SMD = 0.425 nmol/L; 95% CI 0.050–0.801; $P = 0.026$) [68].

High-quality research aimed at investigating the effects of ISDs on improving hirsutism and acne in women with PCOS is scarce. The updated Cochrane review [119] demonstrated no differences between metformin and OCPs in improving severity of hirsutism as assessed by Ferriman-Gallwey (FG) in affected women with BMI <25 kg/m^2 (MD 0.38; 95% CI −0.44 to 1.19; 3 RCTs; n = 134) and BMI >30 kg/m^2 (MD −0.38; 95% CI −1.93 to 1.17; 2 RCTs; n = 85). Metformin was found to be, however, less effective in women with a BMI between 25 and 30 kg/m^2 (MD 1.92; 95% CI 1.21–2.64; 5 RCTs; n = 254). Metformin and OCP monotherapies were less effective in improving hirsutism than combination therapy (MD 1.36; 95% CI 0.62–2.11; 3 RCTs; n = 135) and (MD 0.54; 95% CI 0.20–0.89; 6 RCTs; n = 389), respectively. Only one trial using the visual analog scale (VAS) for the assessment of hirsutism showed that metformin improved hirsutism comparably to OCPs (MD −2.70; 95% CI −4.41 to −0.99; 1 RCT; n = 34) [120]. These findings concur with the results of a systematic review (MD −0.5; 95% CI −5.0 to 3.9; 5 trials), which also found no differences in FG hirsutism scores when metformin was used in combination with the anti-androgen flutamide versus flutamide alone (MD 0.9; 95% CI −0.4 to 2.2; 2 trials) [121].

The effect of ISDs on acne in women with PCOS has been inconclusive. Three clinical trials evaluating 131 women demonstrated that metformin was less effective than OCPs in improving acne symptoms (OR 0.30; 95% CI 0.11–0.79; 3 RCTs; n = 131). Only one RCT showed comparable subjective acne scores between both treatment groups (WMD 0.90; 95% CI −0.40 to 2.20; 34 women) [116, 119].

Effects of ISDs on Metabolic Endpoints in Women with PCOS

Insulin resistance is now recognized as a key player in the pathogenesis of PCOS, and the affected women are deemed at risk for developing T2DM [30, 47]. Women with impaired glucose tolerance (IGT) at baseline are particularly at risk, as 59% of them are expected to develop T2DM [122] over a period of 6 years, a conversion rate of 8.7% per annum [122]. Obesity confers an estimated tenfold increase in the risk of developing this metabolic disorder. Metformin treatment was shown to significantly reduce fasting insulin levels compared to placebo (MD −4.13 mIU/ L; 95% CI −5.67 to −2.58; 14 studies; 573 women), irrespective of BMI stratification [96]. Although the effect of metformin on fasting glucose levels was of statistical significance compared to placebo, the difference was deemed as of limited clinical relevance (MD −0.14 mmol/L; 95% CI −0.21 to −0.07; 15 studies; 849 women) [96]. The Diabetes Prevention Program Research group reported on a lower incidence of T2DM in persons of both genders receiving metformin (7.8 cases per 100 person years) and lifestyle intervention therapy (4.8 cases per 100 person years) compared with placebo [123]. However, lifestyle intervention was associated with significantly better and more sustainable weight reduction compared to metformin [123]. Only one RCT evaluated the development of T2DM in 18 women with PCOS and found no differences when metformin was used compared with OCPs (OR 0.17;

95% CI 0.00–8.54; 18 women) [91]. Based on this limited body of data, the place for long-term prophylactic treatment with metformin in preventing progression to T2DM in women with PCOS is unclear.

A meta-analysis of 6 clinical trials (355 women) compared MI and metformin in PCOS and showed no significant differences in fasting insulin levels, HOMA index, and testosterone and SHBG levels between the two groups [124]. In the absence of data evaluating the benefits of combination therapy, the aforementioned metanalysis highlights the feasibility of using MI in combination with a reduced dose of metformin for the purpose of improving gastrointestinal tolerability [65].

Hypertension has also been described to be associated with PCOS [125]. Metformin was shown to reduce systolic blood pressure (MD −3.59 mmHg; 95% CI −5.13 to −2.04; 7 trials; 379 women) but did not affect diastolic blood pressure (MD −0.14 mmHg; 95% CI −1.35 to 1.07; 6 trials; 292 women) in treated women [96]. Metformin use was shown to have no effect on cholesterol (MD −0.14 mmol/L; 95% CI −0.31 to 0.02; 10 trials; 562 women) and triglyceride levels (MD 0.14 mmol/L; 95% CI −0.05 to 0.32; 7 trials; 309 women) [96].

Numerous markers of cardiovascular disease (CVD) risk are identified to be elevated in women with PCOS, such as serum levels of C-reactive protein (CRP) and homocysteine [126], in addition to increased carotid intima-media thickness [127]. The prevalence of metabolic syndrome among women with PCOS is estimated to be about 43% [128], projecting a cluster of additional risk factors for CVD in this population [129]. Epidemiologic studies, although limited, have however failed to demonstrate evidence for an increased incidence of cardiac events in reproductive age women with PCOS [130]. Although metformin use is suggested to reduce the risk of microangiopathy and macroangiopathy among patients with T2DM [131] and to reverse impaired endothelial function among women with PCOS following 6 months' treatment [132], no RCTs are available to evaluate the impact of metformin use on the outcome measures of stroke and myocardial infarction in women with PCOS.

Chronic anovulation and hence lack of progesterone exposure, as well as hyperinsulinemia, are suggested mechanisms for the increased risk for endometrial cancer in women with PCOS [133]. While in vitro data suggest that metformin therapy may offer protection against the development of endometrial cancer [134], unfortunately, data on the risks of endometrial cancer in women with PCOS who have been placed on metformin therapy are currently nonexistent.

There is little doubt that the management of PCOS in women should extend well beyond the immediate reproductive needs to address the metabolic concerns that hold far-reaching adverse health implications for these women [25, 47]. A systematic review, however, found insufficient data to make any conclusions on the relative efficacy of ISDs for long-term prevention of diabetes mellitus, cardiovascular disease, and endometrial cancer in women with PCOS [135]. Long-term data supporting far-reaching benefits of lifestyle interventions however do exist and underscore a need for sustaining efforts at achieving and maintaining healthier lifestyle in women with PCOS given their risk profile for myriad health hazards.

Summary and Implications for Practice

- For women with PCOS in general, data supporting a role for ISDs in the prevention of T2DM, cardiovascular disease, hypertension, or endometrial cancer are sparse to nonexistent.
- For the management of ovulatory infertility in the nonobese PCOS population (BMI <30 kg/m^2), there is moderate-quality evidence demonstrating that metformin monotherapy improves the odds of ovulation and chance of achieving clinical pregnancy [26].
- For obese women (BMI ≥30 kg/m^2) with PCOS-associated subfertility, there is low-quality evidence showing the failure of metformin monotherapy to improve reproductive endpoints [26]. There is moderate-quality evidence to support a beneficial effect of metformin in combination with CC therapy in increasing the likelihood of ovulation and clinical pregnancies [26].
- For women with CC-resistant subfertility, there is moderate-quality evidence to support that metformin co-treatment increases ovulation rates [26].
- For women with PCOS undergoing IVF/ICSI treatments, there is moderate evidence to support the failure of metformin co-administration to improve the clinical outcomes of live births, clinical pregnancies, or miscarriages. Low-quality evidence showed that metformin might be associated with higher clinical pregnancies in long GnRH agonist protocols but not in the short GnRH antagonist counterpart. Regardless of the stimulation protocol, metformin co-treatment was not associated with any differences in the mean gonadotropin dose, number of stimulation days, and number of oocytes retrieved. There is moderate evidence demonstrating a significant reduction in the risk of OHSS with metformin co-treatment, when hCG is used to trigger final oocyte maturation [115].
- For women with symptomatic PCOS-related androgen excess, there is limited evidence available to support that metformin or inositol monotherapy improves hirsutism and acne, compared with more established anti-androgenic drugs. There is insufficient evidence demonstrating that the addition of metformin to the OCP is more effective than OCP alone in improving hirsutism or acne [119].
- The main proven benefits of inositol are limited to better fasting glucose levels and HOMA indices. Although no high-quality head-to-head comparative studies between metformin vs. inositol monotherapies exist, the concomitant use of inositol in combination with reduced doses of metformin may be used to improve overall tolerability [65].

It should be emphasized that the strengths of the conclusions in this chapter remain limited by weaknesses related to the quality of existing evidence. Despite the availability of meta-analyses, many suffered from significant heterogeneity between studies and from poorly powered clinical trials. Differences in ethnic backgrounds, geographic locations, diagnostic criteria for PCOS, presence of infertility confounders, and influence of fertility co-treatments, inconsistencies in methodology of ISD administration, outcome endpoints, study design, and varying follow-up

periods are some of the factors identified as source of heterogeneity within existing data. The suggested recommendations were based on the current state of evidence, and readers are encouraged to stay attuned to accruing data on the subject.

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Role of Statins and Resveratrol in PCOS Management

13

Anna Sokalska, Israel Ortega, and Antoni J. Duleba

Key Points

- Ovaries of women with PCOS are characterized by hyperplasia of theca cells, which produce excessive amounts of androgens. PCOS is associated with increased oxidative stress and systemic inflammation.
- Statins are inhibitors of the mevalonate pathway and possess antioxidant and anti-inflammatory properties.
- Statins reduce proliferation and increase apoptosis of ovarian theca cells. Statins inhibit androgen production by theca cells by reducing the expression of the CYP17A1 gene.
- In women with PCOS, statins reduce androgen levels, decrease ovarian size, and improve menstrual cyclicity.
- Resveratrol is a natural polyphenol with cardioprotective, anti-dyslipidemic, and antidiabetic effects. In the ovary, resveratrol exhibits antiproliferative and androgen-lowering effects on theca-interstitial cells.
- In women with PCOS, resveratrol reduces androgen levels and fasting insulin level and increases insulin sensitivity index.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_13

Introduction

The use of statins and/or resveratrol as therapy for polycystic ovary syndrome (PCOS) is still experimental. In this chapter, we will present the rationale as well as in vitro, in vivo, and clinical evidence in support for considering statins and/or resveratrol in the management of PCOS. Furthermore, we will discuss limitations and risks of using these two agents, and, finally, we will speculate on future directions of research. In order to justify the concept that statins and/or resveratrol may be useful in the management of PCOS, we will first review the key features of this syndrome and the mechanisms of action of statins and resveratrol.

PCOS is the most common endocrine disorder, affecting 5–10% of women of reproductive age [1–3], although the prevalence of PCOS in overweight and obese women is considerably higher, reaching almost 30% [4]. Women with PCOS present a variety of clinical symptoms, including menstrual dysfunction, features of androgen excess, and infertility. Insulin resistance with resulting hyperinsulinemia is also common among women with PCOS, along with increased risk for dyslipidemia, hypertension, diabetes, and related cardiovascular consequences [5]. In addition, prolonged unopposed estrogen exposure secondary to anovulation represents a risk factor for the development of endometrial hyperplasia and endometrial cancer [6].

Since the description of PCOS by Stein and Leventhal in 1935, several different sets of diagnostic criteria for PCOS have been considered, including those proposed by National Institutes of Health 1990 conference [7], the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine [8], and the Androgen Excess and PCOS Society [9]. All these definitions include at least two of the following three criteria: hyperandrogenism, oligo- or anovulation, and/or polycystic ovarian morphology with exclusion of other diagnoses, such as congenital adrenal hyperplasia, nonclassic adrenal hyperplasia, androgen-secreting tumor, Cushing's syndrome, hyperprolactinemia, and thyroid disorders.

Androgen excess, represented by biochemical and/or clinical hyperandrogenism, is the central defect of PCOS and is present in up to 90% of women with PCOS [10]. Although the ovary represents the main source of androgens, adrenal hyperandrogenism is also documented in PCOS patients [11]; however, neither the mechanisms nor magnitude of adrenal contribution to the androgen excess of PCOS is clearly understood [12]. While the etiology of ovarian hyperandrogenism is still disputed, inherent steroidogenic defects in PCOS theca cells, abnormal stimulation of theca cells (e.g., by insulin, growth factors, oxidative stress), and/or an excessive number of theca cells are suggested mechanisms. In vitro studies indicate that the enhanced steroidogenic potential of PCOS theca cells is associated with increased mRNA expression and activity of the main enzymes involved in androgen biosynthesis, such as side-chain cleavage enzyme (P450scc, encoded by *CYP11A1*), 3-beta-hydroxysteroid dehydrogenase type II (3-β-HSD, encoded by *HSD3B2*), and 17α (alpha)-hydroxylase/17,20-lyase (P450c17, encoded by *CYP17A1*) [13–15]. In particular, enhanced P450c17 enzyme expression and activity may account for

hyperandrogenism in PCOS [16]. In addition, dysregulation of several signal transduction pathways has been shown to play a role in androgen excess in PCOS women. For example, inhibition of mitogen-activated protein kinase 1 (MAPK1) signaling has been demonstrated in cultures of PCOS theca cells and seems to mediate the action on theca androgen production [16, 17].

The etiology of androgen excess in PCOS women is likely multifactorial and may involve both extraovarian and intraovarian factors. First, impaired gonadotropin dynamics may play a role in excessive androgen production in PCOS. Increased luteinizing hormone (LH) pulse frequency and amplitude may directly increase androgen synthesis, whereas relatively reduced follicle-stimulating hormone (FSH) levels, by virtue of lesser stimulation of aromatase, may contribute to decreased conversion of androgen to estrogen and hence worsen ovarian androgen excess [18]. Furthermore, increased levels of insulin and insulin-like growth factor 1 (IGF-1) may stimulate theca steroidogenesis, by inducing the expression of key enzymes involved in androgen synthesis as well as by promoting proliferation and reducing apoptosis of theca-interstitial cells [19]. In addition, insulin further contributes to hyperandrogenemia in PCOS by stimulating adrenal androgen production and by inhibiting hepatic production of sex hormone-binding globulin (SHBG), which in turn leads to an increase in bioavailable androgens [20, 21]. Several intraovarian paracrine factors may also play a role in ovarian androgen excess. For example, anti-Müllerian hormone (AMH) has an inhibitory role during folliculogenesis and thus may indirectly increase androgen production by inhibiting FSH action and/or by blocking aromatase activity [22]. Other intraovarian factors, such as activin and inhibin, may also affect theca steroidogenesis and proliferation [23, 24].

The above findings underscore the need to develop therapy of hyperandrogenism in PCOS that may address excessive androgen production by theca cells as well as excessive growth of ovaries (especially the thecal compartment).

Another important aspect of PCOS involves alterations in metabolic status and associated increase in risk for development of metabolic syndrome. The prevalence of the metabolic syndrome has been reported in 43% of women with PCOS, which is twofold higher than that for age-matched controls [25].

Insulin resistance is a common feature of PCOS observed in the majority of obese and in many nonobese women with PCOS [26]. The molecular basis for insulin resistance, though not yet fully understood, may be related to a post-receptor defect involving reactive oxygen species (ROS)-mediated phosphorylation of the insulin receptor substrates 1 and 2 (IRS-1, IRS-2), which leads to an abrogation of insulin signaling via its receptor [27, 28]. Among women with PCOS, 30% have impaired glucose tolerance (IGT) and an additional 7.5% meet criteria for type II diabetes [29]. Insulin resistance is worsened by the coexistence of obesity, which is also increased in the PCOS population and exacerbates several other metabolic abnormalities in these women. Obese women with PCOS have greater insulin resistance than weight-matched controls or lean PCOS subjects [30, 31].

In the long term, PCOS women are at high risk of dyslipidemia, leading to increased cardiovascular risk and possibly cardiovascular disease and even mortality [32]. The pattern of dyslipidemia in women with PCOS includes elevated total

cholesterol levels, low-density lipoproteins (LDLs), very LDLs (VLDLs), and triglycerides with concomitantly reduced levels of high-density lipoproteins (HDLs) [33]. At least one lipid abnormality is seen in up to 70% of women with PCOS [34]. These derangements in the lipid profile correlate with the hyperinsulinemia and hyperandrogenemia of PCOS, and treatment of these conditions may improve the lipid abnormalities and thus mitigate the associated clinical implications [35].

Endothelial structure and function is impaired even among young patients with PCOS without additional risk factors for cardiovascular disease [36]. A recent meta-analysis showed that carotid intima-media thickness (CIMT), a marker of subclinical atherosclerosis, is higher in women with PCOS compared with controls [37]. In addition, homocysteine levels are also higher in women with PCOS, representing another independent risk factor for cardiovascular disease by inducing cytotoxic effects on vascular endothelium via mechanisms involving increased inflammatory cytokine expression, altered nitric oxide bioavailability, and induction of oxidative stress [38, 39]. Other markers of endothelial function, such as endothelin-1, are also altered in PCOS women [36]. In addition, women with PCOS have increased daytime blood pressure after adjusting for BMI, body fat distribution, and insulin resistance, which may indicate a prehypertensive state and thus a further risk factor for cardiovascular disease in these women [40]. In view of the above findings, it is apparent that comprehensive management of PCOS should include reduction of long-term cardiovascular risks.

Women with PCOS exhibit elevation of several biomarkers of oxidative stress, including malondialdehyde (MDA; a marker of lipid peroxidation), superoxide dismutase (SOD) activity, and protein carbonyl content [41, 42]. Furthermore, reduced glutathione and decreased level of haptoglobin, a protein with antioxidant properties, are described in women with PCOS suggesting a decreased antioxidant reserve in this population [42–44]. Oxidative stress may aggravate androgen excess in PCOS via at least two mechanisms. First, modest oxidative stress induced by hypoxanthine and xanthine oxidase stimulates the proliferation of theca-interstitial cells [33] and may thus contribute to hyperplasia of ovarian theca and stroma. Second, oxidative stress impairs insulin signaling resulting in compensatory hyperinsulinemia, which, in turn, further stimulates theca-interstitial cell steroidogenesis [45].

Increased oxidative stress and decreased antioxidant capacity may also contribute to the increased risk of cardiovascular disease in women with PCOS [42]. For example, advanced glycation end products (AGEs), the products of nonenzymatic glycation and oxidation of proteins and lipids, activate the production of endothelin-1 (ET-1), a peptide that causes endothelial dysfunction and cardiovascular sequelae, in women with PCOS [46]. Furthermore, oxidative stress and chronic inflammation are closely interrelated; indeed, growing evidence supports the concept of a vicious circle, whereby inflammation generates ROS, while oxidative stress promotes inflammation [47]. Such a vicious circle has been reported in the endothelium and in adipose tissues [48]. Notably, elevated C-reactive protein (CRP), a marker of systemic inflammation, is commonly encountered in women with PCOS; CRP is a risk factor for cardiovascular events and correlates with increasing plaque vulnerability and propensity to thrombosis [49].

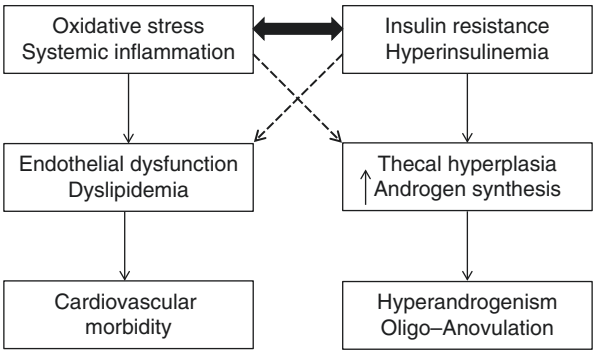

Fig. 13.1 Proposed pathophysiology and sequelae of polycystic ovary syndrome. Oxidative stress, systemic inflammation, and insulin resistance are interrelated processes involved in stimulation of theca-interstitial cell compartment and endothelial dysfunction. Cardiovascular morbidity, hyperandrogenism, and oligo- and anovulation are final consequences

Additionally, other markers of low-grade chronic inflammation, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), have been related to PCOS [50] (Fig. 13.1).

Ideally, therapy of PCOS should reduce hyperandrogenism, normalize ovarian physiology and morphology, as well as improve metabolic profile and reduce cardiovascular risks without affecting fertility. However, despite a growing number of studies evaluating pharmacological interventions in PCOS, currently available treatments are not fully satisfactory and have significant limitations. Thus, there is an urgent need for more effective therapies aimed at the broad spectrum of endocrine and metabolic aspects of PCOS. The following sections will discuss how statins and resveratrol may fulfill these requirements. To this end, we shall review the mechanism of action of statins and resveratrol and subsequently will discuss how these actions may ultimately benefit women with PCOS.

Statins

Mechanisms of Action of Statins

Statins are competitive and reversible inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway (Fig. 13.2) [51]. Statins are effective in improving lipid profile and reducing risk of cardiovascular diseases, being the first-line treatment of choice in patients with elevated cholesterol and those diagnosed with coronary heart disease [52]. In addition to their cholesterol-lowering effect, statins also exert many other potentially beneficial effects, including improvement of nitric oxide-mediated endothelial function, as well as exhibit anti-inflammatory and antiproliferative actions [53, 54].

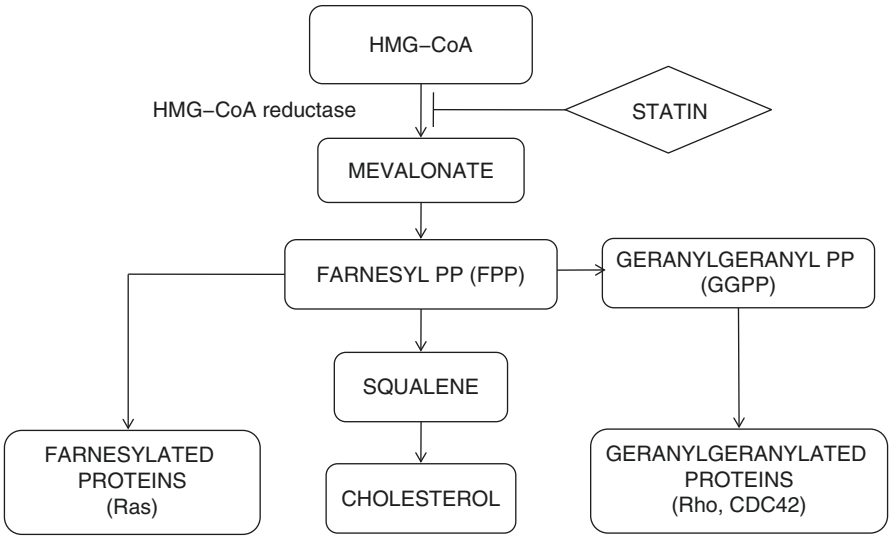

Fig. 13.2 Outline of the mevalonate pathway and its major products. Statins competitively inhibit HMG-CoA reductase, the rate-limiting step of cholesterol synthesis. FPP and GGPP are the intermediate downstream products of mevalonic acid, involved in farnesylation and geranylgeranylation of proteins, respectively. (Adapted from Banaszewska et al. [84])

The mevalonate pathway is responsible for the synthesis of cholesterol and other important biomolecules such as coenzyme Q, dolichols, and isoprenoids; these biomolecules are involved in several cell functions, such as membrane integrity, protein isoprenylation, and energy homeostasis [55]. Inhibition of this pathway leads to decreased production of several biologically active downstream products, including cholesterol and substrates of isoprenylation (farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP)) [56].

Isoprenylation consists of the covalent addition of two types of isoprenoids: FPP (farnesylation) or GGPP (geranylgeranylation) to cysteine residues at or near the carboxyl terminus of many proteins, especially small G proteins, facilitating their membrane attachment and function [57]. These proteins are monomeric G proteins with molecular masses of 20–40 kDa [58] and are referred to as "small GTPases." Particularly relevant small GTPases are members of the Ras superfamily, such as Ras, Rho, Rac, and Cdc42; their function depends on their association with the cytoplasmic leaflet of cellular membranes, a process that requires farnesylation of Ras and geranylgeranylation of Rho, Rac, and Cdc42. These proteins are involved in many key cellular functions, such as cell growth, differentiation, cytoskeletal function, and vesicle trafficking [59–61]. Hence, statins, by reducing the synthesis of isoprenoids, may alter a wide range of intracellular processes.

Interestingly, crosstalk between the insulin signaling pathway and the mevalonate pathway has been reported. Insulin stimulates the phosphorylation and activation of farnesyl transferase and geranylgeranyl transferases I and II [62, 63], increasing the isoprenylation of Ras and other small GTPases. This elicits

phosphorylation of the MAPK cascade through activation of the Ras-Raf-Erk1/2 signaling pathway, leading to mitogenic responses [64]. In addition, oxidative stress shares the same signal transduction pathways with insulin and IGF-1, specifically the Erk1/2 and mTOR/p70s6k pathways [65, 66]. A convergence of the actions of insulin/IGF-1 and ROS at the Erk1/2 and mTOR/p70s6K pathways may explain the similar effects of these compounds on cell growth [19].

Isoprenylation also plays a role in oxidative stress, as isoprenylated proteins affect generation of ROS by NADPH oxidase. This multicomponent electron transport system consists of two known membrane-bound components, gp91-phox and p22-phox, and two other well-characterized oxidase components, p47-phox and p67-phox, both found in the cytosol fraction. Previously, isoprenylated Rac1 and Rac2, members of the Rho family, have been implicated in the regulation and assembly of the components of NADPH oxidase [67]. Therefore, disruption of isoprenylation may lead to cellular disturbances, owing to a decreased generation of intracellular ROS.

In Vitro Studies of Statins

In vitro, statins exert antiproliferative actions on different tissues and cell types, such as vascular smooth muscle cells [68], thyroid cells [69], and cardiomyocytes [70], as well as on a broad range of cancer cell types, including melanoma [71], meningioma cells [72], and lung and breast cancer cells [73, 74]. Studies on ovarian rat cells have shown that the statin mevastatin induces a concentration-dependent inhibition of theca-interstitial cell DNA synthesis as assessed by radiolabeled thymidine incorporation assay [75, 76]. This inhibition was observed in the presence and in the absence of serum, suggesting that decreased supply of cholesterol is unlikely to be the primary mechanism of the mevastatin-induced block of proliferation. Furthermore, inhibitory effects of mevastatin were partly abrogated by mevalonic acid, indicating that mevastatin-induced inhibition of theca-interstitial cell proliferation may be due to decreased isoprenylation [76]. Similarly, another statin, simvastatin, induced an inhibition of theca-interstitial cell DNA synthesis, and this effect was abrogated by FPP and GGPP, but not by squalene or cholesterol. In addition, direct inhibitors of farnesyl transferase and geranylgeranyl transferase reduced cell proliferation [77]. Thus, these findings indicate that statins inhibit theca-interstitial cell proliferation, at least in part, by reduction of isoprenylation.

The above inhibitory effects of statins on cell proliferation were verified in human theca-interstitial cells [78]. Both mevastatin and simvastatin induced an inhibition of DNA synthesis in cells obtained from women with and without PCOS; these effects were observed both in the absence and in the presence of 22-hydroxycholesterol, indicating that statins inhibit the proliferation of human theca-interstitial cells irrespective of the availability of cholesterol in normal and PCOS ovaries.

In addition to the inhibition of cell proliferation, statins also reduce steroidogenesis. Several reports have demonstrated the statin-induced inhibition of steroid

production in steroidogenic cells, such as Leydig cells [79] and adrenocortical cells [80]. Mevastatin has been shown to inhibit the production of progesterone and testosterone in cultures of rat theca-interstitial cells [75]. Simvastatin decreased androgen levels in a time- and concentration-dependent fashion, and this inhibitory effect correlated with a decrease in *Cyp17a1* mRNA levels, the gene encoding the key enzyme regulating androgen biosynthesis [81]. The addition of 22-hydroxycholesterol increased androgen levels as well as *Cyp17a1* mRNA expression and abrogated the inhibitory effects of simvastatin. Furthermore, the addition of substrates of isoprenylation (GGPP and FPP) significantly reduced simvastatin-induced suppression of *Cyp17a1* mRNA expression and androgen levels. Thus, the inhibitory actions of simvastatin on androgen production may be mediated by several mechanisms related to the inhibition of HMG-CoA reductase and, hence, a decreased production of several biologically active downstream products of the mevalonate pathway, including cholesterol and substrates of isoprenylation.

The total amount of steroids secreted by the ovary depends on two factors: the total number of steroids producing cells in the ovary and the steroidogenic capacity of individual cells. As demonstrated by in vitro studies, statins inhibit both proliferation and steroidogenesis of theca-interstitial cells and consequently may be relevant to treatment of conditions associated with thecal hyperplasia and hyperandrogenism, such as seen in PCOS.

Clinical Studies of Statins

Several studies evaluated the effects of statins on the clinical, endocrine, and metabolic profiles of women with PCOS. The first prospective, randomized clinical trial demonstrating the inhibitory effect of a statin on androgens in women with PCOS was published in 2006 [82]. In this study, patients were randomly allocated to a Statin Group (simvastatin, 20 mg daily plus oral contraceptive pill (OCP); $n = 24$) or an OCP Group (OCP alone; $n = 24$). After 12 weeks of treatment, serum testosterone levels decreased by 41% in the Statin Group and by 14% in the OCP Group. Furthermore, there was a greater decline of LH in the Statin Group compared to Control Group (43% vs. 9%), resulting in a greater decrease of LH/FSH ratio (44% vs. 12%). In several studies, simvastatin also improved clinical hyperandrogenism, characterized by both hirsutism and acne [82–84].

This inhibitory effect of statins on androgen production is in agreement with other clinical trials evaluating different statins. For example, Kaya et al. carried out a randomized clinical trial in PCOS patients comparing effects of two statins, simvastatin and atorvastatin, on hyperandrogenism and other clinical/metabolic aspects; although both statins were effective in reducing hyperandrogenemia, simvastatin had a greater effect on decreasing the level of testosterone in PCOS women [85]. Furthermore, metformin-induced inhibition of ovarian steroidogenesis has been shown to be potentiated by a combined therapy using atorvastatin in conjunction with metformin [86]. In another study, comparable and significant decreases of total testosterone and free testosterone levels were observed following treatment with

simvastatin or treatment with metformin; however, a significant decrease of DHEA-S was observed only following treatment with simvastatin [83].

It should be noted that the statin-induced improvement of ovarian function appears not to be mediated by improved insulin sensitivity as determined by fasting and post-glucose challenge levels of insulin and glucose. Indeed, simvastatin had no significant effect on fasting insulin and glucose levels as well as the fasting measure of insulin sensitivity (QUICKI) [82, 83]. Nonetheless, these observations should be interpreted with caution since another statin, atorvastatin, at a dose of 20 mg daily for 12 weeks improved insulin resistance measured by homeostasis model assessment for insulin resistance (HOMA-IR) by 20% compared to a Control Group, and this effect correlated with the reduction in free androgen index (FAI) [87]. Further long-term studies comparing different statins are needed to resolve this issue.

The effects of statins on lipid profile are consistent and highly significant. In the first report evaluating simvastatin plus OCP (Statin Group) in comparison to OCP alone (OCP Group) in women with PCOS, total cholesterol declined by 10% in the Statin Group, whereas in the OCP Group it increased by 8% [82]. Low-density lipoprotein cholesterol levels decreased in the Statin Group by 24% but not in the OCP Group, whereas high-density lipoprotein cholesterol levels increased by 9% in the Statin Group and by 13% in the OCP Group. Finally, triglyceride levels did not change in the Statin Group but were significantly increased in the OCP Group [82]. These findings are consistent with subsequent studies, whereby simvastatin and atorvastatin markedly improved lipid profile in PCOS women [83, 87].

In addition to improvement of lipid profile, statins also reduce several other cardiovascular risk factors in women with PCOS, including improvement of markers of endothelial dysfunction and indices of low-grade chronic inflammation. Banaszewska et al. carried out a prospective crossover trial demonstrating that in women using OCPs, simvastatin decreased high-sensitivity C-reactive protein (hs-CRP), a marker of systematic inflammation, and levels of soluble vascular cell adhesion molecule 1 (sVCAM-1), a marker of endothelial dysfunction [84]. These findings are consistent with subsequent studies, whereby a similar statin-induced improvement of systemic inflammation in PCOS women has been shown after simvastatin as well as atorvastatin therapies [7, 83, 85, 87]. In addition, atorvastatin decreased malondialdehyde (MDA) concentrations, a marker of oxidative stress in patients with PCOS [88]. Taken together, these findings provide convincing evidence that statin therapy significantly reduces cardiovascular risk factors even in this young, but at-risk, population.

According to the Cochrane Review, statins in PCOS patients improve lipid profiles and reduce testosterone levels with no evidence of improvement in resumption of menstrual regularity, spontaneous ovulation, hirsutism, or acne [89]. Only four trials with a limited number of participants and short duration of treatment were included in this analysis. The trials included in this review comprised a total of 244 women with PCOS receiving 12 weeks or 6 weeks of treatment. Two trials (184 women randomized) studied the effects of simvastatin, and two trials (60 women randomized) studied the effects of atorvastatin. The most recent meta-analysis on statins in PCOS women (including 5 studies on statins vs. placebo and 4 comparing

statins vs. combination of statins and metformin) concluded that combined statin and metformin therapy can improve lipid and inflammation parameters, but cannot effectively improve insulin sensitivity and reduce hyperandrogenism [90].

Little is known about long-term clinical and biochemical effects of statins on PCOS. To date, the two longest studies, not included in the abovementioned Cochrane Review and meta-analysis, evaluated the effects of up to 6-month long use of statins on clinical and biochemical abnormalities in subjects with PCOS. The first one was carried out by Banaszewska et al., who randomized 139 patients with PCOS to simvastatin alone, metformin alone, or simvastatin plus metformin groups. Clinical, endocrine, and metabolic parameters were evaluated after 3 and 6 months of treatment [83]. Subjects in all groups experienced a significant improvement of menstrual regularity, with the greatest increase in the number of spontaneous menses observed in women receiving simvastatin. Similarly, ovarian volume decreased significantly in groups treated with simvastatin, whereas the decline of ovarian volume in women treated with metformin alone was of borderline statistical significance. This simvastatin-induced decline in ovarian volume occurred in parallel with improvement of hyperandrogenism. Overall, this study indicated that in the long term, simvastatin may be superior to metformin in actions on clinical and biochemical aspects of PCOS. Comparable results were obtained in the most recent, randomized, double-blind, placebo-controlled study conducted by Seyam et al. [91]; 200 patients with PCOS were randomized (1:1) to receive 20 mg simvastatin daily or placebo for 6 months. Simvastatin therapy resulted in decreased levels of testosterone (by 28%), LH (by 40%), LH/FSH ratio (by 43%), total cholesterol level (by 26%), LDL (by 39%), and triglycerides (by 23%), improved menstrual regularity and spontaneous ovulation (in 10% of patients in the simvastatin group compared to 0 in the placebo group), and decreased hirsutism, acne, and ovarian volume. Insulin resistance did not show a significant difference.

In summary, current, however limited, literature suggests that statins and combination of statins and metformin are promising options for PCOS patients; however, large-scale randomized controlled studies on divergent populations of women with PCOS should be performed to ascertain the long-term effects of proposed new therapeutic strategies.

Limitations of the Use of Statins

Interesting and promising studies on the effects of statins on PCOS should be viewed with caution and with recognition of potential risks and limitations. While statins are usually considered to be safe, their use, especially in the long term, is not risk-free. Despite an overall favorable risk profile, statins, especially at high doses, may increase the rate of myopathy defined as muscle pain, tenderness, or weakness that is accompanied by substantial increases in blood creatine kinase concentrations, rhabdomyolysis, diabetes mellitus and potentially increase the rate of hemorrhagic (but not ischemic) stroke more frequently observed in individuals with pre-existing cerebrovascular disease [92]. Furthermore, serious adverse effects have

been reported with concomitant use of statins with other drugs, including fibrates, macrolide antibiotics, antiarrhythmics, cyclosporin, and protease inhibitors [93]. In view of these concerns, the FDA reduced the recommended simvastatin doses.

One of the greatest concerns regarding the use of statins in reproductive age women is the potential for teratogenicity, although these concerns have not been conclusively substantiated to date in humans. Based on animal data, several studies failed to demonstrate teratogenicity of statins [94], whereas others found that at high doses, statins may be associated with impairment of placental and skeletal formation [95, 96]. In humans, several studies evaluated the effects of statin exposure during the first trimester of pregnancy and did not demonstrate any significant increase in the risk for malformations [97–99]. However, since statins are currently listed by the FDA as pregnancy category X medications, their use is contraindicated in pregnancy, and sexually active women of reproductive age should use reliable contraception when considering statin therapy.

Another important area of concern is the potential for statins to affect glucose metabolism, particularly for a population that is innately at risk for developing diabetes, such as women with PCOS. The JUPITER randomized trial, including 17,603 patients without a history of vascular disease or diabetes, revealed slightly increased concentration of glycated hemoglobin after 2 years of rosuvastatin 20 mg daily treatment compared to placebo (5.9% vs. 5.8%). Four hundred eighty-six participants developed diabetes during follow-up: 270 on rosuvastatin vs. 216 on placebo (HR 1.25, 95% CI 1.05–1.49, $P = 0.01$) [52]. Importantly, this excess of diabetes diagnoses appeared mainly among patients who had risk factors for diabetes (e.g., elevated body mass index or HbA1c, or impaired fasting glucose), and did not appear to get larger as treatment continued [52, 100].

Statins – Future Directions

The likely key mechanism responsible for pleiotropic actions of statins is related to inhibition of synthesis of isoprenylation substrates, leading to changes in many important biological pathways. Individual statins, however, have different pharmacokinetic and pharmacodynamic properties as well as different therapeutic dose ranges. These differences are due to their chemical structure, ring attached to the pharmacophore, polar or nonpolar substituents, and lipo- or hydrophilicity. Cerivastatin, simvastatin, fluvastatin, lovastatin, atorvastatin, and pitavastatin are classified as lipophilic, while pravastatin and rosuvastatin as hydrophilic [101, 102]. Hydrophilic statins tend to be more but not exclusively hepatoselective [103] secondary to their tissue-specific active transport system involving organic anion transporting polypeptide (OATP) [104]. In contrast, lipophilic statins enter cells by passive diffusion through the cell membrane and hence achieve higher concentration in the extrahepatic tissue. In addition, different statins also undergo metabolism by different cytochrome P450 isoenzymes, and hence affecting the risk of clinically relevant drug interactions [101]. These considerations raise the question, which statins are the most effective and appropriate to achieve a given biological effect in

specific tissue, for example, ovary? Thus, future studies should be focused on the evaluation of a new generation of statins with high potency, better side-effect profile, and different pharmacokinetic profiles. It should be emphasized that while lipophilicity of the statins is at least partially responsible for their pleiotropic effects, due to ease of tissue penetration, it also contributes to higher risk of side effects like myositis and myopathy as well as rhabdomyolysis, impaired insulin secretion, and insulin resistance.

Resveratrol

Mechanism of Action of Resveratrol

There is a growing interest in the development of new adjuvant therapies that could successfully address the myriad endocrine and metabolic concerns in PCOS and in identifying strategies that may potentiate beneficial effects of statins at lower doses and, hence, improve tolerance while mitigating possible adverse effects such as on glucose metabolism. One candidate for such adjuvant therapy is resveratrol (*trans*-3,5,4′-trihydroxystilbene), a natural polyphenol synthesized by several plants as a phytoalexin in response to pathogens such as bacteria or fungi and found in grapes, nuts, berries, and red wine. This phytoestrogen appears to have a potential for a broad range of beneficial actions, including anticarcinogenic, cardioprotective, neuroprotective, anti-inflammatory, and antioxidant activities [105–110]. Resveratrol interacts with multiple cellular targets and signaling pathways, but many effects, including DNA repair, transcriptional silencing, as well as glucose and lipid metabolism, are attributed to its activation of sirtuins, a family of nicotinamide adenine dinucleotide^{+}-dependent deacetylases [111, 112]. SIRT1, the mammalian homologue of yeast Sir2 (silent information regulator 2), deacetylates several targets in mammalian cells, such as p53, FOXO1, PGC-1α, liver X receptor, and hypoxia-inducible factor 2α. This property may account for resveratrol-induced beneficial effects on lifespan extension and cell survival supporting the hypothesis that sirtuins are universal regulators of the aging process [113]. Crucial roles at the level of the ovary for SIRT1 and SIRT3, the main components of the sirtuin family, are the regulation of the redox state in oocytes and granulosa cells and potential protection of oocytes from age-related oxidative stress [114]. While activation of SIRT1 seems to be an important mechanism of action of resveratrol, it was also demonstrated that resveratrol may possess antiproliferative properties by downregulation of phosphoinositide kinase-3 (PI3K)/protein kinase B (Akt) signaling pathways crucial for cell survival and proliferation [115, 116] including cancer cell lines [117–119]. Similarly, resveratrol inhibits the survival and proliferation of estrogen-sensitive cells by interfering with an estrogen receptor (ER) α-associated PI3K pathway [120]. Resveratrol's antiproliferative, anti-inflammatory, and anticarcinogenic effects are also attributed to its ability to interfere with tumor necrosis factor α-induced activation of NF-κB [121]. The other mechanisms of action of resveratrol include inhibition of cyclooxygenase (COX) and induction of nitric oxide synthase,

explaining its anti-inflammatory, antiplatelet, and cardioprotective actions [122, 123]. Moreover, resveratrol was shown to inhibit the rate-limiting step of the mevalonate pathway, by reducing expression and activity of HMG-CoA reductase, leading to decreased availability of downstream products, including FPP, GGPP, and cholesterol, and interfere with key cell functions [61].

In Vitro Studies of Resveratrol

Resveratrol has been shown to promote apoptosis and reduce theca-interstitial cell proliferation as well as inhibit insulin-induced theca-interstitial cell growth using cultures of rat theca-interstitial cells. Similarly, resveratrol counteracted the anti-apoptotic actions of insulin, as shown by a concentration-dependent increase in caspase-3/7 activity and DNA fragmentation. Resveratrol also induced progressive time- and concentration-dependent morphological changes in theca-interstitial cells consistent with apoptosis, including nuclear shrinkage with condensed chromatin and cytoskeleton degradation [124]. Therefore, resveratrol emerges as a modulator of the insulin signaling pathway in theca-interstitials cells; these properties are of potential clinical relevance in pathological conditions associated with insulin resistance, hyperinsulinemia, and theca-interstitial cell hyperplasia, such as seen in PCOS.

Furthermore, it has been demonstrated that resveratrol reduces theca-interstitial androgen production primarily by inhibiting *Cyp17a1* mRNA expression. This inhibition is independent of mechanisms involving isoprenylation and activation of sirtuins and may be mediated, at least in part, by blocking the activity of the serine-threonine kinase/protein kinase B pathway [125]. Thus, resveratrol may decrease both the number of steroid-producing cells and the steroidogenic capacity of each individual theca-interstitial cell by exerting its inhibitory effects on proliferation and steroidogenesis, resulting in a decrease of the total amount of androgens secreted by the ovary. Importantly, resveratrol's action on ovarian steroidogenesis is selective and has no effect on progesterone production by theca cells. In granulosa cells, resveratrol has cytostatic but not cytotoxic effect and reduces the expression of vascular endothelial growth factor but not anti-Müllerian hormone [126]. Moreover, resveratrol was found to inhibit protein expression and enzyme activities of CYP17 and CYP21 in human adrenal H295R cells. This modulation of steroidogenic activity was sirtuin independent [127].

Recently, resveratrol has been demonstrated to potentiate simvastatin-induced inhibition of theca-interstitial cell proliferation in a dose-dependent manner by blocking the mevalonate pathway via distinctly different mechanisms than statins; simvastatin reduces HMG-CoA reductase activity while indirectly inducing HMG-CoA reductase mRNA and protein expression, whereas resveratrol inhibits HMG-CoA reductase mRNA and protein expression as well as HMG-CoA reductase activity (Fig. 13.3) [61].

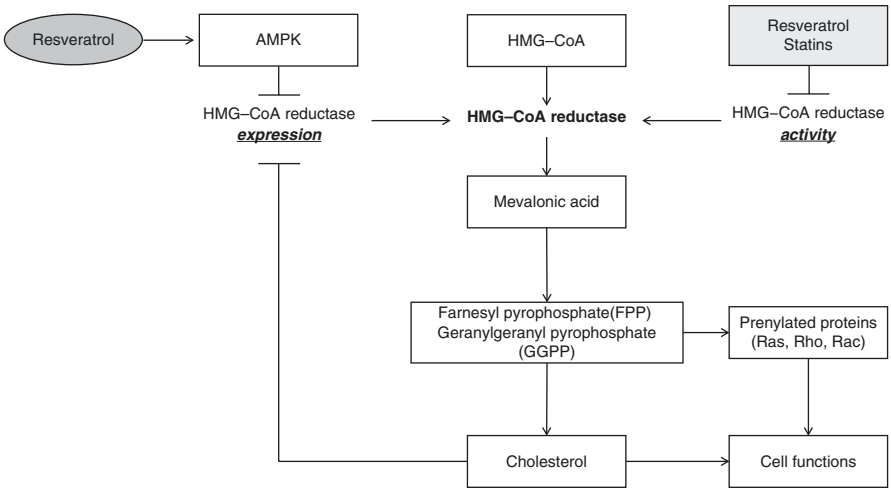

Fig. 13.3 Proposed diagram for the inhibition of HMG-CoA reductase by resveratrol (at the level of HMG-CoA reductase expression and activity) and by statins (at the level of competitive inhibition of HMG-CoA reductase activity). Products of the mevalonate pathway, such as cholesterol, inhibit HMGCR expression

In Vivo and Clinical Studies of Resveratrol

Benrick et al. studied the effects of resveratrol on reproductive and metabolic parameters using a dihydrotestosterone (DHT)-induced PCOS rat model. Five to six weeklong resveratrol treatment resulted in reduction of the size of adipocytes, increased estrogen-related receptor α gene expression in subcutaneous fat, and improved estrus cyclicity. Resveratrol had no effect on insulin sensitivity and ovarian steroidogenesis in this model of PCOS. However, this outcome may likely be related to the use of DHT in the animal model and hence the direct induction of hyperandrogenism [128]. Similarly, Ergenoglu et al. evaluated in vivo effects of resveratrol in a PCOS rat model induced by subcutaneous DHT pellets. Resveratrol treatment resulted in a significant decrease in both the antral follicle count and levels of anti-Müllerian hormone and insulin-like growth factor 1. Additionally, resveratrol exerted antioxidant effects, reducing superoxide dismutase activity and increasing glutathione peroxidase content [129]. Moreover, in an androgen-induced PCOS rat model, resveratrol decreased oxidative stress and ovarian fibrosis, by activation of sirtuin type 1 (SIRT1), which lead to reduced expression of p66Shc, the protein which plays an important role in the production of mitochondrial ROS [130]. In vivo studies have also demonstrated that resveratrol may interfere with the mevalonate pathway, reducing serum cholesterol by downregulating hepatic HMG-CoA reductase mRNA expression in hamsters fed a high-fat diet [131]. Another study, suggested that combined therapy of metformin and resveratrol may ameliorate the weight gain, hormone profile and normalize folliculogenesis by inducing

antioxidant and anti-inflammatory systems via SIRT1 and AMP-activated protein kinase (AMPK) activation in DHEA-induced PCOS rats [132]. The serum testosterone level was especially decreased, and the lipid peroxidation markers improved in the group treated with resveratrol alone.

To date, there is only one clinical study published evaluating the use of resveratrol to treat conditions associated with ovarian dysfunction [133]. In this double-blind, placebo-controlled randomized trial, 30 patients diagnosed with PCOS based on Rotterdam criteria were assigned to receive for 3 months 1500 mg of micronized trans-resveratrol or placebo. Resveratrol treatment led to a significant decrease of total testosterone by 23.1% ($P = 0.01$). The leaner patients had a significantly greater decline of testosterone level than subjects with higher BMI. In parallel, resveratrol induced a 22.2% decrease of dehydroepiandrosterone sulfate ($P = 0.01$), a decrease of fasting insulin level by 31.8% ($P = 0.007$), and an increase of the insulin sensitivity index (Matsuda and DeFronzo) by 66.3% ($P = 0.04$). This significant reduction of the level of the ovarian and adrenal androgens may be at least partially related to the improvement of insulin sensitivity and decline of insulin level. Another possible mechanism was reduction of the enzymatic activity of 17α-hydroxylase/C17,20-lyase. The magnitude of improvement of hyperandrogenemia observed in response to resveratrol was comparable to or greater than that found in response to OCPs or metformin, with the exception of preparations containing cyproterone acetate, which are not available in the USA. Levels of gonadotropins, BMI, ovarian volume, the lipid profile, as well as markers of inflammation and endothelial function were not significantly altered. This clinical trial conducted on a small group of patients brought promising results. Especially, marked reduction of hyperandrogenemia after only 3 months of treatment and no adverse effects on metabolic aspects of PCOS were encouraging. Further and larger clinical studies are needed to reliably evaluate the efficacy and safety of resveratrol in the treatment of PCOS and establish optimal dose and length of therapy.

Limitations of the Use of Resveratrol

Available safety data on resveratrol use are limited. Only few human studies on a small number of participants were conducted to evaluate side effects. The main reported adverse effects were nausea, diarrhea, flatulence, and stomach pain. They were related to long-term use of resveratrol in high doses (500 mg and above) [134]. The levels of resveratrol in the food vary significantly and are considerably low. For example, red wine contains between 0.2 and 5.8 mg of resveratrol per 1 L. Additionally, bioavailability of oral resveratrol is about 0.5% secondary to rapid hepatic glucuronidation and sulfation. The half-life of resveratrol is only 8–14 min and its metabolites about 9 hours [135, 136]. Extensive metabolism of resveratrol may have clinical implications and affect efficacy of resveratrol administered as a supplement.

Resveratrol – Future Directions

Relatively limited bioavailability and rapid metabolism of resveratrol may be the main reason of lower-than-expected efficacy of resveratrol in various clinical studies. Hence, there is a need for further studies focusing on improving methods of delivery of resveratrol to the tissues at therapeutic doses.

There is an emerging interest in evaluating the potential beneficial effect of combined therapy using statin in conjunction with resveratrol in anticipation that such therapy may improve endocrine, metabolic, and cardiovascular aspects of PCOS, allow for lower doses of therapeutic agents, and decrease the risk of potential side effects. For example, in vivo study using an ischemic rat heart model evaluating simvastatin and resveratrol demonstrated superior cardioprotective profile of combined therapy compared to simvastatin alone [137].

Conclusions

Hallmarks of PCOS include menstrual cycle irregularities, androgen excess, enlarged polycystic ovaries, and a broad range of metabolic and cardiovascular risks. Statins and resveratrol emerge as promising therapeutic agents targeting most of the endocrine and metabolic underpinnings of PCOS (Fig. 13.4). Despite the promise however, routine clinical use of statins and resveratrol in reproductive age women with PCOS cannot be yet recommended, and longitudinal studies that comprehensively evaluate benefits and safety of these agents in diverse populations of women with PCOS are urgently needed to assess and ensure long-term safety. Furthermore, in view of a potential for teratogenicity of statins, reproductive age women must ensure concomitant use of reliable contraception when considering the use of this class of medications.

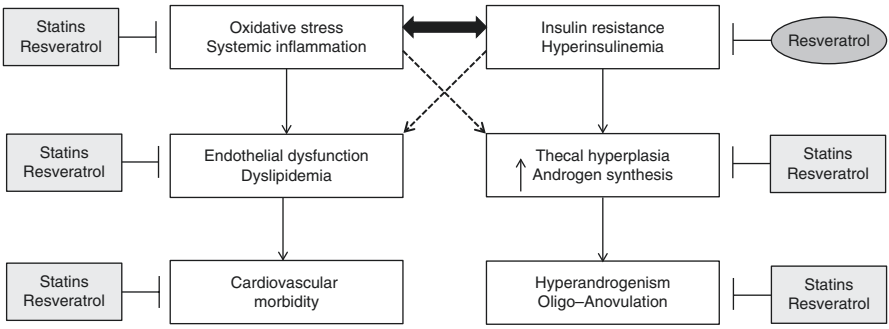

Fig. 13.4 Rationale for the use of statins and resveratrol for the treatment of polycystic ovary syndrome. ↑indicates increased; solid line indicates established cause and effect; dashed line indicates proposed pathway

Acknowledgments This study was supported by grant R01-HD050656 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (to AJD).

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Role of Lifestyle, Diet, and Exercise in the Management of Polycystic Ovarian Syndrome

14

Amy Plano

Key Points

- Lifestyle modification has been shown to be effective in improving body weight as well as the metabolic, reproductive, and psychological features of polycystic ovarian syndrome (PCOS).
- Abnormalities in insulin-induced glucose optimization consequently resulting in hyperinsulinemia are well known in women with PCOS.
- Hyperinsulinemia can promote fat storage (and hence weight gain), increase cravings for carbohydrates, cause hypoglycemia, and make weight loss challenging.
- Long term these metabolic adaptations can increase an individual's risk for developing obesity, type 2 diabetes, hypertension, and cardiovascular disease.
- Hyperinsulinemia responds remarkably well to weight reduction through diet, exercise, and lifestyle modifications.
- Therefore, diet therapy is without question a most important and effective management strategy managing the symptoms of PCOS.
- While there is much debate about the optimal balance of macronutrients that is uniformly beneficial to women with PCOS, evidence supports that both the quality and quantity of dietary carbohydrates are important for a woman with PCOS.
- Evidence does not support the benefits of drastically reducing or eliminating dietary carbohydrates in this population.
- Instead, a diet with an emphasis on consuming a moderate amount of unrefined carbohydrates qualified as low to medium on the glycemic index scale is supported.
- Additionally, the diet should be low in saturated fat and sodium and high in fiber from whole grains, fruits, and vegetables and modest in lean sources of protein.
- A diet of this quality can improve short- and long-term symptoms of PCOS, as well as decrease the risk of chronic diseases associated with insulin resistance.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_14

- Given their propensity to experience reactive hypoglycemia, women with PCOS are an ideal population to benefit from frequent meals and snacks.
- Physical activity can reduce insulin levels, improve insulin sensitivity, and optimize lipid profiles as well as improve self-esteem, *decrease* depression, and *reduce* anxiety in women with PCOS.
- Regular exercise must be encouraged and incorporated in the management paradigm of PCOS.

Introduction

With insulin resistance and compensatory hyperinsulinemia central to the pathogenesis of polycystic ovary syndrome (PCOS) and obesity and dyslipidemia being common accompaniments to the clinical picture, lifestyle interventions aimed at reducing insulin levels and improving insulin sensitivity are critical to the overall management of this disorder. While there is strong agreement that a hypocaloric diet is beneficial for overweight and obese women with PCOS, there is much debate about the balance of dietary macronutrients that could be construed as optimal for this population. The inclusion of regular exercise has also demonstrated a positive impact on metabolic and clinical parameters in women with PCOS. Management strategies must prioritize body weight optimization and improving insulin sensitivity. This chapter provides an overview of lifestyle interventions that can successfully address the spectrum of metabolic derangements that are commonly encountered in PCOS and are modifiable through dietary modification and regular physical activity.

Why Diet Makes a Difference in Women with PCOS

Abnormalities in insulin-induced glucose utilization are well recognized in PCOS [1–3]. The inability of the secreted insulin to optimally drive the cellular machinery through glucose transportation and utilization results in a reactionary increase in pancreatic output of insulin; higher levels of insulin are thus needed to facilitate insulin actions, a phenomenon called *insulin resistance*. In the presence of hyperinsulinemia and impaired insulin signaling, energy efficiency is impaired and glucose, instead of being utilized as fuel, gets diverted towards lipogenesis and hence fat storage [4]. Hyperinsulinemia, in turn, encourages fatty acids to be deposited as body fat while also inhibiting its release from fat stores. The metabolic processes are subtle and yet translate into progressive weight accrual despite reportedly regular physical activity and even caloric restriction. Over time, these processes can exhaust the pancreatic beta cells; ability to adequately maintain euglycemia is thus impaired, setting a stage for the development of type 2 diabetes, a disorder commonly seen in women with PCOS [5, 6]. Adiposity worsens the innately disturbed insulin signaling. Body mass excess is evident in almost two-thirds of women with PCOS and is recognized to worsen several features of PCOS, such as

hyperandrogenism, hirsutism, infertility, and pregnancy-related complications. Insulin resistance is associated with an increased risk for several other disorders, including hypertension, dyslipidemia, elevated markers of inflammation, endothelial dysfunction, and heart disease. Impaired glucose tolerance or type 2 diabetes develops by the age of 30 in 30–50% of obese patients with PCOS [5, 6].

Progressive weight gain, cravings for carbohydrates (CHO) and sweets, and symptoms of hypoglycemia (such as dizziness, fatigue, shakiness, irritability, headaches, postprandial exhaustion, and somnolence) are common in women with PCOS. Episodes of hypoglycemia are typically encountered when an individual goes long periods of time without eating. More typically, however, patients manifest features of *reactive hypoglycemia* following ingestion of refined carbohydrates, e.g., candy or a large serving of sweets. An outpouring of pancreatic insulin in response to an abrupt rise in glucose, with lingering hyperinsulinemia and delayed onset of insulin action, is contributory to a precipitous drop in circulating blood glucose levels. A few hours after ingestion of a CHO load, instigating cravings for sweets ensues. Indeed, many women with PCOS report strong, almost compulsive, uncontrollable urges for "sweets" and CHO-rich foods [7–9].

Reassuringly, insulin resistance of PCOS responds remarkably well to weight reduction through dietary change, exercise, and improved lifestyle [10]. An optimal diet for a woman with PCOS must encompass several facets. It must be calorie appropriate to support the specific needs of the particular woman (weight maintenance, weight loss, or weight gain). Proportioning meal size and ensuring a timely intake of nutritionally balanced snacks and meals are strategies that can successfully abort the viscous cycle of insulin resistance-driven carbohydrate cravings. Lowering of dietary sugar load and choice of complex dietary CHO reduces the postprandial excursion in serum glucose and hence lessens the magnitude of insulin secretion in response to dietary load. Reducing postprandial insulin levels has demonstrated an increase in fat oxidation for several hours following a meal and reduced hunger and hence predisposition to overeating and weight gain [5]. A balance in respective amounts of dietary carbohydrate, protein, and fat can further ensure homeostasis while mitigating insulin resistance. *Eating patterns in combination with lifestyle modification that include consistent exercise that support lower insulin levels and should be central to the treatment of the symptoms of PCOS.*

Dietary Carbohydrates

The primary role of dietary CHO is to serve as fuel. Carbohydrates in diet are almost exclusively of plant origin. Milk is the only animal-derived food that contains a significant amount of CHO. Fruits, vegetables, whole grains, and legumes (beans, lentils, peas) are major sources of whole, unprocessed CHOs; fruit drinks, soft drinks, cookies, ice cream, and candy bars too are examples of dietary CHO, albeit these latter represent more processed and less nutrient-dense versions of carbohydrates. Although foods containing protein and fat affect insulin secretion, ingested CHO content has a more pronounced effect on pancreatic insulin release and

circulating insulin levels. The rapidity of CHO breakdown and gastrointestinal absorption following ingestion depends on the *type* of ingested CHO. The more refined and processed the CHO content of a meal, the less work the body has to exert to digest and absorb the nutrient. The glycemic effect (also called the *glycemic response*) refers to the effect a particular food has on a person's blood glucose and insulin response. The more refined varieties of dietary CHO evoke a higher glycemic response, and this phenomenon represents the basis for how many CHO-containing foods are classified.

Glycemic Index of Foods

The glycemic index (GI) of foods is a numerical system of measuring how much of a rise in circulating blood sugar the food triggers – the higher the GI number, the greater is the expected excursion in blood sugar response following ingestion of that particular food. Foods containing CHOs that are broken down easily and raise the blood sugar quickly are referred to as *high glycemic* foods; both glucose and white bread are currently considered reference foods with a GI of 100. Foods with a GI index between 70 and 100 are considered *high*, whereas values between 55 and 70 and <55 are deemed of medium and low GI index value [10, 11]. The fiber content of various edibles influences their respective GI index; higher dietary fiber content slows the rapidity of glucose absorption and hence offers a more controlled rise in blood sugar levels. Food items containing a high fiber content (>5 grams/serving) are classified as *low glycemic* foods, which also promote an increased level of satiety, thus helping control appetite and decrease hunger, and are ultimately helpful for weight management. Table 14.1 presents GI values for common food items.

There are many proponents of a low-GI diet as a means to improve insulin sensitivity and possibly improve the androgen profile of women with PCOS [12, 13]. Because the majority of women with PCOS demonstrate marked compensatory hyperinsulinemia after CHO ingestion, there may be specific metabolic and cardiovascular benefits of consuming low-GI value diet. However, while numerous studies have assessed the impact of a low-GI diet in the general population (non-PCOS women), few studies have specifically examined the role of a low-GI diet on women with PCOS [14–16].

In a 2010 study performed by Marsh et al., researchers compared the changes in insulin sensitivity and clinical outcomes after similar weight losses after the consumption of a low-GI diet compared with a conventional healthy diet in women with PCOS [16]. Both diets were designed as reduced-energy, moderate-to-high fiber with similar macronutrient distribution. Only the quality of carbohydrates (i.e., GI) varied between the two diets. Overweight and obese women with PCOS were assigned to one of two groups: (1) an ad libitum low-GI diet or (2) a macronutrient-matched healthy diet. Both groups were followed for 12 months or until they reached a 7% weight loss. With only a modest weight loss of 4–5% of body weight, the group of women following the low-GI diet experienced a threefold greater improvement in whole-body insulin sensitivity. In addition, women following the

Table 14.1 Glycemic indices of common dietary items

High glycemic index foods
Watermelon
White bread
Certain cereals (Cheerios, Corn Flakes, Rice Krispies)
Mashed potatoes
Honey
Soft drinks
Moderate glycemic index foods
Watermelon
Sourdough or rye breads
Cream of wheat and instant oatmeal
Orange juice
Pineapple
Low glycemic index foods
Bran cereal
Old-fashioned oatmeal
Peaches
Lentils
Milk
Sweet potatoes

low-GI diet demonstrated improved menstrual cycle regularity (95%) versus those women following a conventional healthy diet (65%). While not a long-term study, this research nonetheless supports the capacity of dietary carbohydrates to increase postprandial glycemia, which ultimately when manipulated may be an important consideration for managing the symptoms associated with PCOS.

Another study performed by Mehrabani et al. examined the impact of the glycemic load on overweight and obese women with PCOS [17]. A total of 60 overweight and obese women with PCOS were recruited and randomly assigned to 1 or 2 hypocaloric diet groups for a single-blind clinical trial. Group 1 included a conventional hypocaloric diet (CHCH – with 15% of dietary calories coming from protein). Group 2 included a modified hypocaloric diet (MHC) with a high protein, low glycemic load (30% of daily energy from protein plus low-glycemic load foods selected from a list). Both diets were prescribed via 12-weekly counseling visits. While weight loss and androgen reduction were similar between both diet groups, the group adhering to the MHCD presented with a significant reduction in insulin level and an overall decrease in high-sensitivity C-reactive protein when compared with the conventional diet. While research in this area does appear promising, more research regarding the long-term efficacy of low-GI diets in this population is clearly needed.

Limitations of Using the Glycemic Index for PCOS Patients

Despite the potential dietary advantages noted above for metabolic benefit in chronically hyperinsulinemic and insulin-resistant populations, an overenthusiastic focus on dietary GI has several disadvantages worth noting. First, in assigning a numerical value to a food item, GI assumes the food is eaten alone, which is often not the case. When foods are eaten in combination, indices of consumed

items may influence the overall GI of the meal. Dietary fat and fiber slow the process of meal digestion and, therefore, can lower the GI of an individual food item. For example, an apple eaten alone produces a rapid rise in blood glucose. However, the rise is not as substantial if you add a tablespoon of natural peanut butter; the apple is digested at a slower rate.

The GI of the food is also affected by its acidity, ripeness, processing, the length of storage, cooking methods, and its variety. For example, a yellow banana has a lower GI than a brown banana. Fully cooked pasta has a lower GI than al dente pasta; boiled potatoes have a lower GI than a baked potato. Even beverages such as soymilk, depending upon the brand, can dramatically vary in the CHO content and, hence, GI.

Lastly, the GI does not tell us anything about the nutritional content of the food. *A low-GI food does not equate to a healthy food*. Also, just because a particular food has a low or moderate GI does not mean that food can be consumed in abundance. The GI simply tells us how quickly a particular CHO affects blood glucose levels and thus pancreatic insulin response to the consumed food. Therefore, by strictly avoiding certain foods with high GI, individuals may deprive themselves of some nutritious foods. For example, **watermelon has a GI of 72 and is considered a high-GI food**, while a chocolate bar has a GI of 68 and potato chips have a GI of 58, making classifying them both as moderate-GI foods. It is evident watermelon is nutritionally superior choice to a chocolate bar or potato chips. Therefore, while the GI deserves some consideration, it should not be the only criterion when selecting appropriate meal choices. The total amount of dietary CHO, the food's caloric density, the amount and type of fat, dietary fiber, and salt contents are additional dietary considerations that merit attention.

Guidelines for Dietary Carbohydrate

As discussed, diets high in CHO and refined and processed forms of CHO are particularly detrimental for the insulin-resistant PCOS population. However, evidence does not support the benefits of drastically reducing or eliminating carbohydrates in the diet either [18, 19]. Given that the majority of women with PCOS have underlying issues with insulin resistance, the increased likelihood of obesity, and risk factors for developing type 2 diabetes, a diet that is moderate in CHO with a focus on low-GI foods is best suited for this population. Women with PCOS should aim for around 40% of their total calories coming from CHO (Table 14.2). This amount is appropriate to optimize blood glucose levels while still providing many of the benefits of low-GI diet. For a woman consuming 1500 kcals per day, this would equate to a maximum value of 150 g of CHO spread consistently throughout the day (e.g., 20–30 g of low-GI CHO with breakfast, 35–40 g of low-GI CHO with lunch, and 30–35 g of low-GI CHO with dinner). The remainder should be consumed in two to

Table 14.2 Recommended daily carbohydrate consumption for women with PCOS based on daily caloric intake

Daily caloric level	Daily maximum carbohydrate level (g/day)
1400	140
1500	150
1600	160
1700	170
1800	180
1900	190
2000	200

three low-GI snacks spaced equally between the three meals. A specific meal plan fitting these criteria is provided later in this chapter.

Fruits and PCOS

All fruits in their natural form are nutritious, and regular consumption should be encouraged by women with PCOS. Despite the "healthy" connotations, all fruits are predominantly composed of CHOs. Typically, one serving of fruit contains about 15–20 g of CHO, no protein or fat, and 60 calories. Therefore, while fruit (quantified as one small whole fruit such as apple, orange, or banana; ¾ to 1 cup of cubed fruit such as melon, strawberries, or grapes; or ¼ cup of dried fruit such as raisin, prunes, or apricots) contains many positive benefits, such as fiber, vitamins, minerals, and antioxidants, an individual with PCOS needs to be mindful of the quantity consumed; women should aim for consuming two servings of fruit per day.

Given fruit items are poor in fat or protein, they may be best paired with a lean protein choice or modest amount of fat, such as low-fat cheese, yogurt, or nuts, to stabilize blood sugar and promote increased satiety. Women with PCOS should particularly avoid fruits in canned heavy syrup; when purchasing canned fruit, the words "extra light syrup" and "packed in juice" are optimal.

Dried fruit can be a nutritious addition to the diet of an individual with PCOS. Dried fruit is high in fiber, vitamins, and minerals such as potassium and iron as well as antioxidants. However, due to the fact dried fruit has been dehydrated, the natural sugars and calories are much more concentrated compared to those found in fresh fruit. In addition, some manufacturers add sugar in the processing. Therefore, while women with PCOS do not need to avoid dried fruit, they do need to be mindful of the quantity they consume just as they should with all fruits.

All fruit juices represent a concentrated source of sugars and low amount of fiber and are best avoided, as even small amounts can spike insulin, making weight loss difficult. It is always preferable to choose whole fruit over fruit juice, even if the juice label states "100 percent juice."

Vegetables and PCOS

Vegetables are high in fiber and abundant in vitamins, minerals, and antioxidants. There are two main categories of vegetables: non-starchy and starchy vegetables. Non-starchy vegetables include spinach, lettuce, broccoli, green beans, onions, mushrooms, zucchini, and peppers and promote satiety. They are low in calories and in CHO and contain fiber and numerous vitamins, minerals, and antioxidants, so an individual can consume them without worrying about gaining weight or worsening insulin resistance. Women with PCOS can typically consume non-starchy vegetables in unlimited amounts and should aim for *at least* three servings of non-starchy vegetables (each serving defined as one-half cup of cooked vegetables, one cup of raw vegetables, or one 8 ounce cup of vegetable juice) daily.

Starchy vegetables have a higher CHO content and, therefore, a more significant impact on insulin levels than the non-starchy ones and include corn, peas, winter squashes, plantains, and all types of potatoes. Although excursions in insulin levels are much higher following ingestion of starchy vegetables, the goal however is not to eliminate starchy vegetables from the diet. One serving of starchy vegetables contains 15 g of CHO, 0–3 g of protein, 0–1 g of fat, and 80 calories in contrast to approximately 5 g of CHO, 2 g of protein, and 25 calories per non-starchy vegetable serving. Therefore, women with PCOS when consuming starchy vegetables should practice caution.

Dietary Fiber and PCOS

Dietary fiber is the indigestible part of plant-based foods and passes through the gastrointestinal tract in its original form. In spite of the numerous benefits of consuming adequate fiber, research supports that women with PCOS consume a diet lower in fiber compared to their non-PCOS counterparts [20, 21]. Fruits, vegetables, nuts, seeds, beans, and whole grains all contain fiber. In the stomach, fiber content of consumed food conveys a feeling of fullness, delays gastric emptying, and promotes a feeling of satiety. Additionally, in appropriate amounts, dietary fiber has been demonstrated to have a cholesterol-lowering effect [22, 23]. The products of bacterial digestion of fiber in the colon are absorbed into the body and may inhibit the body's production of cholesterol as well as enhance the clearance of cholesterol from the blood. Lastly, fiber has been demonstrated to improve the way the body handles glucose by slowing the digestion and absorption rate of CHO. This promotes a slower, more stable rise of blood glucose and helps prevent the symptoms of reactive hypoglycemia and reactionary sugar cravings that are commonly experienced by women with PCOS [24]. In addition, the more gradual the release of blood glucose, the longer it will take for the body to feel hungry again.

Box 1 Strategies for Optimizing Dietary Fiber Intake

- For breakfast, choose a high-fiber breakfast cereal. Look for cereals with at least 5 or more grams of fiber per one cup serving.
- Add a few tablespoons of unprocessed wheat bran, flaxseed, or chia seed to your favorite cereal.
- Consume breads that list the words "whole wheat," "whole-wheat flour," or other whole grain as the first ingredient on the label.
- Purchase brands of bread with at least 3 g of dietary fiber per slice.
- Add beans such as lentils, black-eyed peas, lima beans, great northern beans, and chickpeas to your diet.
- Sprinkle crushed walnuts or slivered almond into your hearty salads.
- Snack on raw non-starchy veggies like celery, cucumbers, and peppers by themselves or dipped in a bean dip like hummus.

Recommendations for Fiber and PCOS

Women with PCOS should aim to consume 25–30 g of fiber per day. Products containing 3 g or more per serving are considered a good source of fiber, whereas diets containing 5 or more grams of fiber per serving are considered as *high* fiber source. *Just because a food label may state "100% wheat" or "multigrain" does not mean it is a good source of fiber*. Products that have the words *whole wheat*, *whole oats*, *whole rye*, *bulgur*, *graham flour*, *oatmeal*, or *wild rice* listed as the first ingredient on the food's label represent a high-quality source of fiber. Table 14.3 offers tips for including more fiber in the diet.

Protein

Protein has many vital roles in the body. About 20% of our total body weight is protein. The body uses protein for building and maintaining tissues, to make important enzymes, hormones, neurotransmitters, tendons, ligaments, antibodies, and other body chemicals. Women with PCOS should do their best to select lean rather than high-fat sources of animal protein. Women with PCOS are advised to substitute a portion of their dietary CHO for protein as a strategy to reduce overall dietary CHO content. Protein takes longer than CHO to digest, and dietary protein content confers a sense of satiety; thus, inclusion of lean protein in meals and snacks can improve the overall insulin response in women with PCOS [25]. An optimal intake for protein for women with PCOS would be between 25% and 30% of total calories consumed. For a woman following a 1500-calorie diet, this would equate to around 100–110 g of protein per day, spread equally among meals and snacks. A helpful

hint is every 1 ounce of lean protein is equal to 7 g of protein. Therefore, a 4 ounce chicken breast (equivalent in size to small computer mouse or deck of cards) contains roughly 28 g of protein. By consuming regular amounts of protein throughout the day, women with PCOS can help stabilize blood sugar and prevent hypoglycemia and subsequent cravings.

Box 2 Considerations When Choosing Dietary Protein Sources

- 3 ounces of protein is equal to the size of the palm of your hand or a deck of cards.
- Fish and shellfish, fresh, frozen, or canned in water, are excellent protein choices. Experts recommend heart-healthy benefits of consuming at least two servings of fish per week.
- Meat, chicken, and fish are all excellent sources of protein, as well as iron, zinc, and vitamin B_{12}. Women with PCOS should focus on the low-fat varieties as well as the leanest cuts of meat, such as the following:
  - Flank steak, round steak, sirloin, tenderloin, or extra-lean ground beef (90% lean or greater).
  - Lean ham, Canadian bacon, pork tenderloin, and center-cut loin pork chops.
  - Chicken, turkey, or game hens without the skin; extra-lean ground turkey or chicken breast.
  - At the deli counter, select low-fat, low-sodium options such as lean ham, turkey, chicken, or lean roast beef.
  - Eggs are also an excellent protein choice. Choose organic, cage-free eggs whenever possible. They are higher in omega-3 fatty acids. Egg substitutions, although they do not supply any of the healthy fats or many of the beneficial nutrients found in whole eggs, can also be consumed. One-quarter cup of egg substitute is equal to one egg.
  - Beans are a good source of fiber as well as good source of protein. All beans do contain carbohydrates, so it is important for women with PCOS to be mindful of the quantity. One-quarter cup of any of the following is equivalent to 1 ounce of meat: kidney beans, lentils, chickpeas, white beans (cannellini), black beans, or pinto beans.

Lipid Abnormalities in PCOS and Relevance of Dietary Fat

Abnormalities in lipid profile are encountered in almost two-thirds of women diagnosed with PCOS [26]. Hyperandrogenemia, central obesity, insulin resistance, and hyperinsulinemia acting in conjunction are recognized as mechanisms for the observed spectrum of dyslipidemia in women with PCOS. Lipid abnormalities commonly encountered in PCOS patients are similar to those observed in diabetics and include elevated levels of low-density lipoprotein (LDL) cholesterol and triglycerides and lower levels of high-density lipoprotein (HDL) cholesterol [27].

Hyperinsulinemia and hyperandrogenemia have been thought to cause adipocytes to undergo increased catecholamine-induced lipolysis and release of free fatty acids (FFA) into the circulation [27]. Increased FFA in the liver stimulates the secretion of triglyceride-rich very-low-density lipoprotein (VLDL), ultimately contributing to the commonly encountered hypertriglyceridemia in this population [28].

Dietary Fat and PCOS

The type and amount of dietary fat consumption have implications for weight management, insulin resistance, and cardiovascular risk. By consuming the appropriate qualities and quantities of fat in their diet, women with PCOS can actually help enhance the absorption of healthful fat-soluble vitamins, decrease their overall risk of cardiovascular disease, and even gain reproductive benefit (as the majority of the sex hormones are controlled by dietary fat) [29–31]. Fat also takes far longer to metabolize than dietary CHO, ultimately promoting a sense of fullness and satisfaction.

Fats and oils are made up of basic units called fatty acids. Each type of fat or oil is a mixture of different fatty acids, and independent of the type of fat consumed, all types of dietary serve as equitable sources of energy, yielding 9 calories per gram. An optimal intake for fat for women with PCOS should be between 25% and 30% of total calories consumed. For a woman following a 1500-calorie diet, this would equate to around 40–50 g of fat per day, with an emphasis on food sources containing higher concentrations of monounsaturated and polyunsaturated fatty acids.

Monounsaturated fatty acids (MUFAs) are found mainly in vegetable oils (vegetable, olive, rapeseed, and peanut), nuts and nut butters, seeds, olives, and avocados. Studies support eating a diet rich in MUFAs can improve cholesterol levels, optimize insulin levels, and aid in blood glucose control [32].

Polyunsaturated fatty acids (PUFAs) are found mainly in vegetable oils, almonds, cashews, pecans, avocados, fish, and seafood. Omega-3 and omega-6 fatty acids are types of PUFAs and are considered essential fatty acids because humans are dependent on dietary sources, as these cannot be synthesized in vivo.

Omega-3 fats are particularly relevant for women with PCOS given that a balanced intake of these fatty acids confers anti-inflammatory benefit in a condition that is associated with low-grade systemic inflammation, as evidenced by elevation of multiple clinical markers such as C-reactive protein, interleukin-18, as well as endothelial dysfunction [33]. Adequate omega-3 consumption supports healthy cardiovascular, brain, mood, skin, and immune function as well as decreases cholesterol and triglycerides and improves insulin sensitivity [34–36]. The three major types of omega-3 fatty acids are EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), and ALA (alpha-linolenic acid). EPA and DHA are found in fatty cold-water fish (like salmon, mackerel, blue fish, albacore tuna, sardines, lake trout, and herring) as well as high-quality supplements (krill, cod liver, and algal oil). ALA is found in seeds, nuts, beans, green leafy vegetables, flaxseed, and canola and soybean oil. In response to the well-demonstrated positive health qualities of omega-3s

and their low occurrence in frequently consumed foods, some manufactures have started adding omega-3 fatty acids to more commonly consumed foods whose inherent omega-3 values are low. Certain brands of eggs, yogurt, juice, and milks have been fortified with omega-3s in an effort to increase the general population's consumption of these integral fats.

Although *omega-6* fatty acids also play an integral role in health, however, their extreme prevalence in the Western diet – in vegetable oils, animal fats, and bakery goods (donuts, cookies) – is contributory to systemic inflammation. *The optimal ratio of omega-6 to omega-3 in healthy diet is 4:1*, whereas the typical American diet presents with a ratio of 20:1. This imbalance triggers a genetically programmed inflammatory response in the body. Therefore, most women with PCOS could greatly benefit from decreasing their intake of omega-6 fatty acids and increasing their intake of omega-3 fats. Experts recommend a minimum of 200 mg of DHA per day from either fatty fish or fish oil pills [32]. This can also be obtained by consuming at least 12 ounces of fatty fish per week, whereas vegetarians or vegans can take flaxseed oil, a rich source of ALA. Strategies for maximizing dietary intake of omega-3 fatty acids are summarized in Box 3.

Box 3 Tips for Increasing the Omega-3 Content of the Diet

- Add salmon or tuna to a salad with chopped walnuts.
- Select oils that provide omega-3 fatty acids such as canola or walnut oil.
- Sprinkle ground flax, hemp, or chia seeds on your yogurt, cereal, or salad.
- Blend a teaspoon of cod liver oil into your smoothie.
- Spread peanut butter with added omega-3 fatty acids on whole-wheat toast.
- Grill, bake, or broil your favorite seafood with a bit of olive oil (mackerel, salmon, tuna, etc.).

Saturated fatty acids are found mainly in foods from animal sources like high-fat cuts of meat, dairy products made from whole milk (butter, ice cream, cheese, and sour cream), and chicken skin. Some vegetable oils, such as coconut, palm kernel, and palm oil, also contain saturated fat. Saturated fats are reported to raise both total and LDL cholesterol. These foods should be eaten sparingly. National dietary guidelines recommend that saturated fat intake be less than 10% of total calories consumed. For example, if a person consumes 1500 calories per day, she should consume less than 17 g of saturated fat.

Trans-fatty acids are liquid vegetable oils that have been chemically processed to become semisolid at room temperature through the addition of hydrogen atoms. Trans-fatty acids, also called "partially hydrogenated" oils, are used in some margarines, fried foods, cakes, cookies, and processed snack foods to improve the flavor, texture, and shelf life. Surprisingly, many commercial peanut butters also contain trans fats. Trans fats are easily oxidized to form free-radical chain reactions that, in turn, can damage cell membranes and body tissues and compromise immune

function. Research confirms consumption of trans fat may promote inflammation, premature aging, and promotion of various cancers [37–39]. Dietary trans fat content can adversely affect the risk of coronary disease by raising LDL cholesterol levels, lowering HDL cholesterol levels, and raising triglyceride levels [40]. Indeed, in a large, prospective study conducted on more than 800,000 women enrolled in the Nurses' Health Study, researchers found a higher dietary intake of saturated fat and trans fat was associated with an increased risk of coronary disease [40]. By replacing the saturated and trans fat content of the diet with unsaturated fats, there appears to be a clear benefit on blood lipids [41, 42]. Dietary restriction of trans fats should be a priority in women with PCOS who are inherently deemed at an enhanced risk for premature atherosclerosis.

Dietary Calcium

Low-fat dairy-rich foods are excellent sources of protein, calcium, and other important nutrients. Beyond their benefit for skeletal health, adequate dietary calcium is suggested to hold implications for blood pressure lowering and relevant for insulin signaling as well as suggested to have a role in fat metabolism [43, 44]. Table 14.3 presents calcium-rich foods and their relative calcium content. Women with PCOS should aim for 1200–1500 mg of calcium per day, which equates to about three servings per day with dietary sources being preferable to supplementation strategies. Sufficient vitamin D is necessary for the optimal absorption of calcium.

Part-skim mozzarella, farmer's cheese, feta, goat, and low-fat cheeses of all varieties are all excellent low-fat dietary options; these choices are lower in saturated fat and calories than their full-fat counterparts (less than 5 g of fat per ounce).

Vitamin D and PCOS

In contrast to a prevalence of 20–48% among the general adult population, a relative higher prevalence of vitamin D deficiency is observed among women with PCOS (approximately 67–85% of women with PCOS have low levels of vitamin D) [45, 46]. The current research regarding vitamin D levels and PCOS appears inconclusive. Several studies support a higher incidence of lower serum 25(OH)D in women

Table 14.3 Foods rich in calcium

Food	Amount	Calcium content
Milk (1% or nonfat)	8 ounces	300 mg
Yogurt (low-fat)	8 ounces	350–400 mg
Cheese	1 ounce	200 mg
Canned sardines (with bones)	3 ounces	375 mg
Canned salmon (with bones)	3 ounces	170 mg
Leafy greens	1/2 cup	100–150 mg

with PCOS when compared to healthy controls [47, 48], while other studies support no differences in metabolic or endocrine parameters [49–51]. A compromised vitamin D status has been associated with an increased incidence of insulin resistance, metabolic syndrome, type 2 diabetes, and an unfavorable lipid profile [52–54]. In spite of promising studies, inconsistencies in study design, same size populations, as well as differences in the quantity and quality of vitamin D supplementation appear to affect the comparability and ability to reproduce consistent reliable results.

Vitamin D is the only nutrient the body produces when exposed to sunlight. There are two known forms of vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D3 is only found in animal-sourced foods, whereas D2 mainly comes from plant sources and fortified foods. Both forms can be commonly found in supplements. Due to the fact many individuals avoid exposure to the sun and spend the majority of their time indoors, their ability to generate sufficient vitamin D is compromised. In addition, vitamin D is only found in a select variety of foods including fortified milk and cereals and fatty fish.

The daily value for vitamin D is 800 IU (20 mcg) per day. Table 14.4 presents vitamin D-rich foods and their relative content. The highest sources are found in the fatter fish like wild-caught salmon and halibut with a modest amount in fortified foods like milk, eggs, and orange juice. While the correlation with vitamin D and PCOS still appears unclear, it remains important to encourage women with PCOS to consistently consume adequate vitamin. If they are unable to meet their dietary needs, supplementation should be considered.

There has been a widespread perception that vitamin D2 and vitamin D3 are equally efficacious when it comes to raising vitamin D status [55–57]. Both forms of the vitamin are known to be effectively absorbed into the bloodstream. However, the liver metabolizes each form of the vitamin differently. The liver metabolizes vitamin D2 into 25-hydroxyvitamin D2 and vitamin D3 into 25-hydroxyvitamin D3. Collectively these metabolites are known as calcifediol. Calcifediol is

Table 14.4 Food rich in vitamin D

Food	Amount	Vitamin D content
Cod liver oil	1 teaspoon	448 IU
Wild salmon	3.5 oz.	988 IU
Trout (rainbow)	3.5 oz.	752 IU
Farm-raised salmon	3.5 oz.	526 IU
Tuna (canned), light, packed in oil	3.5 oz.	315 IU
Mushrooms exposed to UV light	3.5 oz.	130–430 IU
Halibut	3.5 oz.	384 IU
Mackerel	3.5 oz.	360 IU
Milk, 2%	8 oz.	120 IU
Milk, skim	8 oz.	120 IU
Soy, almond, or oat milk	8 oz.	100–120 IU
Orange juice	8 oz.	100 IU
Tuna (canned), light, packed in water	3.5 oz.	93 IU
Egg	1 each	40 IU

considered the predominant form of vitamin D. Therefore, its blood levels reflect the active stores of the nutrient.

Over the past two decades, a number of trials have been completed comparing the relative efficacy of vitamin D2 with vitamin D3 in increasing serum total 25-hydroxyvitamin D (25(OH)D). In a 2020 meta-analysis performed by Chakalian et al., researchers examined nine randomized controlled trials that used equivalent dosages of vitamin D2 or D3 in healthy adult participants. The study concluded that both vitamin D2 and vitamin D3 effectively raised vitamin D serum levels. While vitamin D3 led to a slightly higher increase, the difference between the two was not statistically significant [58].

In a 2011 systematic review and meta-analysis performed by Tripkovic et al., researchers found that vitamin D2 supplementation yielded less calcifediol than an equal amount of vitamin D3 [59]. D3 proved to be more effective than vitamin D2 at raising blood levels of calcifediol. This challenges the concept that both forms of the vitamin are equally advantageous relative to supplementation [59, 60]. While the widespread data appears inconclusive, the specific source of vitamin D may be a deciding factor in selecting a supplement. Additional research is needed to determine the specific metabolic pathways involved in hydroxylation as well as the effects across age, sex, and ethnicity. Furthermore, dosing schedules and dosing amounts are all factors requiring more investigation. While both oral vitamin D3 and vitamin D2 have the ability to raise serum concentrations of 25(OH)D, additional studies are needed to determine if supplementation derived from D3 does in fact have a significant positive impact over D2 [61, 62].

Dietary Sodium and PCOS

Given a predisposition to premature atherosclerosis and propensity for endothelial dysfunction, attention to dietary sodium may be relevant for women with PCOS. While sodium occurs naturally in most foods such as milk, beets, and celery, it is a common additive to various food products in forms such as monosodium glutamate, sodium nitrite, sodium saccharin, baking soda (sodium bicarbonate), and sodium benzoate. Sodium is also found in condiments such as ketchup, Worcestershire, teriyaki and soy sauce, onion salt, garlic salt, bouillon cubes, and soup stocks and broths. Processed meats, such as bacon, sausage, and deli meats, and canned soups and vegetables are all examples of foods that contain high amounts of added sodium. Due to their processed nature, fast foods are generally also high in sodium. Sodium intake should be consistent with the dietary guidelines for healthy Americans and be no more than 2300 mg (one teaspoon table salt) per day. Adults with high blood pressure should have no more than 1500 mg per day and are encouraged to choose foods labeled as “reduced-sodium,” “no-salt-added,” or “unsalted” products.

Defining Obesity, Overweight, and Normal Body Weight

It is well documented that obesity and overweight are prevalent characteristics among the PCOS population [63]. While the actual definition of obesity appears to vary, the terms *overweight* and *obesity* imply a weight greater than what is considered healthy. An individual's body mass index (BMI) is often used in defining this criteria.

BMI is calculated by using a person's weight in kilograms (kg) and dividing it by their height in meters (m) squared. An adult who has a BMI of 25–29.9 is considered overweight, while an adult who has a BMI greater than or equal to 30 is obese. A person with a BMI of 18.5–24.9 is considered to be normal weight. A person is morbidly obese (extreme obesity) if his or her BMI is over 40.

Excess weight exacerbates all reproductive and metabolic symptoms of PCOS including clinical and biochemical hyperandrogenism, insulin resistance, glucose intolerance, and an atherogenic lipid profile [64]. The International Evidence-Based Guidelines for the Assessment and Management of PCOS suggest lifestyle modification targeting weight management as the first-line treatment for women with PCOS regardless of presenting symptoms [65]. It has been noted that PCOS symptoms may improve with even a modest weight loss of 5–10% of one's body weight [66]. However, in situations where the individual is morbidly obese, a 25–50% weight loss may be required to experience similar improvements [67].

Caloric Needs and PCOS

An individual's caloric needs are based on a variety of factors including activity level, age, sex, body composition, and their own metabolism. Direct and indirect calorimetry is considered the gold standard for calculating resting energy expenditure (REE) [55]. REE is defined as the amount of calories needed to maintain basic body systems and body temperature at rest. However, due to the expense, time needed to achieve an accurate measurement, and the need for trained personnel to perform this measurement, this method is not commonly used. Therefore, prediction equations are the main clinical method for determining REE.

Currently, there are no validated prediction equations for determining the REE in women with PCOS. However, numerous studies have demonstrated relative accuracy of the Mifflin-St Jeor prediction equation when compared against other equations in calculating REE in obese and overweight people [56, 57]. In a 2018 study conducted by Broskey et al., researchers confirmed that the Mifflin-St Jeor equation was accurate within 10% of the gold standard [58]. In addition, the Academy of Nutrition and Dietetics endorses the Mifflin-St Jeor equation for calculating REE in individuals who are overweight or obese population [59]. Given the high incidence of overweight and obesity in the PCOS population, it would therefore be plausible to apply the Mifflin-St Jeor equation when calculating REE in women with PCOS [60].

The Mifflin-St Jeor equation for calculating REE is as follows:

$$(10 \times \text{weight in kg}) + (6.25 \times \text{height in cm}) - (5 \times \text{age in year}) - 161$$

Therefore, if we applied the Mifflin-St Jeor equation to calculate the REE of a 28-year-old female who is 5 feet 3 inches, weighs 180 pounds, and has a BMI of 32, the calculation would be as follows:

$$(10 \times 81.81\,\text{kg}) + (6.25 \times 160\,\text{cm}) - (5 \times 28\,\text{year in age}) - 161 = 1{,}662\,\text{calories / day}$$

Once REE is established, next an individual's activity level must be applied to the REE.

- Sedentary = 1.2 (no activity)
- Lightly active = 1.375 (light exercise/sports 1–3 days/week)
- Moderately active = 1.550 (moderate exercise/sports 3–5 days/week)
- Very active = 1.725 (intense exercise/sports 6–7 days/week)
- Extra active = 1.9 (intense exercise + physical job or 2 × per day training 6–7 days/week)

If the individual noted in the example was lightly active, we would therefore:

$$1{,}662\,\text{calories} \times 1.375 = 2{,}285.25\,\text{calories}$$

For this woman to maintain her current weight of 180 pounds, she would need to consume around 2285 calories. However, based on her BMI of 32, she is obese and would therefore likely benefit from weight loss. Weight loss can be achieved in theory by deducting 500 calories from her maintenance calories. This is based on the assumption that 1 pound of body fat is equal to about 3500 calories. Therefore, by deducting 500 calories per day for 7 days theoretically should result in a loss of 1 pound of body fat per week.

It is important to appreciate that the Mifflin-St Jeor equation only provides an estimate of calories. More studies in women with PCOS are needed to develop and validate a unique equation for women with PCOS that is reliable given the underlying potential influence of the hyperinsulinemia and hyperandrogenemia prevalent in this population. Many overweight, insulin-resistant women have a challenging time losing weight. They may benefit from seeking the guidance of a registered dietitian to establish a more personalized dietary regime that takes into account their unique metabolic needs and profile.

Behavior Modification in PCOS Management

Snacking and Optimal Meal Patterns

Eating small, frequent meals and snacks not only decreases food cravings and prevents binges, but it also provides a steady flux of energy and helps prevent low blood sugar. Given an inherent tendency towards reactive hypoglycemia and CHO

cravings, women with PCOS are an ideal population to benefit from consumption of small and frequent snacks spaced every 2–4 hours. The optimal meal pattern is to consume breakfast within 1 hour of waking and a snack 2–3 hours later, eat lunch 2 hours later, consume another snack 2–3 hours later, and then eat dinner after another 2 hours. If hungry after dinner, a third snack can be consumed, if needed.

Box 4: Items to be considered as snack substitutes:

Box 4 List of Recommended Snack Substitutes

- 6 ounce plain nonfat Greek yogurt (90 calories)
- 1 small piece fresh fruit with 1/4 cup of low-fat cottage cheese (100 calories)
- 1 small apple sprinkled with cinnamon and 1 tablespoon sugar-free syrup, baked (80 calories)
- 2 graham crackers squares with 1 teaspoon peanut butter (90 calories)
- 12 almonds or 20 peanuts (83 calories)
- 1/2 cup whole-grain cereal and ½ cup low-fat milk (100 calories)
- 1/2 cup cooked edamame beans (100 calories)
- 2 tablespoons hummus with 12 grape tomatoes, 2 stalks celery (71 calories)
- 6 medium shrimp with cocktail sauce (60 calories)
- 1 hard-boiled egg (75 calories)
- 1 part-skim mozzarella string-cheese stick (70 calories)
- 1 medium tomato stuffed with 1/3 cup reduced-fat cottage cheese (100 calories)

The guidelines for snacks allow for flexibility. A snack should roughly be around 60–100 calories, 0–20 g of CHO, 0–7 g of fat, and a modest amount of protein (2–8 g), if possible.

Importance of Exercise for PCOS Management

Regular exercise has long been accepted as an effective part of weight management and overall maintenance of physical and mental health. Physical activity can reduce insulin levels, improve insulin sensitivity, and optimize lipid profile as well as may also improve self-esteem, decrease depression, and reduce anxiety in women with PCOS. Therefore, exercise can play an integral role in managing the symptoms of PCOS.

It is well known exercise improves glucose homeostasis associated with insulin resistance [60]. Exercise improves glucose homeostasis by promoting an upregulation of the expression and/or activity of proteins involved in insulin signal transduction in the skeletal muscle [60]. Numerous studies have examined the impact of exercise specific to women with PCOS. In a study performed by Vigorito et al., 90 young women with PCOS were randomly subdivided into two groups, each composed of 45

subjects. The PCOS-T (trained) group underwent a 3-month structured exercise training program, whereas the PCOS-UnT (untrained) group maintained an unaltered lifestyle. This study demonstrated that a 3-month structured exercise training program induced a significant improvement in cardiopulmonary functional capacity and insulin sensitivity and also led to a significant reduction in BMI and CRP levels in a wide overweight PCOS population [61]. In yet another study by Brown et al., researchers demonstrated that moderate-intensity exercise without significant weight loss improved several components of the lipoprotein profiles of women with PCOS. There were significant reductions in the concentration of large VLDL/chylomicrons and medium/small HDL, and increased large HDL and average HDL size in the exercise group compared to controls. Further, there were reductions in calculated triglycerides and VLDL triglycerides in the exercise group compared to controls [62]. These findings support the recommendation to increase physical activity in women with PCOS to obtain improvements in the overall metabolic picture and to reduce the overall risk for cardiovascular morbidity in this population.

However, in spite of the ongoing research supporting evidence-based exercise recommendations aimed at women with PCOS, a finite modality has not been established. Without conclusive population-specific results, the recommendations for exercise and physical activity, therefore, must be applied from the general population data. More data from exercise intervention literature needs to be evaluated to garner a better understanding of the specific health impact of various forms of exercise in women with PCOS. Overall, a stronger body of evidence is required to better assess what exercise modalities and in what combinations are necessary to achieve optimal health outcomes in women with PCOS.

Putting It All Together

Sample Menu Plans for PCOS

1200- to 1400-Calorie Sample Menu

Breakfast

- 1 1/4 cup of strawberries
- 1 container (5.1 oz.) plain nonfat Greek yogurt
- 1/2 cup high-fiber cereal
- 6 walnuts

Lunch

- 4 ounces of grilled chicken
- 2 cups of Romaine lettuce with 1 cup of assorted non-starchy vegetables
- 1 tablespoon of olive oil
- 1 tablespoon of balsamic vinegar

- 1 small orange

Dinner

- 4 ounces of grilled salmon
- 1 cup of green beans with 2 teaspoons butter
- 1 cup of cooked wild rice
- 8 oz. 1% milk

Snacks

- 1 ounce of low-fat cheese (<3 g of fat)
- 1 whole-grain granola bar

Totals 1440 calories, 120 g of carbohydrates (33% total calories), 25 g of fiber (100% of needs), 52 g of fat (33% of total calories), and 122 g of protein (34% of total calories)

Exercise Recommendations

Currently, there are no PCOS specific exercise guidelines. The Physical Activity Guidelines for Americans supports the recommendations for physical activity should be no less than 150 minutes total per week of moderate-intensity exercise. Activities such as brisk walking, water aerobics, ballroom and line dancing, general gardening, tennis (doubles), or sports in which one catches and throws (baseball, softball, volleyball) are all considered to reflect moderately intense physical activity. When exercising moderately, an individual should be able to talk but not sing. If the individual chooses to increase the intensity of exercise (while decreasing the duration), recommendations are for 75 minutes total per week. Vigorous-intensity activities include race walking, aerobic dance, biking faster than 10 miles an hour, hiking uphill, heavy gardening, jumping rope, martial arts (such as karate), jogging, running, swimming fast laps, tennis (singles), and any sport that involves a significant amount of running (basketball, soccer, field hockey). During vigorous activity, an individual should not be able to say more than a few words before breathing deeply. Activities that strengthen the muscles, improve balance, and preserve bone, such as weight training, using resistance bands, weight-bearing aerobics, and heavy gardening, are recommended to be included 2 or more days per week.

In spite of the numerous benefits of exercise for women with PCOS, getting started can be challenging. Therefore, starting with low-impact exercise like walking and swimming is encouraged. While 150 minutes of moderate exercise per week is the goal, movement of kind and duration should be encouraged and supported in this population.

Summary

The majority of women with PCOS are overweight and therefore likely to benefit from weight reduction strategies. Successful weight loss can be achieved through a combination of dietary modifications and restrictions. Minimizing intake of simple CHOs, saturated fats, and omega-6 fatty acids, optimizing dietary fiber and omega-3 fatty acid content, ensuring against spells of starvation, and encouraging intake of frequent and small meals are strategies that will facilitate improvements in metabolic as well as phenotypic burden of PCOS. Regular physical activity of moderate intensity in conjunction with the specified dietary modification is sure to further enhance the overall health benefit.

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Part IV

Management of Polycystic Ovary Syndrome Related Infertility

Managing PCOS-Related Infertility: Ovulation Induction, In Vitro Fertilization, and In Vitro Maturation

15

Saioa Torrealday and Pasquale Patrizio

Key Points

- PCOS accounts for approximately 80% of women with ovulatory infertility.
- Lifestyle modification with caloric restriction and physical exercise should be considered as first-line intervention when managing infertile women with an overweight/obese PCOS phenotype.
- Oral ovulation induction agents are first-line treatment for anovulatory PCOS women with proven tubal patency and no contributing male factor.
- Both aromatase inhibitors (AI) and clomiphene citrate (CC) are suitable ovulation induction agents. However, the use of AI is preferred due to its increased live birth rate and ovulation rates when compared to CC.
- Gonadotropin use is an option for patients who have failed or are resistant to oral ovulation induction medications.
- Treatment-related risks of ovarian hyperstimulation and multiple pregnancies may be exaggerated in the PCOS population; therefore, gonadotropin therapy should be reserved as a second-line strategy for women with PCOS and approached with caution and close monitoring.

The views expressed in this chapter are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or U.S. Government.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_15

- In vitro fertilization (IVF) is a reasonable option for women with PCOS who have additional factors for infertility (tubal occlusion, male factor) or for those who have failed prior treatment with oral agents or gonadotropins.
- The risk for OHSS is high in PCOS patients, but manageable, with consideration of gonadotropin dosing, the specific protocol selected, and the use of "freeze all" or elective single embryo transfer cycles in those of highest risk.
- Metformin co-treatment, dopamine agonists and a GnRH agonist to trigger ovulation are pharmacologic adjuncts that are likely to lessen the risk of OHSS in high-risk patients.
- In theory, in vitro maturation (IVM) is a potentially useful strategy for the management of PCOS-related infertility; in reality however, the reported pregnancy rates with IVM are lower than those reported with conventional IVF. More research is needed before IVM can be offered as a reliable treatment option for women with PCOS.

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting up to 9–18% of all reproductive-age women [1]. Among women with anovulatory infertility, PCOS accounts for nearly 80% of the cases and affects approximately 20% of couples trying to conceive [2]. A diagnosis of PCOS is based on features of oligo-ovulation and/or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic-appearing ovaries on ultrasound [3]. Endocrine abnormalities of the reproductive axis include hypersecretion of pituitary luteinizing hormone (LH) that is attributed to an accelerated gonadotropin-releasing hormone (GnRH) pulsatile activity, ovarian theca-stromal cell hyperactivity, and hypofunction of the follicle-stimulating hormone (FSH)— ovarian granulosa cell network [2]. These abnormalities result in higher ovarian androgen secretion, arrest of follicular growth, and intermittent or lack of ovarian cyclicity. Given these endocrine abnormalities, infertility is a common feature that often prompts medical evaluation. This chapter aims to address the different management options available for treating ovulatory infertility in women affected by PCOS.

Infertility Evaluation

Although ovulatory dysfunction/anovulation is the likely culprit for infertility in women with PCOS, a thorough evaluation must be undertaken to rule out any additional contributing factor(s). Evaluation should include assessment of tubal patency and a semen analysis, in addition to testing the endocrine and metabolic milieus. Disproportionately elevated serum levels of pituitary gonadotropin LH are seen in up to 40% of women with PCOS [4]. The elevated serum LH levels are attributed to an increase in the amplitude and frequency of LH pulses, which is suggested to be

detrimental not only to the success of the ovulation induction cycle, but have also been inconsistently associated with an increased incidence of miscarriage [4]. Serum levels of anti-Müllerian hormone (AMH), a glycoprotein hormone of the transforming growth factor beta superfamily secreted by granulosa cells and an established marker of ovarian reserve, are higher in women with PCOS compared to healthy controls and attributed to the larger cohort of pre-antral and small antral follicles typical of PCOS [5, 6]. Thyroid-stimulating hormone (TSH) and prolactin levels should also be obtained to rule out any underlying thyroid or pituitary disorder contributing to ovulatory infertility.

Endocrine and metabolic evaluations of PCOS have been addressed in distinct chapters within this text; suffice it to state that beyond attainment of a successful pregnancy, overall well-being of the woman and the future conceptus should be the primary goal. Optimization of the metabolic picture as well as normalization of glycemic and dyslipidemic profiles through lifestyle changes and indicated pharmaceutical interventions should be considered prior to initiating targeted fertility medications. Additionally, obese and insulin-resistant women with chronic anovulation are deemed at risk for endometrial pathology such as endometrial hyperplasia or occult endometrial cancer; therefore, an endometrial biopsy should be considered in these women to rule out underlying endometrial pathology before proceeding with infertility treatment. Preconception counseling is additionally encouraged for those considered at an enhanced risk for pregnancy-related complications. A multidisciplinary approach is thus an optimal paradigm for managing PCOS-related infertility.

Lifestyle Modifications and Weight Loss

In women, body mass index (BMI) correlates with increased rate of cycle disturbances and infertility [4, 7–9]. Central obesity manifested as an increased waist-hip ratio (WHR) appears to have a negative association with the probability of conception per cycle (hazard ratio 0.706, 95% CI 0.562–0.887) than overall body mass excess or age [10]. Observational studies have shown that weight loss is associated with improved spontaneous ovulation rates in women with PCOS, and pregnancies have been reported after losing as little as 5% of the initial body weight [11–13].

In addition to influencing the time to conception, which is often delayed in obese women, excess body mass is also recognized to adversely affect the response to fertility treatments [14]. Indeed, higher doses of ovulatory medications are needed to achieve an ovulatory response in the setting of obesity [15, 16]. Given that almost two-thirds of women with PCOS are overweight or obese, optimization of body mass and improving metabolic consequences through lifestyle modifications (caloric restriction, portion size, food choices, and regular physical activity) should be considered as first-line intervention in women with the overweight/obese PCOS phenotype. Although the ideal amount of weight loss for an individual woman prior to attempting infertility treatment remains unclear, strong consideration should be made for obese women to lose 5–10% of body weight prior to starting any medical therapy.

Medical Strategies for Achieving Ovulation

Clomiphene Citrate (Clomid, Serophene)

Clomiphene citrate (CC), a selective estrogen receptor modulator (SERM), has historically been a first-line ovulation induction agent and is a good alternative when aromatase inhibitors fail or are unavailable or due to provider/patient preference [2]. The mechanism for the ovulatory effect of SERMs lies in the blockade of estrogen-mediated negative feedback on the hypothalamic-pituitary axis. Abrogation of the estrogen-mediated suppression results in an increased secretion of FSH from the anterior pituitary, which in turn is responsible for initiating the cascade of events that culminate in follicular growth, development, and selection to dominance [17]. An alternative SERM, tamoxifen, has also been used with similar effects, although it is generally not utilized as a first-line therapy [2].

CC is a racemic mixture composed of two stereoisomers, en- and zu-clomiphene, both of which are thought to contribute to the estrogen receptor antagonism [18]. En-clomiphene is the more potent isomer and the one responsible for its ovulation-inducing action. The half-life of en-clomiphene is relatively short; therefore serum concentrations rise and fall quickly after treatment [19, 20]. Zu-clomiphene has a long plasma and tissue retention, with measurable concentrations in plasma for at least 1 month after a single 50 mg dose of CC [21]. These properties are reflected in the significant accumulation of zu-clomiphene from cycle to cycle across multiple cycles of CC administration [21]. The cost of CC is low, the oral route of administration is patient-friendly, and its safety is well established when properly utilized. The goal of treatment is to mimic a physiological ovulatory cycle with monofollicular development and ovulation from a single follicle while minimizing the risks of ovarian hyperstimulation syndrome (OHSS) and multiple gestations [22].

Given the prolonged half-life of its isomers, a short course of CC is recognized to suffice in achieving folliculogenesis. The standard practice is to administer CC for 5 days starting early in the menstrual cycle after onset of a spontaneous or progestin-induced menstrual bleed; commonly utilized treatment protocols in clinical practice consist of daily CC from cycle day 3–7 or from cycle day 5–9. Ovulation, conception rates, and pregnancy outcomes are similar with either treatment start protocols [23]. The starting dose of CC is 50 mg/day and may be incrementally increased to 150 mg/day (recommended maximum dose by the American College of Obstetricians and Gynecologists (ACOG) and ESHRE/ASRM Committee) until ovulation occurs [2, 14, 24]. The US Food and Drug Administration (FDA) recommends not exceeding 100 mg/day of CC. Some consider higher doses (200–250 mg), particularly in obese patients; however the benefit of these high doses is not proven [2, 25]. Most conceptions occur within the first six ovulatory cycles, and over 75% of patients ovulate at a dose of less than 150 mg/day [26].

Approximately 20–25% of women are CC-resistant (i.e., remain anovulatory despite using maximum CC dosages), requiring an alternative approach to their management [27]. In CC-resistant patients, some advocate extending the treatment period to greater than 5 days or using a step-up approach (or redosing) in which a

second administration of CC at a higher dose is provided without interrupting the stimulation plan [28, 29]. The addition of metformin, a biguanide anti-hyperglycemic agent, has been suggested as an adjunct strategy for women resistant to CC, although the data are equivocal, and current recommendations are that use of metformin be for metabolic indications as there is no direct benefit of using metformin for induction of ovulation or to achieve live birth [30–32].

Although up to 85% of women ovulate with CC, only 30–40% of women will conceive following CC therapy after six cycles [33–35]. Degree of obesity, magnitude of hyperandrogenemia, hirsutism, and the patient's age have all been related to likelihood for treatment success [26]. Persistent local anti-estrogenic effect of CC on the endometrium (secondary to the accumulation of the isomer zu-clomiphene) and the prolonged depletion of estrogen receptors may create an adverse milieu for implantation, thus explaining reduced live birth rates with CC despite successful ovulation; alternatively, anti-estrogenic effects of CC at the level of the endocervical glands may contribute to a hostile cervical milieu which hinders sperm penetration [33–36]. It is estimated that 15–50% of patients using CC may be affected by poor cervical mucus and suboptimal endometrial development as assessed by transvaginal ultrasound [36].

Success of CC cycles has not shown to be impacted by the stringency of cycle monitoring nor by the ovulatory stimulus (exogenous hCG administration versus endogenous LH surge). While ultrasound surveillance of ovarian response is not necessary to predict cycle success, evidence of a mono-follicular response and information on endometrial thickness may be particularly meaningful for individual patients. The addition of an ovulatory trigger dose of human chorionic gonadotropin (hCG) has not been shown to improve CC-related ovulation, pregnancy, or miscarriage rates [37]. However, for individual patients, an ovulatory trigger allows for timely scheduling of adjunct procedures such as intrauterine insemination, and the timeliness and predictability of subsequent events may mitigate treatment-related stress for others.

The risk of multiple pregnancies with CC is between 7% and 10% with the majority being twin gestations; the risk of triplets is 0.3–0.5%, for quadruples 0.3%, and for quintuplets 0.1% [38]. The increased risk of multi-fetal gestation seen with ovulation induction medications is yet another reason to treat at the lowest effective dose. The incidence of OHSS in CC cycles is difficult to determine confidently because definitions in the literature differ among studies. Mild OHSS symptoms (transient abdominal discomfort, mild nausea, slight distention) with use of CC are not uncommon; however, expectant management is typically only required. The risk of severe OHSS resulting in ovarian enlargement, ascites, oliguria, and intractable nausea and emesis is rare (<0.1%) [17]. Other adverse side effects associated with CC include hot flushes, abdominal distention, and, rarely, visual disturbances (blurred vision, scotomata, or light sensitivity) [39, 40].

There has been considerable concern on whether CC use increases the incidence of ovarian and breast cancer. The incidence of ovarian cancer is increased in nulliparous women and decreased in parous women and in those who use oral contraceptive pills, suggesting that *incessant ovulation* may predispose to development of

ovarian cancer [41]. Therefore, apprehension on whether treatment that induces ovulation may cause an increase in the risk of ovarian cancer became of heightened concern. Initial case-control studies in the 1990s raised credence to this notion; however, there were methodological flaws in the studies [42, 43]. More recently, numerous studies have confirmed that while infertility per se is associated with an increased risk of ovarian cancer, there is no evidence that ovulation-inducing drugs, such as CC, enhance that risk any further [44–49]. The risk of developing breast cancer with CC use has been conflicting, with some studies showing no association with CC or other ovulation-inducing medications, whereas others show a possible increased risk in certain subgroups [46, 49, 50]. A meta-analysis of 23 published case-control (n = 8) and cohort (n = 15) studies did not show an increased risk of breast cancer associated with CC or other ovulation induction medications (RR 1.08, 95% CI 0.98–1.19) [51].

The existing data are thus reassuring regarding the risk of ovarian or breast cancer in relation to use of ovulation induction medication; prolonged treatment (>6 cycles), however, should be avoided, principally because it offers little benefit for success. Patients who are CC-resistant should be offered an alternative treatment modality, whereas those unable to achieve a pregnancy despite multiple ovulatory cycles (3–6) should be encouraged to consider more definitive fertility treatment options (discussed further in this chapter).

Aromatase Inhibitors

Over the past decade, aromatase inhibitors (AI) have become the preferred initial treatment for PCOS women. Aromatase is part of the cytochrome P450 enzyme family and is found in highest concentrations within the ovarian granulosa cells of premenopausal women, in addition to other sites such as adipose tissue, brain, and skin fibroblasts [52]. Inhibition of the aromatase enzyme decreases the aromatization of androgens to estrogen; thus, AIs are a therapeutic option for estrogen-dependent conditions such as breast cancer and endometriosis [52]. Following AI exposure in premenopausal women, the profound decline in circulating and tissue levels of estrogens releases the hypothalamic-pituitary axis from estrogenic negative feedback and subsequently increases the pituitary release of FSH [53]. The increased FSH stimulates follicular development and is the basis for the use of AIs for ovulation induction. Similar to CC, the AIs are efficacious following oral administration, and the relatively low cost makes them an attractive choice.

Letrozole, a type 2a third-generation aromatase inhibitor, is the most commonly used AI for ovulation induction. In a randomized controlled trial (RCT) by Legro et al. comparing pregnancy outcomes between CC and letrozole in PCOS infertile patients, the investigators found that patients who utilize letrozole have higher liver birth rates (27.5% vs. 19.1%, P = 0.007) and ovulation rates (61.7% vs. 48.3%, $P < 0.001$) when compared to those women who used CC [54]. A Cochrane systemic review of 14 RCTs similarly confirmed letrozole with timed intercourse was associated with a higher live birth rate (OR 1.68, 95% CI 1.42–1.99) and a higher

pregnancy rate (OR 1.56, 95% CI 1.37–1.78) when compared to the CC cohort [55]. Furthermore, miscarriage per pregnancy (OR 0.94, 95% CI 0.7–1.26) and multiple pregnancy rates (OR 0.69, 95% CI 0.41–1.16) were similar between letrozole and CC [55]. These studies highlight that letrozole should be the first choice in the algorithm for ovulation induction in PCOS women.

Dosing paradigm of AIs is similar to that of CC [56, 57]. The dose of letrozole is incrementally increased from a starting dose of 2.5 mg daily to a maximum of 7.5 mg/day for 5 consecutive days until ovulation is achieved. Letrozole can be initiated from cycle day 3–5 following a spontaneous or a progestin-withdrawal menses. In women with anovulatory amenorrhea, letrozole can be started without the need for a menses, once pregnancy has been excluded. Similar to CC, monitoring follicular growth with ultrasound and/or the use of hCG to trigger ovulation can be utilized to augment the treatment protocol; however the necessity of those adjuvant modalities is unclear. Two meta-analyses of six RCTs compared letrozole with CC in women with PCOS who were therapy-naïve, deemed CC resistant, or CC failures. Per cycle ovulation rates and per patient pregnancy rates were comparable between patients treated with AI compared to CC, even though letrozole was associated with significantly fewer mature follicles and significantly lower estrogen concentrations per cycle [27, 58, 59]. Thus, letrozole has the advantage of greater mono-follicular growth, and given its short half-life (2 days) there are less untoward anti-estrogenic effects on the endometrium than can often be seen with CC [60]. It should be highlighted that despite evidence of effectiveness and safety in well-designed studies, letrozole has not been approved by the U.S. FDA, and thus its use for ovulation induction is an off-label indication.

Letrozole is generally well tolerated with minimal side effects. The greatest risk with the use of AI, similar to CC, is the risk of twins and higher-order multiples. Although as previously mentioned AI are prone to more frequent mono-follicular development, the twin pregnancy rate of 4–6% is still higher than what is seen in spontaneous conceptions [55]. Given this risk, counseling and cycle cancellation should be encouraged if the risk is deemed too high. A RCT of PCOS women who took letrozole found that the most commonly reported side effects include headache (41%), fatigue (22%), and abdominal pain/cramping (19%) [61]. It should be noted that early in AI use as ovulation induction agents, an initial report had suggested concerns for AI-related teratogenicity; an abstract presented in 2005 described an increase in fetal cardiac and bone abnormalities with letrozole use in premenopausal women [62]. However, subsequent larger investigations have failed to show an association between AI exposure and congenital malformation risk, and the initial paper has since been retracted [63, 64].

Gonadotropins

The first gonadotropins for ovarian stimulation were purified from the urine of postmenopausal women in the 1960s and labeled as human menopausal gonadotropin (hMG) [65]. Although human menopausal gonadotropins, which contain equal

amounts of FSH and LH activity, are still widely used today, an increasing trend toward using recombinant human FSH (rFSH) is evident.

Failure to respond to common ovulation induction strategies such as AI or CCs is an indication for a trial of gonadotropin therapy in anovulatory patients, including women with PCOS. Anovulatory PCOS patients who have failed to ovulate with oral agents, failed to become pregnant after 4–6 ovulatory cycles of AI and/or CC, or had other issues (i.e., thin endometrial lining or intolerance of medication) are candidates for gonadotropin therapy [66]. Gonadotropin therapy, whether with administration of rFSH, hMG, or urinary purified FSH (uFSH), is given for a transient period to initiate and maintain follicular growth of few follicles. A recent Cochrane review found little or no difference in live birth, incidence of multiple pregnancy, clinical pregnancy rate, or miscarriage rate between urinary-derived gonadotrophins and recombinant follicle stimulating hormone in women with polycystic ovary syndrome [67]. Recognizing that the ovarian response with the latter strategy is "less controlled' than resulting from an endogenous gonadotropin release as is achieved with CC and AI, it is important to closely monitor the ovulatory response with serial transvaginal ultrasound and laboratory hormonal parameters. Therefore, the use of gonadotropins comes at the expense of an escalation in risks for multifollicular ovarian response, multiple pregnancy, cycle cancellation, and OHSS compared to oral agents.

A cautious approach to ovarian stimulation with gonadotropin use in PCOS patients is reflected in the two commonly employed *step-up* and *step-down* treatment protocols. The *step-up* protocol is based on the principle of small increments in gonadotropin dosing until follicular development ensues. Typically, a low starting dose (25–75 IU) is chosen, which is then adjusted by small increments after 5–7 days, only if there is no evidence of follicular response (as evidenced by rise in circulating estradiol (E2) level and/or growth of ovarian follicle/s on serial transvaginal ultrasound monitoring). Once follicular response is observed, the same gonadotropin dose is maintained until attainment of a single dominant follicle [2]. In contrast to the cautious *step-up* approach, the *step-down* regimen starts with a higher gonadotropin dose (100–150 IU), with rapid decrease in dosing once follicular development is observed (typically with attainment of a leading follicle of about 12–13 mm diameter size). The rationale is to deprive the non-selected smaller follicles in the cohort by decreasing the gonadotropin dose and thus causing arrest of their growth [2]. Both protocols have similar rates of mono-follicular development [2].

During ovulation induction with gonadotropins, serial transvaginal ultrasound and serum E2 level assessments are commonly utilized to assess the magnitude of ovarian response. Patients must be counseled regarding the risk of multiple pregnancies and for the development of OHSS; cycle cancellation should be considered for patients deemed at a disproportionate risk for either.

While absolute guidelines are lacking, the risk for high-order multiple pregnancy must be individualized in relationship to the number of developing follicles and the patient's age. The rate of mono-follicular ovulation using the low-dose

gonadotropin regimens is approximately 70%, with a pregnancy rate of 18–20% per cycle [68]; patients must be counseled regarding risks of multiple pregnancy (6–36%) and severe OHSS (<1–5%) [2]. Although specific normative cut-offs vary, in 2006 the ASRM Practice Committee suggested that caution was indicated when there was a rapidly rising serum E2 level or if the E2 concentration reached in excess of 2500 pg/mL [69]. Once the follicle(s) size approaches 16–18 mm, ovulation is triggered with exogenous hCG followed by intrauterine insemination or timed intercourse. In previous studies, cycle cancellation was considered if three or more follicles 16 mm or larger were observed, whereas other more stringent studies recommend no more than two follicles greater than 14 mm in order to prevent the risk of multiple pregnancies and OHSS [70–73]. An alternative means to save a cycle with too many follicles is to convert the patient to an IVF cycle in order to better control the risk of multiple pregnancies (i.e., transferring a single embryo). As the goal for fertility treatment is to mitigate risk and achieve a singleton pregnancy, PCOS patients as well as their providers may opt to proceed with IVF instead of using gonadotropins due to the beforementioned risks of multiple gestation and cycle cancellation due to overresponse in these women with an exaggerated follicular pool.

In Vitro Fertilization

In vitro fertilization (IVF) is the most efficacious of the existing fertility treatment strategies, albeit it is also the most costly and invasive approach. While IVF is clearly indicated for women with PCOS with concomitant tubal disease or severe male factor, this strategy should be considered for women failing to achieve successful pregnancy following multiple attempts at ovulation induction.

Women with PCOS undergoing IVF treatment have similar pregnancy, miscarriage, and live birth rates compared to those of non-PCOS patients, as evidenced by a large systematic review and meta-analysis of nine observational studies comparing 793 cycles of women with PCOS with 1116 cycles of matched controls [69, 73]. IVF cycles undertaken in the PCOS population are characterized by slightly longer stimulation (1.2 days longer), higher number of developing follicles, higher E2 levels, and retrieval of higher number of cumulus-oocyte complexes (COC) (2.9 more COC) than in women without PCOS [74]. Of note, cycle cancellation rates are higher in patients with PCOS undergoing IVF compared to those with other infertility etiologies (12.8% vs. 4.1%), and these higher cancellation rates can be attributed to either absent or limited ovarian response to exogenous gonadotropins or to an exaggerated response with escalated risk of developing severe OHSS [2].

Treatment strategies employed during IVF aim at achieving “controlled” growth and development of a cohort of ovarian follicles that is available and responsive to exogenous gonadotropin stimulus while concomitantly suppressing the hypothalamic-pituitary axis. In the absence of a suppressive strategy, escalating

serum E2 levels concomitant with development of ovarian follicles can initiate an endogenous LH surge with subsequent ovulation, thus defeating the purpose of the management strategy. There are two primary GnRH *agonist* protocols, the long luteal and the short *GnRH* agonist (GnRHa) protocols. In the long luteal GnRHa protocol, suppression of the hypothalamic-pituitary axis is initiated during the late luteal phase of the preceding menstrual cycle followed by initiation of controlled ovarian hyperstimulation with use of gonadotropins initiated after the onset of menses (Fig. 15.1). In contrast, in the short GnRHa protocol, GnRHa is administrated at the beginning of the menstrual cycle in parallel with the start of exogenous gonadotropins.

Recent years have seen a sharp decline in the use of GnRH *agonist* and an upsurge in the utilization of *GnRH antagonist* protocols, particularly in women with PCOS, and currently this is the preferred protocol for this subset of women. In the GnRH antagonist protocol, the hypothalamic-pituitary suppression is achieved with a GnRH antagonist that is initiated a few days after starting gonadotropin therapy (Fig. 15.2). In the fixed protocol, GnRH antagonist is started either around day 5–6 of gonadotropin stimulation, regardless of the follicular size or E2 level; in the flexible protocol however, GnRH antagonist is introduced when a leading follicle of about 13 mm is visualized on ultrasound and estradiol levels of about 200–400 pg/mL are reached. Four randomized controlled trials have compared the fixed (on day 6) versus a flexible (by a follicle diameter of 13 mm) protocol of GnRH antagonist administration and found no significant difference in live birth outcomes [75]. A meta-analysis of eight randomized control trials comparing the GnRH antagonist protocol to the long GnRHa protocol in PCOS women undergoing IVF with intracytoplasmic sperm injection (ICSI) found no difference in ongoing pregnancy rate (OR = 0.91, 95% CI, 0.67–1.22) or clinical pregnancy rate (OR = 0.87, 95% CI, 0.64–1.19) [75].

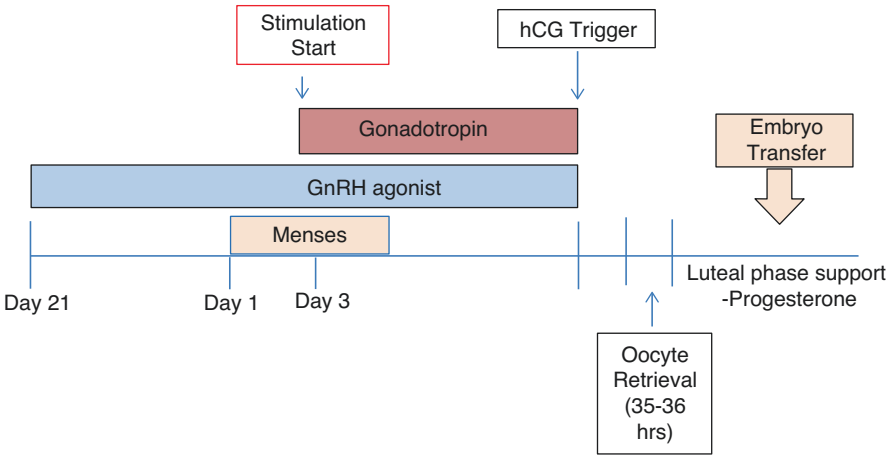

Fig. 15.1 Luteal (long) GnRH agonist protocol wherein ovarian suppression is initiated in the late luteal phase of the preceding menstrual cycle

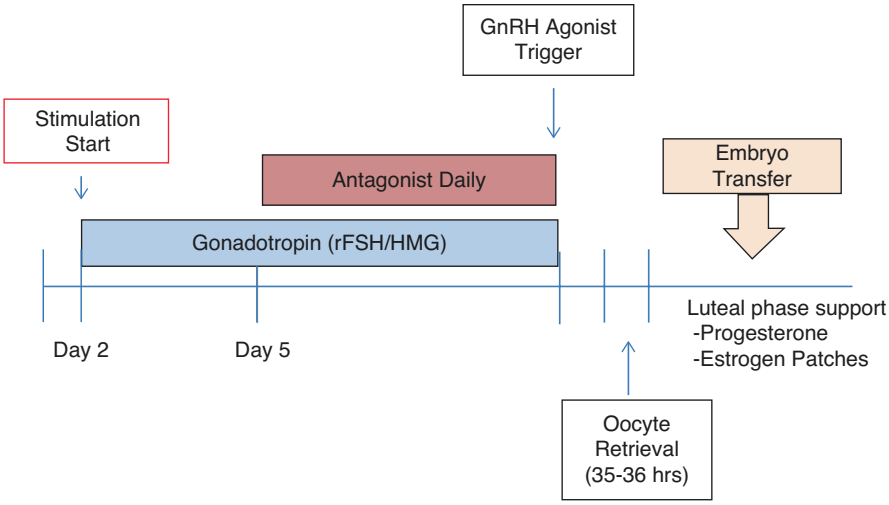

Fig. 15.2 GnRH antagonist protocol with GnRH agonist ovulatory trigger

Ovarian Hyperstimulation and PCOS

The risk of OHSS is particularly elevated in women with PCOS [76]. Given that OHSS is an iatrogenic disease that if left untreated can lead to severe morbidity and can even be life-threatening, prevention is paramount. There are several known risk factors that heighten the OHSS risk among this subgroup to include younger age, low BMI, vitamin D deficiency, high antral follicle count, and elevated anti-Müllerian hormone levels. Noting these elements in a patient allows one to tailor treatment in attempts to mitigate the already elevated risk of OHSS. Several risk factors are modifiable and therefore should be optimized before undergoing IVF to prevent OHSS complications. Vitamin D deficiency, a common condition seen in reproductive age women, has been associated with poor treatment success in ovulation induction and IVF cycles as well as poor reproductive outcomes among PCOS women [77, 78]. Secondary analysis of the pregnancy in polycystic ovarian syndrome (PPCOS) I and II RCTs revealed that vitamin D status was a predictor for both ovulation and live birth [77, 78]. Upon evaluation of women in the PPCOS I RCT, vitamin D deficiency (25(OH)D <30 ng/mL) was found to have a reduction in live birth by 44% (OR 0.58, 95% CI 0.35–0.92) in PCOS women [77]. Similarly, in the PPCOS II RCT, women with PCOS and vitamin D deficiency (25(OH)D <20 ng/mL) were less likely to ovulate (OR 0.82, 95% CI 0.68–0.99) and experienced a 40% lower chance of live birth (OR 0.63, 95% CI 0.41–0.98); intriguingly, unlike PCOS women, these findings were not substantiated in women with vitamin D deficiency and unexplained infertility [78]. Furthermore, vitamin D deficiency has been shown to increase vascular endothelial growth factor (VEGF), which is the major culprit in the pathophysiology of OHSS [79]. Thus, vitamin D-replete women may

have less occurrence or severity of OHSS [79]. Although more research is needed to fully grasp the role of vitamin D in PCOS infertile women, given the potential benefits and low expense of maintaining normal levels, it seems prudent to screen women for vitamin D deficiency and remedy when indicated.

One strategy to lessen the OHSS risk has focused on the use of metformin, an insulin-sensitizing agent, which as previously underscored is used to alleviate the insulin resistance and associated compensatory hyperinsulinemia often associated with the PCOS milieu. There have been a multitude of RCTs which have investigated the impact of metformin use on ovulation, live birth, and clinical pregnancy rates among PCOS women. More recent attention has focused on whether the use of metformin co-treatment prior to and during IVF stimulation has any impact on the development of OHSS. Wu et al. performed a systematic review and meta-analysis of 12 RCTs evaluating the association of metformin with pregnancy outcomes in women with PCOS undergoing IVF or intracytoplasmic sperm injection with subsequent embryo transfer [80]. The meta-analysis included 1123 women with PCOS who were either randomized to receive metformin or placebo/no treatment [80]. The risk of OHSS collectively in women randomized to receive metformin co-treatment before and during the IVF cycle was lower than the control cohort (OR 0.43, 95% CI 0.24–0.79); yet this difference was not significant when a subgroup analysis compared women with a BMI <26 compared to those with a BMI of 26 or greater (OR 0.67, 95% CI 0.30–1.51) [80]. Furthermore, there was no significant difference seen in clinical pregnancy rate (OR 1.24, 95% 0.82–1.86) or live birth rate (OR 1.23; 95% CI 0.74–2.04) among the collective population evaluated, yet in women with a BMI of 26 or greater, the metformin arm was associated with an increased pregnancy rate (OR 1.71; 95% CI 1.12–2.60) [80]. This suggests that perhaps the greatest benefit of metformin utilization as co-treatment is in PCOS women with a higher BMI. Similarly, a 2020 Cochrane review of 13 RCTs involving 1132 women in which one cohort received metformin co-treatment and the other cohort received placebo or no treatment found no clear evidence that metformin improves live birth rates or clinical pregnancy rates [81]. However, the study revealed overall metformin co-treatment may reduce the risks of OHSS from a 20% risk without metformin co-treatment to between 6% and 14% risk in the metformin-treated cohort [81]. More data is needed to conclusively state the impact of metformin co-treatment on pregnancy outcomes and OHSS risks given that many of the studies analyzed had data collection limitations. Additionally, the treatment protocol selected, the duration and dose of metformin utilized, patient demographics, and other factors may play a role on pregnancy outcomes and thus should be explored.

Attention to dosing regimen and to stimulation protocols are the main strategies to reduce the risk of severe OHSS in women with PCOS undergoing IVF. Individualized FSH dosing based on the woman's ovarian reserve testing (AMH, antral follicle count) should be prioritized to minimize the risk of OHSS. Women with elevated AMH levels should start at a lower FSH dose with escalation as indicated; fixed FSH dosing paradigms is discouraged in these high-risk women. Although lower FSH regimens may decrease the mean number of

oocytes retrieved, pregnancy rates have not been shown to be compromised [82, 83]. The GnRH antagonist protocols are associated with a reduced risk for OHSS compared to the long GnRHa regimen in high-risk patients [75]. Ragni et al. showed that antagonist cycles have a shorter stimulation period with fewer numbers of follicles and a lower E2 level on the day of HCG administration [84]. These findings are consistent with several other studies comparing mid-luteal GnRHa and antagonist cycles [85–87]. In addition to decreasing the risk of OHSS, the number of cycles that were cancelled in high responders was significantly less when the antagonist protocol was utilized [84].

The main advantage for using GnRH antagonist protocol is the option to use a GnRHa trigger to induce oocyte maturation, instead of hCG. This strategy has been shown to markedly decrease the rates of OHSS in these high-risk patients (see Fig. 15.2) [88, 89]. Exogenous hCG has routinely been used for final oocyte maturation due to its LH homology. Unlike the natural mid-cycle LH surge, the hCG-mediated LH activity has a long half-life and spans several days (8–10 days) into the luteal phase [90]. The supraphysiological LH activity of hCG stimulates the multiple corpora lutea which leads to high progesterone and E2 levels and upregulation of VEGF, fibroblast growth factor (FGF2) , and numerous cytokines [90]. VEGF is a key substance that induces vascular hyperpermeability, making capillaries "leaky" which results in a fluid shift from the intravascular system to the abdominal and pleural cavities [91]. Supraphysiologic production of VEGF due to the prolonged effect of exogenous hCG is thought to be the main process underlying OHSS [91]. For patients that receive an hCG trigger and are at high risk for OHSS, recommendation to delay transfer and subsequently cryopreserve all embryos is encouraged. In contrast, when GnRHa are used during an antagonist protocol, the GnRHa displaces the GnRH antagonist in the pituitary, activating the GnRH receptor which leads to a flare of gonadotropins release. This surge in gonadotropin release is similar to what is evident in a natural cycle. The main difference between a GnRHa-induced surge and a natural cycle is that the GnRHa surge is of shorter duration lasting 24–36 h compared to the 48 h seen in a natural cycle [92–94]. GnRHa trigger nearly eliminates the risk of OHSS because the luteolysis induced by the short half-life of endogenous LH is significantly shorter than the prolonged half-life of hCG [95]. The advantage of a GnRHa trigger cannot be overstated since it has drastically reduced the incidence of OHSS and its associated complications within this susceptible group.

For all patients who receive a GnRHa for oocyte maturation, there is concern for insufficient luteal phase support given the previously mentioned short half-life of the medication. A RCT found a significant reduction in clinical pregnancy rate (36% vs 6%, $P = 0.002$) and an elevated risk of pregnancy loss (4% vs 79%, $P = 0.005$) in patients receiving a GnRH agonist trigger versus hCG trigger [96]. Therefore, additional steps are necessary to ensure that appropriate luteal hormonal support is attained to adequately support a pregnancy. Although an optimal protocol has not been established, methods to compensate for the hormonal deficit include a microdose of hCG at oocyte retrieval or the addition of estrogen with the progesterone for luteal support.

A secondary prevention intervention for women with PCOS at high risk for OHSS includes the use of a dopamine agonist (i.e., cabergoline) to lessen the risk of moderate to severe disease [97, 98]. DA agonists are believed to inhibit vascular endothelial growth factor receptor 2 (VEGFR-2) phosphorylation and signaling. To investigate the impact of dopamine agonist on OHSS risk, a Cochrane review meta-analysis of 16 RCTs involving 2091 women at high-risk for OHSS was performed to measure the incidence of moderate to severe OHSS and live birth rates among this population [98]. When compared with placebo or no intervention, dopamine agonists were effective in the prevention of moderate or severe OHSS (OR 0.27, 95% CI 0.29–0.39; 1022 participants, 8 studies); however there was no evidence that pregnancy outcomes were influenced in women who underwent a fresh embryo transfer [98]. This meta-analysis suggests that if women taking placebo or no treatment have a 29% risk of moderate or severe OHSS, then women taking a dopamine agonist will only have between a 7% and 14% risk of moderate or severe OHSS [98]. Similar to with the use of metformin previously described, the use of a dopamine agonist may cause some mild side effects (such as gastrointestinal distress). Although there is no consensus on when to start the dopamine agonist, the medication has typically been started at the time of hCG trigger or at oocyte retrieval. Given that vascular permeability and VEGF levels increases around this time in the presence of hCG as previously emphasized, there have been some studies that have suggested starting the dopamine agonist even earlier during the stimulation cycle; however details still need further clarification and investigation in this arena [99].

Lastly, another effective and often employed strategy for reducing the risk of OHSS is to avoid a fresh embryo transfer, “freeze all” embryos, and subsequently do a transfer in a thaw cycle. This strategy of postponing an embryo transfer eliminates the risk of late-onset OHSS; a process which can arise secondary to the pregnancy-related HCG production.

Undertaking of IVF in women with PCOS thus involves particular attention to pretreatment serum AMH levels and the choice of treatment protocol (GnRHa versus antagonist), dosage of gonadotropins, choice of regimen to achieve final oocyte maturation (avoidance of hCG trigger in favor of GnRHa trigger), close monitoring of ovarian response, and preempting the risk for severe OHSS by avoiding fresh embryo transfer in favor of a frozen embryo transfer cycle. Table 15.1 highlights some strategies to consider in order to minimize the risk of OHSS among PCOS women. The success rates of IVF for women with PCOS are reassuring, and limiting the number of embryos transferred can mitigate the risk of multiple pregnancies. Thus, young women with PCOS are ideal candidates for consideration of elective single embryo transfer.

In Vitro Maturation

In vitro maturation (IVM) entails a process somewhat similar to a traditional IVF cycle except that immature oocytes are retrieved and allowed to attain maturity in vitro over a 24- to 48-h period. Access to a laboratory with expertise in gamete

Table 15.1 Strategies to minimize the risk of OHSS among PCOS women

Strategies to minimize the OHSS risk in patients undergoing IVF
Recognize high-risk patients
Younger patients
Low BMI patients
Suboptimal vitamin D levels
High anti-mullerian hormone values
High antral follicle count
First in vitro fertilization cycle
Preload with metformin prior to stimulation start
Proper selection of treatment protocol
Low-dose gonadotropin regimen
Close monitoring with medication adjustments as indicated
Consideration for step-up protocol versus coasting (withholding gonadotropins)
Utilizing gonadotropin-releasing hormone agonist (GnRHa) trigger
Withholding embryo transfer (freeze-all cycle)
Elective single embryo transfer
Cycle cancellation if risk deemed too high
Adjunct treatment: cabergoline (at time of trigger), antagonist (post-retrieval)

biology and to special culture media is requisites for this undertaking. Since the first reported human pregnancy with IVM in 1991 by Cha et al., there have been successful pregnancies reported, and over 5000 babies have been born with this alternative approach [100, 101]. Compared to conventional IVF cycles, the treatment protocol for IVM is more convenient, requiring less medications and minimal monitoring. Given that exposure to exogenous gonadotropins is limited, IVM has fewer side effects and a reduced risk for OHSS when compared with conventional IVF protocols.

An IVM treatment cycle typically begins after either a naturally occurring or induced menstruation (Fig. 15.3). A baseline ultrasound is recommended on cycle day 1–3 to assess ovarian morphology and evidence of early follicular recruitment; baseline hormone parameters (FSH, E2) are also obtained. A second ultrasound is routinely done on cycle day 6–8 in order to monitor early selection of the dominant follicle and to assess the endometrial thickness. Transvaginal oocyte retrieval, of both mature and small immature follicles, is usually performed on days 8–10 depending on the size of the spontaneously achieved leading dominant follicle, which should not exceed 12–14 mm [102, 103]. Once the dominant follicle has reached an appropriate size, hCG is typically administered for oocyte maturation; hCG in IVM cycles has been shown to improve the oocyte retrieval rate during aspiration as well as improve IVM cycle outcomes in women with PCOS [104]. Exogenous endometrial priming is essential in IVM protocols; typically, E2 supplementation is initiated at the time of egg retrieval, and luteal progesterone support is then added 1–2 days later. Both E2 and progesterone supplementation are continued until 8–10 weeks of gestation, a point when luteo-placental shift in production of these steroid hormones is established.

Pretreatment with gonadotropins prior to oocyte retrieval in an IVM cycle remains a matter of debate [105–110]. There are conflicting data on IVM from natural cycles compared with gonadotropin-primed cycles; however, it appears that FSH

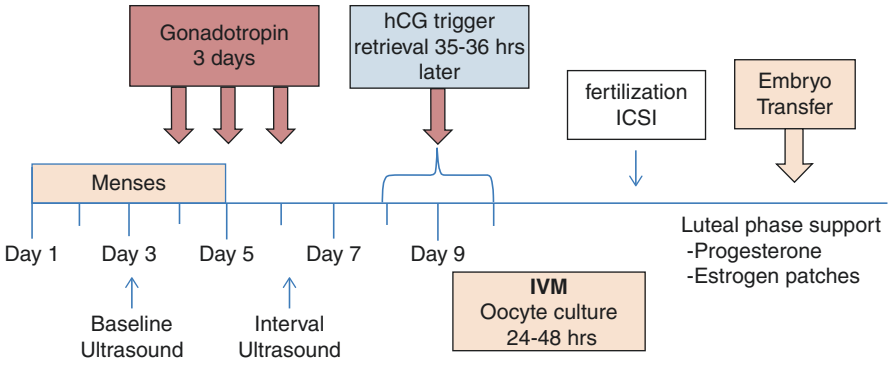

Fig. 15.3 In vitro maturation (IVM)—IVF protocol utilizing low gonadotropin dose priming, shortened follicular phase with harvesting of immature eggs followed by IVM of oocytes

priming may be of some benefit. Gonadotropin-primed cycles consist of a short course (3 days) of low-dose gonadotropin administration (75 IU hMG) initiated on day 3–4 of the cycle (see Fig. 15.3) [105]. Cha and Chian have shown more rapid progression of meiosis I and increased first polar body extrusion after FSH-primed IVM cycles when compared to natural cycles [105]. Furthermore, several other studies have demonstrated improved meiotic maturation after 48 h in culture with gonadotropin priming in IVM cycles compared to non-primed cycles [106–109]. Mikkelsen et al. found that the percentage of oocytes reaching metaphase II was significantly higher in the FSH-primed group compared to the non-primed group; however there was no difference in rate of oocyte fertilization or cleavage between the two groups [110]. In contrast, a study of 60 women randomized to either FSH priming or no priming found that there was no significant difference in the maturation rate (76.5% vs. 71.9%), fertilization rate (75.8% vs. 69.5%), or pregnancy rate (31.4% vs. 36.4%) between the two groups [111].

There is no randomized controlled trial that compares IVM and conventional IVF treatment cycles. The few case-control studies that compared IVM to IVF show that although IVM may be safer and simpler than conventional IVF, the success rates are significantly lower in IVM. Gremeau et al. compared 97 PCOS women undergoing IVM with 97 PCOS women undergoing conventional IVF and found a significantly lower clinical pregnancy rate (50.5% vs. 19.6%) and live birth rate (44.3% vs. 16.5%) in the IVM group [112]. These findings are consistent with other case-control studies, which show pregnancy rates of 22–27% with IVM [113, 114]. Thus, while IVM may be an appealing strategy for younger women with PCOS because it nearly eliminates the risk for developing OHSS, enthusiasm for this option is somewhat dampened by the lesser success rates when compared with conventional IVF. Table 15.2 summarizes some of the pros and cons of the various treatment modalities described within the chapter.

Table 15.2 Comparisons of the various treatment modalities

	Clomiphene citrate	Letrozole	Gonadotropins	In vitro fertilization	In vitro maturation
Route	Oral	Oral	Subcuticular injections	Subcuticular injections	±Subcuticular injections
Dosages	50–250 mg	2.5–7.5 mg	Varies	Varies	Varies
Medication start (days)	Cycle day 3–5	Cycle day 3–5	Cycle day 2–3	Cycle day 2–3	Varies
Typical duration of medication use (days)	5 days	5 days	Varies	Varies	Varies
Monitoring required	No	No	Yes	Yes	Yes
Cost	$	$	$$$	$$$$	$$
Multiple pregnancy risk	Moderate	Moderate	High	Low (single embryo transfer)	Low (single embryo transfer)
OHSS risk	Low	Low	Moderate	High	Low
Common side effects	Hot flashes, abdominal distention	Headache, fatigue, abdominal cramping	Abdominal discomfort, injections site bruising	Abdominal distention and pain, nausea, injection site irritation	None

Summary

Management of ovulatory infertility in PCOS women requires a detailed assessment and risk quantification, and choice of therapy needs to be individualized to the clinical profile of each patient. Patients should be reassured and counseled regarding the spectrum of available options. Serious consideration must be given to achieve optimization of health parameters and risk reduction prior to initiating therapy; importance of lifestyle modification for the overall well-being of the patient cannot be minimized. For patients with ovulatory dysfunction as the primary mechanism for infertility, a stepwise approach is recommended with the use of AI or CC for ovulation induction being the first-line approach. The goal of infertility management is to achieve a singleton pregnancy while minimizing the risks of OHSS and multiple pregnancies. Consideration for the long-term health of the patient and for the safety of the pregnancy should be the primary goal of all health-care providers.

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Considerations and Challenges for Pregnancy in Polycystic Ovary Syndrome

16

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Key Points

- PCOS is associated with an increased risk of gestational diabetes, hypertensive diseases of pregnancy, fetal growth abnormalities, preterm birth, and operative delivery.
- PCOS diagnosis is associated with an increased risk for perinatal mortality.
- Some studies suggest an increased risk of sporadic miscarriage in women with PCOS.
- PCOS does not appear to affect rates of congenital anomalies.
- Given an increased risk of adverse pregnancy outcomes, preconceptional and antenatal counseling and stringent antenatal surveillance of pregnant women with PCOS are warranted.
- Postpartum counseling and care should focus on improving the long-term health of patients with PCOS by encouraging and supporting lifestyle modifications that diminish longterm risks of diabetes, hypertension, and cardiovascular outcomes.
- Breastfeeding is associated with greater postpartum weight loss, offers a physiological approach to metabolic wellness, and should be prioritized for mothers diagnosed with PCOS.
- Well-designed prospective studies using standardized definitions are necessary to elucidate the true effects of PCOS on adverse outcomes in pregnancy.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_16

Scope of the Problem

Although the relationship of polycystic ovarian syndrome (PCOS) with subfertility is well established, postconception effects of PCOS on pregnancy remain debated. Obstetrical complications associated with PCOS may include miscarriage, gestational diabetes mellitus (GDM), hypertensive diseases of pregnancy, preterm birth, birth-weight alterations, perinatal mortality, and increased risk of operative delivery (Table 16.1) [1]. Unfortunately, the heterogeneity of the syndrome and the paucity of large randomized controlled trials studying pregnancy in PCOS limit our understanding of causal relationships between PCOS and adverse pregnancy outcomes. This chapter reviews the pathophysiology of PCOS in pregnancy, updated literature regarding the association with adverse pregnancy outcomes, and the effect of PCOS treatments on pregnancy outcomes.

Effects of PCOS on Pregnancy

Early Pregnancy Loss

The prevalence of early pregnancy loss, defined as miscarriage during the first trimester, has been reported in women with PCOS to be as high as 30–50% [2]. This rate is approximately threefold higher than reported rates of 10–15% for women without PCOS [2]. Conversely, in women who have a history of prior miscarriages, polycystic ovaries (PCO) were identified on ultrasound imaging in up to 40–82% of the subjects, compared to 23% of unselected women [3, 4]. However, the presence of morphologically abnormal ovaries on imaging may not translate to an increased risk of miscarriage in a subsequent pregnancy [4].

Although the association of PCOS with early pregnancy loss has been repeatedly reported in earlier studies, the relevance of PCOS for recurrent pregnancy loss remains contested. The broad variance in reported rates of ovarian findings in these studies can be attributed to the use of non-standardized diagnostic criteria, discrepant modes of imaging (transabdominal vs. transvaginal), and variable subject selection criteria [5].Furthermore, it is important to note that these studies reported only the findings of PCO and not the presence or the absence of other clinical and biochemical alterations that define the syndrome [6].

Table 16.1 Possible obstetrical complications associated with PCOS

Early pregnancy loss
Gestational diabetes mellitus
Hypertensive disorders of pregnancy
Alterations in birth weight
Preterm birth
Increased risk for operative (cesarean) delivery
Perinatal morbidity and mortality

The pathophysiology of early pregnancy loss in PCOS has been attributed to the various metabolic and endocrinologic abnormalities commonly encountered in women with PCOS, including obesity, hyperinsulinemia, hyperandrogenemia, abnormal pituitary gonadotropin profile with elevated luteinizing hormone (LH) concentrations, and endometrial dysfunction [1, 5]. We will evaluate the roles of each of these putative mechanisms in pathophysiology of early pregnancy loss and potential management directed at each metabolic perturbation.

Body mass excess is commonly encountered with more than one-third of PCOS patients having body mass index (BMI) greater than 30 kg/m^2 [7]. In a retrospective study evaluating women with PCOS who conceived on ovulation induction therapy utilizing low-dose gonadotropins, overweight body habitus (BMI between 25 and 27.9 kg/m^2), compared to individuals of normal BMI, was associated with an increased risk in miscarriage at or below 8 weeks gestation (60% vs. 27%, $p < 0.05$) [7]. The negative impact of obesity on maintenance of pregnancy in the PCOS population was also described in a prospective observational cohort study of 270 women with PCOS and infertility from Kuwait with 121 pregnancies [8]. Live birth was noted in 97.2% of women with BMI 18–24 kg/m^2, compared to approximately 60% for BMI >30 kg/m^2. A large cohort study further argues that obesity may be the primary mechanism underlying early pregnancy loss in women with PCOS; the authors observed that the risk of pregnancy loss in PCOS fades after adjusting for obesity [9]. Additional meta-analyses have demonstrated a higher risk of early pregnancy loss in women with POCS after spontaneous conceptions (RR = 2.87; 95%CI:1.65–4.98) and in pregnancies conceived with in vitro fertilization (IVF) (OR = 1.41, 95%CI:1.04–1.91) [10–12].

This association of BMI with adverse pregnancy outcomes in both PCOS and non-PCOS patients results in the empiric recommendation of pre-conception weight reduction and lifestyle modifications. Although a 2011 Cochrane review found insufficient well-designed literature on the effect of lifestyle interventions on reproductive outcomes, newer data have shown potential benefits. A randomized controlled trial in a PCOS population demonstrated improved ovulation and live birth rates with delayed fertility treatment with clomiphene citrate when preceded by lifestyle modification with weight loss compared with immediate treatment. In non-PCOS populations, however, a randomized trial showed that women who successfully lost weight prior to pregnancy were more likely to have greater weight gain later in pregnancy [13]. Further research is needed on the impact of prepregnancy weight optimization in PCOS patients on adverse pregnancy outcome other than miscarriage.

Hyperinsulinemia is common to PCOS and has been proposed as an independent risk factor for miscarriage, irrespective of BMI [14]. Proposed mechanisms of hyperinsulinemia-induced early pregnancy loss include excess transplacental transport of glucose to the fetus [15], and alterations in levels of serum glycodelin [16], insulin growth factor-binding protein-1 (IGFBP-1) [16], and plasminogen activator inhibitor 1 (PAI-1) [17]. Glycodelin and IGFBP-1 are major endometrial secretory proteins that may play important roles in endometrial receptivity during the implantation period and in the maintenance of pregnancy [16]. PAI-1 activity is the major

determinant of hypofibrinolysis, and overexpression may result in higher rates of venous thromboembolism and adverse pregnancy outcomes, including recurrent miscarriage, intrauterine growth restriction, placental abruption, preeclampsia, and intrauterine fetal demise. Treatment with metformin may reverse the thrombophilic state induced by PAI-1 overexpression, thereby preventing adverse pregnancy outcomes resulting from thrombosis-induced placental insufficiency [18]. Despite the putative mechanistic contributions, it is important to note that there is no current role for screening for levels of these proteins in pregnancies with or without PCOS.

Hyperandrogenemia has been described in subjects experiencing recurrent early pregnancy loss, both with and without PCOS [5]. Comparison between studies is difficult due to measurements of different androgens, including total testosterone, free testosterone, and calculated free-androgen index [6]. Abnormal endometrial development [19], reduced expression of endometrial protein PP14 [19], and detrimental effects on oocyte quality [20] are proposed as mechanisms relating androgen excess to early pregnancy loss. Endometrial protein PP14 correlates well with luteal phase endometrial dysfunction [19]. Negative correlation between plasma androgen concentrations and uterine PP14 concentrations in the women with recurrent miscarriages suggests that high androgen concentrations may result in an abnormal secretory endometrium and hence a suboptimal milieu for successful implantation. In a small study of women with PCOS who were treated with ethinylestradiol/cyproterone acetate (EE/CPA) for 3 months prior to conception, lower rates of preterm delivery, GDM, and gestational hypertension were noted than women with PCOS who did not undergo pretreatment, further supporting the association of hyperandrogenism with poor pregnancy outcomes [21]. The rate of miscarriage remains higher in women with PCOS and a hyperandrogenic phenotype when controlling for obesity as demonstrated in a recent meta-analysis [22].

Another common feature of PCOS that has been implicated in early pregnancy loss is the abnormally elevated circulating LH. Elevated LH is a neuroendocrine hallmark of PCOS which results from persistent rapid pulsatile secretion at an exaggerated amplitude [23]. Proposed theories regarding the pathophysiology of early pregnancy loss in PCOS include the premature maturation and aging of oocytes and dysfunctional endometrial development secondary to the abnormally elevated LH. Although early studies suggested a link between LH hypersecretion and miscarriage [24], recent data have been unable to corroborate the earlier findings [5, 25]. Variations in findings can be attributed to the difference in experimental assays and design [25]. Suppression of elevated LH levels before conception in a subset of women with a history of recurrent pregnancy loss and PCO does not appear to improve pregnancy outcomes [26].

Ovulation induction strategies are commonly utilized for the management of ovulatory infertility in women with PCOS and include clomiphene citrate, letrozole, gonadotropin, laparoscopic ovarian drilling (LOD), and use of metformin [5]. Clomiphene citrate, a mixed estrogen agonist-antagonist, until recently has been considered as a first-line strategy to induce ovulation in PCOS with anovulation [27]. However, in patients who experience recurrent miscarriage after successful conception, it is uncertain whether clomiphene offers any protective advantage [5].

Letrozole, an aromatase inhibitor, has become the first-line agent of choice for ovulation induction for women with PCOS given its association with a higher live birth rate (OR 1.64; CI, 1.32–2.04) as well as an increased clinical pregnancy rate (OR, 1.40; CI, 1.18–1.65) [27]. Gonadotropin treatment is commonly utilized to induce ovulation in anovulatory women who are clomiphene resistant. However, there have been no studies evaluating the effects of gonadotropins in the management of recurrent miscarriage. Ovarian drilling, a surgical procedure thought to decrease androgen-producing tissue in the PCOS ovary, is an accepted alternative therapy in clomiphene-resistant patients [28]. Use of LOD has also been shown to decrease testosterone, LH, and the LH/FSH ratio while increasing FSH levels. Ovulation rates and clinical pregnancy in women with PCOS are thereby improved [29]. The ongoing pregnancy rate after LOD, followed by clomiphene citrate or gonadotropin therapy (if anovulation persists), seems equivalent to rates seen with the use of recombinant follicle-stimulating hormone alone, although pregnancies after ovarian drilling carry a lower risk of multiple gestation [28]. Rates of miscarriage at less than 12 weeks gestation were comparable between LOD and gonadotropin therapy arms. Lastly, metformin, an oral anti-hyperglycemic medication, initially held promise in decreasing miscarriage risk and increasing live birth rate in women with PCOS, compared to clomiphene citrate and LOD [30, 31]. However, this latter impression was not confirmed by a meta-analysis that failed to establish an efficacy of metformin in reducing miscarriage rate in women with PCOS [32, 33]. A randomized controlled trial (PregMet2) also failed to demonstrate a benefit to metformin use in preventing late miscarriage [34].

Women who have PCOS and have failed ovulation induction strategies alone may go on to in vitro fertilization (IVF). A multi-center randomized control trial compared frozen embryo transfer to fresh embryo transfer in infertile women with PCOS undergoing IVF cycles. Women with PCOS who underwent frozen embryo transfer had a higher rate of live birth after the first transfer (49.3% vs 42.0%) giving a rate ratio of 1.17 (95% CI: 1.05–1.31; $p = 0.004$). The rate of early pregnancy loss was also lower in women with frozen embryo transfer (22.0% vs 32.7%) with a rate ratio of 0.67 (95% CI: 0.54–0.83; $p < 0.001$) [35]. This finding of decreased rates of early pregnancy loss was further seen in a secondary analysis of a randomized control trial looking at women with PCOS undergoing IVF with frozen vs fresh embryos [36].

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is defined by the American Congress of Obstetrics and Gynecologists as carbohydrate intolerance that begins or is first recognized during pregnancy [37]. Rates of GDM in pregnancies in the general US population are currently reported to be 3–9%, although this number is expected to continue to escalate secondary to the parallel obesity epidemic, sedentary lifestyles, urbanization, and older maternal ages at conception [38]. Complications of GDM in pregnancy include fetal macrosomia, polyhydramnios, birth trauma, instrumental

vaginal delivery, cesarean section, and higher perinatal mortality. Although universal screening for GDM at the beginning of the third trimester is standard protocol for most institutions within the United States, screening for GDM early in pregnancy is recommended in women with PCOS who are overweight or obese, if not performed prior to conception [37]. A recent large randomized clinical trial evaluating the efficacy of GDM treatment showed that dietary counseling, home blood glucose monitoring, and insulin therapy effectively reduce serious perinatal morbidity and may also improve the woman's health-related quality of life [39].

More than 50% of nonpregnant patients with PCOS exhibit resistance to the action of insulin [40]. Up to 40% of all women with PCOS will develop type 2 diabetes mellitus or impaired glucose tolerance during their reproductive years or by the age of 40 [41]. During gestation, the inherent insulin resistance of PCOS is further compounded by physiologic changes of pregnancy designed to accommodate and prepare for the nutritional requirements of the developing fetus, especially in the third trimester. Placental hormones, such as human placental lactogen, cortisol, progesterone, and estrogen, all alter maternal glucose homeostasis by inducing peripheral insulin resistance [42].

Women with PCOS demonstrated a significantly higher chance of developing GDM (OR 2.94, 95% CI 1.70–4.08) in a meta-analysis [43]. A subgroup analysis of five higher validity studies from the same meta-analysis further reinforced the increased risk (OR 3.66, 95% CI 1.20–11.16) [43]. Conversely, in a study comparing women with a previous history of GDM with those without such a history, a higher prevalence of PCO by ultrasound (41% vs. 3%, $p < 0.0001$) and other clinical and endocrinologic stigmata of PCOS (hirsutism, $p < 0.01$; irregular menstrual cycles, $p < 0.01$; higher BMI, $p < 0.001$; and higher concentrations of androstenedione, $p < 0.01$; testosterone, $p < 0.01$; and LH/FSH ratio, $p < 0.01$) were apparent in women with a history of GDM [44].

As with early pregnancy loss, a potential confounder in studies relating GDM to PCOS is obesity [45]. In a population-based retrospective study in the United States, pregnant women with PCOS were more likely to be obese (22.3% vs 3.5%, $p < 0.001$) than women without PCOS and more likely to have pregestational diabetes (4.1% vs 0.9%, $p < 0.001$) [46]. In a meta-analysis of studies evaluating the effect of BMI on development of GDM, the risk of carbohydrate intolerance is significantly positively correlated with prepregnancy BMI [47]. A later meta-analysis of pregnancy complications in women with PCOS in China demonstrated that when controlling for BMI, the rates of GDM were significantly higher in women with PCOS who were overweight/obese prior to pregnancy. Women with PCOS who had insulin resistance prior to pregnancy were also shown to have higher risks of GDM [48]. Likelihood for developing GDM in the overweight, moderately obese, and morbidly obese women was observed to be linear with odds ratio for developing GDM of 1.97 (95% CI 1.77–2.19), 3.01 (95% CI 2.34–3.87), and 5.55 (95% CI 4.27–7.21), respectively. Nonetheless, even after controlling for BMI, the increased risk of developing GDM remained evident in women with PCOS [43]. Obese women with PCOS who develop GDM have also been found to have lower homeostasis models assessment of ß-cell function (HOMA-ß) at preconception than those

without GDM, and measurement of this at preconception has been proposed as a predictor of the development of GDM in women with PCOS [49].

Metformin, an insulin sensitizer and an inhibitor of gluconeogenesis, is a commonly used pharmacotherapy in nonpregnant patients with PCOS as a strategy for improving insulin resistance and hyperandrogenemia of PCOS; addition of metformin to ovulation induction strategy is suggested to improve treatment success with clomiphene citrate, especially in women with PCOS who are also obese [27, 45, 50].

The frequent use of metformin in the patients with diabetes and PCOS, and its favorable safety profile, therefore led to a hypothesis that metformin may decrease the risk of GDM in patients with PCOS [14]. Metformin treatment in early pregnancy, however, has not been shown to reduce incident GDM rates compared to placebo controls (17.6% vs. 16.9%, $p = 0.87$) [51]. This lack of improvement was further confirmed in two more recent studies [34, 52]. It is recommended that women with PCOS should continue metformin until the end of the first trimester if they were taking it prior to pregnancy, but studies regarding this recommendation are limited [37]. Metformin has been used as a management strategy for GDM and has demonstrated a reassuring safety profile with perinatal complication rates comparable to those treated with the gold standard, insulin [37].

Additionally, women with GDM managed on metformin demonstrated less weight gain from enrollment to term compared to term (0.4 ± 2.9 vs. 2.0 ± 3.3, $p < 0.001$) [53]. In a randomized trial, women treated with metformin had lower mean glucose levels and neonates with lower rates of hypoglycemia than those randomized to insulin [54]. A recent meta-analysis also demonstrated that women treated with metformin had a higher rate of preterm birth (risk ratio 1.5), but had a lower rate of gestational hypertension (risk ratio 0.53) when compared to women treated with insulin [55]. Metformin does cross the placenta and as such is not recommended as a first-line treatment. When considering its use in pregnancy, it is recommended that women be counseled about the increased rate of preterm birth, placental transfer, and the lack of long-term safety data [37].

Hypertensive Disorders of Pregnancy

Hypertensive disorders during pregnancy are the second leading cause of maternal mortality in the United States (after thromboembolism). Accounting for almost 15% of maternal deaths [56], these disorders are estimated to affect 2–8% of all pregnancies [57]. Hypertensive disorders in pregnancy are categorized into chronic hypertension, preeclampsia/eclampsia, preeclampsia superimposed on chronic hypertension, and gestational hypertension [57]. Some known risk factors for hypertensive disorders of pregnancy include diabetes, autoimmune disease, renal disease, and obesity [58].

Women with PCOS demonstrated a significantly higher chance of developing a hypertensive disorder of pregnancy in a meta-analysis (OR 3.67, 95% CI 1.98–6.81) [43]. A subgroup analysis of two higher validity studies also revealed a significant increased risk of any hypertensive disorder of pregnancy (OR 3.71, 95% CI

1.72–17.49) and preeclampsia (OR 3.47, 95% CI 1.95–6.17). However, many earlier studies in which preeclampsia was an endpoint reported a lower parity, higher BMI, or more multiple pregnancies among women with PCOS versus controls [58]. A recent retrospective cohort study comparing a diverse group of pregnant women with PCOS to women without PCOS demonstrated that women with PCOS were at higher risk of gestational hypertension, but this was not independent of weight status. The same study demonstrated that nulliparity and higher prepregnancy BMI was associated with increased risk of gestational hypertension, with or without PCOS [59]. A more recent cross-sectional analysis showed an increased risk of hypertensive disorders in women with PCOS who were underweight or obese, but this was not seen in women with PCOS who were of normal weight or overweight [60]. A meta-analysis performed in 2016 demonstrated that women with PCOS have higher rates of hypertensive disorders of pregnancy (RR = 2.46; 95% CI:1.95–3.09, $p < 0.001$) as well as preeclampsia (RR = 2.79, 95% CI 2.29–3.38, $p < 0.001$) even after controlling for age and BMI [10]. Additional risk factors contributing to hypertensive disorders include nulliparity, obesity, gestational diabetes, multiple gestations, hyperandrogenism, and IVF [61].

Preterm Birth

There are approximately 12% of births in the United States that are preterm, defined as occurring at less than 37 weeks gestation [62]. A recent meta-analysis demonstrated a moderate association between PCOS and preterm birth (RR = 1.52; 95% CI:1.22–1.9, $p < 0.001$), but the studies were heterogenous and a subgroup analysis controlling for BMI did not show an association of PCOS and preterm birth when the prepregnancy BMI is >25 kg/m^2 [10]. Prior meta-analysis also showed a twofold increased risk of preterm birth, but the studies did not differentiate between spontaneous preterm birth and iatrogenic preterm birth related to medically indicated deliveries [1, 43]. In 2014, a cohort study showed an increased risk of preterm birth in women with PCOS, but was confined to those with hyperandrogenism [61].

Alterations in Birth Weight

The increased risks of gestational diabetes and preeclampsia for women with PCOS have led to hypotheses that the offspring in this population may be at an enhanced risk for a spectrum of growth aberrations that range from small-for-gestational age to large-for-gestational age [1]. Existing literature however is equivocal, and a lack of consistency in observed relationships can in part be attributed to differing definitions of macrosomia and SGA utilized across published studies. In a meta-analysis, pooled results from 12 studies showed statistically, albeit not clinically, significantly lower neonatal birth weight among infants of women with PCOS (mean weight difference, −38.4 g; 95% CI −62.2 to −14.6) [43]. Subgroup analysis of four studies in which controls were matched for confounders showed no significant difference in neonatal birth weight. PCOS babies also showed no significant increase in the

incidence of macrosomia or growth restriction. In a more recent meta-analysis, there was again no association between PCOS and large for gestational age (LGA), fetal growth restriction, fetal macrosomia, or congenital malformations [10]. However, in a subgroup analysis with prepregnancy BMI, there was an association between small for gestational age (SGA) and PCOS when the prepregnancy BMI was >25 kg/m^2.

Perinatal Morbidity and Mortality

In a meta-analysis of 5 studies with 162 pregnancies with PCOS and 725 control pregnancies, a statistically and clinically significant increase in perinatal mortality was observed in the offspring of women with PCOS compared to controls (OR 3.07; 95% CI 1.03–9.21) [43]. Reported causes for perinatal mortality in offspring of pregnancies affected by PCOS include lethal malformations, cervical insufficiency, sepsis, and placental abruption. However, as with many other pregnancy complications, most studies showed a higher BMI in women with PCOS compared to controls, which is a recognized independent risk factor for perinatal mortality [63].

A large Swedish study utilizing the national birth registry, published after the previous meta-analysis, reported that women with polycystic ovary syndrome are at increased risk of adverse pregnancy and birth outcomes that cannot be explained by the increased use of assisted reproductive technologies in this population. Infants born to mothers with PCOS were more prone to be large for gestational age (aOR 1.39; 95% CI 1.19–1.62), meconium aspiration (aOR 2.02; 95% CI 1.13–3.61), extreme prematurity (aOR 2.21; 95% CI 1.69–2.90), and Apgar scores <7 at 5 min (aOR 1.41; 95% CI 1.09–1.83) [64].

The exposure of the offspring of women with PCOS to insulin resistance and hyperandrogenism may be associated with increased risk of developmental disorders. In a recent population-based prospective study, the diagnosis of PCOS was associated with an increased risk of the offspring failing the fine motor domain area of the Ages and Stages Questionnaire (ASQ) assessed at 4,8, 12, 18, 24, 30, and 36 months of age [65]. Women who did not receive treatment for their PCOS had a stronger association with failing ASQ than among the offspring of women who reported receiving treatment for their PCOS.

Mode of Delivery

In some meta-analyses, women with PCOS were noted to have a higher incidence of delivery by cesarean section compared to controls (OR 1.56; 95% CI 1.20–2.02) [43], but this was contradicted by more recent meta-analyses showing no association [58]. The total number of deliveries, proportion of cesarean delivery, and indications for cesarean delivery were not reported in the meta-analysis. However, when a subgroup analysis was performed on three higher validity studies, no significant increased risk of cesarean delivery was observed (OR 0.92; 95% CI 0.54–1.58). This increased risk in surgical delivery in some studies was attributable to the

differences in body habitus between PCOS and control groups. There were no differences between rates of spontaneous vaginal and forceps or vacuum-assisted vaginal deliveries (OR 1.37; 95% CI 0.80–2.35).

Breastfeeding

Breastfeeding is a major focus of global strategies to improve infant nutrition and is considered to be the most effective single factor influencing the worldwide infant death rate [66]. The health benefits of breastfeeding are well established in the neonate, but there are maternal benefits as well [67]. Breastfeeding is associated with greater postpartum weight loss which can contribute to an increase in metabolic rate which can extend to 24 months after delivery [68]. This is especially important in women with PCOS who are also obese or overweight. Additionally, breastfeeding has shown to decrease the risk of type 2 diabetes and cardiovascular disease [69].

In a prospective observational cohort study in Northern California, women who were exclusively breastfeeding and mostly breastfeeding had lower adjusted mean group differences in fasting plasma glucose (mg/dL) of −4.3 (−7.4 to −1.3) and −5.0 (−8.5 to −1.4), fasting insulin (μU/mL) of −6.3 (−10.1 to −2.4) and −7.5 (−11.9 to −3.0), and 2-h insulin of −21.4 (−41.0 to −1.7) and −36.5 (−59.3 to −13.7) (all $p < 0.05$), respectively [69]. Given that women with PCOS have a higher risk of having insulin resistance and of developing diabetes mellitus, breastfeeding is of special importance for optimizing their postpartum long-term health benefits [40, 41]. Unfortunately, the insulin resistance and hyperandrogenism that are often seen in women with PCOS have been proposed to impair successful breastfeeding [67]. Insulin enhances the effect of prolactin on breast tissue during the process of milk synthesis, and maternal androgen levels have been negatively correlated with breastfeeding success in some studies [70, 71].

Summary

PCOS is associated with an increased risk of early pregnancy loss, gestational diabetes, preeclampsia, and preterm birth. Other reported adverse outcomes, such as alterations in birth weight, increased risk of perinatal mortality, and operative delivery, remain debated due to conflicting data between studies. Additional well-designed prospective studies using standardized definitions are necessary to elucidate the effects of PCOS on adverse outcomes in pregnancy and should control for other confounders, such as BMI. Nonetheless, the reported increased risk of adverse pregnancy outcomes warrants preconceptional and antenatal counseling and stringent antenatal surveillance of pregnant women with PCOS. Postpartum counseling and care should focus on improving the long-term health of patients with PCOS by encouraging and supporting lifestyle modifications that diminish the risks of diabetes, hypertension, and cardiovascular outcomes. Practical considerations for management of the patient throughout the reproductive continuum are summarized in Table 16.2.

Table 16.2 Practical pregnancy considerations for the patient with PCOS

Period	Potential risks	Considerations
Trimester zero: preconception [5, 7, 13, 27, 34]	Oligoovulation Hyperandrogenism Infertility Elevated BMI Pregestational diabetes or pre-diabetes	Recommend lifestyle and healthy dietary changes that facilitate weight loss Refer to infertility experts regarding role of ovulation induction or IVF if indicated Refer to endocrinology for optimization of disorder, including discussion of metformin, if indicated Consider combined oral contraceptive if hyperandrogenic, not yet ready for pregnancy, and without contraindications Assess hemoglobin A1c prior to pregnancy, if possible
First trimester [2–36]	Early pregnancy loss Insulin resistance spectrum	Early ultrasound for viability and dating after 5–6 weeks of gestation Assess/reassess hemoglobin A1c or glucose challenge test (unless recent assessment performed); Initiate dietary counseling, home blood glucose monitoring, and insulin therapy, if indicated Start aspirin for preeclampsia prophylaxis at 12–16 weeks GA if the patient has any additional risk factors for hypertensive disorders in pregnancy Apply shared decision-making regarding risks/benefits of metformin in pregnancy
Second and third trimesters [37–65]	Gestational diabetes Hypertension Fetal growth abnormalities Preterm birth Perinatal mortality	Ultrasound surveillance: Anatomical survey at 20 weeks Consider a growth ultrasound in third trimester Antenatal testing as indicated for other comorbidities Monitor maternal blood pressure closely after 20 weeks' gestation Perform glucose challenge testing at 24–28 weeks if no prior evidence of diabetes Management of comorbidities with obstetrician/maternal-fetal medicine, as indicated Apply shared decision-making regarding risks/benefits of metformin in pregnancy
Delivery [58]	Cesarean delivery	Counsel patients regarding potential for increased risk of cesarean delivery to establish appropriate expectations
Postpartum [27, 40, 41, 66–71]	Difficulties with lactation	Shared decision-making via discussion of risks and benefits of breastfeeding Early integration of supportive strategies, including lactation consultant services, if breastfeeding desired Discuss appropriate contraception based on the patient's needs
Interpregnancy interval [27, 72]	Progression of comorbid conditions Unintended pregnancies	Consider bariatric surgery if morbid obesity present in PCOS patient Continuation of care with appropriate healthcare professional for monitoring for insulin resistance, dyslipidemia, weight, cardiovascular disorders, and endometrial cancer

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Part V

The Role of Surgery in the Management of Polycystic Ovary Syndrome

Role of Surgery in the Management of PCOS: Rationale and Considerations for Bariatric Surgery

17

Héctor F. Escobar-Morreale

Key Points

- Obesity is the major factor responsible for metabolic disorders in patients with PCOS.
- Lifestyle modification is effective for prevention of metabolic disorders in obese patients with PCOS, but it is rarely effective in the long term due to difficulty in sustaining the newly adopted lifestyle by the majority.
- Bariatric surgery in severely obese women usually resolves the clinical picture of PCOS.
- Fertility may be restored after weight loss in women with PCOS submitted to bariatric surgery.
- Avoidance of the pregnancy-related and lifetime risks associated with severe obesity compensates for the innate procedure-related risks in appropriately selected patients.

Introduction

Polycystic ovary syndrome (PCOS), the most common endocrine disorder in premenopausal women [1, 2], is characterized by the association of androgen excess with chronic oligoovulation and/or polycystic ovarian morphology, provided that other disorders such as hyperprolactinemia, nonclassic congenital hyperplasia, and androgen-secreting tumors have been excluded [3, 4].

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_17

Metabolic disorders and classic and nonclassic cardiovascular risk factors cluster in women with PCOS from very early in life [5]. A disproportionately higher risk of early onset type 2 diabetes and evidence of premature atherosclerosis are well recognized in this population, underscoring the need for timely institution of metabolic prevention strategies in women with PCOS. This chapter focuses on the major role that obesity plays in the association of PCOS with metabolic disorders and the scarce, but promising, evidence suggesting that bariatric surgery may be a very useful tool for the management of PCOS and associated metabolic conditions.

Role of Obesity in the Pathophysiology of PCOS and in the Development of Metabolic Disturbances

McAllister and colleagues [6] demonstrated two decades ago that exaggerated androgen secretion by ovarian theca cells is a primary feature of PCOS. After several passes in primary culture, theca cells from women with PCOS secreted increased amounts of androgens, and their steroid precursors, compared with cells obtained from women without androgen excess disorders, and PCOS theca cells showed increased expression of all the enzymes involved in androgen synthesis [6]. The fact that these cells maintained an increased capacity for androgen synthesis when isolated from the endocrine and metabolic milieu characteristic of women with PCOS strongly suggests that androgen excess is a primary ovarian defect in this syndrome [6].

However, insulin resistance and compensatory hyperinsulinism also contribute to the pathogenesis of PCOS, because insulin acts as a co-gonadotropin at the ovary, facilitating androgen secretion [7].

We hypothesized that women with PCOS suffer from a vicious circle, whereby androgen excess favoring the abdominal deposition of fat further facilitates androgen secretion by the ovaries and adrenals in PCOS patients (Fig. 17.1) [8, 9]. The possibility that androgens influence body fat distribution and visceral adipose tissue dysfunction is supported by recent studies. Women with PCOS present with increased thickness of intraperitoneal and mesenteric fat depots, and, when considered together with non-hyperandrogenic women and with men, the thickness of visceral adipose tissue depots correlated positively with serum androgen concentrations and negatively with serum estradiol levels [10]. Moreover, both genomic and proteomic nontargeted studies of visceral adipose tissue indicated substantial differences in the gene expression profiles and in the proteomes of severely obese patients with PCOS compared with control women [11, 12]. Of note, the promoter regions of several of the genes dysregulated in visceral adipose tissue of women with PCOS contain putative androgen response elements, suggesting that androgen excess might influence adipose tissue dysfunction in these patients [13]. The impact of androgen excess in women with PCOS may also extend to muscle proteomes [14] and circulating microRNA profiles [15].

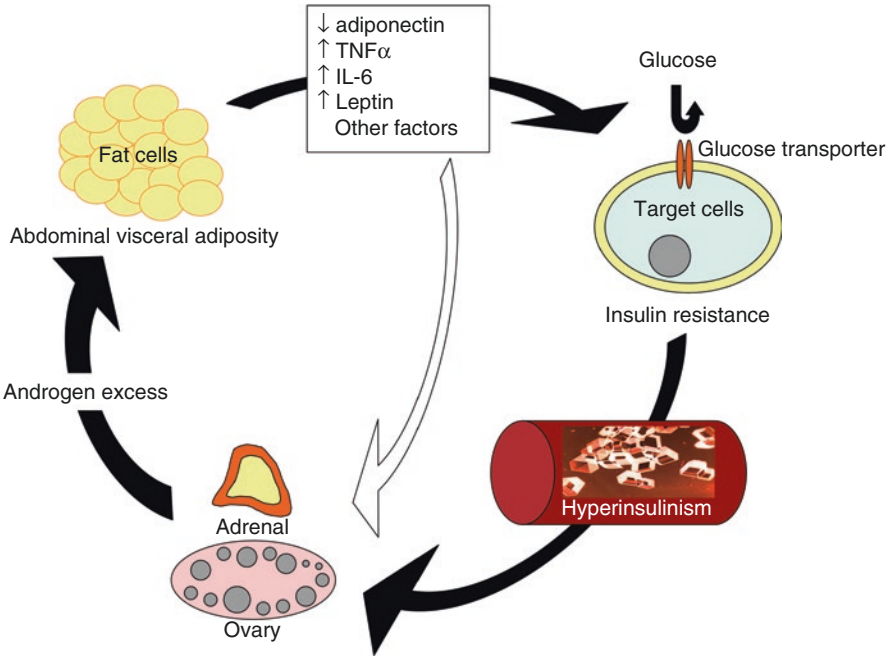


Fig. 17.1 Unifying hypothesis explaining the interplay between the polycystic ovary syndrome and abdominal adiposity as the result of a vicious circle represented by the black arrows: androgen excess favors the abdominal deposition of body fat, and visceral fat facilitates androgen excess of ovarian and/or adrenal origin by the direct effects (white arrow) of several autocrine, paracrine, and endocrine mediators or indirectly by the induction of insulin resistance and hyperinsulinism. (Reprinted with permission from Escobar-Morreale and San Millan et al. [8], Copyright 2007, Elsevier)

The aforementioned vicious circle might start very early in the life of affected women, even during fetal development [16]. Animal models of prenatal androgenization demonstrated that, in mammals, in utero exposure to excessive androgen concentrations favors the development of abdominal adiposity and endogenous androgen excess after birth [17, 18]. The familial aggregation of PCOS suggests an inherited basis for this disorder [19]. Therefore, female fetuses from PCOS mothers may be exposed to excessive androgen levels during pregnancy if they inherit the abnormal mechanisms leading to androgen excess, and this exposure may condition the development of PCOS and abdominal adiposity after birth, as suggested by animal studies.

Girls born from mothers with PCOS may present with increased umbilical vein androgen levels at birth [20], supporting the hypothesis that androgen excess begins during fetal life in humans; yet other studies failed to reveal increased androgen levels in such girls [21–23]. The samples used in these studies (venous or mixed cord blood obtained at delivery) may account for these discrepancies as such

samples may contain a certain amount of androgens of maternal origin from the mother. Further studies using umbilical artery blood, which comes from the fetus, or actual blood samples from the newborns are definitely needed to resolve this controversy. However, anogenital distance is similar in girls born to mothers with PCOS or to normoandrogenemic women, casting doubt upon the occurrence of a relevant prenatal exposure to androgen excess in the former [24].

Of note, the development of exogenous obesity aggravates the vicious circle of androgen excess, abdominal adiposity and adipose tissue dysfunction, insulin resistance, and further androgen excess in women with PCOS, increasing markedly the metabolic and cardiovascular risks of affected women [9].

However, our metabolomic data indicate that insulin resistance is not universal in PCOS [25]. Even if patients with PCOS as a group were hyperinsulinemic and insulin-resistant compared with the controls, nonobese patients with PCOS showed a metabolic profile consisting of suppression of lipolysis and increased glucose utilization in peripheral tissues. PCOS patients as a whole showed decreased 2-ketoisocaproic and alanine concentrations, suggesting utilization of branched-chain amino acids for protein synthesis and not for gluconeogenesis [25]. These metabolic processes required effective insulin signaling; hence, insulin resistance was not present in all tissues of these women, and different mechanisms, such as a decrease in insulin clearance, possibly contributed to their hyperinsulinemia. On the contrary, in obese women with PCOS, the increase in plasma long-chain fatty acids, such as linoleic and oleic acid, and glycerol suggests increased lipolysis, possibly secondary to impaired insulin action at adipose tissue [25]. Therefore, obesity appears to be the major determinant of metabolic heterogeneity in PCOS.

The metabolic heterogeneity of PCOS may be explained by the existence of a continuum in the relative contribution of androgen excess, on the one hand, and of abdominal adiposity and insulin resistance, on the other [8, 9]. In one extreme of this spectrum, women presenting with severe androgen excess may develop PCOS without the participation of any other pathophysiological mechanism (Fig. 17.2). In the other extreme of the spectrum, women with mild androgen excess only develop PCOS when another pathophysiological mechanism, such as abdominal adiposity, obesity, insulin resistance, and/or hyperinsulinemia, is also present (see Fig. 17.2). Obviously, the most severe phenotypes are observed in patients presenting with both severe androgen excess and severe obesity. But in order to develop PCOS, a primary defect in androgen secretion, from mild to severe, is needed. In the absence of such a defect, PCOS cannot develop, explaining how approximately 40% of morbidly obese women do not suffer the syndrome, even in the presence of massive abdominal adiposity and severe insulin resistance [26].

Although abdominal adiposity, metabolic dysfunction, and markers of subclinical atherosclerosis may also be present in nonobese women with PCOS [27–29], obesity is clearly related to the development of metabolic disorders in women with PCOS [30], explaining why not every women with PCOS is at increased risk of metabolic and cardiovascular disease [5]. Accordingly, the

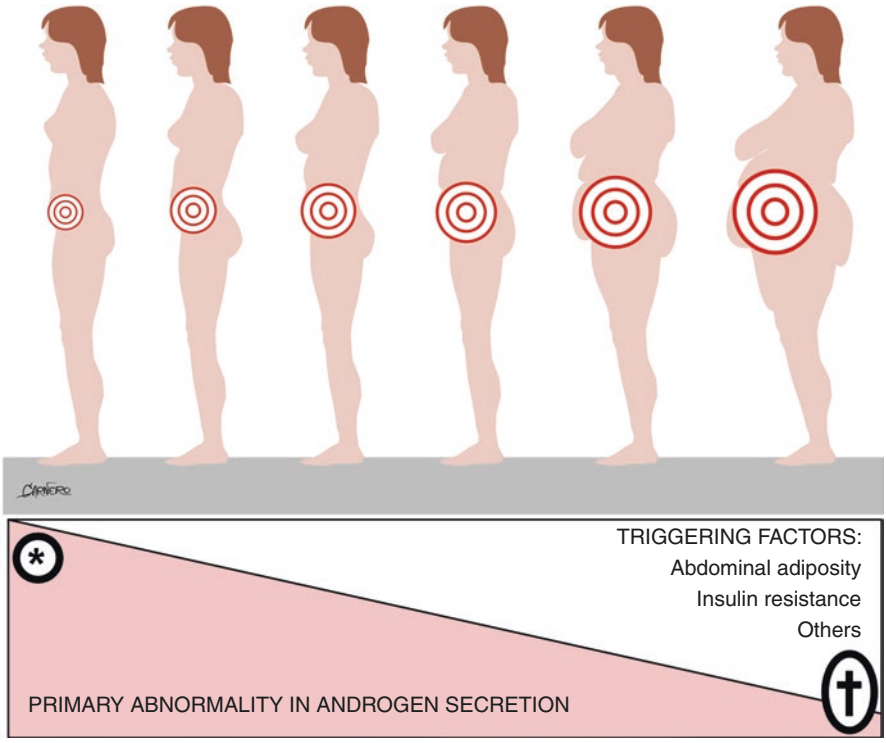


Fig. 17.2 The polycystic ovary syndrome as the result of the interaction of a primary abnormality in androgen synthesis, manifesting as androgen excess, with environmental factors such as abdominal adiposity, obesity, and insulin resistance. At one extreme (*), in some patients, the disorder is severe enough to result in PCOS, even in the absence of triggering environmental factors. At the other extreme (†), a very mild defect in androgen secretion is amplified by the coexistence of abdominal adiposity, obesity, and/or insulin resistance. Between the two extremes, there is a spectrum in the severity of the primary defect in androgen secretion, explaining the heterogeneity of PCOS patients with regard to the presence of obesity and metabolic comorbidities. Yet all patients share a primary defect in androgen secretion. (Reprinted with permission from Escobar-Morreale and San Millan et al. [8], Copyright 2007, Elsevier)

frequency of dyslipidemia and disorders of glucose tolerance in women with PCOS increases markedly with obesity [31–33]. In Spaniards, abdominal adiposity is an intrinsic characteristic of PCOS; yet obesity increases markedly the amount of fat in the visceral adipose tissue depots of these women [10]. In fact, it is obesity that is actually responsible for the association of PCOS with the metabolic syndrome [34], hypertension [35], hyperuricemia [36], and decreased health-related quality of life [37].

Therefore, obesity is a major player in the association of PCOS with metabolic dysfunction, and its prevention and management must be a priority when designing strategies for the long-term management of PCOS [5, 38].

Role of Surgery in the Management of Metabolic Dysfunction of PCOS

The cornerstone for management of metabolic dysfunction in PCOS is lifestyle modification, especially diet-induced weight loss [5, 38]. Indeed, evidence supports that lifestyle intervention improves body composition, hyperandrogenism, and insulin resistance in women with PCOS [39]. However, the magnitude of the positive effects of lifestyle intervention on such outcomes according to a recent meta-analysis is too small to actually represent any clinical advantage. Although statistically significant, average improvements after intervention of 1.7 kg of body weight, of 0.3 kg/m^2 in BMI, of 1 cm in waist circumference, of 1 point in the modified Ferriman-Gallwey score, or 0.1 nmol/l in serum testosterone [39] are irrelevant for most obese women with PCOS. Moreover, there is not enough evidence for the effect of lifestyle intervention on improving glucose tolerance or lipid profiles and no literature assessing, in women with PCOS, clinical reproductive outcomes, quality of life, and treatment satisfaction in the relation to lifestyle modification strategies [40].

Furthermore, long-term efficacy of lifestyle intervention in patients with PCOS is still to be proven. In general, the magnitude of weight loss usually attained after caloric restriction combined with increased physical activity is usually moderate, in the range of 5–10% of the initial body weight, and is frequently not maintained for long periods of time [41, 42].

The evidence supporting the efficacy of pharmacological treatment for metabolic dysfunction in PCOS is unfortunately far from conclusive, with the possible exception of the use of metformin for glucose intolerance and diabetes and of lipid-lowering drugs in dyslipidemic patients [43]. However, even metformin has limited efficacy in women with PCOS when compared with lifestyle intervention [44].

In this context, surgical management of obesity has emerged as an alternative therapeutic approach for metabolic dysfunction in PCOS when lifestyle intervention and drug treatment fail. It must be highlighted that there is not enough evidence at present to universally recommend bariatric surgery for patients with PCOS and metabolic dysfunction. Hence, the indications for bariatric surgery in women with PCOS are the same that apply for any other obese patient: grade 3 obesity as defined by a BMI equal or above 40.0 kg/m^2 or grade 2 obesity (BMI 35.0–39.9 kg/m^2) in the presence of a comorbidity that may be ameliorated or cured by weight loss [45], provided that diet, exercise, and drug therapy failed to attain weight loss goals.

The available data for bariatric surgery in obese patients with PCOS however are very promising, especially considering that the prevalence of PCOS diagnosis is reportedly higher in women submitted to bariatric procedures compared with the general population [26, 46–48]. In fact, PCOS diagnosis and menstrual abnormalities are possibly the most common accompaniments of morbid obesity when the analysis of comorbidities is restricted to adolescents [48–50].

Bariatric surgical procedures induce weight loss by inducing restriction of caloric intake through reduction of the reservoir capacity of the stomach, by inducing malabsoption through bypass of the small bowel absorptive surface area or diversion of

the billiopancreatic secretions that facilitate absorption or a combination of above mechanisms [51]. The most commonly used bariatric procedures nowadays include laparoscopic vertical banded gastroplasty and Roux-en-Y gastric bypass (Fig. 17.3). Bariatric procedures usually result in effective weight loss, and a substantial majority of patients with diabetes, hyperlipidemia, hypertension, and obstructive sleep apnea experience complete resolution or improvement of these complications [51].

Years ago, we screened for PCOS in a series of 36 morbidly obese premenopausal women who elected to undergo bariatric surgery at our hospital. Seventeen of the 36 met the 1990 National Institute of Child Health and Human Development criteria for PCOS [52], 5 had idiopathic hyperandrogenism, and 14 had no evidence of androgen excess or ovarian dysfunction [26]. These study groups were comparable in terms of BMI, waist circumference, waist to hip ratio, blood pressure, and pulse rate [26]. One patient with PCOS presented with diabetes and hypertension, another had diabetes and dyslipidemia, and two were hypertensive, but their glucose tolerance and lipid levels were normal. Only one of the regularly menstruating

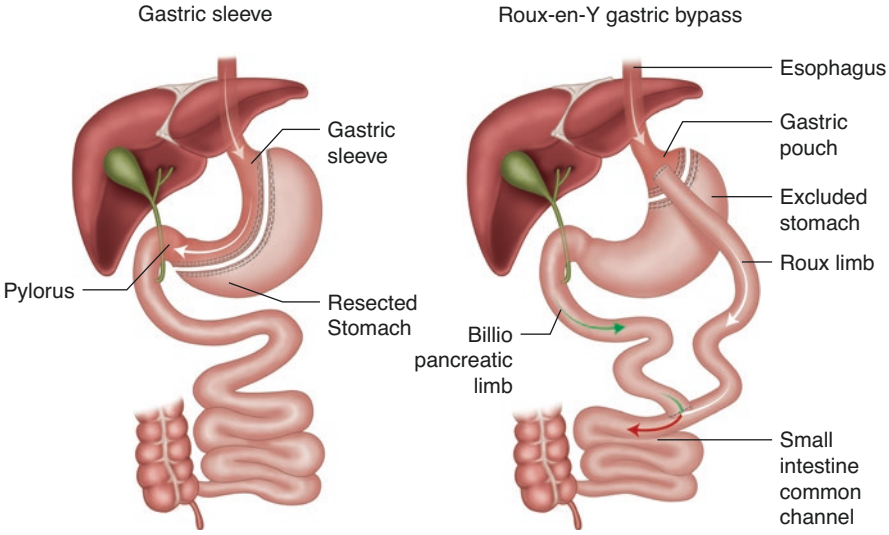


Fig. 17.3 Diagrams of the bariatric procedures most commonly used nowadays. Vertical sleeve gastrectomy (left panel) reduces the stomach to about 15% of its original size, by surgical removal of a large portion of the stomach along the greater curvature. This restrictive procedure leaves a stomach of approximately 150 ml where food (white arrows) circulates, but given its much smaller size, premeal hunger is reduced and satiety is facilitated. Roux-en-Y gastric bypass (right panel) combines a restrictive procedure in which the stomach is reduced to a 15–30 ml pouch with a malabsorptive procedure. The small pouch is connected by a 80–150 cm small intestine Y limb to a distal common channel–thereby excluding most of the small intestine from absorbing nutrients (white arrows) – whereas the longer stomach pouch remains connected to the duodenum forming the other Y limb that drains both stomach and billiopancreatic secretions (green arrows) into the shorter small intestine common channel, where food absorption is preserved (red arrows). Free clip arts downloaded from http://hiclipart.com, last accessed January 11, 2021

hyperandrogenic women had hypertension, whereas three of the nonhyperandrogenic women had hypertension, one had diabetes, and one had dyslipidemia.

Of the women with PCOS, 11 were submitted to billiopancreatic diversion and 4 were treated by laparoscopic gastric bypass [26]. The choice of procedure was dictated by the increasing expertise with laparoscopic gastric bypass of the surgeon performing bariatric surgery. We were able to follow-up 12 of these patients after weight loss stabilization and a mean loss of weight of 41 ± 9 kg [26]. The diagnosis of PCOS could not be sustained in any of these women after weight loss: hirsutism improved and even normalized; androgen levels returned to the normal range in all but one women (Fig. 17.4); all the patients regained regular menstrual cycles; and in the 10 patients in whom we were able to measure serum progesterone concentrations during the luteal phase of the menstrual cycle, these levels indicated recent ovulation [26]. These favorable changes paralleled the improvement in insulin resistance, as homeostasis model assessment of insulin resistance values returned to the normal range after weight loss (see Fig. 17.3) and was accompanied with improvement or resolution of diabetes and hypertension [26].

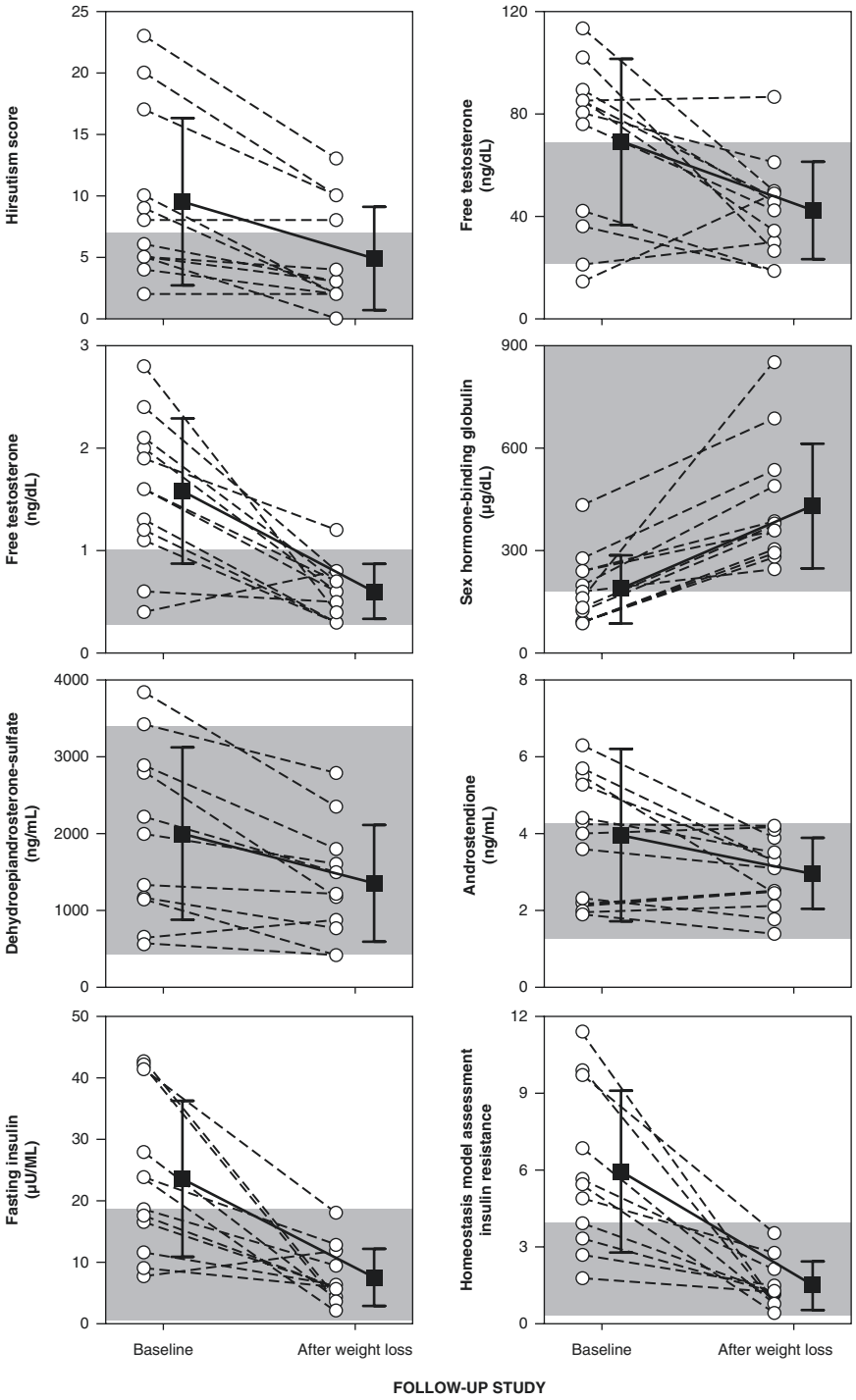
These results have been confirmed by a recent meta-analysis of all studies addressing the outcomes of severely obese women with PCOS submitted to bariatric procedures [48]. The prevalence of PCOS in women submitted to bariatric surgery was 36% with a 95% confidence interval of 22–50 [26, 48, 50, 53–55].

Surgically induced weight loss resulted in a 96% resolution rate of PCOS in these women with a 95% confidence interval of 89–100 [26, 48, 55–58] (Fig. 17.5). Resolution of hirsutism in 53% (95% confidence interval 29–76%) and menstrual dysfunction in 96% (95% confidence interval 88–100%) explained why these women did no longer meet criteria for the diagnosis of PCOS after bariatric surgery [26, 48, 55–58] (Fig. 17.5). Later studies did nothing but confirm these findings [59–61] and also showed resolution of polycystic ovarian morphology in about 50% of cases [60].

One of the most heartening effects of bariatric surgery is the potential of restoring fertility given that PCOS is a major contributor to anovulatory infertility. In parallel to the resolution of androgen excess and ovulatory dysfunction abovementioned, bariatric surgery in obese women with PCOS also normalizes elevated antimüllerian hormone concentrations, an important indirect ovarian marker of normal folliculogenesis and fertility [62].

In conceptual agreement, our very recent data show that women with PCOS submitted to bariatric surgery have fertility rates and maternal and fetal pregnancy outcomes that were not different from those of women without androgen excess submitted to the same surgery [63]. Pregnancy and live birth rates in PCOS patients with earlier bariatric procedures seeking fertility were very high (95% and 68%, respectively) with few maternal and neonatal complications [63].

Fig. 17.4 Clinical and biochemical characteristics of the morbidly obese PCOS patients submitted to bariatric surgery, before and after surgical weight loss. White circles are individual values, and black squares are mean ± SD. The shaded areas represent the reference range for each variable. All paired comparisons are statistically significant ($P < 0.02$). (Reproduced with permission from Escobar-Morreale et al. [26]. Copyright 2005, The Endocrine Society)


Hirsutism score
25
20
15
10
5
0
Free testosterone (ng/dL)
120
80
40
0
Free testosterone (ng/dL)
3
2
1
0
Sex hormone-binding globulin (µg/dL)
900
600
300
0
Dehydroepiandrosterone-sulfate (ng/mL)
4000
3000
2000
1000
0
Androstendione (ng/mL)
8
6
4
2
0
Fasting insulin (µU/ML)
50
40
30
20
10
0
Homeostasis model assessment insulin resistance
12
9
6
3
0
Baseline
After weight loss
Baseline
After weight loss
FOLLOW-UP STUDY

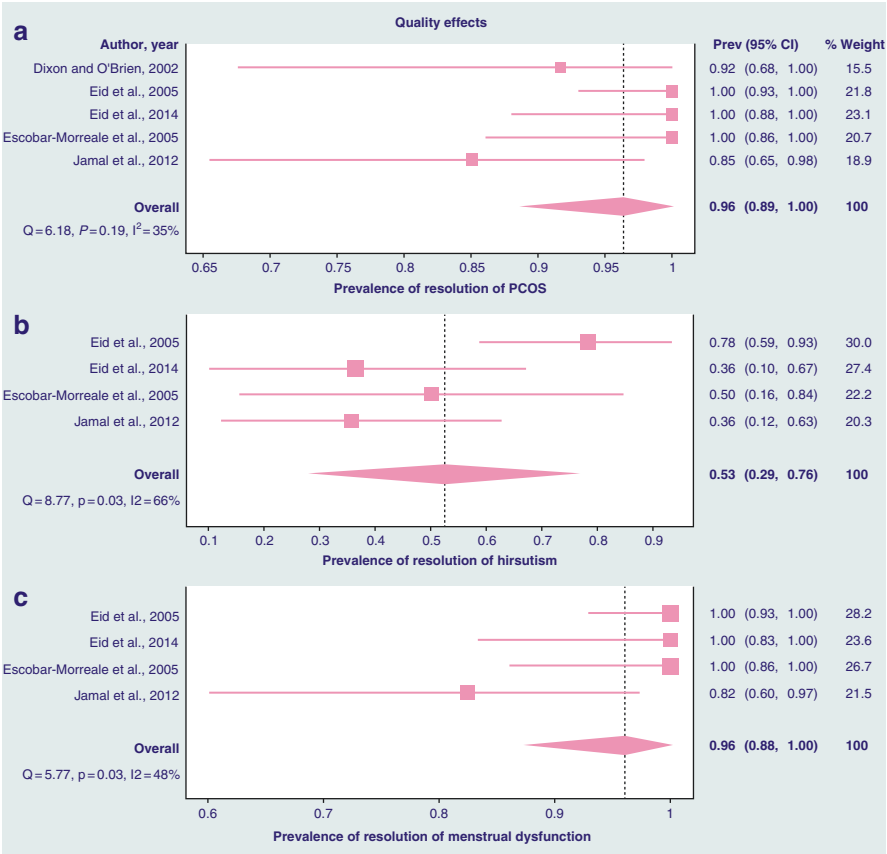


Fig. 17.5 Meta-analysis of studies reporting the resolution rates of PCOS (panel **a**), hirsutism (panel **b**), and menstrual dysfunction (panel **c**) after weight loss in severely obese women with PCOS submitted to bariatric surgery. A quality effects model was used for meta-analyses. (Modified with permission from Escobar-Morreale et al. [48]. Copyright 2017, Oxford University Press)

Earlier studies focused on pregnancy outcomes in women who had undergone bariatric surgical procedures regardless of androgen excess. These studies suggested concerns regarding a potential increased risk of intrauterine growth restriction, possibility of complications of surgery, including internal hernia and need for strict nutritional supplementation during pregnancy; however, recent data including ours are reassuring with theoretical risk being more than counterbalanced by the avoidance of the many pregnancy-associated risks that are well recognized in the setting of severe maternal obesity [63–65]. Beyond facilitation of ovulation and attainment of pregnancy, beneficial effects of surgery-induced weight loss for a future pregnancy include reduced rates of maternal complications such as gestational diabetes and preeclampsia and reduced incidence of premature delivery, low birth weight, and macrosomia compared with obese women; indeed, pregnancy outcomes of

women who attained successful weight loss following bariatric surgery were similar to those of nonobese controls [64, 65]. Furthermore, assisted reproductive techniques, such as in vitro fertilization, are safe and effective in women with prior bariatric surgery [66]. The current recommendations include delaying pregnancy for at least 1 year after the bariatric procedure in order to avoid the initial period of rapid weight loss following surgery, nutritional supplementation tailored to the individual patient and the type of bariatric procedure performed, and close follow-up of fetal growth during pregnancy [65].

While the bulk of evidence derives from the effects of bariatric procedures in severely obese women, a recent clinical trial compared bariatric surgery with conventional treatment in moderately obese (body mass index 30–40 kg/m^2) patients recently diagnosed with type 2 diabetes and suggested that the beneficial effects of bariatric surgery on metabolism are not restricted just to severely obese subjects [67]. These data introduce the possibility of exploring a role for bariatric surgery in moderately obese women with PCOS who manifest evidence of metabolic dysfunction, are deemed at an enhanced lifetime risk for chronic conditions such as diabetes and cardiovascular disease, who are either unable to sustain lifestyle modification strategies or in whom such interventions fail to significantly impact on the overall clinical picture.

Despite the promise of bariatric surgery, as outlined earlier, one cannot minimize the importance of appropriate patient selection for surgical consideration as morbidities and even mortality relating to bariatric procedures are well described. Of the 36 patients reported by our group, a single mortality event occurred, and one of the 36 patients submitted to bariatric surgery died as a consequence of postoperative surgical complications followed by sepsis of abdominal origin and multiorgan failure [26]. In the past, perioperative mortality has been reported in as many as 1.5–2% of bariatric surgical cases, yet these figures have been reduced to less than 0.3% from more recent registries involving many thousands of patients [68]. Moreover, serious perioperative complications reportedly occur in 1–4% of patients [68].

Summary

Obesity aggravates all the manifestations, risks, and metabolic comorbidities of PCOS. While lifestyle modification and dietary interventions constitute a first-line management strategy, these approaches are unlikely to be effective in the long term in a significant proportion of this population. Given that most pharmacological approaches are, at best of modest benefit, bariatric surgery offers a promising alternative therapeutic approach in obese to morbidly obese patients with PCOS and for those with evidence of metabolic comorbidities. Despite the promise however, existing data on bariatric surgical management of PCOS are limited, and large-scale prospective studies addressing not only the amelioration of PCOS symptoms and reproductive success but also the long-term consequences are definitely needed before bariatric surgery gets considered in the routine management paradigm for PCOS.

Acknowledgments Supported by Instituto de Salud Carlos III (Plan Estatal de I+D+I 2013-2016), grants PI11/00357, PI15/01686, PIE16/00050, and PI18/01122, and cofinanced by the European Development Regional Fund 'A way to achieve Europe' (EDRF). CIBERDEM and IRYCIS are also initiatives of the Instituto de Salud Carlos III.

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18 Surgical Management of Polycystic Ovary Syndrome: A Contemporary Viewpoint on Place of Ovarian Surgery in PCOS Management

Melissa Taylor-Giorlando and Lubna Pal

Key Points

- The mainstay in PCOS management is predominantly medical therapy.
- Consideration of ovarian surgery should be reserved as a second-line approach for those anovulatory women who remain unresponsive to conventional medical interventions (such as those exhibiting resistance to clomiphene or aromatase inhibitors).
- Surgical approach may also be considered for those anovulatory women with PCOS who are unable to undertake medical therapy for ovulation induction (due to clinical or financial constraints).
- Surgical approach may be considered for those in whom treatment-related risk for multiple pregnancy is to be avoided.
- The surgical approaches utilized in PCOS management range from the conventional procedure of ovarian wedge resection that has long been superseded by the more prevalent and acceptable minimally invasive techniques (ovarian drilling utilizing microlaparoscopy, fertiloscopy, and ultrasound-guided ovarian drilling).
- Rates for ovulation following ovarian surgery are almost comparable to those achieved with use of gonadotropins.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_18

- Ovarian drilling reduces thecal/granulosa cell mass and decreases the surplus of LH, androgens, inhibin, and AMH. This can restore ovulation in the majority of cases of PCOS.
- In contrast to the conventional strategies for inducing ovulation, the risks for multifetal gestation and ovarian hyperstimulation are markedly reduced with surgical approach.
- Existing surgical techniques (ovarian wedge resection or ovarian drilling) should be individualized based on the expertise of the practitioner, access to surgical tools, and individualized risk profile as well as preferences of the patient.
- In some situations, ovarian surgery may be more cost-effective than trial of gonadotropins for addressing ovulatory dysfunction of PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a complex disorder that is characterized by an array of clinical, endocrine, and metabolic manifestations. In order to accommodate the heterogeneity of this disease, the diagnostic criteria have been generalized and therefore are broadly inclusive, despite differences in severity of the clinical picture [1, 2]. In general, PCOS patients present with symptoms and laboratory anomalies of hyperandrogenism (hirsutism, acne, elevated testosterone levels) and ovulatory dysfunction (oligoovulation/anovulation) with ensuing menstrual aberrations (oligomenorrhea/amenorrhea). Ovulatory dysfunction is an obvious contributor to infertility in this population. Given the high prevalence of PCOS in the general population, it is the most common cause of ovulatory infertility. Treatment of PCOS is aimed at mitigating or even reversing the progressive endocrine and metabolic disturbances that are recognized as hallmarks of this disorder (discussed extensively in other chapters of this textbook). Although ideally the treatment should focus on targeting the inciting endocrine events, the exact pathophysiology of PCOS still remains unclear. Therefore therapeutic approaches empirically attempt to override abnormal signaling pathways.

While medical treatment is the mainstay in PCOS management, surgical elimination of ovarian tissue has been thought to be efficacious (further detailed in next section). The ovarian stroma, specifically the theca, is a primary source of hyperandrogenemia of PCOS [3]. Destruction or removal of populations of theca cells is thus expected to decrease synthesized and secreted androgens, resulting in amelioration of hyperandrogenemia of ovarian origin. Indeed, improvements in the abnormal endocrine and metabolic milieu of PCOS following partial removal of ovarian tissue are well described [4]. A decline in the circulating androgen levels associated with "de-bulking" of the polycystic ovaries is hypothesized to restore the sensitivity of the hypothalamic–pituitary–ovarian axis, and this in turn leads to the resumption of spontaneous ovulation, normalization of menstrual regularity, and restoration of fertility, phenomenon that are well described following surgical interventions discussed in this chapter.

History

Reports from as early as the 1920s describe an association between obesity and menstrual irregularity as well as between hirsutism and infertility. Polycystic ovary syndrome was first described by I.F. Stein and M.L. Leventhal in 1935 [5–7], and the authors published their findings in a landmark paper entitled "Amenorrhea Associated with Bilateral Polycystic Ovaries" in the American Journal of Obstetrics and Gynecology in that same year [8]. Practicing in an age when laparotomy had facilitated surgical approach to the ovaries, Stein and Leventhal operated on these patients only to discover that their ovaries were enlarged to 2–4 times the normal size, with numerous small fluid-filled cysts. Whereas some of the ovaries were flat and "oyster-like," in general, they all appeared dull, grayish, and polycystic. Thus, ever since the initial description of the syndrome, an association was made between the characteristic symptomatology and the presence of enlarged and polycystic ovaries. Stein and Leventhal initially performed wedge biopsies of the ovaries to more fully elucidate the underlying pathology. Although no hallmark pathological lesion was identified, they serendipitously noticed that their biopsied patients resumed regular menstruation within a few months of the procedure; many of whom also spontaneously conceived thereafter. Of the 96 patients subjected to ovarian wedge resection by Stein and Leventhal, 71 had experienced infertility, and in this subset, 63 achieved spontaneous pregnancy. Additionally, from the entire cohort, all except three patients had a resumption of regular menstrual cyclicity [6, 8, 9]. Ovarian wedge resection thereafter became a therapeutic option rather than a diagnostic modality.

However, despite the initial enthusiasm based on high success rates, ovarian wedge resection fell out of favor with time due to a high incidence of postoperative adhesions and concerns about a significant loss of endocrinologically active normal ovarian tissue [10, 11]. In its place, medical therapy, which was considered a safer and more efficacious option, became the mainstay for induction of ovulation in women with PCOS.

Contemporary Modes of Ovarian Surgery in PCOS

Given the concerns of adhesion formation and ovarian tissue destruction after wedge resection, a less invasive approach of laparoscopic ovarian drilling (LOD) was developed by Gjønnæss in 1984 [12]. Using minimally invasive surgical concepts, focal destruction of the ovarian stroma is achieved utilizing ablative strategies that have ranged from diathermy to laser tissue vaporization. In the initial report, diathermy was utilized to create up to ten individual punctures into the ovarian cortex; Gjønnæss treated 62 women with PCOS, of whom 92% ovulated successfully within 3 months following the procedure; regular menstrual cyclicity was established in 86% (51/62 patients). Of the 35 patients presenting with infertility, 24 became pregnant (69%); interestingly, 7 of 9 patients determined to be clomiphene-resistant ovulated spontaneously, whereas the remaining two became responsive to clomiphene in the

postoperative period. In a subsequent larger series of 212 patients, the ovulation and pregnancy rates following were 92% and 84%, respectively [13]. As observed in their initial cohort, patients that remained anovulatory after surgery did respond to clomiphene and eventually achieved pregnancy. Inclusion of this group increased the overall pregnancy rate to 89%. Although lean individuals had higher spontaneous ovulation rates (96–97%) compared to obese patients (70%), pregnancy rates were similar once ovulation was established (92–95%).

The reproductive outcomes of PCOS patients who underwent LOD were evaluated in a Cochrane database analysis comprising 38 trials and 3326 women [14]. They compared LOD with and without medical ovulation induction to medical ovulation induction alone. Pooled results suggested that the live birth rate may be lower in women who undergo LOD than those who undergo medical ovulation induction alone (28–40% vs 42%, respectively). However the risk of multiple pregnancies was lower in those undergoing LOD alone compared to medical ovulation induction (0.9–3.4% vs 5%, respectively). Overall the quality of the data was found to be low, and therefore it is currently difficult to use these studies to guide clinical management.

Operative Techniques

In general, in order to minimize morbidity and reduce the likelihood of pelvic adhesion formation, minimally invasive approach is preferred for ovarian drilling unless contraindications to laparoscopic approach exist (Fig. 18.1). Traditional laparoscopy has been used in the majority of reports and trials on LOD. There does not appear to be a difference in either the postoperative hormone profile or ovulation

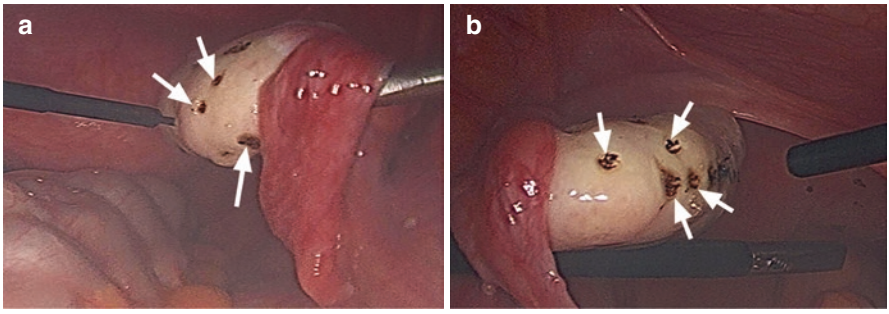

Fig. 18.1 Surgical management of PCOS-related ovulatory infertility by laparoscopic ovarian drilling. Electrocautery was delivered to the ovarian cortex laparoscopically to the right (**a**) and left (**b**) ovaries in a 23-year-old nulliparous patient. Ovarian cortexes were punctured with a monopolar needle cautery set at 30 W coagulation for approximately 3–4 s at each puncture site. The patient had been experiencing oligoovulation/anovulation with irregular menstrual cycles. Although she desired regular cycles and wanted to have the option of conceiving, she was not willing to undertake treatment by medical ovulation induction secondary to ethical and religious beliefs. Individualized treatment by laparoscopic ovarian drilling was therefore the option of choice in this carefully selected and counseled patient. *White arrows* indicate ovarian drilling sites

and pregnancy rates with regard to the type of thermal technique (harmonic scalpel, monopolar cautery, bipolar cautery, or laser coagulation) employed for ovarian thecal decompression [15, 16]. Each of these minimally invasive approaches creates focal areas of damage in the ovarian cortex and the stroma; the extent of ovarian tissue damage with LOD approach is minimal compared to seen with the originally described ovarian wedge resection technique.

A significant concern with any pelvic surgery is the potential risk for excessive, unintended ovarian tissue destruction that spreads beyond the area intended for surgical ablation. Excessive ovarian cortical destruction could potentially result in diminished function with consequent adverse sequelae on ovarian endocrinology and fertility. In order to determine the minimal energy requirement for effective ovarian stromal destruction and resumption of ovulation, Amer et al. treated 30 clomiphene-resistant PCOS patients with LOD [17]. Using a specially designed monopolar electrode, 1–4 punctures were made to a depth of 8 mm each into the ovarian stroma, delivering 150 J of energy per puncture site. Ovulation and pregnancy rates were highest (67% each) in the subgroup of patients that received four punctures per ovary—an average of 600 J/ovary or 1200 J/patient. In contrast, in patients receiving only one puncture per ovary, the corresponding rates were 33 and 0%, respectively. The authors, therefore, recommend that an ovarian dose of 600 J is required for optimal tissue response [17]. In another study, 26 patients were treated with 5 punctures and 37 patients with 10 punctures per ovary [18]. The clinical and laboratory outcomes of both groups were similar. The authors concluded that the efficacy of five punctures per ovary was sufficient treatment. From these studies, it appears that there is a threshold effect that peaks at 4–5 punctures per ovary.

Monopolar diathermy is probably the most commonly employed strategy for achieving ovarian stromal reduction. Caution is warranted regarding site of ovarian puncture; site of ovarian puncture, which should be remote from the fallopian tube in order to avoid subsequent fimbrial adhesions and thereby consequent tubal factor infertility [19].

Additionally, unilateral LOD has been compared to bilateral LOD in efforts to minimize overall ovarian destruction and adhesion formation. There is no clear evidence that bilateral LOD has superiority in terms of menstrual cycle regulation [20], live birth rate, clinical pregnancy rate, or miscarriage [21]. However, there is some evidence that unilateral LOD may be equivalent in terms of achieving pregnancy [20, 22].

In line with previous efforts to pursue minimally invasive techniques, ultrasound-guided transvaginal needle ovarian drilling (UTND), a procedure that can be safely undertaken in office settings, has also been evaluated as a method of inducing controlled ovarian damage. In a technique that mimics that of transvaginal oocyte retrieval, a needle connected to continuous vacuum pressure is inserted transvaginally. Ovarian follicles are then punctured repeatedly and from different angles. One study evaluated UTND and found while it did result in improvements in ovulation, pregnancy, hirsutism, and acne, it was not superior to LOD. The duration of UTND was found to be shorter than LOD however [23, 24]; other investigators have

successfully used an ultrasound-guided laser technique for ovarian drilling [25]. However, there are concerns that the cortical blood flow may be disrupted with this technique [26].

Use of microlaparoscopy, a procedure that can be performed in the office setting under a combination of intravenous sedation and local anesthesia, to achieve ovarian drilling is also described. A malleable 2.2-mm fiber-optic laparoscope introduced through a metal sleeve that fits over a Veress needle [27] and a high-frequency 2.2-mm electrocautery probe have been effectively utilized for the ovarian drilling procedure. With technological advances and increasing prioritization of low-cost treatments, previously used diagnostic modalities such as fertiloscopy or transvaginal hydrolaparoscopy are being revived as relatively less invasive methods that can permit reliable ovarian access for focal decompression. Transvaginal hydrolaparoscopy was initially used in the 1960s and 1970s but was abandoned because transabdominal laparoscopy provided a more panoramic view of the pelvic and abdominal cavity and better access for surgical treatment. This technique uses a small culdotomy incision with a 4-mm endoscope that has a 30° angle [28–32]. While less invasive than conventional laparoscopy, these minimally invasive approaches are not currently in widespread use.

Repeated LOD in PCOS

Given concerns of adhesion formation and of detriment to ovarian reserve consequent to focal destruction of the ovarian tissue, it is reasonable to question the safety of repeated LOD procedures in the management of PCOS. To answer this question, 20 patients who had previously undergone LOD 1–6 years prior to the study (to which 12 had and 8 had not responded) underwent repeat LOD intervention [33]. Following the repeat procedure, the ovulation and pregnancy rates were 83 and 66%, respectively, in the 12/20 who had achieved resumption of ovulation following the first procedure, whereas in the remaining eight (nonresponders to first intervention), the respective rates were 25% (2 out of 8) and 29% (2 out of 7). As suggested by this single study, although there appears to be a short-term benefit from repeat LOD, especially in those who had achieved ovulatory response following the first intervention, long-term effects of repeated LOD procedures both with respect to risk of pelvic adhesions and for ovarian detriment remain unclear. In the absence of robust data, caution is advised regarding repeat LOD, which should be considered only after exhaustion of the existing repertoire of medical management options.

Unilateral Versus Bilateral Ovarian Drilling

Some have investigated the necessity of performing LOD on both ovaries versus unilateral drilling. One systematic review and meta-analysis by Hashim et al. [34] found that there is no difference in clinical outcomes including rates of ovulation,

pregnancy, live birth, or miscarriage when comparing unilateral and bilateral LOD. They found reductions in AMH levels, but they were equivalent between groups. However the bilateral LOD group did have lower antral follicle counts. Therefore, it does not seem that bilateral drilling is necessary for improved clinical outcomes, but may pose a greater threat to ovarian reserve. Therefore unilateral ovarian drilling should be considered when pursing surgical intervention.

Endocrine Events After Ovarian Surgery

Dysregulated endocrinology with an overall androgen excess is the underlying driver of PCOS-associated pathophysiology. Androgen excess, either relative or absolute, is a hallmark of PCOS, and the magnitude of hormonal imbalance is often reflected in the severity of symptomatology. Although numerous theories abound about the signaling event(s) responsible for the panoply of hormonal aberrations, the instigating mechanisms remain far from clear. Disproportionately elevated levels of luteinizing hormone (LH) [35, 36], hyperandrogenemia [37, 38], hyperinsulinemia, and insulin resistance [39, 40] are commonly encountered in women with PCOS and recognized to underlie the common symptoms as well as comorbidities of PCOS [41, 42].

In most studies, there is an immediate decrease in circulating levels of androgens and androgen precursors (testosterone, dihydrotestosterone, androstenedione, and 17-hydroxyprogesterone) of ovarian origin following ovarian drilling, notable as early as within 24 h of the procedure [43–54]. In contrast to the consistent reduction in serum androgen levels after ovarian surgery, the overall effect on estradiol levels is less clear. This is partly attributable to differences in size and heterogeneity of studies, but more importantly also to the lack of consistent menstrual cycle phase-dependent monitoring. In studies where measurements correlated with cycle phase, there was either no change in the early and mid-follicular estradiol levels, or some increase in the late follicular and luteal levels, the latter evident in those who responded with resumption of menstrual cyclicity. Lower estradiol levels in the early proliferative phase may be due to decreased tonic LH stimulation of theca cells resulting in a lower intra-ovarian androgen load. In turn, the lower thecal androgen output results in diminished substrate for granulosa cell aromatase and therefore lower estradiol levels. Higher estradiol levels later in the menstrual cycle thus likely reflect resumption of ovarian folliculogenesis following surgical intervention. Ovarian surgery however did not have any significant impact on progesterone concentration [55].

A transient elevation in serum LH levels is commonly noted on the day after ovarian surgery, with a more consistent and sustained decline in levels thereafter. The decrease in LH levels (except for the preovulatory surge) can reportedly persist for several years following ovarian surgery [56]. The decline in LH levels following ovarian surgery is attributable to decreased LH pulse amplitude; LH pulse frequency reportedly remains unchanged [57]. The magnitude of decline in circulating LH was noted to be greater in those achieving spontaneous ovulation and/or conception

following ovarian surgery compared to the nonresponders [43]. Similar to LH, FSH levels also increased in most studies in the immediate postoperative phase and then declined over time to pretreatment values [44, 58]. Pituitary FSH secretion is driven by hypothalamic GnRH, which in turn is responsive to feedback regulation by the sex steroids. FSH is also inhibited by inhibin, which is produced by ovarian granulosa cells. Although the data on the impact of ovarian surgery on inhibin are limited, inhibin levels appear to decline in the immediate postoperative period followed by a gradual return to baseline levels within a few weeks following the procedure [58]. Destruction of the ovarian stroma and granulosa cells can thus be hypothesized to underlie the decline in inhibin levels notable immediately following the procedure, thereby explaining the rise in endogenous FSH levels and creation of a more physiological milieu that is conducive to follicular growth.

In contrast to the amelioration in hyperandrogenemia of ovarian origin following ovarian surgery, serum levels of adrenal dehydroepiandrosterone sulfate (DHEAS) are essentially unchanged [59].

Long-Term Implications

There have been a few studies that have evaluated the effects of LOD on pregnancy rates and on long-term ovarian endocrine function. There is evidence that in clomiphene-resistant women, LOD decreased future need for ovulation induction strategies and increased the chances of a second pregnancy [60]. A single study has demonstrated persistent effects of LOD on reproductive hormone profile; circulating levels of androgens, and ratio of pituitary gonadotropins LH and FSH may be significantly decreased as many as 9 years later [61]. In this same study, there were no cases of premature menopause in the 9-year follow-up. However there is evidence that AMH declines after the procedure [62]. It is unknown if this is a true reflection of a decrease in ovarian reserve or rather a normalization of pathologically elevated AMH associated with PCOS.

Most of the studies that reported persisting endocrine benefit following ovarian drilling utilized electrocautery and comprised fewer than 20 patients. Existing data are limited by small sample sizes, by heterogeneity in populations, in study designs, and in the chosen methodology for ovarian drilling; it is thus difficult to draw definitive conclusions regarding long-term effects of this strategy.

Mechanism(S) Underlying Effectiveness of Ovarian Surgery in PCOS

There are many proposed mechanisms to explain the alteration in endocrine function following surgical treatment of PCOS. Those with PCOS are found to have increased ovarian stromal area, and destruction of this tissue, particularly of the androgen producing theca cells, may be directly responsible. Alternatively, removal of the follicular fluid by aspiration or boiling consequent to thermal effects of

electrocautery may contribute. The fluid within the small ovarian follicles in women with PCOS contains higher levels of proinflammatory biomarkers and decreased levels of anti-inflammatory molecules [63–66]. Thus removing this "unhealthy" fluid may improve the local environment of the ovary. Some evidence also suggests that alterations in ovarian vasculature and in the local expression of vascular endothelial growth factor (VEGF, discussed further in Chap. 24) may be implicated. At baseline, patients with PCOS have increased intraovarian vascularity, and LOD can at least transiently normalize the angiogenic processes that may underlie the ovarian dysfunction of PCOS [67, 68].

Conclusion

Surgical intervention such as LOD is an effective treatment option for select patients with PCOS who fail to respond to common ovulation induction agents (such as clomiphene or aromatase inhibitors) and who are not deemed optimal candidates for trial of gonadotropin therapy either due to treatment related risks (such as for multiple pregnancy or of ovarian hyperstimulation syndrome) or because of treatment-related financial burden. Almost 20% of patients with PCOS demonstrate resistance to oral ovulation inducing agents such as clomiphene [69]. Rates are slightly improved when using an aromatase inhibitor [70]. However, this subgroup of anovulatory women is particularly at risk for developing ovarian hyperstimulation syndrome as well as multiple pregnancy with gonadotropin exposure; these patients may represent an ideal population to benefit from LOD. Indeed, pregnancy rates with LOD are comparable to seen with ovulation induction and gonadotropin therapy, albeit with minimal iatrogenic risk of multiple pregnancy. For patients not seeking fertility who are also not candidates for hormonal therapy, LOD may be considered as a management strategy but only after detailed discussion on the risks and benefits relating to this approach. In an appropriately selected patient, LOD can be considered as a relatively safe and financially viable option that offers a potential for long-term benefit for PCOS-related symptomatology. There are a wide variety of techniques that can be employed for LOD and with no formal recommendations by professional societies. Based on existing data and our own clincial experience, we would recommend a monopolar needle approach, set between 20 and 30 W, with approximately 5–10 punctures lasting 4 seconds per puncture depending on size of the ovary.

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Part VI

Health Risks and Burden of Polycystic Ovary Syndrome

PCOS and Its Association with Mental Health

19

Snigdha Alur-Gupta and Anuja Dokras

Key Points

- PCOS is associated with several mental health problems including depression, anxiety, and eating disorders.
- Women with PCOS have a high prevalence of depressive symptoms with a three-fold increased odds of depressive symptoms after matching for BMI.
- Women with PCOS have an increased risk of both perinatal and postpartum depression.
- Women with PCOS have a fivefold increased risk of anxiety compared to controls.
- The etiology of this association between PCOS and depression and anxiety is likely multifactorial and may be related to insulin resistance, high androgens, obesity, and body image distress.
- All women with PCOS should be screened for depression and anxiety at the time of diagnosis.
- Treatment of women with PCOS with depression should take a multi-pronged approach and include lifestyle modification, cognitive behavioral therapy (CBT), OCPs, and antidepressants. The negative impact of some antidepressant on weight should be considered.
- Proposed treatments for anxiety include lifestyle modifications, CBT, and anxiolytics, as indicated.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_19

- The associations between PCOS and anxiety and depression appear to persist across from adolescence into adulthood, with some studies suggesting increased risk in offspring.
- Women with PCOS have a threefold increased risk of abnormal eating disorder (ED) scores and ED diagnosis.
- Anxiety, depression, high androgens, insulin resistance, and body image distress may all contribute to the increased risk of ED.
- There are currently no studies evaluating treatment of eating disorders specifically in women with PCOS; however, an integrative model with comprehensive CBT may be beneficial.

Introduction

PCOS is the most common endocrine disorder affecting reproductive-aged women, with a prevalence ranging from 8% to 13% depending upon the population studied and criteria used [1].(Table 19.1) PCOS is a lifelong disease associated with multiple risk factors and predispositions across a woman's lifespan. These include metabolic syndrome, obesity, type 2 diabetes, dyslipidemia, infertility, and pregnancy complications [2].

The association between PCOS and mental health disorders has gained more prominence in the past two decades as evidence emerges identifying women with PCOS to be an increased risk for depression, anxiety, eating disorders, and more recently perinatal and postpartum depression and body image distress. This chapter seeks to explore these findings and summarize the potential impact of PCOS on mental health, evaluate potential treatments, as well as identify gaps in the literature for future research.

Table 19.1 PCOS definition (Rotterdam Criteria), 2 out of 3 criteria

1. *Oligo-anovulation:* 1 to <3 years post menarche: <21 or > 45 days 3 years post menarche to perimenopause: <21 or > 35 days or < 8 cycles per year 1-year post menarche: >90 days from any one cycle Primary amenorrhea by age 15 or > 3 years post thelarche
2. *Hyperandrogenism:* Modified Ferriman-Gallwey score ≥4–6, account for ethnic variation and only use terminal hair for consideration AND/OR Biochemical in the form of calculated free testosterone, free androgen index, or calculated bioavailable testosterone. If these are unavailable, consider DHEAS or androstenedione
3. *Polycystic appearing ovaries as assessed by US with a frequency of 8 MHz:* ≥20 follicles measuring 2–9 mm per ovary AND/OR Increased ovarian volume ≥ 10 mL on either ovary in the absence of cysts or dominant follicles

PCOS and Depression

Prevalence and Longitudinal Risk

Depressive disorders are one of the major causes of global disease burden and the leading cause of years lived with disability in 2010 [3]. Depression is also nearly twice as common in females, with a prevalence of 10.4% in women [4]. Several studies have evaluated the association of depression in women with PCOS. A meta-analysis by Cooney et al. found that of 18 included studies, the median prevalence of depressive symptoms was 36.6% (interquartile range [IQR] 22.3–50.0%) and women with PCOS had increased odds of depressive symptoms, even after matching for BMI (OR 3.25, 95% CI 1.73–6.09) compared to those without PCOS. The prevalence of moderate or severe depressive symptoms was also increased (OR 4.18, 95% CI 2.68, 6.52) [5]. This association has been preserved in more recent meta-analyses as well, with one including 24 studies and 167,912 women showing that those with PCOS were more likely to have a clinical diagnosis of depression (OR 2.79, 95% CI, 2.23–3.50) [6]. When comparing women with PCOS to controls, those with PCOS were also noted to have a higher odds of abnormal depression scores (OR 4.03, 95% CI 2.96–5.50) [7].

This risk appears to persist across all age groups. A cross-sectional study in Turkey of 28 adolescents with PCOS compared to 31 controls found that the prevalence of psychiatric disorders was significantly higher in those with PCOS, with depression being the single most common disorder (21% vs 3%) [8]. In a longitudinal community-based cohort study of over 11,000 young adults aged 18–23 years, surveys conducted 1 year following diagnosis showed the odds of moderate to severe depression were significantly higher in women with PCOS compared to controls (OR 1.62, 95% CI 1.21–2.18) [9]. A prospective longitudinal study of 60 women with PCOS, average age 32 years, found that after approximately 2 years of follow-up, there was a 19% conversion rate to new depressive disorders or other depressive symptoms [10]. In a 15-year follow-up study of a Finnish cohort, women with PCOS as diagnosed by irregular menses and hirsutism were noted to have increased prevalence of depression symptoms at age 31 (9.6% vs 5.3%) and 46 (25.9% vs 14%) compared to controls [11]. The risk of women with PCOS self-reporting depression between age 31 and 46 was higher than controls after adjusting for BMI as well (OR 2.02, 95% CI 1.12–3.64) [12]. A population-based cohort evaluating women participating in the Coronary Artery Risk Development in Young Adults study found that of 83 subjects meeting criteria for PCOS, depression scores assessed every 5 years were high compared to controls across the 25-year lifespan of the study [13].

In line with the persistent prevalence of depression, a recent population-based cohort study in Finland of 1,097,753 births to 590,939 women found that maternal PCOS was associated with an increased hazards ratio of any psychiatric diagnosis in offspring (HR 1.32, 95% CI 1.27–1.38), including mood disorders (HR 1.27, 95% CI 1.18–1.35). This suggests that perhaps PCOS may have adverse effects on the mental health throughout a woman's life span and on future generations [14].

Perinatal and Postpartum Depression

More recently, the impact of PCOS on depression in the postpartum period has also been explored. Perinatal depression is one of the most common disorders affecting pregnancy, occurring in 1 in 7 patients [15]. A cross-sectional Australian study of 566 parous women found a positive although statistically non-significant association between PCOS and postnatal depression (OR 1.6, 95% CI 0.9–2.9) [16]. A larger cross-sectional study of self-reported data of 5239 women in the Australian Longitudinal Study on Women's Health found the prevalence of postnatal depression was higher in those with PCOS compared to those without (26.8% vs. 18.6%, $p < 0.001$); however this association failed to reach significance in adjusted analyses [17]. We published a retrospective cohort study using the nationwide claims database Optum of 42,391 unique women with polycystic ovary syndrome and 795,480 women without PCOS and found that women with PCOS were at a significantly higher odds of having both perinatal and postpartum depression compared to women without PCOS, even after adjusting for prior depression history and socioeconomic factors (aOR for perinatal 1.27, 95% CI 1.22–1.33, aOR for postpartum 1.46, 95% CI 1.36–1.57) [18]. In a recently published cross-sectional analysis using 3906 postpartum women completing the Utah Pregnancy Risk Assessment Monitoring System questionnaire, clinical PCOS was associated with a 1.76 (95% CI 1.03–3.00) higher adjusted prevalence ratio for postpartum depressed mood or anhedonia, and interestingly prenatal depression and anxiety mediated 20% and 32% of this effect, respectively [19].

Potential Reasons for the Association Between PCOS and Depression

The etiology of the association between PCOS and depression is not clear but may be related to insulin resistance, hyperandrogenism, obesity, and body image distress. A cross-sectional analysis of 301 women with PCOS found that those with positive depression screens had higher measures of insulin resistance by HOMA-IR compared to those with normal screens even after stratifying by BMI and evaluating only obese women (HOMA-IR: 7.4 vs 4.1, $p = 0.05$) [20]. A secondary analysis of a multicenter trial involving women with PCOS found that of 738 women studied, elevated HOMA-IR was associated with increased odds of depression after controlling for age and BMI (OR 2.23, 95% CI 1.11–4.46) [21].

Others however have suggested a greater role for hyperandrogenism in depression. A Japanese study comparing 25 women with PCOS to 31 eumenorrheic women found that Hospital Anxiety and Depression Scale (HADS) survey scores correlated significantly with androstenedione levels for both anxiety ($r = 0.533$, $p = 0.006$) and depression ($r = 0.487$, $p = 0.014$) in women with PCOS [22]. A longitudinal cohort study following women through the perimenopausal transition found that in Caucasian women, higher testosterone levels were associated with increased depressive symptoms, even after controlling for age, obesity, and hot flushes (RR 1.09, 95% CI 1.00–1.17, $p = 0.042$) [23].

Another major factor may be weight and obesity. A prospective longitudinal analysis of 163 women with PCOS followed over 5.5 years found that obese women had a fivefold increased odds of depression risk compared to women with normal weight at baseline (aOR 5.07, 95% CI 1.07–24.0) [24]. In a meta-regression of 14 studies evaluating the pooled standard mean differences (SMDs), women with PCOS and concurrent depression had higher BMIs, FG scores, and HOMA-IRs compared to women with PCOS without concurrent depression, although effect sizes were small [5].

Tying into the concept of weight is the role of body image distress on mood disorders. Body image is defined as an individual's psychological experience of the appearance and function of his/her body [25]. Several studies show that women with PCOS are more likely to have body image dissatisfaction compared to those without PCOS. In one cross-sectional study of 177 women with PCOS compared to 109 controls, women with PCOS had more negative body image, and a lower appearance evaluation on body image surveys was associated with depression independent of BMI. [26] A more recent analysis of 94 women with PCOS found that they experienced body dissatisfaction and distorted self-perceived body image and those with higher BMI were more dissatisfied with their body image [27]. In a cross-sectional analysis of 189 women with PCOS with 225 controls, we showed that women with PCOS had worse body image distress scores in adjusted analyses, and the association between PCOS and higher depression scores was mediated either completely or in part by different body image subscales. These findings suggest that therapeutic interventions targeted at improving body image may decrease depressive symptoms in this population [28].

Proposed Treatments

Based on the mechanisms identified above, few studies have examined the role of lifestyle management on depression symptoms in women with PCOS. One randomized controlled trial of 104 overweight/obese PCOS women found that lifestyle interventions of dietary restriction, diet and aerobic exercise, or diet and combined aerobic-resistance exercise all resulted in improvements in depression scores after 20 weeks, with no inter-group differences [29]. A recent randomized controlled trial in Sweden evaluating 68 women with PCOS and BMIs $\geq$27 kg/m^2 found that after 4 months in a behavioral modification program involving structured training and lifestyle coaches to achieve long-term weight control, those in the intervention group expressed less anxiety (p = 0.035), higher general health (p = 0.012), and lower depressed mood (p = 0.033) [30]. Exercise may also improve depression through its effects on body image distress. A larger controlled clinical trial of 87 women with PCOS found that those in the continuous aerobic training arm had improvements in body image dissatisfaction compared to the control group and total depression survey scores improved in those receiving exercise interventions. In addition, there were positive correlations between scores for dissatisfaction and depression [31].

OCPs are used as first-line treatment for symptom management of irregular menses and hirsutism in PCOS. A prospective observational study of 36 women with PCOS did not find change in depression scores with OCP use [32]. However, in data from the Oral Contraceptive and Weight Loss (OWL)-PCOS trial, OCP use was associated with a significant decrease in abnormal depression score prevalence (13.3–4.4%) [33]. Given the role of insulin resistance on depression, insulin-sensitizing agents have also been evaluated in the treatment of depression with mixed findings. A pilot trial of 19 adolescents and 25 adults with PCOS found that after a 3-month trial of metformin, anxiety and depression scores decreased significantly ($p < 0.001$) as measured by the Beck Anxiety and Depression Inventories (reduction of 3.4 and 3.3 points, respectively) [34]. Although not an RCT, a prospective multicenter cohort study in Saudi Arabia found that when comparing 53 women with PCOS receiving metformin and lifestyle modifications to 33 receiving lifestyle modifications only for 3 months, those receiving metformin had a significantly lower odds of having major depression (OR 0.302, 95% CI 0.093–0.976) [35]. However, a 6-week double-blinded RCT comparing the effect of metformin versus pioglitazone on depression scores found that pioglitazone was superior to metformin and resulted in a 38.3% vs 8.3% reduction in baseline depression scores ($p < 0.001$). This effect appeared to be independent of its insulin-sensitizing properties, given that HOMA-IR values were not significantly different between groups ($p = 0.888$) [36]. In the general population as well, random effects meta-analysis of RCTs have shown pioglitazone to improve depression symptoms compared to controls (pooled effect size = −0.68 (95% CI -1.12 to −0.24), $p = 0.003$), although this was not seen with metformin (pooled effect size = +0.32 (95% C.I. −0.23 to 0.88), $p = 0.25$) [37]. However larger RCT studies in the PCOS population are needed.

Cognitive behavioral therapy (CBT) is a form of psychotherapy that focuses on changing the dysfunctional thoughts that lead to negative mood states and is recommended by the American Psychological Association as a first-line treatment of depression [38]. Given the previously mentioned role of body image on mood, it is perceivable how CBT may positively impact depression risk. A 16-week randomized clinical pilot investigating the use of CBT along with lifestyle interventions found that those with the addition of CBT lost more weekly weight (−0.35 kg/wk. vs. −0.16 kg/wk) compared to just lifestyle interventions. Interestingly however, measures of depression and anxiety were not significantly different between the two arms [39].

A combination of therapies is likely the most effective route. A recent secondary analysis of a RCT examined the effect of 20 CBT lifestyle sessions combined with a healthy diet and physical therapy compared to care as usual in 155 overweight women with PCOS. Depression scores decreased significantly in the intervention program ($p = 0.045$), and self-esteem scores significantly improved ($p = 0.027$). Interestingly, while weight loss mediated the effects on self-esteem, it did not for depression or body image. Androgen and insulin resistance parameters also did not mediate this effect [40]. With regard to antidepressants, there are no RCTs evaluating their use specifically in women with PCOS. However, the technical report of the international guidelines recommends considering selective serotonin reuptake

inhibitors (SSRIs), noradrenergic reuptake inhibitors (NARIs), norepinephrine-dopamine reuptake inhibitors (NDRIs), or melatonin agonists among the first-line antidepressants after using physical examination and laboratory investigations to rule out other comorbid conditions. Tolerability to side effects is an important area of assessment in determining the best treatment option [1].

In summary, women with PCOS are at increased risk for depression. The etiology of this risk is likely multifactorial with insulin resistance, elevated androgens, and body image distress proposed as possible mechanisms. Treatment of depression therefore must also take a multi-faceted approach focusing on lifestyle changes in the form of diet and exercise along with therapies, such as CBT, to modify negative perceptions. The impact of OCPs or insulin-sensitizing medications on depression has yet to be determined fully. Antidepressants may also be needed though caution is urged when considering agents and their side effects.

PCOS and Anxiety

Association Between PCOS and Anxiety

Closely tracking with depression is the prevalence of anxiety in women with PCOS. Anxiety disorders contribute significantly to financial burden related to healthcare, with one study estimating that generalized anxiety disorder is associated with 1.5–5.4 days of work impairment per month [41]. Early studies found that the odds of anxiety symptoms were significant greater in women with PCOS compared to controls (OR 6.88, 95% CI 2.5–18.9) [42]. A recent meta-analysis of 1400 subjects also found women with PCOS to be at increased risk for anxiety compared to controls (OR 5.62, 95% CI 3.22–9.80). The odds of moderate and severe anxiety symptoms were similarly high in this group (OR 6.55, 95% CI 2.87–14.93) [5]. A cross-sectional analysis from the Australian Longitudinal Study of Women's Health, a population-based study, comparing 478 women with self-reported PCOS to 8134 women without, found the prevalence of anxiety symptoms was higher in those with PCOS (50% vs 39.2%) and remained more elevated after controlling for BMI, infertility, and sociodemographic factors [43]. Data linkage studies of statewide hospital admission morbidity databases in Western Australia have also demonstrated increased clinical anxiety in women with PCOS (14%) compared to those without (5.9%) [44].

As with depression, prevalence of anxiety appears to be higher in all age groups studied. In a cross-sectional analysis of 28 adolescents with PCOS compared to 31 controls, the second most common psychiatric disorder diagnosed in girls with PCOS was social anxiety disorder (diagnosis rate of 17.8%) with 42% of PCOS adolescents having some form of anxiety disorder [8]. A larger cross-sectional study in Turkey comprised of 153 adolescents with PCOS and 161 controls (mean ages approximately 15.6 and 15.7 years, respectively) found that those with PCOS scored significant higher on depression and anxiety surveys compared to controls ($p < 0.001$ for both) [45]. In the 15-year follow-up study of a Finnish cohort mentioned previously, women with PCOS had higher anxiety scores at both age 31 ($p < 0.001$) and

46 ($p = 0.002$) compared to controls. The coexistence of anxiety and depression in women with PCOS was also higher compared to controls at both age 31 and 46 ($p < 0.001$) [12]. In an age-matched population cohort study of 5431 women with PCOS and 21,724 controls in Taiwan, women with PCOS had a significantly higher risk of subsequent anxiety disorder compared to controls over a median of 5 years of follow-up (HR 1.392, 95% CI 1.121–1.729) [46]. Similar to the findings of increased mood disorder in the offspring in the Finnish population-based cohort study by Chen et al. [14], a prospective birth cohort study of a total of 1915 mother-child dyads in the United States found that children born to mothers with PCOS had higher risk of anxiety (aRR 1.62, 95% CI 1.02–2.57) compared to children born to mothers with PCOS [47].

Potential Reasons for Association Between Anxiety and PCOS

As with depression, several underlying risk factors are felt to contribute to the increased risk observed with PCOS and anxiety. In the general population, a recent meta-analysis of 25 studies reported increased anxiety in obese individuals as compared to normal weight individuals (pooled OR 1.30, 95%CI 1.20–1.41) [48]. As it is estimated that up to 80% of women with PCOS in the United States are obese [2], this factor could certainly play a contributing role. In a meta-regression of pooled SMDs, women with PCOS and concurrent anxiety had a higher mean BMI, Ferriman Galleway score, and free testosterone/free androgen index, but not HOMA- IR, compared to women with PCOS who did not have anxiety [5]. A Swedish study conducted in dermatology clinics found that higher levels of hirsutism was significant correlated with lower quality of life as well as increased symptoms of both anxiety and depression [49]. However, in the Finnish longitudinal cohort study, neither BMI nor hyperandrogenism were associated with anxiety symptoms, with high anxiety noted both in women with a BMI <25 kg/m^2 as well as 25–30 kg/m^2 as well as decreased anxiety scores noted in the highest free androgen index quartile [11].

Body image distress also correlates with anxiety, with a retrospective cohort study finding that Hospital Anxiety and Depression Scale (HADS) anxiety scores were significantly associated with body image subscales of appearance orientation, overweight preoccupation, self-classified weight, and body areas satisfaction. The higher anxiety scores seen in the PCOS population as compared to the control population was either completely or partially mediated by body image satisfaction as well [28]. Similarly, a cross-sectional study of 177 women with PCOS and 109 controls that anxiety in PCOS was predicted by negative body imagine including self-worth and lower perceived quality of life [26].

Proposed Treatments

Given the potential role of obesity and high androgens on anxiety risk, proposed treatments have included lifestyle management. In a Cochrane systematic review of the

impact of lifestyle changes in women with PCOS, lifestyle intervention was shown to improve the free androgen index (MD -1.11, 95% CI -1.96 to −0.26), although the quality of the evidence was low [50]. As mentioned previously, in the RCT by Oberg et al. evaluating the impact of a behavioral modification program consisting of structured training and lifestyle coaches, those in the intervention arm expressed less anxiety ($p = 0.035$) after 4 months compared to the control group [30]. In the secondary analysis of OWL-PCOS, the prevalence of anxiety decreased significantly from 15.9% to 4.7% in the lifestyle group (OR 0.30, 95% CI 0.10–0.85). OCPs do not seem to play a role however, since while the prevalence of anxiety decreased from 6.7% to 2.2% in the OCP group, this was not significant (OR 0.32, 95% CI 0.06–1.64) [33]. A small prospective observational study of 36 women with PCOS found that after 6 months of therapy with OCPs, while hirsutism and emotion domains of the Polycystic Ovary Synrome Health-Related Quality of Life Questionnaire (PCOSQ) improved, mean anxiety scores did not significantly change [32].

Since CBT can be used for the treatment of both depression and anxiety, the use of CBT to treat anxiety in women with PCOS also warrants exploration. However, a 16-week randomized clinical pilot investigating the use of CBT along with lifestyle interventions found that the addition of CBT did not significantly alter anxiety in women with PCOS, although stress responsiveness as measured by cortisol levels did improve [39]. Further trials are needed however to explore this strategy more closely. When looking at alternative therapies, a secondary analysis of a Swedish RCT found that after 16 weeks of acupuncture, exercise or neither, anxiety scores decreased modestly within the acupuncture group, although this did not differ from the control group [51]. A prospective pilot RCT evaluating the efficacy of electroacupuncture found decreases in serotonin levels, increases in neuroendocrine levels, and significant decreases in anxiety scores ($p = 0.003$) after 16 weeks of intervention compared to controls [52]. As with depression, there are no studies specifically evaluating the use of anxiolytics in women with PCOS. However, the international guidelines technical report recommends avoiding long-term use of benzodiazepines because of addiction issues, urge caution with use of low-dose antipsychotics due to problems with weight gain and metabolic issues, and suggest consideration for SSRI if first-line psychotherapeutic treatment has not succeeded [1].

PCOS and Eating Disorders

Association as Seen in Literature

Eating disorders (EDs) which include anorexia nervosa (AN), bulimia nervosa (BN), binge eating disorder (BED), and otherwise specified feeding and EDs are a class of DSM-5 disorders associated with unhealthy preoccupations with eating, exercise, and body weight [53]. Body image disturbances are central to eating disorders as often people with ED will have large discrepancies between their ideal and perceived body shape and weight. EDs are relatively common in the general population, with a female lifetime prevalence of 3.5% for BED and 1.5% for BN. There is

also a strong relationship between EDs and anxiety as well as depression, for example, up to 50% of individuals with BN have experienced major depression and 12% also have generalized anxiety disorder [54]. Because women with PCOS are already at risk for body image distress, anxiety, and depression as described earlier, the association with EDs is an important area for further investigation.

In one cross-sectional study of 148 women with PCOS and 106 controls, women with PCOS were at increased risk for overall abnormal eating disorder questionnaire scores OR 4.75, 95% CI 1.36–16.58 [55]. In an Australian cross-sectional study of 501 women with PCOS and 398 without, disordered eating, though not eating disorders, was more common in women with PCOS [56]. In another cross-sectional study of women enrolled in the Australian Longitudinal Study comparing 875 women with PCOS versus 7592 without, after adjusting for confounders such as socioeconomic factors and BMI, women with PCOS were more likely to report eating disorders (aOR 1.6, 95% CI 1.2–2.0) [57]. A recent systematic review and meta-analysis of 470 women with PCOS and 390 controls found that of 7 studies included in the meta-analysis, odds of an abnormal ED score (OR 3.05, 95% CI 1.33–6.99) and the odds of any ED diagnosis (OR 3.87, 95% CI 1.43–10.49; four studies) were higher in women with PCOS compared to controls [58].

Potential Reasons for Association

As mentioned earlier, increased weight as well as depression and anxiety may all confer a greater risk for eating disorders. In unadjusted analyses of the cross-sectional study by Lee et al., BMI (OR, 1.06, 95% CI 1.01–1.11), elevated depression score (OR 5.43, 95% CI 1.85–15.88), and elevated anxiety score (OR 6.60, 95% CI 2.45–17.76) were associated with abnormal global scores on the eating disorder questionnaire [55]. In the study by Pirotta et al., increased BMI elevated the odds of both disordering eating and eating disorders (OR 1.03, 95% CI 1.01–1.05 and OR 1.04, 95%CI 1.02–1.06, respectively), but PCOS diagnosis did not (OR 1.43, 95%CI 0.96–2.13 and OR 1.38, 95%CI 0.97–1.95, respectively) [56]. Interestingly in the Australian Longitudinal Study, being obese was not associated with an increased odds for eating disorders (aOR 0.8, 95% CI 0.6–1.0), although being underweight was (aOR 2.5, 95% CI 1.8–3.4) [57]. However, in a prospective cohort study of 164 women with PCOS, eating disorder questionnaire scores were higher in those with PCOS compared to the normative population, and obesity at baseline was associated with a 6.9-fold increase in the odds of elevated scores (aOR 6.89, 95% CI 2.70–17.62), while a positive depression screen conferred 3.6-fold increased odds (aOR 3.58, 95% CI 1.74–7.35) [59]. Depression may also play a mediating role for the relationship between body dissatisfaction and disordered eating, as suggested by a Norwegian study using structural equation modeling, which found that of the 320 students in the general population it evaluated with various survey tools, the effect of body dissatisfaction on restrained eating, binge eating, and compensatory behavior may be completely mediated by self-esteem and depression [60].

Hyperandrogenism and insulin resistance have also been proposed as potential hypotheses for the relationship between PCOS and eating disorders. Elevated testosterone, for example, may promote food cravings and promote bulimic behaviors via its effects on impulse control [61]. One small study in the general population found that normal-weight women with bulimia nervosa displayed significantly higher levels of free testosterone compared to age-matched controls ($p = 0.03$) [62]. Others have also studied use of anti-androgenic therapies in the form of OCPs to improve bulimic behavior with some improvement noted in relation to decreased androgen levels [63]. It has been postulated that the hyperinsulinemia known to independently be associated with PCOS creates increased food cravings, particularly for foods high in carbohydrates [64]. This may then lead to the development and maintenance of binge eating. Interestingly, young adults with type 1 diabetes have also been shown to have higher rates of eating disorders, though mechanisms are still being elucidated and often are associated with insulin restriction [65, 66]. Hyperinsulinemia also promotes hyperandrogenism thereby creating a vicious cycle with significant psychological implications [67].

Proposed Treatments

An important point raised in the literature is the complexity of managing the metabolic and mood disorder risks associated with PCOS through lifestyle management. Even though obesity has been shown to be associated with multiple physical and mental health consequences in this population, due to the association with eating disorders, counseling about weight loss may have unintended consequences that should be carefully considered. Obese women with eating disorders report higher shame and guilt as well as lower self-esteem, which PCOS women are also independently predisposed to, further complicating therapy strategies [68].

There are currently no studies evaluating treatment of eating disorders specifically in women with PCOS. One case report suggested an integrative model with comprehensive CBT to treat the anxiety, depression, and disordered eating in a 19-year-old with PCOS [69]. An RCT of 52 women with PCOS found significant improvements in body image and self-esteem with the use of acceptance and commitment therapy. In this study, Iranian women aged 18–45 were allocated to a control arm or intervention arm, which consisted of eight 90-minute weekly sessions focusing on goals ranging from creating creativity from hopelessness and identifying and removing obstacles to commitment actions. Body image concern and self-esteem scores were significantly lower within the intervention group after intervention ($p = 0.001$ and $p < 0.001$ respectively) and significantly lower compared to the control group for body image concern ($p = 0.03$) [70]. In the general population, various forms of psychotherapy have emerged including family-based therapy, cognitive remediation therapy, and interpersonal psychotherapy to assist those with eating disorders [71]. These suggest the importance of taking a complementary and creative approach to helping this population.

Gaps in the Literature

Although great strides have been made to understand the relationship between PCOS and mental health issues, much remains to be determined. It is yet unclear which direction some findings are in, for example, if the depression seen in women with PCOS predisposes to body image distress or if perhaps body image issues result in the increased depressive symptoms observed. The emergence of an association between PCOS and postpartum depression has also gained attention more recently; however confirmation of findings in a prospective nature is necessary as well as evaluation of current treatments for postpartum depression in PCOS women. Given the significant impact of postpartum depression on maternal health, further exploration is urgently needed. As described above, although the association of PCOS with eating disorders is gaining prominence, the reasons for this association in the PCOS population and treatment strategies need to be evaluated in further detail. Data in a wider age range of PCOS patients is also needed, such as larger studies evaluating prevalence estimates in adolescents and menopausal women as well as intervention trials in these age groups.

While medication use has been evaluated as they impact metabolic parameters and quality of life, larger prospective RCT studies focusing specifically on treatment interventions within the PCOS population are lacking. Limitations include inadequate studies to evaluate effectiveness of proposed interventions such as insulin-sensitizing agents on depression, CBT on anxiety and eating disorders, and antidepressant or anxiolytic medication use in this population. As several studies have been cross-sectional or observational in nature, selection bias in PCOS versus control inclusion, misclassification bias in PCOS designation, and absence of adequate mechanisms to account for potential confounding can all impact the accuracy of observed intervention findings.

Conclusion

PCOS is a complex reproductive disorder with multiple metabolic consequences. However, its impact reaches beyond just fertility or metabolic syndrome. PCOS's association with mental disorders is becoming well-known, with significant implications on lifetime risk for depression and anxiety. It has also been shown that women with PCOS are at a greater risk for certain types of eating disorders. (See Table 19.2.) The etiology behind these associations is likely multifactorial, with body image issues, hyperandrogenism, insulin resistance, and obesity all thought to contribute. Therefore, the treatment likely needs to take a similar multi-pronged collaborative approach as well between reproductive endocrinologists, primary care providers, gynecologists, dieticians, and mental health providers. Lifestyle management, CBT, and treatment of the symptoms of PCOS are all important, though much remains to be determined on the best way to provide holistic care to this unique population.

Table 19.2 Summary of results

Disorder	Measures of association	Proposed treatments
Depression	*Major depression:* OR 3.25, 95% CI 1.73–6.09 (5) *Perinatal:* OR 1.27, 95% CI 1.22–1.33 (18) *Postpartum:* OR 1.46, 95% CI 1.36–1.57 (18)	Lifestyle modification CBT OCP Pioglitazone Antidepressants
Anxiety	OR 5.62, 95% CI 3.22–9.80 (5)	Lifestyle modification CBT Acupuncture SSRIs
Eating disorders	Abnormal ED score (OR 3.05, 95% CI 1.33–6.99) [58] ED diagnosis (OR 3.87, 95% CI 1.43–10.49) [58]	Psychotherapy

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Obstructive Sleep Apnea and Polycystic Ovary Syndrome

20

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Abbreviations

AHI	Apnea-hypopnea index
BMI	Body mass index
CPAP	Continuous positive airway pressure
OSA	Obstructive sleep apnea
PCOS	Polycystic ovarian syndrome

Key Points

- The prevalence of OSA among women in the general population is estimated to be 6%. In comparison, the prevalence of OSA in women with PCOS is estimated to be 35%, with the risk for OSA in adults with PCOS being approximately 5–10-fold higher than weight-matched adult controls without PCOS.
- Sex hormones play a role in the pathogenesis of OSA in PCOS. Progesterone and estrogen are thought to be protective hormones in OSA development, whereas androgens (including testosterone) may promote OSA development.
- Standard screening tools for OSA include STOP, STOP-BANG, the Berlin questionnaire, and the Epworth Sleepiness Scale.
- Treatment options include lifestyle measures for select patients (e.g., weight loss for overweight patients, non-supine sleep positioning for those with supine-dependent OSA, avoidance of alcohol and sedatives before sleep time), continuous positive airway pressure (CPAP) therapy, mandibular advancement devices, and orthognathic and soft tissue upper airway surgeries for select patients.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_20

- Early treatment of PCOS may reduce the risk of developing OSA. OSA treatment can reduce OSA severity and improve sleepiness, sleep-related quality of life, and blood pressure in the general population. Treatment of OSA in PCOS may have a role in reducing cardiovascular and metabolic risk.

Background of Obstructive Sleep Apnea

Obstructive sleep apnea (OSA) is a common sleep-breathing disorder in which there are cessations or near-cessations in breathing during sleep due to repetitive upper airway obstruction [1] (see Fig. 20.1a). OSA diagnosis is defined by at least five upper airway obstructive events (apneas and hypopneas) per hour of sleep, resulting in an apnea-hypopnea index (AHI) ≥ 5 [2]. The repetitive upper airway obstruction during sleep may result in arousal from sleep, oxygen desaturations, and heightened sympathetic drive [3]. Without treatment, there are adverse neurocognitive, endocrine, and cardiovascular outcomes. OSA is associated with numerous comorbid conditions, including hypertension; cardiovascular events such as strokes, myocardial infarcts, and death [4, 5]; cardiac arrhythmias [6]; diabetes; cognitive dysfunction [7]; mood disorders [8]; and all-cause mortality [9].

OSA is associated with a doubling of medical expenses, primarily driven by increased cardiovascular morbidities costs [10]. Excessive daytime sleepiness, one of the classic symptoms of untreated OSA, has been associated with increased societal burdens with increased motor vehicle accidents, workplace accidents resulting in disability, loss in work productivity, and absenteeism [11].

Epidemiology of OSA

Based on data from the Wisconsin Sleep Cohort Study consisting of 1520 randomly selected participants, the estimated prevalence of moderate-severe OSA (AHI $\geq$15) is approximately 13% in men and 6% in women between the ages of 30 and 70. Given the increasing prevalence of obesity in the United States, the prevalence of OSA is predicted to increase as well [12]. The prevalence is highest in older adults, men, and higher body mass index (BMI). Premenopausal women are the lowest risk group, with an estimated prevalence of 3% [13].

OSA in PCOS

In a meta-analysis of 17 studies involving 648 PCOS participants, the prevalence of OSA in PCOS is estimated to be 35%, with a higher prevalence in obese women with PCOS than lean women with PCOS (limited data from 3 studies with 40 lean women with PCOS) and a higher prevalence in PCOS patients than healthy controls (odds ratio = 3.83, 95%CI 1.43–10.24, 8 studies, 957 participants [349 PCOS and 608 controls]) [14]. The prevalence of OSA is higher in adults with PCOS (32%)

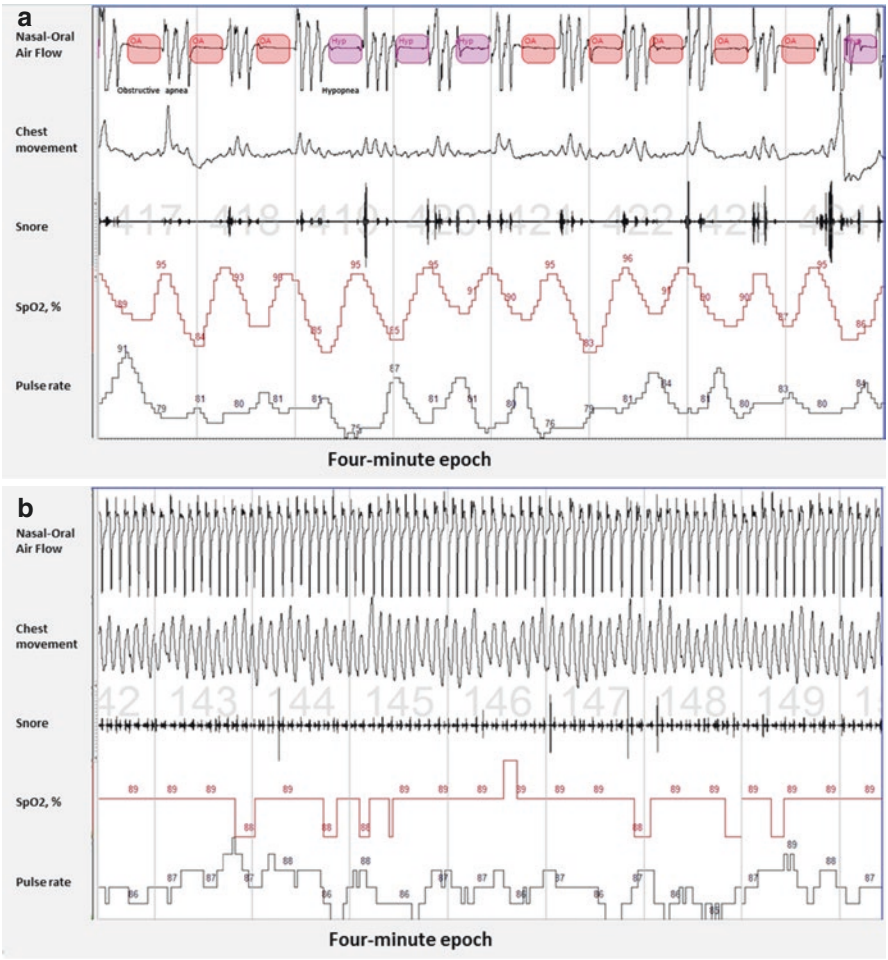

Fig. 20.1 (**a**) Example of a home sleep study tracing of a 39-year-old woman with a history of untreated PCOS who presented with symptoms of habitual snoring and gasping awakenings and was found to have severe OSA with an apnea-hypopnea index (AHI) of 66 events/hour. The 4-minute window tracing above demonstrates back-to-back obstructive respiratory events associated with snoring, cyclic oxygen desaturations, and heart rate variability. (**b**) This tracing of the same patient shows a period of stable breathing with non-apneic snoring

compared to adolescents with PCOS (~8%) [13]. There is no significant difference in AHI on polysomnography in adolescents with PCOS with and without elevated free androgen index compared to healthy age- and BMI-matched controls suggesting that OSA arises later in life in patients with PCOS [15–17]. Interestingly the risk for OSA is 5–10-fold higher in adults with PCOS when compared to controls with similar BMI, suggesting that obesity alone is not the sole driver in the pathophysiology of OSA in this population [18, 19]. Furthermore, the AHI in women with PCOS

is higher than in their age- and BMI-matched controls. AHI correlated with waist-hip ratio and serum testosterone levels and unbound testosterone in women with PCOS [20]. This finding points to the critical role of androgen excess and the android (apple-shaped) distribution of visceral fat in disease development.

Role of Sex Hormones in the Control of Breathing

The sex hormones progesterone, estrogen, and androgens all play a role in breathing regulation [21]. Progesterone and estrogen are thought to be protective hormones in OSA development, whereas androgens (including testosterone) may promote OSA development [22–25]. Changes in hormones during the menstrual cycle have been shown to affect sleep. During the luteal stage of menstruation, there is a surge and peak in the level of progesterone. There is evidence that heightened progesterone levels during the luteal phase are associated with increased upper airway dilatory muscle activity [26] and reduced upper airway resistance compared to the menstrual cycle's follicular phase [27], thereby promoting patency of the upper airway and preventing obstructive respiratory events during sleep. Medroxyprogesterone improves nocturnal ventilation with increased CO2 removal during sleep [28, 29]. Administration of medroxyprogesterone also reduced obstructive apneas and improved daytime sleepiness in patients with OSA [30]. During pregnancy, the level of serum progesterone positively correlates with gestational age. It negatively correlates with maternal weight, and when adjusted for gestational age and maternal weight, the serum progesterone level is significantly lower in pregnant women with OSA [31]. This finding suggests that progesterone plays a protective role in stabilizing breathing during pregnancy.

The prevalence of OSA increases post-menopause when there is a loss of progesterone and estrogen protective sex hormones. Various studies have shown that post-menopausal women experience more nighttime awakenings and worse sleep quality, which can be due to undiagnosed and untreated sleep apnea [32, 33]. The relationship between decreased estrogen and progesterone levels and clinically significant sleep-disordered breathing is likely impacted by other factors such as age, obesity, and menopausal symptomatology. In one study, administering gonadotropin-releasing hormone agonist Lupron in healthy, non-obese young women to induce "medical menopause" did not result in sleep fragmentation or clinically significant sleep-disordered breathing [34].

The role of androgens in the development of sleep-disordered breathing is based on the link between androgen and central adiposity. In a longitudinal study, children with an increased waist circumference at baseline, or central adiposity, were more likely to have a higher AHI later in life [35]. Early case reports describe a strong link between testosterone therapy and the development of OSA in young men [36]; however, more recently, it has been shown that testosterone therapy has a time-limited effect on OSA. Testosterone administration in obese men with severe OSA seems to worsen oxygen desaturation index and nocturnal hypoxia at 7 weeks but not at 14 weeks [37, 38].

Role of Adiposity in OSA

The distribution of adipose tissue plays a role in gender differences in the development of OSA. In one prospective study, dual-energy absorptiometry was used to measure the percentage of fat and lean body mass in 60 men and 36 women recruited from a sleep clinic. The android or central distribution of fat was a strong predictor of elevated AHI in both men and women. However, in women only, the percentage of fat distribution in the neck was correlated with AHI severity. In women, increased adiposity accounts for the increase in neck circumference, resulting in a narrower airway. In contrast, in men, increased neck circumference typically comprises a higher percentage of lean muscle to adipose tissue [39].

Pathophysiology of OSA in PCOS

Hormonal Dysfunction Theory

Given the link between sex hormones and OSA, patients with PCOS are at an increased risk for developing OSA. Women with PCOS are known to have low progesterone and estrogen levels as they are functionally anovulatory or oligoovulatory. The lack of sufficient levels of these protective sex hormones and the relative or absolute excess of androgens may predispose women with PCOS to have increased upper airway collapsibility and reduced ventilatory drive resulting in the obstructive airway and possible hypoventilation during sleep.

Signs and/or biochemical evidence of hyperandrogenism and central adiposity are common features of PCOS. In a study of 18 women with PCOS and 18 age- and BMI-matched controls, there was a positive correlation between total serum testosterone level and the severity of OSA as measured by the AHI ($r = 0.52$, $p < 0.03$). There was also a positive correlation between waist-to-hip ratio and AHI ($r = 0.51$, $p < 0.03$) [20]. This study suggests that BMI alone is not the sole driver of the severity of OSA in patients with PCOS and that androgen levels may play a significant role in disease severity.

Association with Metabolic Syndrome

Retrospective studies have shown that among teens with PCOS, those with OSA had significantly higher proportions of metabolic syndrome (56.3% vs. 8.3%, $p = 0.03$), higher insulin resistance (81.3% vs. 41.6%, $p = 0.03$), lower HDL (38.6 ± 8.7 vs. 49 ± 10.9, $p = 0.01$), and elevated triglycerides (149.7 ± 87.7 vs. 93.3 ± 25.8, $p = 0.03$) compared to those without OSA [40, 41].

Other studies have also shown that women with PCOS and OSA, especially those with a clinically significant disease that warranted treatment, have increased insulin resistance and a worse lipid profile than women without OSA [42, 43].

OSA has a bidirectional link with type 2 diabetes, and this relationship may be further mediated by PCOS. OSA via intermittent hypoxemia, sleep debt, and sleep fragmentation can lead to dysregulated glucose metabolism and increased risk of type 2 diabetes. On the other hand, studies looking at how type 2 diabetes may lead to OSA suggest that diabetic neuropathy may lead to impaired control of respiratory and upper airway neural reflexes [44].

Diagnosis of OSA

Symptoms

The classic symptoms of OSA are habitual (i.e., loud, disruptive) snoring, witnessed apneas (often reported by the patient's bed partner), gasping or choking awakenings, sleep maintenance insomnia, excessive daytime sleepiness, and nonrestorative sleep. Patients may also report waking up with a dry mouth or a morning headache [1] (see Table 20.1). Less recognized OSA symptoms are nocturia in adults and enuresis in pediatric patients, which is partly due to atrial stretch and the release of

Table 20.1 Symptoms and signs of OSA and other sleep-related conditions to consider in the differential diagnosis

	Symptoms and signs of OSA	Differential diagnosis
Breathing disruption	Loud habitual snoring Witnessed apnea Nocturnal gasping/choking	Nonapneic snoring Laryngospasm Nocturnal asthma Nocturnal reflux Paroxysmal nocturnal dyspnea
Sleep-related symptoms	Nocturnal awakenings Nocturia/enuresis	Primary insomnia Sleep-related movement disorder Insomnia due to medical factors impacting sleep (e.g., pain, vasomotor symptoms) Insomnia due to medication or substance
Waking symptoms	Nonrestorative sleep Morning headache Excessive daytime sleepiness Decreased energy Decreased focus	Short sleep duration Other sleep or medical disorder fragmenting sleep Hypersomnia due to medical disorder Hypersomnia due to medication or substance Primary hypersomnia (e.g., narcolepsy)
Signs	Retrognathia Crowded oropharynx Nasal obstruction Enlarged tonsils/adenoids Large neck circumference (>16 inches women, >17 inches in men) Obesity	

the atrial natriuretic peptide as a response to negative intrathoracic pressure of breathing against a closed airway [45].

However, women may not present with the classic men's symptoms of habitual snoring, daytime sleepiness, and witnessed apneas. Their primary symptoms may be insomnia, fatigue, or morning headaches and depression. These atypical symptoms are less likely to trigger a referral for OSA evaluation, resulting in underdiagnosis [46–49]. These women are often worked up instead for a primary mood disorder, anxiety, viral or tick-borne infections, or hypothyroidism before being referred for a sleep evaluation.

Screening Questionnaires

Standard screening tools for OSA include STOP [50], STOP-BANG [51], the Berlin questionnaire [52], and the Epworth Sleepiness Scale [53] (see Table 20.2). The effectiveness of these screening tools is extrapolated to the PCOS population. There are no studies to date comparing the use of these various screening tools in the PCOS population.

Table 20.2 The standard screening tools used in evaluating suspected OSA, their sensitivity/specificity and positive/negative predictive values in diagnosing OSA (AHI >5), and other considerations

Screening tool	Sensitivity/ specificity	PPV/ NPV	Considerations
STOP score ≥ 2	66%/ 60%	78%/ 44%	Quickest to administer Most reliable in older men with BMI > 35 and neck circumference >40 cm
STOP-BANG score ≥ 3	87%/ 31%	44%/ 80%	Requires a measuring tape that may not be readily available in the provider's office Higher scores are correlated with increasing severity of OSA Higher scores in surgical patients correlate with more difficult intubation, increased postoperative complications, and increased risk for postoperative ICU admission Less useful in identifying OSA in the veteran population and the patients with renal failure
Berlin questionnaire score as "high risk"	86%/ 77%	89%/–	A more complex questionnaire with segmented scoring that takes more time Highly sensitive with highest PPV
Epworth Sleepiness Scale score >10	–	–	Designed to measure daytime sleepiness, which can be used to differentiate between primary snoring vs. OSA (mean scores 6.5 ± 3 vs. 11.7 ± 4.6) Scores can be abnormal in other sleep disorders, including narcolepsy, idiopathic hypersomnia, and periodic limb movement disorder

STOP-BANG has eight items: snoring, tiredness during the daytime, observed apneas, high blood pressure, BMI > 35 kg/m^2, age (>50 years old), neck circumference (>40 cm, >16″), and gender (male). Of note, later versions of STOP-BANG separated cut-offs for neck circumferences based on gender (i.e., neck circumference >17″ or >43 cm for males and >16″ or >41 cm for females) [54]. One point is assigned to each positive answer, and a score of ≥3 indicates a high risk for OSA.

The STOP questionnaire is the abbreviated version of STOP-BANG with only the first four items. A score of ≥2 indicates an increased risk for OSA [50, 51].

The Berlin questionnaire is another screening questionnaire for assessing OSA risk. It consists of three categories: snoring severity, excessive daytime sleepiness (EDS), and history of hypertension or obesity. The presence of two or more categories increases the risk for OSA [52].

The Epworth Sleepiness Scale is a widely used questionnaire that assesses for excessive daytime sleepiness. Patients are asked to rate the likelihood of their dozing or nodding off in 8 situations (e.g., while being a passenger in a car for more than 1 hour without a break, while watching TV, while sitting after lunch without alcohol, and other situations of inactivity). Each situation is given a score of 0–3, with 0 beings "would never doze off" and 3 being a "high chance of dozing off." A total score of ≥10 is considered abnormal [55].

Testing

Polysomnography is the gold standard in the detection of OSA [2]. This test involves an overnight stay at a sleep center where sleep, breathing, oxygenation, heart rhythm, movements, and other select parameters are measured. Home sleep apnea testing, a form of limited channel testing, is a feasible, cost-effective, and accurate tool in diagnosing OSA in patients with a high pre-test probability of having sleep apnea, e.g., scoring ≥two on STOP or ≥three on STOP-BANG [56, 57]. However, home sleep apnea testing tends to underestimate sleep apnea compared with polysomnography and should be followed up with a more sensitive test if clinical suspicion persists [58–60]. According to the 3rd edition of the *International Classification of Sleep Disorders* (ICSD) manual published by the American Academy of Sleep Medicine (AASM), a diagnosis of OSA is made where there is at least one of the following clinical symptoms or a comorbid condition plus polysomnography or home sleep apnea testing demonstrating five or more predominantly obstructive respiratory events per hour of sleep or an AHI ≥5 (Table 20.3). The clinical symptoms include sleepiness, nonrestorative sleep, insomnia, gasping or choking awakening, habitual snoring, or witnessed apnea and the comorbid conditions include hypertension, mood disorder, cognitive dysfunction, coronary heart disease, stroke, congestive heart failure, atrial fibrillation, or type 2 diabetes mellitus. In the absence of clinical symptoms or comorbidities, a polysomnography or home sleep apnea testing must show 15 or more predominantly obstructive respiratory events per hour during sleep (AHI ≥15) to meet the diagnostic criteria for OSA [1].

Table 20.3 Classification of OSA based on the criteria defined by the American Academy of Sleep Medicine

	Apnea-hypopnea index, events/hour
Mild OSA	5–14.9[a]
Moderate OSA	15–29.9
Severe OSA	≥30

[a]Plus at least one comorbid condition or symptom. Comorbid conditions include hypertension, mood disorder, cognitive dysfunction, coronary heart disease, stroke, congestive heart failure, atrial fibrillation, or type 2 diabetes mellitus. Symptoms include sleepiness, nonrestorative sleep, insomnia, gasping or choking awakening, habitual snoring, or witnessed apnea

Studies have shown gender differences in the effectiveness of these screening tools. A prospective study assessed STOP and Berlin questionnaires in 350 men and 350 women referred to a sleep clinic with OSA symptoms. The STOP questionnaire was more sensitive in women with severe OSA (70.1% vs. 44.1%) compared to men. The Berlin questionnaire was more sensitive in women with mild OSA (80.7% vs. 60%) than men [46].

Screening and Testing in Patients with PCOS

Women with PCOS seem to have higher sleep disturbances rates, including insomnia and hypersomnia, compared to women without PCOS [61–64]. These symptoms may be related to untreated sleep-disordered breathing, and a high level of suspicion must be maintained to allow timely evaluation.

In a study looking at provider practices in screening and testing for OSA in patients with PCOS, findings show that only a minority (0–25%) of patients with PCOS were referred for a sleep study [65] despite the high prevalence of OSA in patients with PCOS. The lack of awareness of the link between PCOS and OSA or the atypical symptoms that women may present with are potential reasons for the low rate of referrals and testing for OSA and other sleep abnormalities in women with PCOS.

Treatment of OSA

Treatment is indicated in individuals with AHI ≥ 5 plus symptoms (e.g., insomnia, daytime sleepiness) or comorbidities known to be impacted by OSA (e.g., hypertension, cardiovascular disease) or in individuals with AHI ≥ 15 regardless of symptoms or comorbidities (see Table 20.3).

Treatment options include lifestyle measures for select patients (e.g., weight loss for overweight patients, non-supine sleep positioning for those with supine-dependent OSA, avoidance of alcohol and sedatives before sleep time), continuous positive airway pressure (CPAP) therapy, mandibular advancement devices, and

orthognathic and soft tissue upper airway surgeries for select patients (see Table 20.4) [54, 66]. Approaching OSA as a chronic disease utilizing a patient-centered approach may improve patient-reported outcome measures [67].

The mainstay of therapy is CPAP, particularly for individuals with at least moderate OSA, because of its high efficacy and minimal side effects [54]. CPAP acts as a positive pressure splint to maintain an open airway during sleep (see Figs. 20.2 and 20.1b). CPAP can be delivered in multiple modes (e.g., continuous positive airway pressure, auto-titrating positive airway pressure, or bilevel positive airway pressure), with nasal or oronasal interfaces. In straightforward OSA cases, a typical starting auto-titrating PAP setting of 5–20 cm H20 can be used as a rule of thumb. Careful monitoring of the patient's data download can assist providers in narrowing the auto-titrating pressure range.

In a recent systematic review and meta-analysis, PAP was associated with significantly improved OSA severity and improved sleepiness, sleep-related quality of life, and blood pressure [68]. The results are mixed in PAP therapy's effect on glycemic control in those with type 2 diabetes mellitus, with some evidence suggesting a 0.4% decrease in HbA1c levels in those with poor glycemic control at baseline [44].

Table 20.4 The strengths and limitations of available OSA treatment options

	Strengths	Limitations
Weight loss	Effective in patients with obesity	May be difficult to achieve and maintain May reduce AHI but not resolve OSA
Positional therapy	Effective in patients with mild-moderate positional OSA	Involves the use of positioner devices, vibrating alarms, etc. which can be challenging to adhere to A side effect of musculoskeletal pain
CPAP therapy	Very effective in all patients Minimal side effects	Issues with adherence
Oral appliance (mandibular advancement device)	Effective in patients with lower BMI, lower AHI, smaller neck circumference, younger age, and female gender	Costly and may not be covered by health insurance May reduce AHI but not resolve OSA A side effect of bite change
Upper airway soft tissue surgery	Effective in ~50% of adult patients	Is a surgical procedure with inherent surgical risks
Maxillomandibular surgery	Very effective in select patients with mandibular retrognathia	Is a surgical procedure with inherent surgical risks
Hypoglossal nerve stimulation	Effective in select patients with BMI <32 and favorable airway findings	Costly and invasive procedure requiring device implantation Long-term outcomes have not been published Contraindication with MR imaging depending on the device

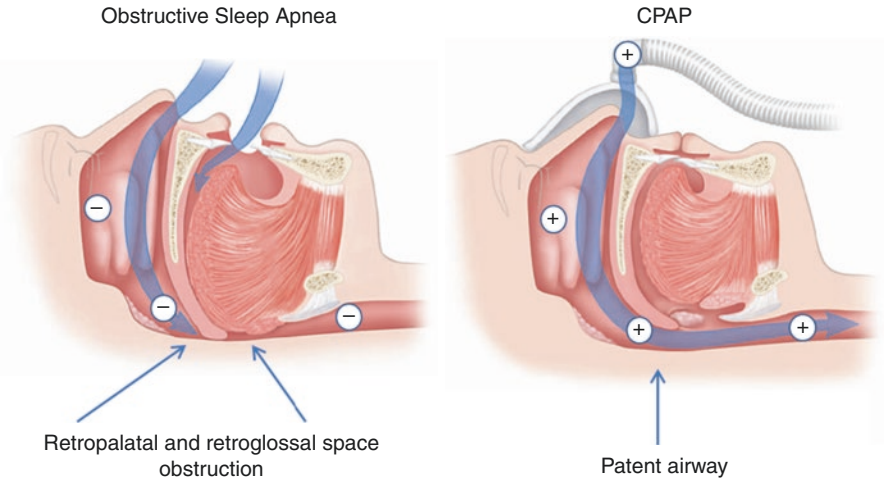

Fig. 20.2 The upper airway on the left demonstrates obstruction at the level of retropalatal and retroglossal space. With the application of nasal CPAP on the right, the upper airway is splinted open with positive air pressure

Despite the effectiveness of PAP therapy, adherence remains a significant barrier to successful therapy. Adherence (defined by the device's use for ≥4 hours for ≥70% of the nights) is estimated to range from 40 to 85%. Predictors of failure include mild OSA, asymptomatic OSA, nasal congestion, poor mask fit, smoking status, and lack of social support [69]. Psychiatric conditions such as PTSD in the veteran population have also been linked to suboptimal adherence [70]. Poor health literacy is associated with undiagnosed OSA and potentially poor treatment adherence resulting from a poor understanding of the disease process [71, 72]. Educational, supportive, behavioral interventions and a patient-centered approach can improve adherence [67, 73]. Often simple measures such as encouraging a patient to try a different mask interface or prescribing intranasal steroids to maintain nasal patency can help mitigate non-adherence. The further phenotyping of OSA, such as low arousal threshold to airway narrowing, unstable ventilatory control (high loop gain), and low responsiveness of the upper airway dilator muscles during sleep, can also be used to personalize the treatment approach [74, 75].

Weight loss is effective in OSA and is recommended for overweight and obese patients with the disorder [76]. A weight loss goal of 10% of body weight has been shown to have a modest effect on AHI and the overall severity of OSA [77]. Both medical and surgical weight loss programs may be beneficial [78, 79]. However, because weight loss is difficult to achieve and may not be maintained, another treatment is often indicated.

Worsening of OSA is common, with 20% of individuals in a recent large series having exclusively positional sleep apnea [80]. Positional OSA is more common in those with mild-moderate OSA compared to ones with the severe disorder. Positional therapy or adjunctive measures to decrease supine sleep (e.g., positioner devices,

vibrating alarms) may benefit these individuals. Based on a Cochrane review, positional treatment effectively reduces the AHI and sleepiness but did not impact the quality of life or cognitive function [81].

Oral appliances that function to protrude the mandible and tongue may reduce upper airway obstruction during sleep. In a meta-analysis comparing oral appliances to CPAP, adherence was significantly better with oral appliance therapy, but AHI reduction was greater with CPAP. There were no differences in quality of life, cognitive, or functional outcomes [82]. Individual patient characteristics such as lower BMI, lower AHI, smaller neck circumference, younger age, and female gender are predictors of response to oral appliance therapy [83]. Oral appliances are recommended as an alternative to CPAP in those who prefer oral appliance therapy or are intolerant to CPAP [84].

Upper airway soft tissue surgery is not as effective as CPAP or oral appliance therapy, with a reported success rate of 50–60% in improving OSA by greater than 50% in adult patients [85]. In contrast, maxillomandibular surgery has a greater than 90% success rate in selected patients with mandibular retrognathia. Newer treatments such as upper airway hypoglossal nerve neurostimulation can be effective in select patients with BMI < 32 and favorable airway findings but is not widely applicable [66, 86].

Considerations in Treatment of PCOS as It Relates to OSA

Prevention of OSA Is Key

Perhaps a key takeaway from the studies discussed earlier comparing polysomnographic findings in adolescents with and without PCOS is that OSA seems to develop later in the adult life of individuals with PCOS. Treatment of PCOS in young individuals may potentially reduce the later incidence of OSA.

El-Sharkawy and colleagues randomized 90 adolescent girls (ages 12 to 18) to 3 groups: control, untreated PCOS, and treated PCOS. PCOS was defined using the Rotterdam criteria. The treatment group received metformin 850 mg twice daily for 3 months. The results showed reductions in body weight, BMI and Epworth sleepiness scores, and improvements in the hirsutism score, fasting and postprandial blood glucose, fasting serum insulin, and HOMA (homeostatic model assessment) index when compared to the untreated group [87].

Longitudinal polysomnographic data at baseline and ~3 years of follow-up in 15 adolescent girls with PCOS treated for hyperandrogenism and insulin resistance showed no difference in the respiratory event index compared to the healthy controls [88]. In other words, early treatment of PCOS may potentially prevent, or at least delay, the development of OSA. While this study was small and did not show comparative longitudinal PSG data on adolescents with untreated PCOS, the results are favorable regarding the early treatment of PCOS.

Role of Weight Loss

Weight loss is a primary therapy in both OSA and PCOS. An average weight loss of 10% of body weight can result in a modest reduction in AHI and an overall improvement in OSA severity [77]. In PCOS, a weight reduction of 5% can restore regular menstrual cycles and improve fertility [89]. Among other benefits of weight loss include decreased adipose and androgen levels and an improvement in insulin resistance [90]. These benefits of reduced androgen and central adiposity can likely impact the pathogenesis of OSA.

There may be a role for bariatric surgery, especially for those who have failed pharmacologic and behavioral weight loss. Several small prospective studies have shown that bariatric surgery may reduce the severity of OSA or even cure OSA. However, the evidence is sparse, and there is yet to be a meta-analysis [91–94]. There are few publications on the effects of bariatric surgery on PCOS, but among those limited cases, an improvement in insulin resistance, hyperandrogenism, and menstrual irregularities was observed [95, 96].

Role of CPAP

Data on the effect of CPAP in PCOS are limited. CPAP therapy can significantly improve OSA severity and symptoms of daytime sleepiness, quality of life, and mood. PAP can improve blood pressure indices in those with hypertension and improves insulin sensitivity indices [68, 97, 98]. Since insulin resistance plays a significant role in developing cardiovascular disease, CPAP's role in improving insulin sensitivity may help reduce the risk of cardiovascular disease in this population.

In a small prospective study comparing CPAP treatment outcomes in women with PCOS and OSA, CPAP adherence resulted in a 25% reduction in plasma norepinephrine levels at eight weeks compared to the pretreatment baseline [97]. Norepinephrine is a neurotransmitter of the sympathetic system, and the heightened sympathetic activity is thought to increase cardiovascular risk and worsen insulin resistance [99, 100]. Therefore, by dampening norepinephrine levels, CPAP may improve the cardiovascular and metabolic risks in PCOS.

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Endometrial Cancer and Precancer in Polycystic Ovary Syndrome

21

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Key Points

- With the increasing prevalence of obesity in the United States, there has been a consequent increase in the risk of endometrial cancer.
- Most cases of endometrial cancer are type I and are estrogen dependent and ER/PR positive, demonstrate microsatellite instability, and tend to present as low-grade tumors often with mutations in KRAS or PTEN oncogenes.
- Endometrial hyperplasia, caused by chronic unopposed estrogen exposure, is generally considered the precursor lesion to type I endometrial cancer.
- Long-term unopposed estrogen stimulation can occur as the result of anovulation, estrogen-producing tumors, and unopposed estrogen therapy and in obese patients.
- Both PCOS and endometrial cancer are characterized by hormonal dysregulation such as hyperandrogenemia, chronic anovulation, and insulin resistance.
- Obesity exacerbates the symptoms of PCOS by worsening low-grade inflammation and insulin resistance.
- There is an increased risk of endometrial cancer in the women with PCOS.
- Both insulin and IGF-1 levels are elevated in PCOS and have been shown to accelerate the growth of endometrial cancer.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_21

- Proteomic studies have identified that the P53 and PTEN genes have the highest number of mutations of in genes shared between PCOS and endometrial cancer.
- Women with PCOS and endometrial cancer have a higher risk of obesity, diabetes, infertility, and cardiovascular disease.
- As the population continues to age, the number of women living with both endometrial cancer and PCOS will increase in size, and as such it will be important to prevent and treat the health problems that arise as a result.

Introduction

Epidemiology of Uterine Cancer

Endometrial cancer (EC) is the most common gynecologic malignancy in the United States with an overall lifetime risk of 2.8% [1]. Most women with EC are peri- or postmenopausal with an average age at diagnosis of 63 years. However, approximately 20% will be diagnosed before menopause, and recent data from the Surveillance, Epidemiology, and End Results program shows a persistent rise in cases among women under 50 years old [2]. As opposed to most other cancers, the incidence and associated mortality of EC continues to rise, most likely due to the increasing prevalence of obesity and metabolic syndrome [3, 4]. It is predicted that by 2030 the incidence of EC will rise to 42.13 cases per 100,000 women, a 55% increase from the incidence in 2010 [5]. Although the rates of EC are rising for all women, the escalation is most dramatic for Black women who also have a higher risk of aggressive endometrial histologies and worse 5-year mortality rates [2].

Endometrial Precancers

Endometrial hyperplasia is a precancerous, non-physiological, noninvasive proliferation of the endometrium that results in endometrial glands of irregular size and shape with an increase in the ratio of glands to stroma. It is caused by chronic excess estrogen stimulation that is unopposed by progesterone. Endometrial hyperplasia used to be classified using the World Health Organization (WHO) 1994 classification system which subdivides the hyperplasia based on the severity of glandular complexity (simple or complex) and cytologic atypia (with or without atypia). Based on this classification system, endometrial hyperplasia exists on a continuum beginning with simple hyperplasia without atypia and progressing to complex hyperplasia with atypia. The severity and presence of cytological atypia is the most important prognostic factor for carcinogenesis [2, 4, 6, 7]. A study by Kurman et al. reported that 1% of patients with simple hyperplasia, 3% of patients with complex hyperplasia, 8% of patients with simple atypical hyperplasia, and 23% of patients with complex atypical hyperplasia progressed to EC [8].

The WHO classification system was simplified in 2014 to include only two categories: hyperplasia without atypia and hyperplasia with atypia (i.e., endometrial

intraepithelial neoplasia (EIN)). Hyperplasia without atypia infrequently progresses to EC (1–3%). EIN is the precursor lesion to endometrioid ECs. Up to 60% of women with EIN will develop, or have already developed occult, invasive disease [9].

Uterine Carcinogenesis

Most cases of EC are associated with long-term unopposed estrogen stimulation. This can be due to chronic anovulation, as seen in PCOS, estrogen-producing tumors, unopposed estrogen therapy, and obese individuals where there is excessive peripheral conversion of androstenedione to estrone and testosterone to estradiol in adipose tissue. EC, compared to all other cancers, has the strongest association with obesity. In the United States 57% of all ECs are associated with obesity. A meta-analysis by the American Institute for Cancer Research showed that for every increase of five BMI units, there was a 50% increase in the risk of developing EC (RR 1.50, CI 1.42–1.59 [10]). Obese patients may also have lower levels of sex hormone binding globulin (SHBG) and insulin resistance which both also contribute to endometrial carcinogenesis. Compared to women with normal BMIs, the relative risk of mortality for women with a BMI of 30–34.9 kg/m^2 is 2.53, and for women with a BMI greater than 40 kg/m^2, the risk increases to 6.25 [5].

Chronic anovulation, common during perimenopause and in women with PCOS, is also a risk factor for EC. It results in unopposed estrogen stimulation and continued endometrial proliferation because there is no corpus luteum to produce progesterone and trigger endometrial decidualization. The risk of EC is also positively correlated with a younger age of menarche and later age of menopause. The underlying mechanism of this association is likely prolonged lifetime duration of exposure to estrogen stimulation. Nulliparity is also associated with an increased risk of disease. A large meta-analysis by Raglan et al. found a 40% reduction in EC incidence among parous women compared to nulliparous women [11]. This is likely due to greater progesterone production during pregnancy which has protective effects on the endometrium.

Unopposed estrogen therapy in postmenopausal women with intact uteri significantly increases the risk of endometrial hyperplasia and carcinoma. After 1 year of receiving unopposed estrogen therapy, 20–50 percent of women will develop endometrial hyperplasia [12]. Oral estrogen therapy is associated with a 2- to 12-fold increased risk of EC, and increased duration and dosage are associated with increased risk [4]. Tamoxifen, a selective estrogen receptor modulator with antiestrogenic effects in the breast, reduces the risk of breast cancer recurrence but doubles the risk of uterine cancer due to its pro-estrogenic effect on the endometrium. This risk may even be quadrupled if tamoxifen is used for more than 5 years [13]. In addition to its agonistic effect on the endometrium, tamoxifen also increases the risk of EC by promoting cytoskeletal remodeling, inducing DNA damage, and causing the migration of EC cells [14].

Hyperestrogenism, Hyperinsulinemia, and Risk of PCOS

Hormonal Dysregulation in PCOS and EC

PCOS is an endocrine-metabolic disorder characterized by ovulatory dysfunction, polycystic ovarian morphology, hyperandrogenism, and insulin resistance. The majority of excess androgens in women with PCOS are produced by the theca cells of the ovary in response to increased levels of insulin and luteinizing hormone (LH). In addition, there are also intrinsic changes in the ovary that lead to increased androgen production. The theca cells of women with PCOS overexpress the LH receptor and have been shown to be more responsive to the stimulating action of LH and insulin than the theca cells of healthy control subjects [15]. In granulosa cells of women with PCOS, there is reduced aromatase activity, resulting in less peripheral conversion of androstenedione to estradiol and higher circulating androgen levels. Patients with PCOS also show higher activity of the enzyme 5-alpha-reductase which converts testosterone into dihydrotestosterone, an even more "potent" androgen [16].

An estimated 65–80% of women with PCOS demonstrate insulin resistance. This decrease in insulin responsiveness is independent of BMI, although obesity has an impact on glucose homeostasis and can worsen hyperandrogenism. Insulin resistance leads to compensatory hyperinsulinemia which both stimulates androgenesis by ovarian theca cells and inhibits the hepatic production of SHBG, thereby increasing the availability of free testosterone [17].

In addition to hyperinsulinemia and hyperandrogenism, women with PCOS also demonstrate abnormal secretion of LH, FSH, and anti-Mullerian hormone (AMH). During a regular ovulatory cycle, progesterone inhibits the secretion of GnRH from the hypothalamus via negative feedback during the luteal phase. However, in women with PCOS, high levels of androgens decrease the negative feedback on GnRH, leading to hypersecretion of LH by the pituitary. These increased LH levels stimulate androgen secretion by ovarian theca cells which, in turn, promote increasing numbers of primordial follicles to grow into antral follicles [15].

At the level of the ovary, the selection of the dominant follicle that proceeds to ovulation does not occur regularly, leading to anovulation. Although FSH levels are usually normal in the PCOS phenotype, the ovarian follicles show resistance to FSH. These follicles arrest in the preovulatory stage and never fully mature, giving rise to the classic polycystic appearance of the ovary in PCOS [18]. It is unclear whether this FSH resistance is fundamental to the disorder or whether it is a consequence of high levels of AMH secreted by the large number of pre-antral follicles. As well as inducing follicular arrest, high levels of AMH also increase androgen levels by inhibiting aromatase activity and blocking the conversion of androgens to estrogens [15].

Many of the hormonal abnormalities that define PCOS are established risk factors for EC. Chronic anovulation, one of the hallmark features of PCOS, causes prolonged exposure to unopposed estrogen and can lead to hyperplastic endometrial changes. It has also been shown that IGF-1, whose levels are raised in PCOS,

also stimulates the growth of EC cells [19]. Insulin resistance is also a risk factor for EC. It has been observed that fasting insulin levels are positively associated with EC risk and that insulin resistance is prevalent among both obese and non-obese women with EC. Excess insulin causes endometrial changes similar to that of unopposed estrogen by binding to insulin-binding sites expressed in the ECs. Insulin promotes endometrial carcinogenesis by increasing androgen production, supplying more substrate for conversion into estrogen. It does this by inhibiting the synthesis of SHBG and by upregulating aromatase activity in endometrial epithelium [20, 21].

Diseases associated with insulin resistance, such as obesity, type II diabetes, and PCOS are risk factors for EC. In a prospective cohort study, the RR for EC among women with diabetes compared to non-diabetic women was 1.94 (95% CI 1.23–3.08). The RR was even higher for obese diabetics (RR 6.39, 95% CI 3.28–12.06) compared to non-obese women without diabetes [22].

Anovulation

The classic ovarian phenotype of PCOS is characterized by increased numbers of early antral follicles, arrested at 5–8 mm diameter size. FSH, AMH, estradiol, and androgens are all necessary for ovarian folliculogenesis. In PCOS altered levels of these hormones lead to ovulatory dysfunction [15]. Increased serum LH causes premature luteinization of follicles, causing the granulosa cells within the follicle to stop dividing. Insulin contributes to antral follicle arrest by sensitizing granulosa cells to LH which increases the chance of premature luteinization and follicular arrest. Although many obese women with PCOS show insulin resistance, the effect of insulin on steroidogenesis is paradoxically preserved and can even be heightened [23]. Excess AMH, secreted by pre-antral and small antral follicles, may also contribute to anovulation in PCOS by inhibiting the maturation of follicles. Several studies have demonstrated that serum AMH concentration is elevated in women with PCOS compared to women without. It has been observed that the granulosa cells of women with anovulatory PCOS secrete approximately 75 times more AMH and show increased AMH mRNA expression compared to normal ovaries [24].

Association of PCOS with Metabolic Syndrome

Metabolic syndrome and PCOS share many similar features and appear to be interrelated. There are many different definitions of metabolic syndrome, but all include central obesity, hypertension, dyslipidemia, and impaired fasting glucose. The prevalence of metabolic syndrome is estimated to be between 2 and 4 times higher in women with PCOS compared with women in the general population with the risk being particularly high in younger women [25]. Essah et al. [26] found that at the time of PCOS diagnosis, 91% of women had at least one component of metabolic

syndrome and that 69% had at least two components. They also showed that low-serum HDL cholesterol was present in 68% of women and high BMI was present in 67% of women with PCOS. Approximately 75% of women with PCOS are overweight and central obesity has been observed in both thin and obese women with PCOS [25]. Obesity exacerbates the symptoms of PCOS by worsening chronic low-grade inflammation and insulin resistance. Increased insulin resistance raises insulin levels which, in turn, worsens the hyperandrogenic state by promoting androgen production by theca cells and inhibiting the production of SHBG. This likely represents the underlying explanation for the greater incidence of hirsutism and menstrual irregularities in obese women with PCOS [25, 26]. Women with PCOS also have metabolic abnormalities that are independent of obesity, showing much higher rates of hypertension, dyslipidemia, glucose intolerance, and type II DM than the general female population [26]. Although the exact pathophysiology that connects metabolic syndrome and PCOS is unclear, it is theorized that insulin resistance is probably the main underlying abnormality that links the two.

Treatment of PCOS

The treatment of PCOS is multifocal, aimed at decreasing androgen secretion and action, protecting the endometrium from unopposed estrogen, improving metabolic status, and improving fertility. Combination oral contraceptive pills are the traditional medical therapy for the long-term management of PCOS. They provide endometrial protection, restore the menstrual cycle, reduce ovarian androgen production, and increase circulating SHBG [27]. Insulin-sensitizing drugs are another long-term treatment option. Metformin is the most widely used and studied insulin-sensitizing agent in the treatment of PCOS. It is a biguanide that is FDA-approved for the management of type II diabetes. It works by inhibiting hepatic glucose production and increasing glucose uptake into muscle. This subsequently increases insulin sensitivity and reduces hyperinsulinemia and insulin secretion. Metformin has also been shown to reduce blood pressure and increase levels of SHBG [28]. A systematic review and meta-analysis of randomized control trials by Costello et al. [29] compared metformin to OCPs in the treatment of PCOS. They found that OCPs were superior to metformin in improving menstrual irregularities and lowering testosterone levels, but that metformin was superior in lowering fasting insulin and triglyceride levels.

Thiazolidinediones (TZDs) are another class of insulin-sensitizing agents that can be used to treat PCOS. They work by binding to the nuclear gamma-peroxisome proliferator activator receptor (PPARγ) and inducing gene activation and transcription. TZDs have been shown to improve insulin resistance and lower androgen levels in women with PCOS. While they are more effective at decreasing insulin levels and improving insulin resistance than metformin, they are not as effective at reducing BMI and triglyceride levels. Despite their effectiveness, TZDs are not currently recommended for the routine treatment of PCOS due to the potential for serious adverse effects [15, 27, 28].

Epidemiology of EC in PCOS

Several studies have demonstrated associations between PCOS and EC, but the causality of this association remains unclear. Ding et al. [30] conducted a population-based cohort study of Taiwanese women to determine the effects of PCOS on the risk of EC. The study included 8155 women, aged 15–49 years, diagnosed with PCOS. After a mean follow-up period of 6 years, they found that 11 patients in the PCOS group and 3 patients in the comparison group developed EC with an incidence of 22.6 and 1.5 per 100,000 person-years in the PCOS and comparison groups. PCOS patients had a significantly increased risk of EC (HR 17.7; 95% CI = 4.9–64.2) compared to the group without PCOS. However, this study is limited by a short follow-up period that did not capture many women over the age of 60 when the majority of ECs are diagnosed.

A similar registry-based retrospective cohort study was conducted by Gottschau et al. [31] that included 12,070 women with PCOS in Denmark from 1977 to 2012. The cohort was followed for a median follow-up time of 5.7 years. Overall, they found a fourfold increased risk of EC in women with PCOS (SIR = 3.9; 95% CI = 2.2–6.3), representing an excess absolute risk of 13.1 per 100,000 person-years. However, this study did not adjust for BMI which is an important risk factor for EC and is highly prevalent among women with PCOS.

A systematic review and meta-analysis of observational studies on this topic by Barry et al. also suggests that women of all ages with PCOS are at an increased risk of EC [32]. Five studies quantifying the risk of EC in women with PCOS were included. Unlike previous reviews on this topic, this review conducted a subgroup analysis stratified by menopausal status as the authors theorized this may alter the outcome. The results suggest that women of all ages with PCOS are at an increased risk of EC (OR 2.79; 95% CI = 1.31–5.95). When studies that included women older than 54 were excluded from the analysis, the risk of EC was even higher (OR 4.05; 95% CI = 2.42–6.76). However, there are several confounding variables that may have exaggerated the increased risk of EC. High BMI and diabetes are both known risk factors for EC. Due to the high prevalence obesity and diabetes in PCOS, it is unclear whether the increased risk of EC is due to PCOS itself or the individual risk factors of diabetes and obesity. This meta-analysis is limited by the variation in diagnostic criteria of PCOS used by the included studies, possibly introducing selection bias.

Most recently, Hanson et al. [33] performed a literature review evaluating the possible association between female infertility, infertility-associated diagnoses, and EC. Overall, they concluded that infertility related to PCOS shows a strong relationship with EC risk. Given the anovulatory nature of PCOS, it is intrinsically linked to infertility. However, it is unclear whether the increased risk of EC arises from PCOS, obesity, their infertility diagnosis, or a combination of these elements. The main limitation of this literature review is that not all of the studies included specified the cause of infertility, with several studies including patients based on an unspecified diagnosis of fertility. It is clearly important to try and clarify the exact diagnosis for a patient's infertility in order to deduce the most appropriate estimated risk of developing EC.

Infertility and the Risk of EC

PCOS is the most common cause of anovulatory infertility. Women with PCOS are 10 times more likely to develop infertility than the general female population [33]. Various studies have also suggested a link between infertility and EC. However, it is unclear if these associations are due to infertility itself or factors underlying the infertility, such as PCOS. A population-based cohort study of Swedish women by Lundberg et al. aimed to clarify the association between infertility and the risk of EC [34]. They found that infertile women had a higher incidence of EC compared to fertile women (aHR 1.25; 95% CI 1.11–1.40). This risk was even higher in women with both infertility and ovulatory disturbances (aHR 2.90; 95% CI 2.05–4.08). These results suggest that the risk of EC among women with infertility is associated with ovulatory disturbances, not that infertility itself is an independent risk factor for EC. The authors posited that PCOS is the likely factor underlying this association.

In a meta-analysis of case control and cohort studies, Skalkidou et al. observed the incidence of EC among 1.9 million women undergoing different infertility treatments [1]. Fifteen studies found an increased risk of EC after exposure to any ovary-stimulating drug compared to a general population control group (RR 1.75; 95% CI 1.18–2.61). Four studies found an increased risk of EC in infertile women treated with clomiphene citrate (RR 1.87; 95% CI 1.00–3.48). This risk is especially notable at higher doses (greater than 2000 mg) and with an increasing number of cycles (greater than 7). Clomiphene citrate is one of the most commonly used medications to treat infertility. It is a selective estrogen receptor and has similar chemical properties to tamoxifen, which is a risk factor for the development of EC. However, this association may be due to the underlying reason for the treatment with clomiphene citrate, such as PCOS, rather than the actual infertility treatment itself.

Genetics/Genomics

A complex interplay of genetics, epigenetics, and environment has been implicated in the pathogenesis of both EC and PCOS (Table 21.1). The molecular basis for EC has traditionally fallen under the histopathologic classification system. Type I ECs, defined histologically as Stage I/II endometrioid ECs, present as low-grade, well-differentiated tumors and tend to be less aggressive in their clinical course. They are commonly estrogen dependent and ER/PR positive, demonstrate microsatellite instability, and are often mutated in KRAS or PTEN oncogenes [35]. Alternatively, type II endometrial carcinomas consist of high-grade endometrioid adenocarcinomas, as well as non-endometrioid histologies such as uterine serous carcinoma (USC) and clear cell carcinoma. These cancers are, by definition, poorly differentiated and portend a worse prognosis than their type I counterparts. Type II ECs are often associated with mutations in CDKN2A, TP53, and ERBB2 [36].

Recently, an alternative classification system was developed based on integrated genomic, transcriptomic, and proteomic characterizations – transcending the

Table 21.1 Hormonal, lipidomic, proteomic, and microbiome features shared between endometrial hyperplasia polycystic ovary syndrome and putative systemic downstream effects increasing the risk of endometrial cancer

	Shared features	Downstream effects
Hormonal dysregulation	Hyperestrogenemia	Increased estrogen receptor expression in the endometrium Increased endometrial progesterone receptor expression/decreased endometrial responsiveness to progesterone
	Hyperinsulinemia	Decreased production of insulin growth factor binding protein Promotion of insulin-like growth factor activity and overexpression of endometrial IGF-1 receptor Increased androgen production and subsequent peripheral aromatization to estrogen Activation of PI3K transduction pathway and increased AKT phosphorylation
	Luteinizing hormone hypersecretion	Overexpression of luteinizing hormone and hCG receptors in the endometrium
Lipidomic dysregulation	Decreased monoacylglycerol 24:0 and capric acid	Unchecked inflammatory response and cancer cell proliferation
	NAD(P)H:quinone oxidoreductase 1 (NQOO1) overexpression	Dysregulation of oxidative stress and lipid metabolism
	Sterol regulatory element binding protein 1 (SREBP1) overexpression	Alterations in lipogenesis Activation of PI3K/AKT pathway leading to cell growth and proliferation
Proteomic dysregulation	Alterations in expression of peptidyl-prolyl cis-transisomerase (FKBP3), cyclophilin A and complement component C4 A	Disruption of immunoregulation and inflammation pathways
	Decreased expression of glycolytic enzymes and mitochondrial activity	Endometrial proliferation mediated through the estrogen receptor pathway
Microbiomic dysregulation	Increased enteric production of active estrogen via beta glucuronidase	Hyperestrogenemia and endometrial proliferation

traditional histo- and clinicopathologic categorization [37] and, thus, further emphasizing the importance of understanding molecular mechanisms in the pathophysiology of endometrial malignancy. While the exact mechanism underlying the relationship between EC and PCOS has yet to be completely elucidated, several similar clinical and metabolic characteristics, including obesity, chronic anovulation, hyperestrogenism, and hyperinsulinemia have been demonstrated indicating the potential for a common pathophysiology. Chronic unopposed estrogen exposure is generally viewed as the precursor to type I EC, and the majority of studies looking

at an association between PCOS and EC focus on this pathologic finding as the intermediary between the two diseases. Given this, estrogen receptor (ER) expression within the endometrium has been a target of investigation. While the expression of ER is known to vary between the secretory and proliferative phases, studies demonstrate an overall increased expression of both ER-α and ER-β in PCOS patients with hyperplasia [38]. It has also been well documented that patients with type I EC exhibit increased levels of ER expression [37, 39]. Increased ER expression results in increased endometrial proliferation and hyperplasia, pathologic characteristics demonstrated in both EC and PCOS. While there have not been any commonly identified genetic ER mutations that facilitate endometrial growth, it is clear that aberrant expression of ER is closely linked to pathophysiology of both diseases.

While estrogen promotes endometrial cell proliferation, progesterone is primarily responsible for endometrial cell differentiation. Patients with PCOS demonstrate impaired endometrial responsiveness to progesterone and a compensatory overall higher endometrial progesterone receptor (PR) expression [40]. It is this progesterone resistance that likely exacerbates endometrial hyperplasia and contributes to uncontrolled endometrial growth [41]. Interestingly, patients with copy-number low EC cluster also demonstrate increased endometrial PR expression [37], implicating steroid hormone dysregulation as an associated component in the neoplastic process. Progesterone therapy, a known treatment for both PCOS and EC, inhibits endometrial ER expression and thus decreases the mitogenic effects of estrogen, preventing uncontrolled and dysregulated endometrial proliferation [38].

There is an intimate relationship between the endocrine and steroid hormone pathways in the pathogenesis of both EC and PCOS. Insulin and insulin resistance have often been touted as the link between EC and PCOS. Hyperinsulinemia ultimately decreases the production of insulin growth factor binding protein (IGFBP) and thereby promotes activity of insulin-like growth factor (IGF-1). This stimulates steroidogenesis and results in increased androgen production, which ultimately aromatizes to estrogen [42]; thus it begins the cascade of promoting dysregulated endometrial cell growth, hyperplasia, and eventually EC. Insulin and IGF-1 play a central role in modulating endometrial proliferation and the downstream pathogenesis of EC [42]. Both insulin and IGF-1 are elevated in PCOS and have been shown to accelerate the growth of EC in vitro [43]. Non-genomic estrogen receptor signaling in the uterus can also cause activation of IGF-1 receptors, which is thought to play a role in EC development [39]. IGF-1 receptors, which are overexpressed in both endometrial hyperplasia and EC [44, 45], have even been shown to directly correlate with histological grade of EC [46]. Shafiee et al. [47] demonstrated that IGF-1, IGFBP-1, and PTEN were all significantly expressed in the endometrium of both PCOS and EC patients compared to control patients. In fact, Kashima et al. [48] demonstrated that EC growth was suppressed when IGF-1 receptors were knocked down in vitro. Together, these studies further solidify the link between endocrine and steroid hormone pathways in the pathogenesis of both EC and PCOS.

In addition to aberrant steroid hormone pathways, the dysregulated HPO axis in PCOS patients results in hypersecretion of LH which has been implicated in the

development of EC in women with PCOS [49, 50]. This is thought to be mediated through an overexpression of LH/hCG receptors in the endometrium, which has been demonstrated in women with EC and in anovulatory women with endometrial hyperplasia, including those with PCOS [51]. A study conducted by Villavicencio et al. [52] demonstrated increased expression of androgen receptor and estrogen receptor β in hyperplastic PCOS endometrium compared to normal PCOS endometrium. This was corroborated in further studies, which demonstrated elevated levels of androgen receptor (AR) expression in proliferative and secretory phases of the menstrual cycle of PCOS endometrium and PCOS endometrium with hyperplasia [53]. While it is hard to assess the individual role of androgens as they are intrinsically linked to insulin (hyperinsulinemia promotes hyperandrogenism), it is clear that they play a role in the pathophysiology of EC and PCOS. Together the aberrant expression of various hormone receptors displays yet another link between EC and PCOS and solidifies the role of the endocrine hormone pathway in the shared etiology of these diseases.

The phosphatidylinositol 3-kinase /protein kinase B (PI(3)K/AKT) pathway is an intracellular transduction pathway that plays an essential role in regulating the cell cycle. Errors in this signaling pathway demonstrate decreased apoptosis with subsequent uncontrolled cellular proliferation and have been implicated in numerous cancer phenotypes [54]. It is well-known that several elements within the PI(3)K/AKT pathway often exhibit mutations in EC [54]. Phosphatase and tensin homolog (PTEN) protein is a known inhibitor of the PI(3)K/AKT pathway [55]. Epigenetic and genetic modifications of the PTEN tumor suppressor gene are the most common mechanism leading to dysregulated PI3K signaling. Alterations in PTEN are the most frequent aberrations seen in type I EC, and they are also commonly observed in endometrial hyperplasia [56] – demonstrated in up to 20% of all endometrial hyperplasia [57]. Meanwhile, similar mechanisms of cell cycle deregulation and uncontrolled proliferation have been demonstrated through the PI(3)K/AKT pathway in PCOS. Villavicencio et al. [58] revealed that PCOS patients with endometrial hyperplasia exhibit increased levels of AKT phosphorylation – signaling dysregulation in the PI(3)K/AKT pathway. Further demonstrating the interdependent relationship between the endocrine and steroid hormone pathways, insulin is a known activator of the PI3K transduction pathway and stimulation of endometrial hyperplasia. Hyperinsulinemia and elevated IGF-1, known pathogenic states in PCOS patients, are thought to promote endometrial mitogenesis through the PI(3)K/AKT pathway [42]. It is clear that the PI(3)K/AKT pathway presents an intermediary in the pathogenesis of PCOS and EC and requires further investigation to more firmly elucidate this relationship.

Lipidomics/Metabolomics/Proteomics

While there has been increasing evidence that metabolomics play an important role in our understanding of disease, there remains a paucity of large-scale lipidomic/proteomic/metabolomic data looking at the association between EC and polycystic

ovary syndrome. Derangements in adipocyte, lipid, and fatty acid metabolism have been correlated with an increased risk of EC – the proposed mechanism thought to be secondary to either inflammation or the increased release of fatty acids from uncontrolled cellular proliferation [59]. One large-scale study assessing the endometrial lipidomic profiles in women with PCOS and EC compared to controls demonstrated two metabolites that were similarly decreased in PCOS and EC, monoacylglycerol 24:0, and capric acid [60]. While the role of monoacylglycerol 24:0 is unknown in the pathogenesis of these diseases, it is thought that capric acid may play an important role in regulating the malignant inflammatory response and even inhibiting cancer cell proliferation in vitro [61]. Another study, conducted by Atiomo et al. [62], assessed the endometrial transcriptome of women with EC and PCOS and identified 94 shared genes, of which NAD(P)H:quinone oxidoreductase 1 (NQO1) was particularly prominent. NQO1 expression is regulated by steroid hormones and has been implicated in numerous cancers. Additionally, NQO1 has been shown to regulate cellular oxidative status and play a role in lipid metabolism [63]. Its overexpression in both PCOS and EC specimens presents it as a possible molecular target linking these two diseases.

In a large systematic review [64], sterol regulatory element binding protein 1 (SREBP1) was noted to be elevated in both EC and PCOS endometrium. SREBP is a gene that helps regulate lipogenesis, and its expression is regulated by insulin and sterol levels in cells. In addition to aberrant expression in the endometrium of EC and PCOS patients, SREBP has been demonstrated to activate the PI3K/Akt pathway – promoting cell proliferation and growth [54] and further implicating a role for steroid dysregulation in the pathogenesis of malignancy.

The overall paucity of robust evidence linking EC and polycystic ovary syndrome on a metabolomic level further supports the need for large-scale bioinformatic analyses. A large review conducted by Yumiceba et al. (2020) [57] undertook significant 'omic evaluation of 264 strongly related PCOS genes in association with EC, ovarian cancer and breast cancer using oncogenic databases. This study revealed that 8% of PCOS-related genes demonstrate hallmarks of cancer. Particularly relevantly, they identified cancer driver genes specifically for EC. Of those, TP53 and PTEN were demonstrated to have the highest number of mutations of in genes shared between PCOS and EC. The identification of PTEN in proteomic studies looking at the association between EC and PCOS, as well as genetic studies focusing on these diseases, highlights this as a potential common mechanism and target for further research. This study also looked at PCOS-related gene expression profiling by matching tumors vs. normal tissue and indicated that 30–40% of genes were downregulated compared to normal; five of these genes (IGFF2, ERBB4, SLP1, CYP1B1, F13A1) were differentially expressed in EC in both the Yumiceba 'omic study [57] and the aforementioned Atiomo 'omic study [62]. The identification of these five genes among both studies demonstrates a shared genetic pathophysiology between EC and PCOS and emphasizes the need for further research to elucidate the exact mechanism. Furthermore, this study [57] identified transcriptional dysregulation in PCOS/gynecologic cancers, with approximately 30 proteins demonstrating altered expression in EC, OC, and BC. In summary, 36 genes were identified that

were involved in cell cycle regulation and steroid hormone pathways, thus supporting their association and promulgation of hormone driven oncogenesis.

There have been few studies to date which conducted thorough proteomic biomarker profiling of PCOS. Galazis et al. (2013) [64] conducted a large systematic review to identify proteomic biomarkers in women with PCOS that may demonstrate EC risk. They identified a total of nine protein biomarkers with similar expression patterns (either under or overexpressed) in women with EC and PCOS. At least 5 of these 9 biomarkers demonstrate some role in immunoregulation/inflammation pathways, and peptidyl-prolyl cis-transisomerase (FKBP3), cyclophilin A, and complement component C4A have all been implicated in both cancer and insulin resistance. Piltonen et al. [65] also implicated the immune response in potential cancer pathogenesis of women with PCOS, as demonstrated by an enhanced expression of cytokines and pro-oncogenic genes. Additionally, an analysis conducted by Wang et al. [66] assessed the role of glycolytic enzymes, ER, AR, and mitochondrial associated factors through protein expression levels. They concluded that patients with PCOS and hyperplasia demonstrated decreased glycolysis and increased mitochondrial activity, suggesting this as a possible role for malignant transformation of ERα-dependent endometrial hyperplasia in the oncogenic pathway.

It is clear that the etiology and relationship of PCOS and EC is multifactorial. One emerging area of research considers the role of the microbiome as a contributing factor in the pathophysiology of these diseases. Gut microbiota has been demonstrated to influence systemic health through both inflammatory and metabolic alterations [67], and dysbiosis of the microbiome has been implicated in several disease states, including obesity and diabetes – two comorbid pathologic states commonly associated with PCOS and EC [68]. The estrobolome, which refers to a collection of enteric bacterial genes that are capable of metabolizing estrogen, has been identified as a non-ovarian contributor of systemic estrogen/estrogen metabolites through its secretion of β-glucuronidase, an enzyme which deconjugates estrogen [69, 70]. This deconjugation renders estrogen unbound and biologically active and provides an additional source of systemic estrogen in already pathologic hyperestrogenic states. This natural pathophysiology introduces the gut microbiome as a targetable source of excess estrogen. Additionally, the diversity of gut microbiota is inversely related to obesity and BMI [70], and surgical intervention with bariatric surgery has been demonstrated to favorably alter the microbiome and metabolic profiles of obese women [67]. Interestingly, the risk of endometrial hyperplasia and subsequent development of EC has also been shown to be reduced after bariatric surgery in obese women [71, 72] – thereby further implicating the estrobolome as a potential key driver in estrogen-dependent states and providing a link between PCOS and EC.

PCOS and EC remain intimately linked and the investigation of large-scale bioinformatic analyses on the genetic, proteomic, metabolomic, lipidomic, and estrobolomic levels remain essential in elucidating the precise shared etiology of these diseases.

Aging/Survivorship/Health Implications After Menopause

While the population continues to age, the long-term health consequences of survivorship become increasingly prevalent. There are more than 1 million survivors of gynecologic malignancy in the United States currently, and this number is expected to continue to rise significantly over the next decade [73]. Meanwhile, PCOS is the most common endocrinopathy in women of reproductive age, and its prevalence is estimated to be anywhere from 6% to 10% [74]. Given this large epidemiologic burden, it is crucial to understand the health repercussions at the intersection of PCOS and EC to maximize the clinical care these patients receive and improve their long-term health outcomes. The comorbidities associated with EC and PCOS are well-known and include obesity, diabetes, infertility, metabolic syndrome, and cardiovascular disease [75].

Obesity is nearly ubiquitous in women with PCOS and EC and presents countless long-term health complications. The prevalence of obesity in women with PCOS is estimated to be up to 61% [74] and demonstrates racial and ethnic distributions, with greater rates of obesity seen in African American and indigenous women [76]. Higher central adiposity, most commonly found in PCOS, is associated with greater rates of insulin resistance [74]. This, in turn, results in the higher levels of T2DM seen in women with PCOS, with up to three times higher risk of T2DM [77]. Menopause, itself, is also associated with a higher likelihood of developing T2DM. Thus, postmenopausal patients with PCOS are at higher risk of developing T2DM and its subsequent downstream health repercussions. Lifestyle modifications have been recommended by the Endocrine Society as first-line therapy for the reduction of obesity and its subsequent downstream health implications in women with PCOS [78]. While there is a paucity of research investigating the role of EC as an individual risk factor for obesity, studies have suggested that lifestyle interventions such as healthy diet and cardiovascular exercise can also improve quality of life and survival [79] in cancer patients as well. It is evident that obesity presents significant long-term health complications for women with EC and PCOS and interventions to decrease obesity should be recommended for the health of these women.

Cardiovascular risk factors and disease are intrinsically tied to obesity and thus PCOS and EC. Dyslipidemia, defined as hypertriglyceridemia, high LDL and low HDL, has been demonstrated in up to 70% of PCOS patients [74]. Subclinical atherosclerosis, an independent risk factor for cardiovascular disease (CVD), has also been identified in women with PCOS [74]. Interestingly, these atherogenic lipid patterns are also seen in postmenopausal women and are commonly the result of, or associated with, insulin resistance [80]. There has been mixed evidence regarding an increased incidence of CVD in postmenopausal women with PCOS [81, 82], and more research is needed regarding the additive cardiovascular risks of PCOS and menopause. Simultaneously, an association between EC and cardiovascular disease has also been demonstrated. The link between hormones and cardiovascular disease is well established, as estrogen plays a cardioprotective role in pre-/early menopausal women [83]. This role is further demonstrated in women who undergo menopause prematurely (either naturally or surgically) have an increased risk of CVD [84]. As the survivorship for early stage EC is quite

high, it has been demonstrated that women with EC are more likely to die from cardiovascular disease than any other illness [85].

As nearly all of the individual risk factors for metabolic syndrome are seen in both PCOS and EC, the cardiometabolic complications of metabolic syndrome (CVD, T2DM, obesity) are also demonstrated to be higher in women with both diseases. Menopause itself also appears to be an independent risk factor for metabolic syndrome [80]. Meanwhile, evidence continues to mount highlighting the negative synergistic relationship between EC and metabolic syndrome. A recent review looking at the relationship between menopause, cardiovascular disease, and gynecologic cancers [86] highlighted the increased risk of EC in women with metabolic syndrome, independent of the confounding obesity risk factor. They demonstrated a nearly twofold increase risk of EC in women with metabolic syndrome and these cardiometabolic risk factors are exacerbated by the hypoestrogenic state of menopause, with worsening rates of metabolic syndrome seen after menopause [87]. The opportunity for prevention of the long-term health repercussions of these diseases lies in the premenopausal period with lifestyle interventions.

As the population continues to age, the number of women living with both EC and PCOS will continue to expand. These diseases not only require immediate attention at the time of diagnosis but also demonstrate lifelong health repercussions including obesity, insulin resistance, cardiovascular disease, and metabolic syndrome that cannot be ignored. It is our duty as clinicians to elucidate these risk factors and intervene to improve the overall morbidity and mortality of our patients with EC and PCOS.

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Economic Burden of Polycystic Ovary Syndrome

22

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Key Points

- Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women, affecting 5–20% of women of reproductive age, depending on the diagnostic criteria used.
- The economic burden of a disease, or cost of illness analysis, quantifies the losses of utility, social welfare, and/or income that ill-health directly or indirectly contributes towards.
- Only two studies thus far have attempted to estimate the economic burden of PCOS, both in the United States (U.S.).
- Considering the costs of the initial diagnostic evaluation, the treatment of menstrual dysfunction/abnormal uterine bleeding (AUB), hirsutism, and infertility, and the costs of pregnancy-related complications (i.e., gestational diabetes, gestational hypertension, and preeclampsia) and long-term health comorbidities (type 2 diabetes [T2DM], myocardial infarction, and stroke), the total economic burden of PCOS in the United States is approximately USD$7.9 billion annually in 2020 dollars.
- Taken together, the economic burden of PCOS-related strokes, menstrual dysfunction/AUB, and T2DM represented approximately 30%, 24%, and 19% of all

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_22

costs associated with the disorder, while the costs of treating PCOS-related hirsutism, infertility, and pregnancy complications accounted for approximately 11%, 10%, and 5% of total economic burden.
- The cost of the diagnostic evaluation represents less than 2% of total costs, suggesting that much of the economic burden of PCOS can be avoided if we simply invested in greater vigilance and assessment for the disorder.
- Of the annual healthcare spending in the United States, approximately 90% of total healthcare expenditure can be attributed to chronic conditions, including PCOS, highlighting the need to prevent or manage symptomatology in order to reduce disease burden.
- While there appears to be little differences in the prevalence of PCOS by race, ethnicity, or geography, it is possible that there are differences in the prevalence of comorbidities, which may impact economic burden estimations.
- We observed an attenuation of disease risk for both the sequelae of PCOS and the associated long-term consequences in the postmenopausal years, which may reflect the much stronger influence of age on the development of these comorbidities, suggesting that the economic burden differential of PCOS is primarily driven by morbidities evident during the reproductive age.
- PCOS research is underfunded by both industry and federal funding sources, considering its large prevalence, economic burden, metabolic morbidity, and negative impact on quality of life.
- In future economic burden research, calculating the economic impact of PCOS should include all indirect costs and other comorbidities affecting physical and behavioral health, as well as assessment of socioeconomic status and race/ethnicity,
- Greater education of industry, the US National Institute of Health (NIH) and other funding agencies in the United States and internationally, other governmental agencies, elected leaders, and the general public education regarding PCOS and its economic burden is critically needed.

PCOS: Diagnostic Criteria, Epidemiology, and Morbidities

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women, affecting 5–20% of women of reproductive age, depending on the diagnostic criteria used [1, 2]. Three sets of diagnostic criteria for PCOS have been proposed over the last 30 years. The first was developed by the National Institutes of Health (NIH) in 1990, which required the presence of both hyperandrogenism/hyperandrogenemia and oligo-ovulation (ovulatory disorder or OD), with or without the presence of polycystic ovarian morphology (PCOM) on ultrasound, and excluding other disorders such as Cushing's syndrome, hyperprolactinemia and congenital adrenal hyperplasia [3]. The NIH criteria result in two potential phenotypes, A and B (Table 22.1), often referred to as "classic PCOS."

The second set of criteria was based on expert consensus reached at a meeting cosponsored by the European Society for Human Reproduction and Embryology

Table 22.1 PCOS diagnostic criteria and phenotypes

	PCOS phenotypes			
Features	A	B	C	D
Clinical and/or biochemical hyperandrogenism	+	+	+	
Ovulatory dysfunction	+	+		+
Polycystic ovarian morphology	+		+	+
Diagnostic criteria				
NIH 1990	+	+		
Rotterdam 2003	+	+	+	+
AE-PCOS Society 2006	+	+	+	

(ESHRE) and the American Society for Reproductive Medicine (ASRM) in Rotterdam, the Netherlands, in 2003 [4]. These criteria, commonly referred to as the Rotterdam criteria, require that two of the following three features to be present for the diagnosis: (1) oligo- or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovarian morphology (PCOM) on ultrasound, after the exclusion of other etiologies. Institution of the Rotterdam criteria broadened the PCOS diagnosis and introduced phenotype C, "ovulatory PCOS," and phenotype D, "non-hyperandrogenic PCOS." Importantly, introduction of the Rotterdam criteria significantly increased the number of patients diagnosed with PCOS.

Finally, in 2006, the Androgen Excess and PCOS (AE-PCOS) Society published the third set of diagnostic guidelines based on recommendations of an expert task force [5]. In contrast to the Rotterdam criteria, the AE-PCOS criteria made androgen excess requisite for the diagnosis, with either ovulatory dysfunction or PCOM as the second criteria. This decision was based in part on evidence that androgen excess is the criterion most predictive of long-term metabolic dysfunction associated with PCOS [6–8]. The AE-PCOS diagnostic criteria therefore do not recognize phenotype D, the non-hyperandrogenic phenotype.

The concurrent use of three different diagnostic criteria, as well as varying access to ultrasound technology globally, contributed to confusion and controversy regarding best clinical practices and research in the area of PCOS. With the goal of establishing evidence-based guidelines for the initial evaluation and management of women with PCOS, the NIH sponsored a consensus conference in 2012 in which the published literature was reviewed by a panel of experts [9]. The recommendation from this conference regarding diagnosis of PCOS was to use the broader ESHRE/ASRM Rotterdam criteria along with specification of the individual patient's phenotype.

Estimating the prevalence of PCOS has been challenging, primarily due to variation in diagnostic criteria used and confounding of selection bias in the study populations. The first study that assessed PCOS prevalence in an unbiased population by Azziz and colleagues reported a prevalence of between 4% and 6.6% among unselected reproductive-age women in the southeast of the United States, using the stricter NIH 1990 criteria [10]. Subsequent population-based studies have estimated that the worldwide prevalence of PCOS ranges between 4% and 21% [11], with the higher rates determined when the ESHRE/ASRM Rotterdam criteria are applied

and the least variability in prevalence estimates across different regions when the NIH 1990 diagnostic criteria are used [11]. Ethnic variation in hirsutism, varying access to ultrasound technology and longitudinal medical care, choice of appropriate reference population, and selection bias are all significant barriers to accurate prevalence estimates and the study of PCOS in general.

Ethnic differences in the prevalence of obesity, metabolic dysfunction, and the pattern and density of terminal hair may influence a patient's presenting PCOS phenotype. While there does not appear to be a difference in PCOS prevalence or in hirsutism prevalence between White and Black women [10, 12], hirsutism is more commonly reported in women of Hispanic, Middle Eastern, and South Asian ethnicity [13, 14]. Additionally, at least preliminarily, the prevalence of PCOS may be higher in Hispanic-Americans [15]. Ethnic differences in the prevalence of obesity and body fat distribution, particularly central adiposity, may affect the risk of pregnancy-related complications, including gestational diabetes, gestational hypertension, labor dystocia and cesarean delivery, as well as long-term metabolic and vascular morbidities.

PCOS is associated with signs and symptoms that most often prompt a woman's first evaluation during the reproductive years, including menstrual irregularity, hirsutism, acne, and subfertility. Increased risk for long-term health consequences including type 2 diabetes (T2DM), metabolic disturbances, cardiovascular disease (CVD), mental health disorders, and neoplasia are also seen with the syndrome. Risks of the long-term health sequelae of PCOS are less clearly defined, due to lack of appropriately designed, adequately powered studies with adequate follow-up.

Increasing evidence suggests that the sequelae of PCOS, including the presenting signs/symptoms (anovulation, hyperandrogenism) as well as long-term metabolic consequences, diminish and may even disappear with age, particularly after menopause. This trend has been seen in a recent, large, long-term prospective population-based study [16], as well as several cohort studies [8, 17, 18]. The attenuation of disease risk in the postmenopausal years may reflect a protective effect of antecedent PCOS diagnosis or, more likely, the much stronger influence of age on the development of these comorbidities [19]. Our analysis confirms the tendency for the difference in the rates of metabolic and vascular dysfunction between PCOS and non-PCOS women to ameliorate as patients age and become menopausal (see below).

Estimating Economic Burden

The economic burden of a disease, or cost of illness analysis, quantifies the losses of utility, social welfare, and/or income that ill-health directly or indirectly contributes towards [20]. Economic disease burden is driven by a number of factors, including various categories of costs as well as the prevalence or incidence of the disease in the population being evaluated. The prevalence-based approach assesses both new and pre-existing illness in a given year and is utilized to calculate the total current economic burden of a disease. Alternatively, the incidence-based approach assesses only new cases in a given year and is used to ascertain expected burden of

a disease in the future [20]. The total economic burden of disease is driven by not only whether the prevalence or incidence approach is utilized but also by the scope of the estimated expenditures due to disease and its comorbidities for a given year. As such, there can be significant heterogeneity in the methodology for calculating the economic impact of a disease.

There are three different types of costs that factor into the economic burden calculation – *direct costs, indirect costs,* and *intangible costs*. *Direct costs* are the monetary value of resources used in prevention, diagnosis, treatment, and rehabilitation of a disease; in other words, these are expenses incurred due to the illness, which can be substantial. Direct costs include healthcare costs (i.e., medical expenditures like co-payments and out of pocket expenses for inpatient visits, outpatient visits, emergency room visits, prescription drugs, medical equipment, etc.) and non-healthcare costs (i.e., expenditures including transportation, childcare, etc. associated with the diagnosis, treatment, or rehabilitation of a disease).

Indirect costs are the value of lost productivity because of reduced working time due to disease-related disability, impacting the individual, family, employer, and society. These costs include the losses from labor force dropout, absenteeism (lost working days due to the illness itself or receiving treatment for the illness), presenteeism (reduced productivity while working compared to individuals without the morbidity in question), and deaths before retirement (at 65 years of age) due to the morbidity being evaluated. Indirect costs, or diminished productivity, are typically measured from patient self-reported data and evaluated against average annual or daily wages.

The illness being studied, accompanied by work loss, can contribute to decreased patient socialization and low self-esteem, such that these direct and indirect costs can lead to further costs known as intangible costs. *Intangible costs* are defined as the disvalue to an individual due to pain and suffering and consists of a wide range of adverse psychosocial factors [20].

All of the aforementioned categories of costs incurred are important contributors to the total economic burden; intangible costs, however, are often not included in economic burden studies due to difficulty of measurement and controversies. As such, the total economic burden of disease is often calculated as the sum of excess health expenditure and the value of forgone productivity due to the disease and its comorbidities, converting costs to the same base year prior to adjusting for current dollars using the consumer price index [21].

Assessing the economic burden of a disease is essential to determining the allocation of resources to potential health interventions and developing and implementing policies, with the ultimate goal of positively impacting patient outcomes. These estimates inform policymakers and decision-makers about the magnitude of economic losses and the distribution of expenditures across various cost categories [21]. Economic impact studies also help decision-makers understand the economic benefits of disease prevention, in other words, the monetary value of what could be saved through prevention, incentivizing stakeholders to take action to reduce disease burden and therefore decrease healthcare expenditure. As such, economic burden analyses are crucial for developing strategies for

both reducing health expenditures through preventive measures or varied treatment approaches and allocating resources and funding. For example, and as we will discuss below, a prior study has shown that PCOS is underfunded relative to other disorders with similar degrees of morbidity and similar or lower mortality and prevalence [22].

As economic burden is one of the factors that informs funding decisions, the scope of the analysis is important to accurately estimate the economic impact of the disease. As we will discuss, we should note that current studies quantifying the economic burden of PCOS tend to underestimate the cost of disease, providing at best a conservative picture of the adverse impact of the disorder. Future economic burden studies of PCOS should strive to address these limitations – by including all indirect costs and intangible costs as well as other comorbidities not included in current analyses – in order to provide a more comprehensive assessment of disease impact.

Economic Burden of PCOS

Only two studies thus far have attempted to estimate the economic burden of PCOS, both in the United States. In the first study, Azziz et al. performed a systematic literature review to obtain epidemiological data on PCOS and its clinical consequences and costs to estimate the economic burden of PCOS during the reproductive years [23]. The investigators chose to use a PCOS prevalence of 6.6%, estimate based on the NIH 1990 diagnostic criteria, for their economic burden calculations, as these criteria defined PCOS phenotypes (A and B) that were most often associated with metabolic consequences. A systematic review of the literature was performed to identify studies evaluating PCOS and its clinical consequences, in order to estimate the relative risks of the related health sequelae as compared to the general population. Direct attributable costs were estimated using data from the 2005 Redbook for drug costs, the 2004 Centers for Medicare and Medicaid Services (CMS) physician fee schedules, CMS clinical laboratory fee schedules, and the consumer price index for inflation.

The estimated cost of the initial diagnostic evaluation of women suspected of having PCOS included costs related to commonly ordered laboratory studies (i.e., total and free testosterone, DHEAS, TSH, prolactin, and 17-hydroxyprogesterone), as well as metabolic evaluation (i.e., basal and 2-h glucose and insulin levels, and a lipid profile). The clinical evaluation was presumed to include transvaginal ultrasonography for all patients and endometrial biopsy in select patients. Overall, the analysis found that the cost of the initial diagnostic evaluation for PCOS for all patients was relatively low, at USD$139 million annually in 2020 dollars.

For estimating the cost of treating menstrual dysfunction or abnormal uterine bleeding (AUB), the investigators assumed that all PCOS patients who suffer from AUB and do not desire pregnancy are treated with an oral contraceptive (OC) and that treatment with an OC will require an additional physician office visit each year. Using the average cost of an OC pack and a physician visit, it was

estimated that the annual cost of treating menstrual dysfunction/AUB is USD$1.9 billion annually in 2020 dollars.

The cost of treating hirsutism associated with PCOS was estimated. This calculation took into account both cosmetic and hormonal therapies, given that the investigators had previously reported that approximately 50% of hirsute PCOS women will undergo electrology at some time in their lives [12]. Based on this assumption and that 70% of PCOS women in the United States are hirsute, the authors estimated that the average annual cost of treating hirsutism in PCOS as being USD$875 million annually in 2020 dollars.

Finally, for the cost of treating infertility, an average of the cost per live birth across different states in the United States was used in order to encompass those couples who conceive after simple ovulation induction as well as those who require advanced reproductive technologies (ART). Estimating that 50% of PCOS patients will seek fertility care at some point in their lives, the average annual cost of infertility care for PCOS patients was estimated at USD$750 million in 2020 dollars.

In total, the cost of the initial diagnosis, evaluation, and treatment of the most common sequelae of PCOS in the reproductive years (menstrual dysfunction/AUB, infertility, and hirsutism) was estimated at roughly USD$3.7 billion in 2020 dollars annually [23].

In a separate follow-up study [24], Riestenberg et al. aimed to estimate the economic burden of PCOS due to pregnancy-related (i.e., gestational diabetes [GDM], gestational hypertension [gHTN], and preeclampsia) and long-term health complications (i.e., type 2 diabetes mellitus [T2DM], myocardial infarction [MI], and stroke) associated with the disorder. As before, the investigators performed a systematic review and meta-analysis of the health outcomes of interest to identify studies that compared a population of PCOS patients with an age and body mass index (BMI)-matched cohort of control women for inclusion in the meta-analyses. The odds ratio (OR) generated by the random-effects meta-analysis was then used to estimate the excess prevalence the health comorbidities attributable to PCOS, which was then used in combination with cost data to calculate total economic burden.

As with the prior study, the investigators chose to use a PCOS prevalence of 6.6% based on the NIH criteria, for the cost calculations in this study. Cost data for medical treatment of the health outcomes of interest were obtained from the Medical Expenditure Panel Survey (MEPS) data from the Agency for Healthcare Research and Quality (AHRQ) where possible [25] and otherwise from review of published literature of healthcare expense in the United States. Inflation was adjusted for using the consumer price index.

For the pregnancy-related comorbidities, the PCOS prevalence estimate was applied to the number of births in 2020 in order to estimate the approximate number of PCOS-related births per year. The OR as generated by the meta-analysis was then used to determine the excess burden of each pregnancy-related health complication. Compared to BMI-matched women without PCOS, women with PCOS were found to have a higher prevalence of GDM, gHTN, and preeclampsia. The OR for GDM, gHTN, and preeclampsia in PCOS was estimated to be 2.78, 2.04, and 2.03 and an excess cost of pregnancy-related care of USD$2265, USD$10,254, and USD

$12,352 per pregnancy, respectively. Taken together, the total excess economic burden of pregnancy-related health consequences in PCOS was estimated in to be $375 million USD annually in 2020 dollars.

Women with PCOS have higher rates of insulin resistance and secondary hyperinsulinemia than weight-matched controls and therefore, and unsurprisingly, a higher lifetime risk of T2DM and metabolic syndrome [26]. Other long-term health morbidities that have been associated with PCOS include obesity, neoplasia, mental health disorders, and possibly CVD and stroke. Given the prevalence of T2DM and vascular disease, particularly in the female population, the investigators included these outcomes in the cost analysis.

Compared to age- and BMI-matched women without PCOS, premenopausal women with PCOS had a higher prevalence of T2DM, with an OR of 2.41 (95% CI 2.1–3.13). Along with previously published age-based baseline prevalence and cost data [27], the excess annual cost of T2DM due to PCOS was estimated to be 1.5 billion USD annually in 2020 dollars.

Alternatively, the meta-analysis of studies comparing the risk of MI in PCOS to BMI-matched control women without PCOS did not find a statistically significant increased risk of this outcome compared to age- and BMI-matched controls (OR 1.46, 95% CI 0.88–2.42). MI was therefore excluded from the calculations of economic burden. Alternatively, the rate of strokes was found to be increased in PCOS women OR: 1.77, 95% CI 1.28–2.44), and the excess direct cost of strokes attributable to PCOS was estimated to be 2.4 billion USD annually in 2020 dollars.

This more recent study [24] estimated the economic burden of PCOS due to pregnancy-related complications and long-term health comorbidities to be USD$4.1 billion annually in 2020 dollars. Considering also the costs of the initial diagnostic evaluation, and the treatment of menstrual dysfunction/AUB, hirsutism, and infertility, the total economic burden of PCOS in the United States is approximately USD$7.9 billion annually in 2020 dollars.

Taken together, the economic burden of PCOS-related strokes, menstrual dysfunction/AUB, and T2DM represented approximately 30%, 24%, and 19% of all costs associated with the disorder (Table 22.2). The costs of treating PCOS-related hirsutism, infertility, and pregnancy complications accounted for approximately 11%, 10%, and 5% of total economic burden. The cost of the diagnostic evaluation represented less than 2% of total costs, suggesting that much of the economic burden of PCOS can be avoided if we simply invested in greater vigilance and assessment for the disorder.

It is critical to note that the economic burden estimates in the above studies should be considered conservative at best for several reasons: (a) use of a lower PCOS prevalence based on the stricter NIH diagnostic criteria; (b) inclusion of only direct costs and some indirect costs, excluding indirect costs due to lost productivity and time-off-work due to illness; and (c) inclusion of only those health consequences which have been most strongly associated with the disorder. Furthermore, these calculations do not include the cost of evaluation and treatment of general metabolic dysfunction, mental health disorders, and neoplastic disease that women with PCOS are at increased risk for [26].

Table 22.2 The overall healthcare-related economic burden of PCOS patients thru age 54 years

	Annual costs in millions 2020 US dollars	% of total costs
Initial evaluation	139	1.8
Treatment		
Menstrual dysfunction/AUB	1900	24.6
Infertility	750	9.7
Hirsutism	875	11.3
Pregnancy-related complications		
GDM	61	0.8
gHTN	187	2.4
Preeclampsia	127	1.6
Long-term morbidities[a]		
T2DM	1500	19.4
Stroke	2400	28.4
Total	7939	

For abbreviations see text

[a]Costs of MI not included. See text for explanation

Economic Burden of PCOS Relative to Other Morbidities

The total annual economic burden of PCOS has been estimated to be $7.9 billion in 2020 USD (Table 22.2). This economic burden is less than that reported for T2DM, systemic lupus erythematosus (SLE), and major depressive disorder (MDD), three disorders with similar prevalence and/or morbidity to PCOS. The total annual estimated cost of T2DM is approximately $350.8 billion in 2020 USD [27]. However, we should note that the economic burden estimated in the latter study included direct costs *and* indirect costs of $96.54 billion in 2020 USD; approximately 28% of the total economic burden of T2DM reported was attributed to reduced productivity.

With a reported prevalence of 3.2–517.5 cases per 100,000 individuals, or on average 260.35 cases per 100,000 individuals, the total estimated annual cost of SLE is estimated to be approximately $43.2 billion in 2020 USD, factoring in both direct *and* indirect costs [28]. Approximately 24% of total economic impact of SLE is attributed indirect costs, amounting to $10.2 billion in 2020 USD. The total estimated cost of MDD is reported to be approximately $253.0 billion in 2020 USD [29]. Of these costs, about 48–50% were attributable to workplace costs or indirect costs. Moreover, 62% of the total costs were attributed to comorbidities other than MDD itself.

Overall, relative to the economic burden analyses of T2DM, SLE, and MDD, PCOS has a lower total economic impact. However, these studies demonstrate that a sizable portion of the estimated economic burden of other diseases are attributed to indirect costs, such as lost productivity and comorbidities. To further contextualize the economic burden of PCOS, the total annual healthcare expenditure in the United States was $3.8 trillion in 2019, accounting for over 17% of gross domestic product (GDP) [30]. Of the significant and increasing annual healthcare spending in

the United States, approximately 90% of total healthcare expenditure, or over $3.4 trillion, can be attributed to chronic conditions [30]. PCOS along with CVD, stroke, diabetes, obesity, and other chronic morbidities contributes to this sizable portion of healthcare costs, highlighting the need to prevent or manage symptomatology in order to reduce disease burden.

Economic Burden and Research Funding in PCOS

As noted, PCOS is a highly prevalent disease with significant negative impact on quality of life [31] and associated with significant short-term and long-term morbidities [28]. Globally, the disorder affects approximately 1 in 10 reproductive age women if the classic presentation (PCOS phenotypes A/B) is considered, and 1 in 7 if the "ovulatory" (PCOS phenotype C) and "non-hyperandrogenic" (PCOS phenotype D) forms are also included [2, 11]. Overall, PCOS is the single most common endocrine disorder of reproductive age women and the single most common cause of subfertility [32]. It is also the most important risk factor for T2DM in young women, stronger than obesity alone [26]. And women with PCOS, manifested as both anovulation and hyperandrogenism, are at increased risk for the development of subclinical CVD [33].

However, despite the enormous economic, medical, and quality of life impact of PCOS, research support has been limited, at best. Private sector drug, biotechnology, and medical device companies provide almost 60% of US biomedical research and development (R&D) [34]. However, investment by industry into new products and treatments for PCOS has been limited. We can recall the troglitazone trial by Parke-Davis [35] which, although yielding positive results, never was approved by the US Food and Drug Administration (FDA) for this disorder as the company voluntarily withdrew the product after reports of severe hepatotoxicity [36]. Preliminary data suggested that D-*chiro*-inositol could be an effective treatment for PCOS-associated anovulation.

Subsequently Insmed Pharmaceuticals embarked on a large multicenter placebo-controlled trial of D-*chiro*-inositol in PCOS, but the company discontinued its internal development of the product for T2DM and for PCOS as the drug did not achieve statistical significance on its primary efficacy measures [37]. Finally, Bristol Myers Squibb attempted to obtain FDA approval for the treatment of PCOS with metformin before its patent expiration of the drug September 2000 but failed to do so. While a number of novel and innovative approaches and products are now being studied for PCOS, most studies are still in Phase 1, and rarely Phase 2, and none is close to being commercially available.

In 2004, federal agencies funded roughly one-third of all US biomedical R&D, with the NIH accounting for three-quarters of this support [34]. However, the NIH also chronically underinvests in PCOS research. We compared funding by the NIH for the years 2006 to 2015 for PCOS and three disorders with similar degrees of morbidity and negative impact on quality of life and similar or lower mortality and prevalence: rheumatoid arthritis (RA), tuberculosis (TB), and SLE [23]. Our

analysis found that PCOS, compared with RA, TB, and SLE, was relatively less funded (total mean 10-year funding was USD$215.1 million vs. USD$454.4 million, USD$773.8 million, and USD$609.5 million in 2015 dollars, respectively).

Diseases of women, in general, tend to be underfunded [38]. Hence, to control for variations in NIH funding for diseases of women, we also studied NIH funding rates for endometriosis (ENDO) and uterine leiomyomas (ULMs). Notwithstanding, the overall funding rates per application remained lowest for PCOS vs. ENDO and ULM, as well as SLE, RA, and TB (12.7% vs. 16.9%, 17.6%, 19.9%, 16.2%, and 17.5%, respectively) (Fig. 22.1).

Assessing the number of Institutes/Centers (ICs) that supported research into the disorders of interest, we observed that for PCOS, ENDO, and ULMs funding arose from one "principal" IC, namely, the NICHD, with very similar funding rates by this IC for all three disorders (16.5%, 16.2%, and 16.4%, respectively). Alternatively, SLE, RA, and TB received significant funding (≥20 funded grants) from at least two "principal" ICs (i.e., the National Institute of Allergy and Infectious Diseases [NIAID] and National Institute of Arthritis and Musculoskeletal and Skin Diseases [NIAMS] for SLE and RA, and the NIAID, National Institute of General Medical Sciences [NIGMS], and National Heart, Lung, and Blood Institute [NHLBI] for TB), with aggregate funding rates for each of these disorders within these ICs of 20%, 15.9%, and 17.7%, respectively. Additionally, funding rates for ICs outside of these "principal" funding ICs was lowest for PCOS vs. ENDO, ULM, SLE, RA, and TB (6.8% vs. 26.7%, 40.0%, 21.5%, and 24.6%, respectively). The effect of the lower funding rate for PCOS by "non-principal" ICs is magnified by the fact that PCOS also had the highest proportion of applications submitted to "non-principal" ICs compared to all other disorders studied (37.3% vs. 9.5–20.7%, respectively) [23].

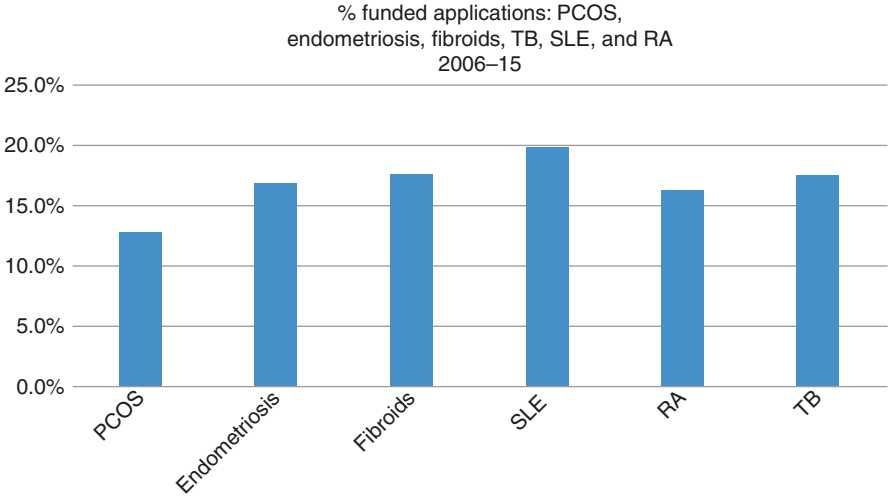

Fig. 22.1 Percent of research applications funded by the US National Institutes of Health, from 2006 through 2015, for PCOS, endometriosis, fibroids, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and tuberculosis (TB)

The challenge of PCOS research funding is further exacerbated by the fact that not only is the NICHD one of the lowest-funded ICs within the NIH, but its mission tends to focus on reproduction, whereas PCOS is also an important metabolic disorder. This discordance is further exacerbated by the general lack of funding for PCOS research by ICs such as the NHLBI or the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), which is surprising considering the important vascular and metabolic dysfunction associated with the disorder.

Assessing the impact of the type of grants awarded, we observed that more individual Research Project Grants were awarded for RA, SLE, and TB (data on grant type for ENDO and ULM was not available) than for PCOS, whereas PCOS research funding was more likely to be through General Clinical Research Centers Program or Specialized Centers Program awards. Of note, funding for individual projects from cooperative sources (P50, M01) provide smaller grants to individual researchers than do individual R01 grants.

It is possible that PCOS receives less funding because there are less grant applications to the NIH for the disorder. In fact, between 2006 and 2015, there were a significantly greater number of applications for TB, SLE, and RA (1460, 742 and 985, respectively) than for PCOS, although PCOS had a greater number of applications than for ENDO and ULMs (126, 118, and 68, respectively) (Fig. 22.2). Hence, lower application numbers alone could not explain the lower funding rate of PCOS.

Finally, and most concerning, was the drop in the number of submitted applications for PCOS research over the period studied, which decreased 42% between 2006–2010 and 2011–2015, compared to a decrease of 11% for ULMs, no change for SLE, and increases of 16–18% for all other disorders studied during the same period. These data suggest that we are losing PCOS investigation and possibly

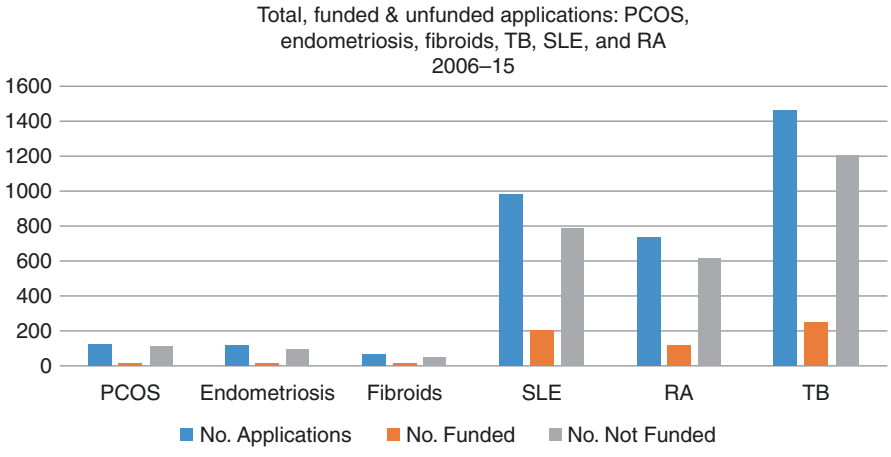

Fig. 22.2 The total, funded, and not funded applications submitted to the US National Institutes of Health, from 2006 through 2015, for PCOS, endometriosis, fibroids, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and tuberculosis (TB)

investigators, at the exact time when the clinical and public health relevance of the disorder is being increasingly recognized.

Overall, our analysis indicates that (a) PCOS is funded by the NIH at a lower rate compared to other disorders studied, including other reproductive diseases; (b) the NICHD is the principal funder of PCOS research, despite the fact that the mission of this IC is focused on reproduction, while PCOS has a strong metabolic and vascular component; and (c) the number of applications for PCOS research is dropping, possibly as a result of the lower funding rates for this disorder, discouraging research into the field [23]. A further worrisome development is the fact that the NICHD, the principal funder of PCOS research, does not even mention the disorder in its 2020 strategic plan [39].

Summary

The reported costs of the diagnostic evaluation, plus the costs of the most common sequelae of reproductive endocrine disorders (menstrual/AUB, hirsutism, and infertility), pregnancy-related complications, and long-term morbidities associated with PCOS from an ongoing study amount to over $7.9 billion annually in 2020 USD [23, 24]. As previously suggested, the attenuation of disease risk of both the sequelae of PCOS and associated long-term consequences in the postmenopausal years may reflect the much stronger influence of age on the development of these comorbidities [8, 16–19]. As such, decreased economic burden in post-reproductive years is consistent with studies demonstrating the dampening of PCOS-related morbidities with age, suggesting that the economic burden differential was primarily driven by morbidities evident during the reproductive age. Consequently, the annual economic burden of PCOS has been evaluated and reported only during the reproductive and early menopausal years.

This economic burden estimate of over USD $7.9 billion annually in 2020 dollars does not include all indirect costs, intangible costs, or all comorbidities associated with the disease, generally underestimating the total economic burden of PCOS. The calculated economic burden of PCOS included only those pregnancy and long-term health complications which have been most strongly associated with the disorder, such as GDM, gHTN, preeclampsia, T2DM, and stroke. Comorbidities less strongly associated with PCOS that were not included in the reported estimate include metabolic dysfunction/dyslipidemia, endometrial neoplasia and cancer, and mental health disorders including depression, anxiety, body image dissatisfaction, and disordered eating [26, 40–42]. Additionally, while there appears to be little differences in the prevalence of PCOS by race, ethnicity, or geography, it is possible that there are differences in the prevalence of comorbidities, which may impact economic burden estimations. In future economic burden research, the economic impact should include all indirect costs and other comorbidities affecting physical and behavioral health, as well as assessment of socioeconomic status and race/ethnicity, to better understand the full extent PCOS impacts healthcare expenditure and ultimately inform the decisions of stakeholders.

Finally, our data suggests that PCOS research is underfunded by both industry and federal funding sources, considering its large prevalence, economic burden, metabolic morbidity, and negative impact on quality of life. However, we recognize that NIH disease-specific research funding generally correlates only modestly (33%) with US disease burden [43], something that elected officials and policymakers should begin to address more deliberately. Greater education of industry; the NIH, including leaders at the NHLBI and NIDDK; other research funding agencies in the United States and internationally; other governmental agencies; elected leaders; and the general public education regarding PCOS and its economic burden is critically needed. Importantly, patient support groups (e.g., *PCOS Challenge* [44]) have begun to advocate with governmental representatives for increased funding for PCOS research. However, much still remains to be done to increase awareness, empathy, and support by the public, industry leaders, elected officials, and policymakers for PCOS care and research.

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Part VII

Emerging Concepts in Polycystic Ovary Syndrome

The Role of Angiogenic Factor Dysregulation in the Pathogenesis of Polycystic Ovarian Syndrome

23

Alice Y. Chen, David B. Seifer, and Reshef Tal

Key Points

- Angiogenesis, the process of new blood vessel formation and stabilization, is crucial for normal follicular development in the ovary.
- Ovarian angiogenesis requires a normal balance of both angiogenic and antiangiogenic factors to ensure proper follicular growth, ovulation, and regression of the corpus luteum.
- PCOS patients are characterized by an increased number of small antral follicles. Numerous studies have shown that this increase is supported by abnormal and increased vasculature in the ovaries and follicles.
- Many angiogenic factors including VEGF, angiopoietins, transforming growth factor-β, platelet-derived growth factor, and basic fibroblast growth factor are dysregulated in women with PCOS and PCOS animal models.
- Angiogenic dysregulation, which includes specific growth factors such as VEGF and angiopoietin-1 and angiopoietin-2 (Ang-1 and Ang-2), likely contributes to the exaggerated risk of developing ovarian hyperstimulation syndrome in women with PCOS.
- Abnormal angiogenesis and angiogenic dysregulation may also create an environment of endometrial dysfunction in women with PCOS.
- There are currently three common therapies for management of PCOS symptoms: hormonal, insulin sensitizing drugs, and laparoscopic ovarian drilling. These therapies have been shown to have normalizing effects on angiogenic factor levels.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_23

Introduction

Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder that affects up to 10% of reproductive age women. First described in 1935 by Stein and Leventhal [1], PCOS is characterized by a variety of symptoms, including anovulation, hyperandrogenism, and polycystic ovarian morphology [2, 3]. The pathophysiology and mechanisms of PCOS are complex and not well understood. This chapter reviews normal ovarian neovascularization to build the foundation for understanding abnormal ovarian angiogenesis and angiogenic factor imbalance characteristic of PCOS. Then, we discuss the implications of this understanding for PCOS pathophysiology and therapy.

Angiogenesis, the process of new blood vessel formation and stabilization, is crucial for normal follicular development in the ovary [4]. Ovarian angiogenesis requires both angiogenic and antiangiogenic factors to regulate blood vessel formation to ensure proper follicular growth, ovulation, and regression of the corpus luteum [5]. Angiogenic factors include vascular endothelial growth factor (VEGF), placental growth factor (PlGF), angiopoietins, transforming growth factor-ß1 (TGF-ß1), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) [6]. Studies have shown that these angiogenic factors vary in gene expression and serum concentration in PCOS patients compared with control patients [5, 7–11]. Thus, there is increasing evidence that there is angiogenic factor imbalance and angiogenesis dysregulation in women with PCOS.

Of all the angiogenic factors, vascular endothelial growth factor (VEGF) is probably the most studied in PCOS. It has been shown that not only are serum levels of VEGF elevated in women with PCOS, but circulating levels of soluble VEGF receptor-1 (sVEGFR1) are also reduced in this population, compared to women without PCOS, further contributing to the increased bioavailability of VEGF in PCOS [7, 12, 13].

Ovulatory dysfunction is a known contributor to PCOS-related infertility, and strategies of ovulation induction (OI) and assisted reproductive technology (ART) are commonly utilized in infertility management. Although conventional thinking is that infertility is related to anovulation in PCOS, there is accumulating evidence that aberrant endometrial receptivity associated with dysfunctional angiogenesis may contribute to endometrial receptivity defects as well as placental dysfunction [14, 15]. Thus, many struggling with infertility turn to fertility treatments including OI and ART regimens. The most serious complication of controlled ovarian stimulation is ovarian hyperstimulation syndrome (OHSS), in which ovarian stimulation medications cause ovarian hyper-response and enlarged ovaries, leading to extravasation of fluid from blood vessels and fluid accumulation in the pelvis and abdomen [16]. Increased vascular permeability caused by VEGF imbalance is considered to be the main mechanism responsible for OHSS [16]. Thus, the angiogenic factor dysregulation exhibited in PCOS may be underlying the increased risk of OHSS in women with PCOS undergoing OI.

Angiogenic Factors in Physiological Ovarian Angiogenesis

Angiogenesis is the process of new blood vessel growth from preexisting vessels. It requires the proliferation and migration of endothelial cells, as well as permeability to the existing vasculature [6]. This process is crucial for tissue development throughout the body during embryogenesis [6]. In adulthood, angiogenesis is typically associated with pathological abnormal conditions such as wound healing and tumor formation and in response to ischemia [5]. However, the female reproductive system characterized by profound cyclic cell turnover is an exception, where, during the reproductive years, the ovaries and uterus regularly undergo physiological angiogenesis as part of the menstrual cycle.

In general, the four dynamic mechanisms of angiogenesis are sprouting, intussusception, vessel elongation, and vasculogenesis, as shown in (Fig. 23.1) [6, 17]. Sprouting is considered the classic mechanism in which enzymes break down capillary basement membranes and new vessels form. Intussusception involves the division of a vessel lumen into two. Elongation is the lengthening of vessels. There are hypotheses that suggest that human endometrial angiogenesis involves non-sprouting mechanisms, whereas corpus luteum vascular expansion involves sprouting angiogenesis [17].

Throughout the later follicular phase and luteal phase of the menstrual cycle, ovarian vasculature delivers nutrients, hormones, and steroid precursors to the developing follicles [5, 18]. Thus, the ovary undergoes cyclic vascular changes, which are tightly regulated by angiogenic factors. When primary follicles first start developing in the follicular phase of the menstrual cycle, they are avascular and

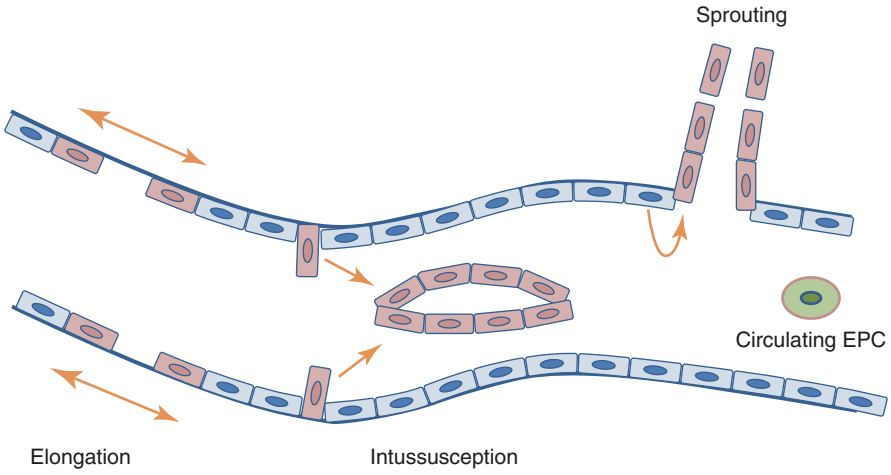

Fig. 23.1 Mechanisms of angiogenesis. The blue cells are dormant endothelial cells. Proliferating endothelial cells are depicted in pink. The green cells represent circulating endothelial progenitor cells. Blood vessel formation, through sprouting, elongation, and intussusception, involves proliferating endothelial cells. (Reprinted with permission from Tal et al. [6] (© 2013 Oxford University Press))

receive their oxygen, nutrients, and hormones by diffusion from the blood vessels in the surrounding ovarian stroma [19]. As thecal cells are recruited to the growing follicle during the secondary follicle stage, new blood vessels generate in the thecal layer, creating a direct link between the follicle and its supply of gonadotropins, growth factors, oxygen, and other nutrients [19]. Angiogenesis, as seen by the proportion of proliferating endothelial cells, markedly increases in the developing corpus luteum after ovulation [20]. Follicular atresia occurs if there is inadequate development of the vasculature or if there is blood vessel regression [21]. While angiogenesis is an essential component of the entire process of follicular development, it is most intense in the postovulatory period. During this time, the corpus luteum is heavily vascularized, supported by high levels of VEGF and other angiogenic factors. This process is shown in (Fig. 23.2). In fact, it has the most blood supply per unit of any tissue in the female body, eight times that of the kidney [5]. If pregnancy occurs, uterine and placental vascularization play a crucial role in supplying nutrients and other substances to the developing embryo [22]. Abnormal or insufficient vasculature of the utero-placental unit can lead to a variety of pregnancy-associated disorders, such as preeclampsia and preterm delivery [22]. When pregnancy does not occur, this increased angiogenesis stops and the corpus luteum becomes avascular and degenerates [23].

Angiogenesis is a dynamic process that requires various regulatory angiogenic factors to remodel existing blood vessels and recruit other cells and angiogenic

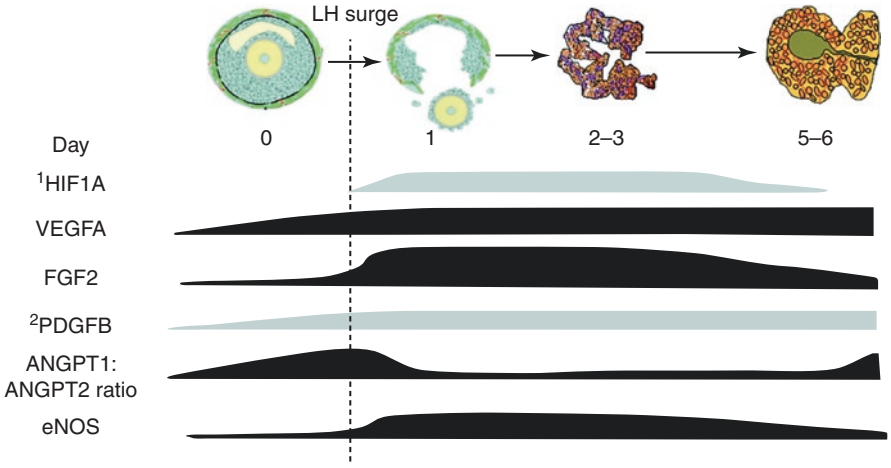

Fig. 23.2 Temporal changes in angiogenic factor levels throughout follicular development and the postovulatory period in cows. (Reprinted with permission from Robinson et al. [66] (© 2009 Society for Reproduction and Fertility)). *Abbreviations*: Angpt1 angiopoietin-1, Angpt2 angiopoietin-2, eNOS endothelial nitric oxide synthase, FGF2 fibroblast growth factor 2, HIF1a hypoxia-induced factor 1α, LH luteinizing hormone, PDGFB platelet-derived growth factor B, VEGFA vascular endothelial growth factor A

[1]Data from marmoset

[2]Data from mouse

factors. In order to achieve this, angiogenic factors vary in temporal and spatial gene expression patterns in a well-orchestrated process. In the section below, we examine individual angiogenic factors with their known role in physiological ovarian angiogenesis.

Vascular Endothelial Growth Factor (VEGF)

One of the main regulators of angiogenesis, as well as one of the most studied angiogenic factors, VEGF, plays a major role in ovarian angiogenesis and follicular development. It is mitogenic in the endothelial cells and increases vascular permeability [24]. This heparin-binding homodimeric protein of 46 kDa has six isoforms: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF) [5]. VEGF binds to tyrosine kinase membrane receptors that mediate cellular signaling cascades. The two VEGF receptors are VEGFR1 (aka fms-like tyrosine kinase: flt-1) and VEGFR2 (or kinase domain-containing receptor: KDR, aka flk-1). All VEGF isoforms bind to both receptors except for PlGF, which only binds to VEGFR1 [25]. VEGFR1 has two isoforms: a membrane-bound receptor that leads to signal transduction and a soluble receptor (sVEGFR1) that inhibits signal transduction. The sVEGFR1 prevents VEGF signal transduction by either sequestering the circulating VEGF or by dimerizing with the membrane receptors. Thus, sVEGFR1 is also a VEGF antagonist [23].

Studies have characterized spatial and temporal expression patterns of VEGF in relation to the menstrual cycle. In healthy humans, follicular fluid VEGF levels are higher than serum VEGF levels [26], indicating substantial local ovarian production. The amount of VEGF in the ovarian granulosa and theca cells changes throughout the human menstrual cycle and across stages of folliculogenesis. The amount in human granulosa and theca cells, as determined by immunohistochemistry, is low during primary follicular development and increases as the follicle matures [27]. After ovulation and during early corpus luteum formation, granulosa and theca cells show strong VEGF protein expression, although the thecal expression is less intense compared to the granulosa cell expression [27]. Similarly, in rats VEGF is very weakly expressed during early follicular development. As follicles mature, immunohistochemistry shows stronger staining for VEGF in granulosa and theca cells of rat ovary [28]. As expected, these VEGF expression patterns in the ovarian tissue follow ovarian angiogenesis patterns throughout the menstrual cycle.

There is substantial evidence indicating that VEGF-mediated angiogenesis plays a critical role in key ovarian processes including folliculogenesis and ovulation. VEGF is heavily involved in follicular and luteal development, as well as ovulation. When VEGFR2 antibody is administered to mice, follicular angiogenesis and development decline, preventing follicle maturation [29]. Injecting sVEGFR1 or another VEGF antagonist slows follicular development in monkeys and impairs ovulation, corpus luteum formation, and progesterone production [21, 23].

Angiopoietins

Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are two frequently studied angiogenic factors involved in vascular stability and in ovarian follicular development. Both Ang-1 and Ang-2 participate in angiogenesis and affect endothelial cells by binding the same tyrosine kinase receptor, Tie-2 [30]. In rats, protein expressions of Ang-1, Ang-2, and their receptor have been shown to increase during ovarian follicular development [30].

Ang-1 and Ang-2 have different effects on vascular stabilization. Ang-1 recruits smooth muscle cells and pericytes, which stabilize blood vessels newly formed by VEGF [31, 32]. When Ang-1 is injected into the primate ovary, there is normal ovulation and luteal development [33]. The effect of Ang-2 on angiogenesis depends on VEGF. When Ang-2 is in the presence of VEGF, there is endothelial proliferation and migration as well as angiogenic sprouting [34]. When there is no VEGF, Ang-2 acts as an Ang-1 antagonist, destabilizing blood vessels by loosening the extracellular matrix and increasing permeability [31]. In bovine and human ovary, Ang-2 normally increases during follicular growth and decreases during follicular maturation, indicating that Ang-2 plays a role in follicular development [35, 36]. The intrafollicular administration of Ang-2 into the primate ovary alters follicular angiogenesis and maturation [33]. It prevents ovulation and corpus luteum generation, as seen by laparoscopic evaluations. Thus, Ang-1 and Ang-2 have opposing effects in the ovary and are important for normal ovarian function.

Ang-1 and Ang-2 differ in location. The concentration of Ang-1 is much greater in serum compared with follicular fluid, whereas the concentration of Ang-2 is greater in follicular fluid compared with serum [36]. During oocyte retrieval, there is a very low Ang-1:Ang-2 ratio in the follicular fluid, further indicating that Ang-2 predominates in the local ovarian environment. There are higher concentrations of Ang-1 in smaller mature follicles (<17 mm), while higher concentrations of Ang-2 in larger mature follicles (>18 mm); thus, the Ang-1:Ang-2 ratio decreases as follicles grow [36].

Transforming Growth Factor-ß (TGF-ß)

Another angiogenic factor involved in ovarian angiogenesis is TGF-ß, which has three isoforms: TGF-ß1, TGF-ß2, and TGF-ß3. These isoforms are expressed in the human ovary in the oocytes, granulosa cells, and theca cells and are involved in follicular development [37]. TGF-ß has a wide range of functions. Not only does it stabilize blood vessels and induce pericyte differentiation and proliferation like other angiogenic factors, but TGF-ß also regulates apoptosis, immune function, and extracellular matrix synthesis [38–41]. When rat granulosa cells are treated with TGF-ß, angiogenic activity increases as shown through angiogenic assays and concentrations of other angiogenic factors [42]. When different aspects of the TGF-ß1 signaling pathway are disturbed in mice, there is reproductive dysfunction such as anovulation and oocyte incompetence [43].

TGF-ß binds to specific receptors to carry out its functions. The TGF-ß type II receptor binds ligands. The TGF-ß type I receptor is a kinase that phosphorylates secondary messengers, Smads, which regulate downstream transcription of genes related to TGF-ß function [44]. In hamsters, the mRNA of these receptors are regulated by gonadotropins [45]. TGF-ß1 and TGF-ß3 can bind a third receptor, soluble endoglin (sENG), which decreases their bioavailability [9]. TGF-ß can also bind to fibrillin, an extracellular matrix protein that is found to be associated with PCOS [46].

Platelet-Derived Growth Factor (PDGF)

The role of PDGF in angiogenesis is well established, and PDGF family members have been found in rat, mouse, and human ovaries, indicating its importance in ovarian vasculature [47, 48]. PDGF is a dimeric growth factor that exists in five different isoforms: PDGF-AA, PGDF-BB, PDGF-CC, PDGF-DD, and PDGF-AB. It acts as a mitogen for smooth muscle cells and fibroblasts [49]. It also recruits smooth muscle cells and pericytes to newly formed blood vessels, contributing to vessel stabilization and maturation [50, 51]. PDGF isoforms bind to two types of tyrosine kinase cell surface receptors: PDGFR-α and PDGFR-ß. PDGFR-α has a high affinity for A, B, and C chains. PDGFR-ß only binds B and D chains, and it plays a large role in angiogenesis regulation [52]. PDGFR-ß is typically found in fibroblasts, pericytes, and vascular smooth muscle cells [53], whereas PDGF-BB and PDGF-DD are widely expressed in endothelial cells and smooth muscle cells [54].

PDGF and PDGFR-ß signaling play an important role in regulating folliculogenesis. Not only has PDGFR-ß been observed in rat granulosa cells from primordial follicles, but PDGF treatment in vitro also aids the primordial to primary follicle transition [55]. Furthermore, Sleer et al. have studied rats and have observed PDGF and PDGFR expression in granulosa and theca cells during the preovulatory period [56]. In rats, after human chorionic gonadotropin (hCG)-triggered ovulation, there is an increase in PDGF-B, PDGF-D, and PDGFR-ß mRNA and protein levels, suggesting that LH induces PDGF gene transcription [47]. In addition, Sleer et al. have also revealed that inhibition of PDGFR-ß in rats results in fewer functional corpora lutea and sometimes widespread hemorrhage [47]. Kuhnert et al. have also found that the administration of a soluble ectodomain of PDGFR-ß in mice led to reduced pericyte recruitment in corpus luteum, less vessel density, and prominent hemorrhage, indicating the importance of PDGF signaling in ovarian angiogenesis and follicular development [57]. Lastly, there is also some evidence indicating that PDGF inhibits VEGF, as PDGF-B and PDGFR-ß knockout mice demonstrate increased VEGF levels [58]. In addition, PDGF-B knockout mice embryos have a lack of pericytes surrounding their vasculature [59]. This further confirms that PDGF regulates angiogenesis. PDGF-A and PDGF-B and their receptors are expressed in human adult oocytes and granulosa cells, suggesting that they may play a similar role in the activation of primordial follicles [48], although their exact role in the human ovary remains to be defined. All in all, PDGF is an important angiogenic factor in ovarian angiogenesis.

Basic Fibroblast Growth Factor (bFGF)

bFGF, aka FGF2, is an angiogenic factor that promotes follicular and corpus luteum development in some species. It is an 18-kDa protein that induces proliferation and differentiation of a variety of neuroectodermal and mesodermal cells [60]. This growth factor promotes angiogenesis by triggering endothelial cell proliferation and inducing matrix remodeling enzymes such as collagenase and plasminogen activator [61, 62]. Expressed in human theca and granulosa cells, bFGF also regulates follicle-stimulating hormone (FSH) action and promotes follicular growth [3]. bFGF binds to two major membrane receptors in order to carry out its activity: fibroblast growth factor type 1 receptor (FGFR-1) and type 2 receptor (FGFR-2) [63].

In cows, bFGF concentration in theca interna cells increases during the late stage of follicular maturation [64]. bFGF mRNA and protein levels in follicles as well as receptor levels continue to increase dramatically after the LH surge [65]. They then decrease as the corpus luteum develops and increase again as the corpus luteum regresses [66]. During the LH surge, bFGF also spatially translocates from theca cells to granulosa cells [65]. When bFGF is inhibited by direct antibody injection into the corpus luteum, there are numerous effects. The corpus luteum decreases in size, progesterone production is impaired, and VEGF and Ang-1 expression is decreased [67]. In humans, it is however unclear how bFGF promotes endothelial cell proliferation and follicular growth. Some research indicates that bFGF is unaffected by LH surge in women [68]. Another study conducted by Yamamoto et al. suggests that bFGF levels increase in granulosa and theca cells as folliculogenesis progresses. Later, during corpus luteum formation and regression, bFGF concentration in granulosa cells decrease while bFGF staining in theca cells remained strong [69]. Thus, despite the existing data suggesting relevance of bFGF for corpus luteum endothelial network formation, more research is needed to determine bFGF's exact role in ovarian angiogenesis.

Angiogenic Dysregulation in PCOS

Ovaries in PCOS have a classic phenotype that is characterized by an increased number of antral and preantral follicles and hyperplasia of the theca interna and of ovarian stroma [70, 71]. Numerous studies have shown that this ovarian mass increase is supported by abnormal, increased intraovarian vasculature. Using Doppler ultrasound, one study showed that women with PCOS have greater ovarian stromal blood flow velocity [72]. Many subsequent investigations comparing PCOS and non-PCOS women, conducted by independent research groups, demonstrated similar findings of increased blood flow and ovarian volume, as well as decreased vessel resistance and abnormal pulsatility in PCOS ovaries [73–75]. These Doppler blood flow–based ovarian abnormalities are correlated with some PCOS characteristics. Women with PCOS who are of a lower body mass index (BMI) or who have milder PCOS symptoms, such as oligomenorrhea instead of amenorrhea, tend to have fewer Doppler abnormalities [19]. Although some studies show little

difference in Doppler indices [73, 76], there is increasing evidence that ovarian blood flow indices differ between PCOS and normal ovulatory women; PCOS patients have greater ovarian vascularization and lower impedance to blood flow.

There is also evidence that current PCOS treatments have normalizing effects on patients' ovarian blood flow, associated with normalization of angiogenic factor levels. Oral contraceptive pills (OCPs) and metformin, both of which treat PCOS symptoms, have been shown to decrease ovarian stromal vascularization and increase Doppler resistance and pulsatility indices [77]. In a study of 101 women with PCOS, 51 women received OCPs and 50 received metformin for 6 months. OCPs were correlated with a greater reduction in stromal blood flow compared to metformin, with each of these agents likely exerting its effects on ovarian vascularization via different mechanisms [77]. The results of this study suggest that OCPs decrease ovarian vascularization via reduction in serum LH and androgens, both of which have a possible role in elevated VEGF levels [12, 77, 78]. The results also suggest that metformin decreases ovarian vascularization by reducing insulin resistance and serum insulin levels [77]. Insulin has been found to promote angiogenesis by activating the vascular endothelium and indirectly inducing VEGF expression [73, 77]. Another research group has demonstrated that patients treated with metformin over a six-month period ($n = 40$) that had ovulated had similar ovarian vascularization to that of healthy controls ($n = 30$), whereas women with PCOS that ovulated with the ovulation induction drug clomiphene citrate (CC) ($n = 38$) had decreased perifollicular and corpus luteum vascularization compared to healthy controls over the same time period [79]. While the underlying mechanism is unclear, the authors suggested that CC alters perifollicular vascularization, which may have detrimental effects on oocyte quality and embryo development by limiting the developmental ability of the oocyte and causing hypoxic damage, potentially leading to cytoplasmic and chromosomal abnormalities [79–81].

Ovarian blood flow also depends on the specific location within the ovary. The blood flow within the stromal compartment of women with PCOS is higher compared to women with normal ovaries. However, perifollicular blood flow of PCOS patients is either slightly lower or no different than normal women [82, 83]. This could be due to different angiogenic environments. The decreased perifollicular blood flow could also be associated with follicular arrest seen in women with PCOS [82]. While the exact causal relationship between arrested follicles and decreased perifollicular blood flow is unclear, abnormal follicular angiogenesis may contribute to anovulation due to the possible role of angiogenic factors in selecting the dominant follicle [84–86]. Interestingly, Ferrara et al. have demonstrated that the expression patterns of VEGF and endocrine gland-derived VEGF (EG-VEGF) are mutually exclusive. While VEGF is found in the granulosa cell layer of follicles, EG-VEGF is found in the ovarian stroma [5]. Thus, there is evidence that blood flow patterns differ based on the local angiogenic environment. Expression of angiogenic factors also vary in different environments, such as ovarian tissue, serum, and follicular fluid (Tables 23.1 and 23.2). Overall, mounting evidence points to ovarian vascular abnormalities and dysregulation of angiogenic factors in women with PCOS.

Table 23.1 Angiogenic factor concentrations in serum and follicular fluid of women with PCOS compared with non-PCOS controls

Angiogenic factor family	Study [Refs]	Design	Population	Reproductive intervention	Outcome measures	Factor	Serum	Follicular fluid
VEGFs	Agrawal et al. 1998 [7]	Prospective observational	15 women with PCOS (Rotterdam criteria); 27 women with PCO; 65 women with normal ovaries	IVF; ovarian gonadotropin stimulation with GnRH long protocol, hCG	Measurement in serum at 5 different points during IVF cycle; measurement in FF after OR	VEGF	↑	↑
	Tulandi et al. 2000 [90]	Prospective	27 CC-resistant PCOS women (Rotterdam criteria); 19 control women	Laparoscopic ovarian drilling	Measurement in serum before and after laparoscopic ovarian drilling	VEGF	↑	–
	Amin et al. 2003 [162]	Prospective	25 women with CC-resistant PCOS (ultrasound, oligomenorrhea); 20 control women	Laparoscopic ovarian drilling	Measurement in serum before and after laparoscopic ovarian drilling	VEGF	↑	–
	Ng et al. 2005 [167]	Prospective	32 women with PCOS (ultrasound, oligomenorrhea); 107 control women	None	Measurement in serum between day 2–4 of menstrual cycle	VEGF	↔	–
	Abd El Aal et al. 2005 [87]	Prospective	50 women with PCOS (ultrasound, oligomenorrhea); 20 control women	None	Measurement in serum during early follicular phase	VEGF	↑	–
	Artini et al. 2006 [11]	Prospective	26 women with PCOS (Rotterdam criteria); 26 control women	IVF, controlled ovarian hyperstimulation with hCG and FSH	Measurement in serum at baseline, day of hCG, and day of OR; measurement in FF after OR	VEGF	↑	↑
	Artini et al. 2009 [89]	Prospective	30 women with PCOS (Rotterdam criteria); 20 control women	IVF, controlled ovarian hyperstimulation with hCG and FSH	Measurement in serum on day of hCG and day of OR; measurement in FF after OR	VEGF	↑	↑
						sVEGFR1	↓	↓
	Savchev et al. 2010 [96]	Prospective	6 women with PCOS (Rotterdam criteria), 11 control women	IVF, controlled ovarian stimulation with GnRH agonist or antagonist, FSH, and hCG	Measurement in FF	$VEGF_{121}$	–	↔
						$VEGF_{165}$	–	↑
						sVEGFR1	–	↔
	Tal et al. 2014 [91]	Prospective	24 women with PCOS (Rotterdam criteria); 14 matched control women	IVF, controlled ovarian stimulation with GnRH agonist or antagonist and hCG	Measurement in serum at baseline, day of hCG, and day of OR; measurement in FF after OR	PlGF	↔	↑
						sVEGFR1	↔	↓

Angiogenic factor family	Study [Refs]	Design	Population	Reproductive intervention	Outcome measures	Factor	Serum	Follicular fluid
Angiopoietins	Sova et al. 2010 [112]	Prospective	50 women with PCOS (Rotterdam criteria); 20 matched control women	None	Measurement in serum	Ang-2	↔	–
	Tal et al. 2013 [8]	Prospective	14 women with PCOS (Rotterdam criteria); 13 matched control women	IVF, controlled ovarian stimulation with GnRH agonist or antagonist and hCG	Measurement in serum at baseline, day of hCG, and day of OR; measurement in FF after OR	Ang-1	↑	↔
						Ang-2	↔	↓
	Scotti et al. 2014 [10]	Prospective	19 women with PCOS (Rotterdam criteria); 27 control women	Artificial reproduction, stimulation protocol not reported	Measurement in FF after OR with ELISA	Ang-1	–	↑
						Ang-2	–	↔
						sTie-2	–	↔
	Wang et al. 2017 [113]	Prospective	44 CC-resistant PCOS patients (Rotterdam criteria); 55 CC-sensitive PCOS patients	Ovulation induction with CC	Measurement in serum with ELISA; PCOS women were divided into those resistant to CC and those not	Ang-2	↓[a]	–
TGF-ß	Raja-Khan et al. 2010 [117]	Prospective	40 women with PCOS without allele 8 polymorphism (anovulation, hyperandrogenism); 40 women with PCOS with allele 8 polymorphism; 40 control women	None	Measurement in serum with ELISA; PCOS women were stratified according to presence of fibrillin allele 8 polymorphism	TGF-ß1	↑[b]	–
						TGF-ß2	↓	–
	Tal et al. 2013 [9]	Prospective	14 women with PCOS (Rotterdam criteria);14 matched control women	IVF, ovarian stimulation with GnRH agonist or antagonist and hCG	Measurement in serum at baseline, day of hCG, and day of OR; measurement in FF on day of OR;	TGF-ß1	↑	↔
						sENG	↓	↔
	Liu et al. 2015 [118]	Prospective	445 Chinese women with PCOS (Rotterdam criteria); 213 controls	Treatment of OCPs with or without metformin	Measurement in serum on days 2–4 of menstrual cycle	TGF-ß1	↑	–

(continued)

Table 23.2 (continued)

Angiogenic factor family	Study [Refs]	Design	Population	Reproductive intervention	Outcome measures	Factor	Serum	Follicular fluid
PDGF	Scotti et al. 2014 [10]	Prospective	19 women with PCOS (Rotterdam criteria); 27 control women	Stimulation protocol not described	Measurement in FF after OR	PDGF-BB	–	↓
						PDGF-DD	–	↓
	Zheng et al. 2018 [129]	Prospective	43 Chinese women with PCOS (Rotterdam criteria; 30 healthy women, BMI and age matched	None	Measurement in serum	PDGF-D	↓	–
bFGF	Artini et al. 2006 [11]	Prospective	26 women with PCOS (Rotterdam criteria); 26 control women	IVF, controlled ovarian stimulation with hCG and FSH	Measurement in serum at baseline, day of hCG, and day of OR; measurement in FF after OR	bFGF	↑	↑

Abbreviations: Ang-1 angiopoietin-1, *Ang-2* angiopoietin-2, *bFGF* basic fibroblast growth factor, *CC* clomiphene citrate, *ELISA* enzyme-linked immunosorbent assay, *FF* follicular fluid, *FSH* follicle-stimulating hormone, *GnRH* gonadotropin-releasing hormone, *hCG* human chorionic gonadotropin, *IVF* in vitro fertilization, *OCPs* oral contraceptive pills, *OR* oocyte retrieval, *PDGF* platelet-derived growth factor, *PlGF* placental growth factor, *sENG* soluble endoglin, *sTie-2* soluble Tie-2 receptor, *sVEGFR1* soluble VEGF receptor 1, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor

[a]Ang-2 was decreased in women with PCOS who were CC resistant when compared with PCOS patients who were CC sensitive

[b]TGF-ß1 was increased in women with PCOS who were negative for fibrillin-3 allele 8 polymorphism (A8–), but not in women with PCOS who were positive for allele 8 (A8+), as compared with women with no PCOS (A8–)

Table 23.2 Angiogenic factor abnormalities in PCOS animal models

Angiogenic factor family	Study	Model	Intervention	Quantitation Method(s)	Factor	Ovarian tissue	Uterine tissue
VEGFs	Abramovich et al. 2012 [97]	DHEA-induced PCOS rat model	Group 1: VEGF inhibitor, trap Group 2: Control, received vehicle	Measurement in protein extracts of ovaries with ELISA and Western blot	VEGF	↑	–
					VEGFR2	↓	–
	Singh et al. 2018 [95]	DHEA-induced PCOS mouse model	Group 1: Control, received vehicle Group 2: PCOS group	Measurement in ovaries with immunoblotting	VEGF	↑	–
	Zhao et al. 2019 [168]	DHEA-induced PCOS rat model	Group 1: PCOS Group 2: Control, no procedures	Gene expression and protein expression (Western blot)	VEGF	↓	↓
	Kelley et al. 2019 [92]	Testosterone-treated PCOS sheep model	Group 1: Testosterone treated Group 2: Control, received vehicle	Gene expression	VEGF	–	↑ (placenta)
	Miller et al. 2020 [94]	DHT-induced PCOS mouse model	Group 1: PCOS Group 2: Control, received vehicle	Gene expression	VEGF	↑	–
Angiopoietins	Abramovich et al. 2012 [97]	DHEA-induced PCOS rat model	Group 1: VEGF inhibitor, trap Group 2: Control, received vehicle	Measurement in protein extracts of ovaries with Western blot	Ang-1	↑	–
					Ang-2	↓	–
					Tie-2	↑	–
	Hu et al. 2019 [114]	DHT- and insulin-exposed pregnant rats	Group 1: DHT and insulin exposed Group 2: DHT exposed Group 3: Control, received vehicle	Gene expression	Ang-1	–	↓
					Ang-2	–	↓

(continued)

Table 23.2 (continued)

Angiogenic factor family	Study	Model	Intervention	Quantitation Method(s)	Factor	Ovarian tissue	Uterine tissue
TGF-ß	Wang et al. 2018 [120]	DHEA-induced PCOS rat model	Group 1: Oil group Group 2: Oil + DHEA induced Group 3: Blank group, saline injection	IHC	TGF-β	↑	–
	Wang et al. 2019 [121]	DHEA-induced PCOS rat model	Group 1: Model group, DHEA injected Group 2: Treatment group, DHEA injected Group 3: Control, oil injection	Measurement of protein in ovarian tissue with IHC and Western blot	TGF-β1	↑	–
	Shen et al. 2019 [122]	DHEA-induced PCOS rat model	Group 1: PCOS, DHEA injected Group 2: Control, saline injection	Measurement in follicular fluid and serum with ELISA and measurement of mRNA with RT-PCR	TGF-β1	↑	–
PDGF	Di Pietro et al. 2015 [128]	DHEA-induced PCOS rat model	Group 1: DHEA, metformin Group 2: No DHEA, metformin Group 3: Control, sesame oil injection	Measurement of protein in ovarian tissue with Western blot	PDGF-B	↓	–
					PDGF-D	↓	–
					PDGFR-ß	↓	–

Abbreviations: Ang-1 angiopoietin-1, *Ang-2* angiopoietin-2, *bFGF* basic fibroblast growth factor, *DHEA* dehydroepiandrosterone, *DHT* 5α-dihydrotestosterone, *ELISA* enzyme-linked immunosorbent assay, *IHC* immunohistochemistry, *PDGF* platelet-derived growth factor, *PDGFR-β* PDGF receptor, *qRT-PCR* real-time quantitative reverse transcription PCR, *Tie-2* Tie-2 receptor, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor, *VEGFR2* VEGF receptor 2

Vascular Endothelial Growth Factor (VEGF)

As mentioned previously, VEGF is one of the main regulators of angiogenesis, contributing to the proliferation of endothelial cells as well as follicular and corpus luteal development. There are numerous studies showing elevated serum and follicular VEGF levels in women with PCOS compared to non-PCOS populations, suggesting a role for VEGF excess in the increased ovarian stromal vascularization in PCOS. In 1998, Agrawal et al. revealed a positive relationship between ovarian blood flow velocity and mean serum VEGF concentration; both were increased in women with PCOS and in women with isolated polycystic ovaries (PCO) [7]. Another research group also demonstrated that PCOS patients have increased ovarian vasculature and elevated VEGF in serum [87]. Not only is VEGF increased in the circulation, but it is also increased in the ovary. It has been shown by immunohistochemistry that VEGF is overexpressed in ovarian stroma of women with PCOS [27, 88]. Several other studies have tracked VEGF levels of women with and without PCOS undergoing fertility treatments. These investigations have found that PCOS patients have higher VEGF levels in serum and follicular fluid at baseline and while undergoing treatment [11, 12, 89, 90]. PlGF, a specific type of VEGF, is elevated in follicular fluid of PCOS patients but not serum [91]. Interestingly, some studies have shown that there is a higher concentration of VEGF in follicular fluid compared to serum, suggesting that the increased systemic VEGF levels in PCOS patients originate in the ovary before entering the circulation [11, 89]. In addition to human studies, a study involving an androgen-induced sheep model of PCOS has shown that VEGF mRNA levels are higher in the placenta of the PCOS group compared to the control group [92]. These PCOS sheep have reduced placental mass and give birth to low-birth-weight female offspring [93]. This rise in VEGF may be due to hypoxia associated with increased testosterone [92]. Another study using a dihydrotestosterone (DHT, a non-aromatizable androgen)-induced PCOS mouse model has also shown that PCOS mice have higher levels of VEGF mRNA in granulosa cells compared to control mice [94]. Furthermore, Singh et al. demonstrated with immunoblotting that mice with dehydroepiandrosterone (DHEA)-induced PCOS have increased VEGF levels in the ovaries [95].

Not only is there an increase in VEGF levels in women with PCOS, but there is also a decrease in its circulating soluble receptor, further contributing to the increased bioavailability of VEGF. Two studies have shown that compared to normal control women, women with PCOS have decreased serum and/or follicular levels of sVEGFR1 [89, 91]. However, others have reported that there is no change in the follicular fluid concentrations of sVEGFR1 or VEGFR2 [96]. The reasons for these differences in results remain to be elucidated. VEGFR2 has been shown to be lower in rat PCOS ovaries [97]. This could be due to a downregulation of this receptor because of elevated concentrations of VEGF.

The exact cause of VEGF upregulation in PCOS is unknown. Possible considerations include LH, androgens, and insulin as players in the elevation of VEGF in PCOS. Each of these three hormones has been shown to increase the secretion of

VEGF by ovarian granulosa cells in vitro [12, 78, 98]. It has been shown that the effect of insulin on the production of VEGF in luteinized granulosa cells is greater in PCOS patients, compared to women without PCOS [98]. Another study has shown that women with PCOS with hyperinsulinemia have greater ovarian Doppler blood flow compared to women with PCOS with normal insulin levels [73]. There have also been studies investigating the effects of androgens on VEGF levels. When DHEA (a precursor to testosterone) is injected subcutaneous into mice, there is an increase in ovarian VEGF and a decrease in VEGFR2 [97]. Besides insulin- and androgen-related theories, there are other explanations for an upregulation of VEGF in PCOS. It is possible that ovaries of women with PCOS have elevated amounts of granulosa cells, although the cause is unclear; alternatively, granulosa cells in PCOS may have an increased capability to secrete VEGF [12]. In addition, granulosa cells overproduce AMH in PCOS patients, perhaps due to increased number of follicles [99], and AMH has been shown to increase VEGF expression in vitro in nonhuman primates [100]. Differing VEGF expression levels can also be due to polymorphisms in the VEGF gene. Numerous single-nucleotide polymorphisms (SNPs) of the VEGF gene have been associated with abnormal VEGF secretion and female reproductive conditions [101–104]. While a systematic review and meta-analysis conducted by Li et al. did not reveal an association between PCOS risk and 3 VEGF SNPs, it did find that Asian women with the specific SNP, +405G>C, had less risk of PCOS [105]. Furthermore, it has been found that in various ethnicities, there are different polymorphisms in the VEGF gene that are associated with an increased risk for PCOS [103, 106, 107].

In support of the notion that elevated concentration of VEGF is involved in PCOS pathogenesis, one research group demonstrated that inhibiting VEGF in a rat model of DHEA-induced PCOS led to a decrease in primary follicles back to control levels, reduced ovarian cysts, and improved follicular development and ovulation, features that are the sine qua non of PCOS [97]. These data suggest that modulation of the VEGF axis may be a potential therapeutic target for PCOS.

OHSS is one of the most common and serious complications of controlled ovarian stimulation. It is caused by increased vascular permeability mediated by increased circulating VEGF levels originating from the ovary. Women with PCOS are more likely to develop this potentially dangerous condition, likely due to abnormalities in ovarian angiogenic factors [5]. Levin et al. have shown that follicular-fluid-induced endothelial cell permeability can be reversed by VEGF antibodies [108]. Furthermore, the VEGF receptor antibody, bevacizumab, has been shown to ameliorate OHSS in a rat OHSS model through lowering VEGF concentrations and ovarian weights [109]. Thus far, only VEGF has been extensively explored in OHSS mechanisms. Further investigations of VEGF as well as more research on other angiogenic factors found to be aberrant in PCOS would help better understand the link between OHSS risk and PCOS. More importantly, it would help elucidate other potential strategies of treating OHSS and PCOS in general. Currently, one of the treatments for OHSS is cabergoline, a dopamine agonist which inhibits VEGFR-2 phosphorylation and signaling [110]. Research has shown that cabergoline and

other dopamine agonists may prevent the increase in vascular permeability that is underlying OHSS [110]. Dopamine agonists are used instead of antiangiogenic drugs because the latter may harm pregnancy development [110, 111].

Angiopoietins

Ang-1 and Ang-2 are important angiogenic factors that are also dysregulated in PCOS ovaries. As described previously, the ratio of Ang-1:Ang-2 in serum is normally higher than the ratio in follicular fluid. Many studies report that this ratio changes in women with PCOS, although these reports have been conflicting. We have shown that in patients with PCOS undergoing IVF treatment, serum Ang-1 levels are increased while serum Ang-2 levels stay the same as control patients [8]. Sova et al. also reported that Ang-2 serum levels in women with PCOS were no different compared to matched controls [112]. Wang et al. have conducted a study involving patients with PCOS that were and were not resistant to clomiphene citrate (CC). They found that CC-resistant women had lower serum Ang-2 levels compared to women who were not resistant to CC. Since lower Ang-2 levels are associated with excessive ovarian angiogenesis, which might subsequently hinder ovulation, they suggest that women with PCOS may be resistant to CC due to lower Ang-2 levels [113]. In follicular fluid, we have found that during oocyte retrieval, Ang-1 levels are similar in PCOS and control patients, but the concentration of Ang-2 is greater in patients with PCOS [8]. This leads to a lower than normal Ang-1:Ang-2 ratio. Because Ang-2 antagonizes Ang-1 and causes blood vessel instability and permeability, this increase in Ang-2 could explain tendency to more "leaky" vessels and why women with PCOS are more likely to develop OHSS. However, Scotti et al. have reported different findings. They found an increase in follicular fluid Ang-1 levels in women with PCOS, while Ang-2 levels remain similar to normal women [10]. They hypothesize that Ang-1 concentration is elevated in order to compensate for VEGF-mediated vessel permeability. These conflicting results could be due to varying stimulation protocols or participant diversity.

In animal studies, Ang-1 has been found to be elevated, while Ang-2 is decreased in the ovaries of rats that had DHEA-induced PCOS [97]. This increase in the Ang-1:Ang-2 ratio is associated with increased vessels and increased vessel stability. Another research group examined pregnant mice with hyperandrogenism and insulin resistance. These pregnant rats were injected with both DHT and insulin daily, and they demonstrated decreased Ang-1 and Ang-2 mRNA expression in uterine tissue compared to controls [114].

There are also opposing results involving angiopoietin receptor concentrations. While Scotti et al. have reported that Tie-2 levels are unchanged in women with PCOS, Abramovich et al. have shown that Tie-2 receptors are increased in a rat model of PCOS [10, 97]. Thus, there are contradicting data, and more research is needed to clarify the abnormalities in expression in angiopoietin and their receptors in women with PCOS as well as their potential role in the pathophysiology of PCOS.

Transforming Growth Factor-ß (TGF-ß)

TGF stabilizes blood vessels and regulates extracellular matrix synthesis. Patients with PCOS exhibit many characteristics of TGF-ß dysregulation, such as increased collagen deposition, extracellular matrix changes, and fibrosis in ovaries [43]. Increased collagen deposition and fibrous tissue can lead to hyperandrogenism and chronic anovulation [43]. Increased TGF-ß levels have also been reported to contribute to hypertension, obesity, insulin resistance, diabetes, and coronary artery disease [5], metabolic manifestations commonly associated with PCOS.

TGF-ß signaling involves fibrillin, an extracellular matrix protein that binds and regulates TGF-ß. Fibrillin-1 mutations are associated with elevated TGF-ß1 levels in serum, which can lead to the abnormal cardiovascular and connective tissue found in Marfan syndrome [115]. The fibrillin-3 gene is linked to PCOS and associated with insulin resistance in women with PCOS [46, 116]. In a study conducted by Raja-Khan et al., PCOS women who do not have a polymorphism in allele 8 of the fibrillin-3 gene (A8-) showed increased serum TGF-ß1 levels compared to healthy individuals; women with PCOS with the polymorphism (A8+) had similar concentrations as control women [117]. In terms of TGF-ß2 concentrations, both A8- and A8+ PCOS women had decreased serum levels compared to healthy individuals [117]. Our lab has also shown TGF-ß dysregulation in patients with PCOS. In a study involving women undergoing ovarian stimulation cycles, women with PCOS had increased TGF-ß1 serum levels and decreased levels of its circulating receptor sENG compared to healthy women, pointing to increased circulating TGF-ß1 bioavailability [9]. Interestingly, there were no differences regarding follicular fluid levels of TGF-ß1 [9]. Liu et al. have also shown increased serum levels of TGF-ß1 in women with PCOS [118]. Notably, treatment of women with PCOS with vitamin D has been shown in a randomized clinical trial to result in a decrease in TGF-ß1 bioavailability, correlating with an improvement in abnormal clinical parameters associated with PCOS [119].

There is accumulating evidence from animal models implicating the role of TGF-ß in PCOS pathogenesis. DHEA-induced PCOS rats have higher TGF-ß1 levels in the ovaries [120–122]. Using DHEA-induced PCOS rat model, it was shown that TGF-ß upregulation is mechanistically linked to ovarian hyperfibrosis, and TGF-βRI inhibition inhibits the downstream signaling molecules of TGF-β and upregulates MMP2, which in turn prevents collagen deposition [120]. The TGF-ß pathway is also implicated in fetal origins of PCOS. Fibrillin-3 is found in human and bovine fetal ovaries [46]. Furthermore, using a model in which primate mothers are androgenized and their offspring develop PCOS characteristics, one study demonstrated that there are different epigenetic alterations on PCOS monkeys compared to control monkeys. Many of the genes that were differentially and epigenetically altered were genes that are involved in the TGF-ß signaling pathway [123]. In addition, microarray gene expression analysis in PCOS granulosa cells revealed that women with PCOS with insulin resistance have differential expression of genes involved in TGF-ß signaling when compared to PCOS women without insulin

resistance [124]. Besides having a role in the fetal origins of PCOS, TGF-ß is also linked with glucose metabolism. Humans with impaired glucose tolerance due to deficient glucose transporter GLUT-10 display excessive TGF-ß activity [125]. In addition, when PCOS-induced rats were treated with sitagliptin, a drug used to treat insulin resistance, there was a decrease in TGF-ß1 protein expression in the ovarian stroma [121].

Lastly, a study conducted by Li et al. has revealed a possible role of microRNA (miRNA) in TGF-ß signaling and PCOS. They found that three miRNA – miR-423, miR-33b, and miR-142 – were dysregulated in granulosa cells of women with PCOS. miR-423, which suppresses Smad7 expression, was downregulated in women with PCOS. miR-33b and miR-142, which target TGF-ß receptor 1, were upregulated in patients with PCOS [126]. This further suggests that dysregulation of various aspects of the TGF-ß signaling pathway can contribute to PCOS characteristics. For a more comprehensive review of TGF-ß effects and the role of TGF-ß in PCOS, please refer to Raja-Khan et al. [43]. Thus, it is clear that TGF-ß expression in patients with PCOS is complex and multifaceted. TGF-ß not only has its own effects, but TGF-ß1 also increases the secretion of VEGF and PDGF-B in rats [42], which leads to angiogenesis upregulation, a characteristic of PCOS patients. More research is needed to understand the effects of these complex interactions.

Platelet-Derived Growth Factor (PDGF)

PDGF may also play an important role in PCOS pathogenesis. Studies have shown that compared to their control counterparts, an OHSS rat model and a DHEA rat model of PCOS have reduced levels of PDGF-B and PDGF-D in ovaries [127, 128]. This has been seen in humans as well. Women with PCOS have decreased serum and follicular fluid levels of PDGF-BB and PDGF-DD [10, 129]. While PDGF-B and PDGF-D levels were altered in the OHSS rat model, there were no differences in the concentration of its receptor, PDGFR-ß [127]. In contrast, the DHEA rat model of PCOS showed a decrease in PDGFR-ß compared to non-PCOS controls [128]. Further research is needed to examine the role of PDGF receptor in PCOS pathology.

This decrease in PDGF may be contributing to the predisposition of patients with PCOS to developing OHSS. PDGF not only prevents an increase in VEGF [58], but PDGF-B and PDGF-D have also been shown to stabilize blood vessels by increasing smooth muscle cell coating of vasculature, thereby stopping the VEGF-mediated "leakiness" in OHSS [130].

Given this PDGF concentration dysregulation in women with PCOS, there are studies investigating the effects of PDGF administration. Di Pietro et al. have introduced PDGF-B to DHEA-injected rats, which resulted in many positive and normalizing effects such as restored AMH, VEGF, and Ang-1 levels similar to those of controls. This PDGF-B injection also increased PDGFR-ß and VEGFR2 receptor levels, which are typically reduced in PCOS [131]. Besides its molecular effects,

PDGF-B administration also had ovarian morphological effects. PCOS rats treated with PDGF-B had a percentage of primordial follicles that was lower than untreated PCOS rats and similar to control rats. Interestingly, PDGF-B did not have a large effect on reducing the percentage of primary follicles. Lastly, PDGF-B-treated PCOS rats have less cysts compared to PCOS rats not treated with PDGF-B [131]. It is clear that PDGF has a regulatory role in both ovarian angiogenesis and folliculogenesis, two altered processes in PCOS. More research into the role of PDGF in PCOS could help illuminate the connection between two common PCOS pathologies: ovarian angiogenesis dysregulation and abnormal folliculogenesis.

Basic Fibroblast Growth Factor (bFGF)

bFGF also contributes to the angiogenic factor imbalance in PCOS. As mentioned previously, bFGF promotes follicular growth and ovarian angiogenesis by stimulating granulosa cells, triggering endothelial cell proliferation, and regulating FSH action [11]. Hammadeh et al. have reported that serum bFGF levels on the day of oocyte retrieval are lower in PCOS patients compared to other infertile patients [132]. In contrast, Artini et al. have shown that on the days of hCG trigger and oocyte retrieval, women with PCOS have higher serum bFGF levels compared to healthy controls [11]. Interestingly, there is no difference between PCOS and healthy women prior to FSH ovarian stimulation, suggesting a relationship between bFGF and FSH [11]. On the day of hCG trigger, there was a positive correlation between the amount of recombinant FSH that was previously administered and serum bFGF concentration, in both the PCOS and non-PCOS population. There was an inverse correlation between follicular-fluid levels of bFGF and percentage of mature oocytes [11].

With regard to follicular fluid bFGF concentrations at time of oocyte retrieval, Hammadeh et al. found no difference between PCOS and control patients undergoing controlled ovarian hyperstimulation [132]. Artini et al. however did see a difference in bFGF concentration in follicular fluid, with PCOS women having higher follicular fluid levels of bFGF [11]. These conflicting results call for more investigations to shed light on the relevance of bFGF in the ovarian angiogenesis dysregulation of PCOS.

Antiangiogenic Dysregulation in PCOS

In addition to the angiogenic factors discussed earlier, antiangiogenic factors also play a role in the dysregulation of ovarian angiogenesis and folliculogenesis that is seen in individuals with PCOS. Angiogenesis is a very complex process, and antiangiogenic factors help regulate blood vessel formation by preventing endothelial cell proliferation and migration [133]. The main antiangiogenic factors are thrombospondins, endostatin, and angiostatin [133], with thrombospondin 1

(TSP-1) being the only one of the three that has been studied in PCOS. Tan et al. demonstrated that serum levels of TSP-1 are lower in PCOS patients, compared to control patients. They have also found that TSP-1 plays a role in limiting in vitro migration and angiogenesis in humans [134]. Liu et al. have similarly found decreased serum TSP-1 concentrations in sera of women with PCOS compared with controls [118].

As mentioned earlier, sVEGFR1 behaves as an antiangiogenic factor by sequestering pro-angiogenic VEGF molecules, thereby limiting VEGF's bioavailability [133]. By binding VEGF, this soluble receptor blocks VEGF signaling pathways that among other processes regulate ovarian vascularization and follicular development. In women with PCOS, levels of sVEGFR1 are decreased in serum and follicular fluid, compared to controls [89, 91], likely exacerbating VEGF bioavailability.

Lastly, SERPINA1 is a protein that exerts antiangiogenic effects. By inhibiting angiogenesis-promoting proteases, SERPINA1 prevents the activation of VEGF and bFGF [135]. In women with PCOS, SERPINA1 transcript levels are elevated in granulosa cells [135]. Furthermore, in a study investigating differences between normal ovulatory women and women with PCOS (with and without evidence of insulin resistance), SERPINA1 was found to be elevated only in the insulin-resistant PCOS population [124]. This suggests that SERPINA1 may be involved in follicular growth as well as metabolic disorders. All in all, SERPINA1 concentrations seem to be dysregulated in women with PCOS.

There is overall less research on antiangiogenic factor dysregulation in women with PCOS when compared with angiogenic factor dysregulation. Considering their role in regulating angiogenesis and the importance of ovarian angiogenesis in PCOS pathophysiology, more research on various antiangiogenic factors is needed to better understand the relevance of a balance of pro- and antiangiogenic factors in the pathogenesis of PCOS.

Role of Abnormal Angiogenesis in Endometrial Dysfunction of PCOS

Women with PCOS have a predisposition for endometrial abnormalities including endometrial hyperplasia and decidualization defects [14, 136]. Women with PCOS have increased rates of implantation failure and poor pregnancy outcomes, and it has been suggested that abnormalities in their endometrium are implicated. In healthy endometrium, angiogenic factors such as TGF-ß are involved in development of endometrial vasculature [137]. Endometrium receptivity to an embryo is dependent on a variety of interactions among cytokines, growth factors, hormones, and cell adhesion molecules [137]. Evidence from experimental and clinical data suggest that the endometrium of women with PCOS displays an array of abnormalities compared to healthy controls [14]. These endometrial aberrations include abnormal expression of sex hormone receptors, impaired endometrial glucose

transport and metabolism, defective decidualization, chronic low grade inflammation and immune dysfunction, and altered endometrial vascularity [14]. Abnormalities in angiogenesis characteristic of PCOS may also have an impact on endometrial receptivity and embryo implantation in women with PCOS, potentially leading to implantation failure and premature labor [138, 139]. There is still no consensus whether women with PCOS have an increased risk of miscarriage; some studies show an association [140–142], while other studies do not [143–145]. Endometrial VEGF expression is increased in proliferative phase endometrial samples of women with PCOS as compared to controls [146]. Interestingly, Zhao et al. have shown that women with PCOS have lower endometrial mRNA and protein levels of hypoxia-inducible factor-1α (HIF-1α), a transcription factor upstream of VEGF that plays a role in endometrial receptivity during embryo implantation [147]. In this study, women with PCOS also had lower VEGF levels in the endometrium during the implantation period compared with control women [147], indicating decreased endometrial angiogenesis. Animal studies suggest that increased androgens may contribute to these endometrial angiogenic abnormalities. In a PCOS rat model study, pregnant mice were injected with varying levels of testosterone and then treated with flutamide. Testosterone administration was associated with increased resorptions and decreased endometrial angiogenesis and uterine NK cells, perhaps through direct endometrial effects [148]. Flutamide, an antiandrogen drug, was found to decrease resorbed embryos, increase CD31 (endothelial cell) staining and NK cell numbers, and restore normal uterine angiogenesis in PCOS rats similar to control mice [148]. Consistent with this, another study in mice found that the hyperandrogenic environment induced by testosterone administration in early pregnancy leads to diminished growth of the utero-placental arterial tree, decreased expression of placental angiogenic factors, and increased placental hypoxia [148]. Studies have shown that women with PCOS and hyperandrogenism have increased uterine artery resistance; these abnormal androgen levels and vascularity can lead to issues in endometrial blood flow and embryo implantation [14]. There is also evidence that hyperandrogenic women with PCOS have increased risk of preterm delivery and preeclampsia [138].

Other studies have shown that the abnormal endometrial receptivity in women with PCOS may also be due to other factors, such as abnormalities in steroid hormones and immune cell migration [149]. Overall, decreased angiogenesis in the endometrium preimplantation and in early pregnancy could contribute to abnormalities described in placentas of women with PCOS [15], leading to pregnancy complications related to abnormal placentation such as preeclampsia [139].

Angiogenic factor dysregulation is also a feature of endometrial hyperplasia, a premalignant condition of increased cellular proliferation. Endometrial hyperplasia is more common in women with PCOS, thought to be largely due to unopposed estrogen conditions [136]. One study has shown that EG-VEGF may be a contributor to angiogenic dysfunction and endometrial hyperplasia in women with PCOS [88]. More research is needed to help elucidate the complex relationship between dysfunctional angiogenesis in women with PCOS and endometrial pathologies.

Targeting of Angiogenic Aberrations in PCOS: *Potential Novel Therapies?*

There is increasing evidence that angiogenic factor imbalance plays a major role in the increased vascularization seen in PCOS pathophysiology. Thus, therapeutic strategies aimed at restoring normal ovarian angiogenesis may be viable targets for PCOS treatment (Table 23.3). Currently, hormonal interventions (e.g., oral contraceptive pills, antiandrogenic drugs, and ovulation inducing agents) and use of insulin sensitizing drugs (e.g., metformin) are the two main classes of therapeutic regimens utilized in addressing symptoms of PCOS.

Combined oral contraceptive pills (OCPs) are typically the first line of treatment for PCOS-related symptoms of hyperandrogenism and menstrual irregularity. Mechanisms for OCP-related benefit against symptoms of hyperandrogenism include a lowering in circulating androgen levels through decreased ovarian androgen synthesis secondary to suppression of pituitary release of LH and a lowering of free androgen levels secondary to increased hepatic synthesis of serum hormone binding globulin levels [150]. Moreover, OCPs have also been shown to reduce ovarian vascularization in both PCOS and control patients as well as contribute to protections against endometrial cancer [151, 152].

Metformin is an oral drug that has been shown to improve menstrual regularity, ovulation, hyperandrogenemia, and live birth rates in women with PCOS [153]. While it is typically used in diabetes due to its effects on endocrine and metabolic abnormalities, this drug also decreases ovarian blood flow [153], as seen through in vivo and in vitro studies. It reduces endothelial migration and angiogenesis, possibly mediated by TSP-1 [134]. Metformin also has molecular effects on angiogenesis. A decrease in VEGF levels in obese diabetic patients after metformin treatment shows metformin's potential for restoring VEGF imbalance in PCOS patients [154]. Metformin has also been associated with a decrease in TGF-ß1 and an increase in TSP-1 in women with PCOS [118]. Furthermore, in a PCOS rat model, metformin led to a decrease in VEGF and Ang-1 as well as an increase in PDGF-B and PDGF-D. This normalization of angiogenic factors was associated with improved folliculogenesis and reduced formation of cysts [128]. Interestingly, metformin use has not only been shown to offer metabolic and endocrine benefit, but it has also been shown to reduce OHSS risk related to gonadotropin use for managing PCOS related infertility, perhaps through inhibiting VEGF [155–158]. A study by Ohara et al. has shown that metformin also reduces androgen receptors in the endometrium of women with PCOS [159]; thus, metformin may have beneficial effects on endometrial receptivity by counteracting the adverse effects of excess androgen on endometrial angiogenesis [148]. There is increasing support to the notion that interventions involving a restoration of angiogenic factor dysregulation are clinically beneficial for patients with PCOS (Table 23.3).

Laparoscopic ovarian drilling (LOD) is a surgical approach to achieving ovulation induction (discussed in detail in Chap. 19 of this textbook) that is uncommonly utilized in anovulatory women with PCOS who are unresponsive to trial of

Table 23.3 Angiogenic factor levels and PCOS characteristics after PCOS therapeutic interventions

Treatment	Study	Population/model	Species	Intervention	Outcome measure	Factor or characteristic	Serum	Ovary
Metformin	Ersoy et al. 2008 [154]	24 patients with type 2 diabetes and a BMI >27 kg/m^2	Human	Week 0–4: Patients continued revised MNT and regular exercise program After 4 weeks: Metformin prescribed for 12 weeks	Measurement in serum before week 0, right before metformin, and 12 weeks after metformin with ELISA	VEGF	↓[a]	–
	Di Pietro et al. 2015 [128]	DHEA-induced PCOS rat model	Rat	Group 1: Received metformin Group 2: Did not receive metformin	Measurement in ovarian tissue with Western blot or ELISA; cystic follicles were large, 4–5 layers of granulosa cells surrounding large antrum	VEGF	–	↓
						Ang-1	–	↓
						PDGF-B	–	↑
						PDGF-D	–	↑
						Ovarian cysts	–	↓
	Liu et al. 2015 [118]	445 women with PCOS (Rotterdam criteria), stratified by PCOS phenotype (obesity, insulin resistance, hyperandrogenism, ovary morphology, ovulatory dysfunction)	Human	Group 1: Received oral cyproterone acetate/ethinyloestradiol (1 tablet per day for 21 days, starting on CD5) Group 2: Received cyproterone acetate/ethinyloestriol (like group 1 and metformin (2–3 times daily)	Measurement in serum on days 2–4 of menstrual cycle with ELISA	TGF-ß	↓[b]	–

Treatment	Study	Population/model	Species	Intervention	Outcome measure	Factor or characteristic	Serum	Ovary
Sitagliptin	Wang et al. 2019 [121]	30 total rats	Rat	Group 1: Control group, just oil injections Group 2: Model group, DHEA injections Group 3: Treatment group, DHEA injections, given Sitagliptin (an incretin) (2 ml daily by gavage for 28 days)	Measurement in serum with ELISA	Testosterone	↓	–
					Measurement of expression and protein levels in ovarian stroma and ovarian tissue with IHC and Western blot	TGF- ß1	–	↓
LOD	Amin et al. 2003 [162]	25 women with CC-resistant PCOS, 20 control women	Human	LOD during early follicular phase	Measurement in serum before and first postoperative cycle after LOD with ELISA	VEGF	↓	–
						Doppler indices	–	↑
	El Behery et al. 2011 [163]	26 women with CC-resistant PCOS, 22 control women	Human	LOD	Measurement in serum before and first postoperative cycle after LOD with ELISA	VEGF	↓	–
						Doppler indices	–	↓

(continued)

Table 23.3 (continued)

Treatment	Study	Population/model	Species	Intervention	Outcome measure	Factor or characteristic	Serum	Ovary
OCPs	Okyay et al. 2014 [151]	200 women, PCOS diagnosis with Rotterdam criteria	Human	Group 1: PCOS, received OCPs Group 2: PCOS, no OCPs Group 3: Control, received OCPs Group 4: Control, no OCPs	Ovarian Doppler measurements at baseline and at end of 3 months of OCPs	Vascularization	–	↓
PDGF Administration	Di Pietro et al. 2016 [131]	DHEA-induced PCOS rat model	Rat	Group 1: PDGF-B injection subcutaneous Group 2: Control, received vehicle	Measurement in ovarian tissue with ELISA 24 h after injection; cystic follicles were bigger than preovulatory follicle	VEGF	–	↓
						Ovarian cysts	–	↓
VEGF Inhibition	Abramovich et al. 2012 [97]	DHEA-induced PCOS rat model	Rat	Group 1: VEGF inhibitor, trap, injected under bursa of both ovaries Group 2: Control, received vehicle	Ovaries were fixed 16 days after beginning DHEA treatment; cystic follicles had 4–5 layers of granulosa cells around a large antrum	Ovarian cysts	–	↓

Treatment	Study	Population/model	Species	Intervention	Outcome measure	Factor or characteristic	Serum	Ovary
Resveratrol	Bahramrezaie et al. 2019 [166]	61 women with PCOS (Rotterdam criteria)	Human	Group 1: 800 mg resveratrol for 40 days Group 2: Oral placebo for 40 days	High-quality oocyte rate was calculated as the number of quality oocytes over the number of retrieved oocytes	Oocyte quality	–	↑
					Measurement of gene expression with RT-PCR	VEGF	–	↓

Abbreviations: Ang-1 angiopoietin-1, *Ang-2* angiopoietin-2, *BMI* body mass index, *CC* clomiphene citrate, *CD* cycle day, *DHEA* dehydroepiandrosterone, *DHT* 5α-dihydrotestosterone, *ELISA* enzyme-linked immunosorbent assay, *IHC* immunohistochemistry, *LOD* laparoscopic ovarian drilling, *MNT* medical nutrition treatment, *OCPs* oral contraceptive pills, *PDGF* platelet-derived growth factor, *RT-PCR* reverse transcription-polymerase chain reaction, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor

[a]VEGF decreased after metformin addition

[b]PCOS women with androgen excess and ovulatory dysfunction and PCOS women with androgen excess and polycystic ovary morphology had a significant decrease

commonly utilized OI strategies [160]. Through multiple ovarian punctures by laser or diathermy, LOD restores LH and testosterone levels, as well as increases spontaneous ovulation and pregnancy rates [161]. There are several theories for its beneficial effects. One is that LOD leads to atresia of follicles, which decreases the amount of thecal cells and subsequently thecal cell androgen production [161]. Another possibility is that LOD decreases VEGF levels and abnormal ovarian vasculature, as demonstrated through Doppler indices [162, 163]. This reduction in VEGF could be due to a reduction in LH and testosterone after LOD. A reduction in angiogenic factors like VEGF could mean less blood supply to the thecal layer, with more normalized androgen production and improved ovarian function.

While the above remain the tried and tested strategies that are utilized in PCOS management, there have been studies in women with PCOS investigating the effects of directly administering or inhibiting angiogenic factors on ovarian angiogenesis and follicle maturation and thereby have examined proof of concept for a new class of therapeutics in PCOS management. Administering PDGF into ovaries of PCOS rats resulted in reduced number of cysts and decreased VEGF levels [131]. Zheng et al. have shown that, in humans, PDGF is positively correlated with progesterone, suggesting that progesterone induces granulosa cell synthesis and secretion of PDGF [129]. There are also studies investigating the effects of VEGF inhibition on PCOS characteristics. When VEGF is inhibited in PCOS rats, there is not only decreased cyst formation in rat ovaries, but also improved ovulation and folliculogenesis [97]. Typically, PCOS women have increased number of oocytes but of poorer quality. It has been shown that elevated follicular fluid levels of VEGF and bFGF contribute to incomplete oocyte maturation, fertilization challenges, and implantation issues [11, 68, 89, 164, 165]. Bahramrezaie et al. have shown that in PCOS population, a reduction in VEGF gene expression due to resveratrol, a polyphenol antioxidant, is associated with higher quality oocytes during IVF cycles and can possibly contribute to lower risk for OHSS [166].

TGF-ß inhibition has also been shown to correct PCOS characteristics in animal models. In a PCOS rat model, Wang et al. demonstrated that oral TGF-β1 inhibition leads to decreased blood glucose levels and decreased serum testosterone levels [121]. This improvement in circulating androgen levels with that TGF-β1 inhibition merits further study in humans as, if safety and effectiveness can be proven, this strategy would add to the existing repertoire of available therapies. Figure 23.3 summarizes the angiogenic factor imbalance and its potential association with various PCOS manifestations (Fig. 23.3).

Conclusion

There is substantial evidence pointing to the premise that dysregulated angiogenesis plays a role in PCOS pathophysiology. Multiple angiogenic factors and their receptors display altered ovarian and circulating concentrations between women with and without PCOS. Not only does this angiogenic factor imbalance play a role in ovarian angiogenesis, but it likely affects many processes that are impacted in PCOS

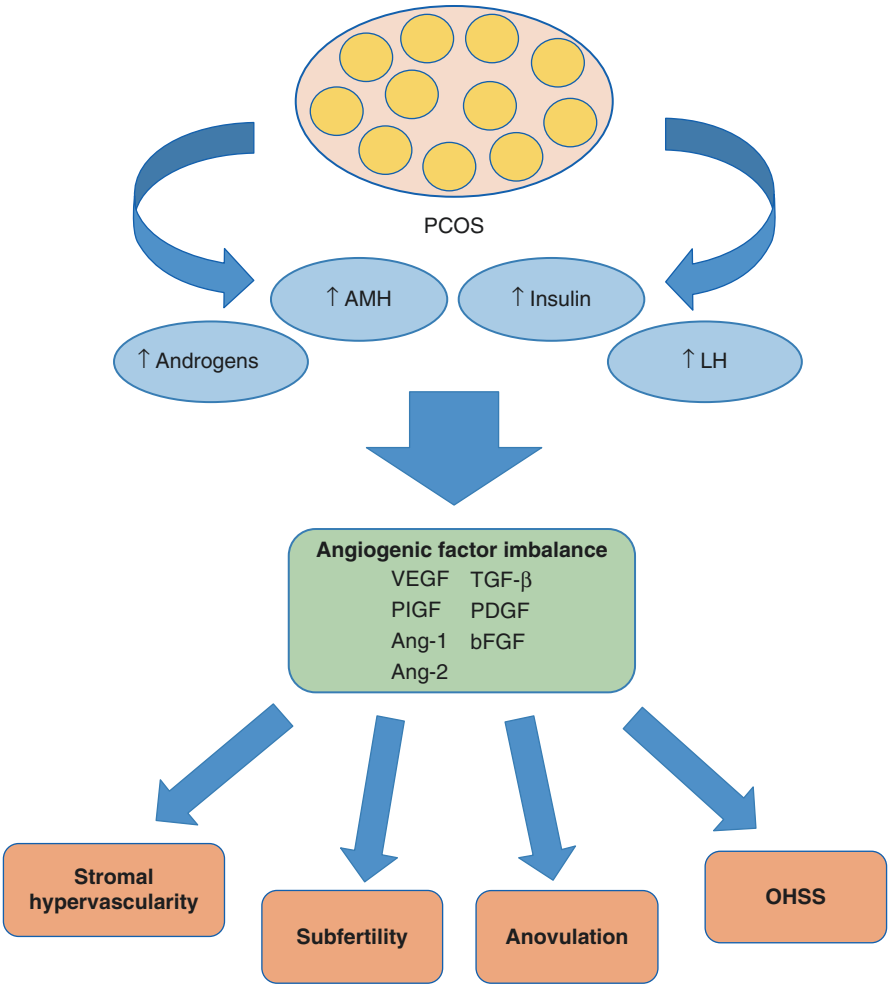

Fig. 23.3 Angiogenic factor imbalance leads to PCOS pathophysiology. Increased androgens, LH, insulin, and AMH in PCOS may lead to increased VEGF and other angiogenic factors. The resulting angiogenic factor dysregulation may contribute to stromal hypervascularity, anovulation, subfertility, and ovarian hyperstimulation syndrome, all of which are characteristic of women with PCOS. (Reprinted with permission from Tal et al. [5] (© 2015 Thieme Publishers)). *Abbreviations*: AMH antimüllerian hormone, Ang-1 angiopoietin-1, Ang-2 angiopoietin-2, bFGF basic fibroblast growth factor, LH luteinizing hormone, OHSS ovarian hyperstimulation syndrome, PCOS polycystic ovarian syndrome, PDGF platelet-derived growth factor, PlGF placental growth factor, TGF transforming growth factor, VEGF vascular endothelial growth factor

including ovarian folliculogenesis, oocyte maturation, ovulation, endometrial receptivity, implantation, placentation, and health of ongoing pregnancy. Abnormal angiogenesis also contributes to PCOS women's higher risk of developing OHSS and possibly to their risk for endometrial pathologies such as endometrial hyperplasia and carcinoma. It is likely that many current PCOS treatments address various

PCOS manifestations, such as irregular menstruation, insulin resistance, and hyperandrogenism, partly by improving the ovarian angiogenic imbalance that characterizes PCOS. Still, more remains unknown than is known about angiogenic factor imbalance and its relevance to the pathophysiology of PCOS. More research is needed to gain a better understanding of the growth factors that are involved in normal and pathological ovarian and endometrial angiogenesis and to assess the potential of angiogenesis-based treatment strategies in PCOS.

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Emerging Concepts: Role of Vitamin D Deficiency in the Pathogenesis of PCOS

24

Samantha Simpson, Lubna Pal, and David B. Seifer

Key Points

- Vitamin D is not a vitamin, but rather a fat-soluble secosteroid manufactured endogenously that mediates its effects through endocrine, autocrine, and paracrine signaling.
- Facilitatory effects of vitamin D extend well beyond the skeletal system. Recent and emerging data identify a relevance of vitamin D for female reproductive physiology.
- 25-Hydroxyvitamin D (25(OH)D) is the major circulating metabolite with a long half-life of 2–3 weeks, and its serum levels reflect the clinical vitamin D status.
- Low vitamin D levels have been documented in women with PCOS with greater frequency than in non-PCOS populations.
- Vitamin D deficiency is associated with obesity, insulin resistance, hyperandrogenemia, dyslipidemia, inflammation, and enhanced risks for diabetes and CVD.
- In women with PCOS, vitamin D deficiency has been related to menstrual irregularity, insulin resistance, hyperandrogenemia, and poorer reproductive outcomes despite fertility treatments.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_24

- Presence of the vitamin D receptor (VDR) in the female reproductive tract identifies the ovarian follicle (oocyte, granulosa cells) and the endometrium as sites of action of vitamin D.
- Presence of 1 alpha hydroxylase, an enzyme that is critical to the activation of this secosteroid, is also described in the female reproductive organs, underscoring a relevance of vitamin D for female reproductive physiology.
- Small clinical trials have demonstrated improvement in insulin sensitivity and menstrual dysfunction as well as a reduction in hyperandrogenemia and CVD risk in vitamin D–deficient women with PCOS following supplementation.
- Possible biological mechanisms by which vitamin D supplementation may offer benefit in PCOS include facilitatory influences upon abnormal folliculogenesis, heightened inflammation, and altered angiogenesis.
- Given the plausibility that vitamin D signaling may modulate processes underlying PCOS, there is a need for large-scale randomized controlled trials (RCTs) to examine the effects of vitamin D supplementation on the endocrinology of PCOS and to determine if fertility outcomes in vitamin D–deficient women with PCOS can be improved through supplementation.
- Until large-scale RCTs are completed, consideration should be given to normalizing vitamin D levels in women with PCOS who have low vitamin D to move towards optimal reproductive health.

Introduction

Polycystic ovary syndrome (PCOS), characterized by clinical and biochemical evidence of hyperandrogenism, ovulatory dysfunction, and polycystic appearance of the ovaries, is the most common endocrinopathy of reproductive age women [1–4]. Insulin resistance (IR) is relevant to the pathophysiology of PCOS [5, 6], with resulting hyperinsulinemia as a mechanism contributory to both the ovulatory dysfunction and hyperandrogenism that characterize this disorder [6]. The endocrine and metabolic milieus of PCOS place this population at enhanced lifetime risk for a spectrum of morbidities including poor reproductive outcomes, type 2 diabetes mellitus (DM), cardiovascular disease (CVD), mood disorders including depression and anxiety, as well as endometrial cancer [1, 2]. Current therapeutic paradigms address the primary clinical concern(s) (e.g. ovulation induction by pharmacotherapy or surgery and assisted reproduction technologies target PCOS-related infertility, whereas hormonal interventions, such as combined hormonal contraceptives and antiandrogens, are preferentially utilized to target symptoms of hyperandrogenism), although attention to the underlying IR through use of insulin-sensitizing agents (i.e., metformin) is a common adjunctive strategy in PCOS management [7].

In the past decade, an accruing body of literature has linked vitamin D deficiency with the endocrine and metabolic milieus as well as with the clinical presentation of PCOS. Vitamin D, which has been simplistically and erroneously misclassified as a "vitamin," is actually a fat-soluble secosteroid; its chemical structure (Fig. 24.1), its mechanism of action via nuclear receptors, and its biological properties all identify vitamin D as a steroid hormone [8].

Vitamin D plays a critical role in calcium and phosphate homeostasis by enhancing their intestinal absorption and is responsible for the maintenance of skeletal health [8]. In addition, a growing body of evidence relates deficiency of vitamin D to a number of nonskeletal sequelae, including obesity, DM, and IR, dyslipidemia, hypertension, inflammation, CVD, autoimmune disease, and cancers [8–13].

The recognition that vitamin D signaling is relevant for female reproductive function in general, and PCOS in particular, is relatively recent. Given that the role of calcium in oocyte activation and maturation is well understood [14–16] and that PCOS is a state of follicular developmental arrest (abnormal folliculogenesis), abnormalities in vitamin D metabolism and action can be linked to the pathogenesis of PCOS. This latter argument is further strengthened by our current understanding of a pathophysiologic role for IR in PCOS. Thus, in PCOS, vitamin D deficiency has emerged as a plausible mechanism to explain many of the metabolic and endocrine features of PCOS in multiple studies, observational as well as randomized controlled trials [17–29]. Accruing data suggest vitamin D insufficiency (<30 ng/mL or <74 nmol/L) and/or deficiency (<20 ng/mL or <50 nmol/L) is linked to PCOS pathophysiology through their associations with obesity, insulin resistance, hyperandrogenism, dyslipidemia, inflammation, as well as features of depression and risk for DM and CVD [18, 20, 22, 29]. While impaired folliculogenesis, steroidogenesis, and reproductive compromise are well described in the animal models of vitamin D deficiency, a relevance of vitamin D in human reproductive biology is

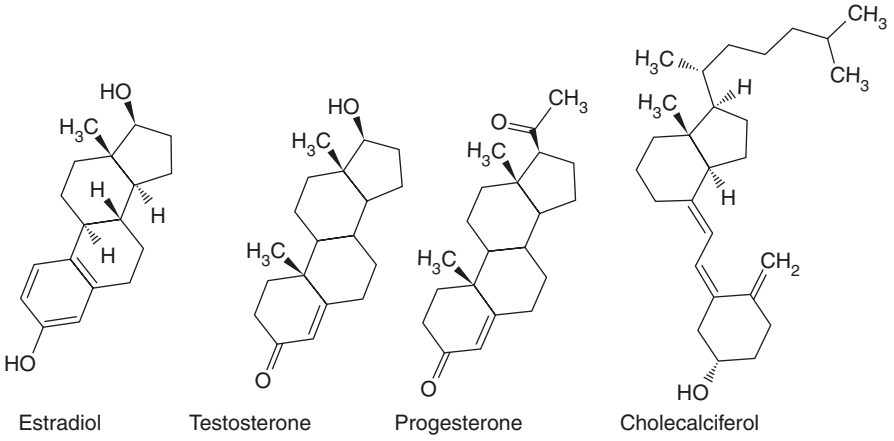

Fig. 24.1 Vitamin D's chemical structure compared to that of the sex steroids

emerging. Herein we provide an overview of our current understanding of the plausible relevance of vitamin D in the pathophysiology of PCOS.

Humans are primarily dependent on endogenous cutaneous synthesis of vitamin D through exposure to solar ultraviolet B (UVB) (290–315 nm); dietary sources account for less than 20% of daily requirements of the vitamin. Exposure to solar UVB converts dehydrocholesterol in the skin to previtamin D_3 which is rapidly converted to vitamin D_3 (cholecalciferol). Dietary vitamin D gets incorporated in the chylomicrons and gets transported via the lymphatics to the circulation. Circulating vitamin D is transported to the liver by vitamin D–binding protein (VDBP) where it undergoes the first step of activation when vitamin D-25-hydroxylase catalyzes the conversion to 25-hydroxy vitamin D3 (25(OH)D3), the major circulating form (Fig. 24.2). The 25(OH)D3 metabolite has a long half-life of 2–3 weeks and reflects the overall clinical vitamin D status. Final activation of 25(OH)D3 occurs in the kidney as well as at target cell level (e.g., in the ovarian follicle), via 1α-hydroxylase to 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), the active vitamin D metabolite that, among many functions, also promotes intestinal calcium absorption through its interaction with the cognate vitamin D receptor (VDR).

Vitamin D acts as a transcription factor via signaling through the nuclear VDR–retinoic acid x-receptor (VDR-RXR) complex [8] and exerts actions across a host of tissues, including the skeleton, pancreas, parathyroid glands, ovary, endometrium, and placenta [31, 32]. Serum levels of calcium, phosphorus, parathyroid hormone

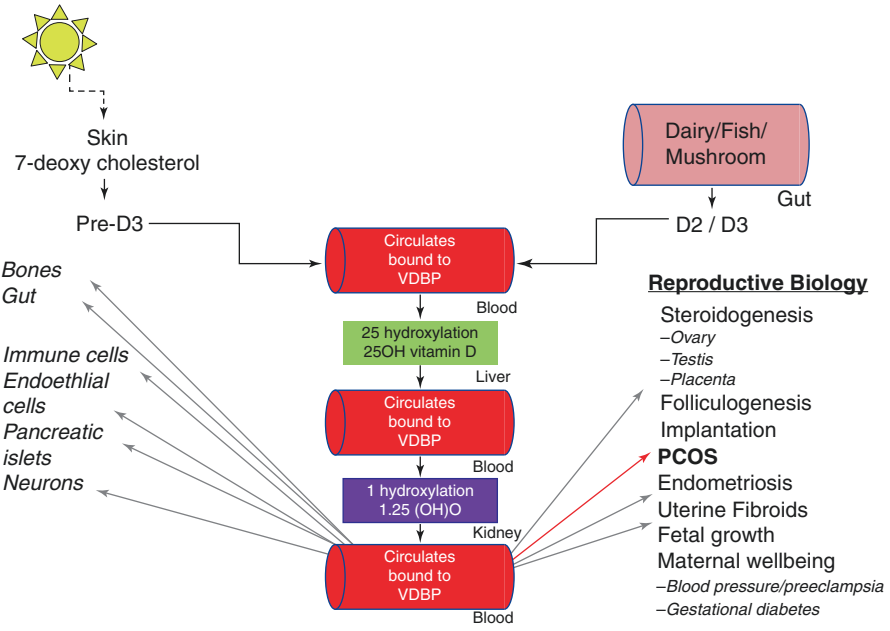

Fig. 24.2 An overview of vitamin D metabolism and salient actions. *VDBP* vitamin D–binding protein, *D2* ergocalciferol, *D3* cholecalciferol. (Modified from [30] Figure Legends)

(PTH), as well as fibroblast-derived growth factor-23 (FGF-23) are recognized modulators of 1α-hydroxylation of 25(OH)D [8].

Obesity, PCOS, and Vitamin D

There is an increased prevalence of body mass excess, including central obesity, in women with PCOS as compared to age-matched controls [1, 6, 33, 34]. An inverse relationship between circulating 25(OH)D levels and parameters of body mass excess such as body mass index (BMI) and waist circumference (WC) is well described across populations including women with PCOS [18, 20–22, 24, 26, 35, 36]. In an observational study of 25 women with PCOS and 27 controls, Li et al. reported an inverse relationship between serum 25(OH)D levels and BMI, with as many as 72% of the PCOS group being vitamin D deficient, of which 44% were severely deficient <10ng/mL or <25nmol/L [18]. A similar association was observed by Wehr et al. in an observational study involving 206 PCOS women wherein 72% of the PCOS population demonstrated evidence of vitamin D deficiency and a significant inverse correlation between 25(OH)D level and BMI was noted [20]. In a cross-sectional study, Joham et al. found the prevalence of vitamin D deficiency to be higher in overweight women with PCOS, compared to overweight controls [37]. Of note, those meeting National Cholesterol Education Program (NCEP) III criteria for metabolic syndrome (MS) [38] had significantly lower 25(OH)D levels compared to women with PCOS who were without MS. A different study specifically linked vitamin D deficiency in PCOS women with central adiposity, as reflected by higher WC indices [39]. Possible rationale for the lower circulating 25(OH)D levels in obesity may include increased sequestration of this fat-soluble secosteroid in the adipose tissue [40]; decreased tendency of obese individuals to seek sunlight may be yet another plausible mechanism [41].

Vitamin D: A Modulator of Insulin Resistance in PCOS

Insulin secretion is a calcium-dependent process [42, 43], and IR and compensatory hyperinsulinemia are well described in the setting of PCOS [5, 6, 26]. The precise mechanism of action whereby vitamin D influences insulin signaling appears to involve genomic stimulation of the insulin receptor mRNA via VDR signaling [44, 45]. Vitamin D signaling appears to promote insulin synthesis and release, enhance insulin receptor expression, and also inhibit pro-inflammatory cytokines that are recognized to play a role in the pathogenesis of IR [46]. The latter mechanisms may explain the observed associations between vitamin D deficiency with impaired glucose and insulin metabolism [47–50] and the recognized high prevalence of hypovitaminosis D in populations with type 2 DM [51–53].

A high prevalence of IR and coexistent vitamin D deficiency has been described in women with PCOS [21, 22, 25, 26, 54]. Various observational studies looking at upward of 700 women with PCOS have demonstrated inverse correlations between

serum levels of 25(OH)D and indices of IR such as the homeostatic model assessment for insulin resistance (HOMA-IR) [22, 54, 55]. On subgroup analysis stratified on BMI, significantly lower 25(OH)D levels were observed in the obese and the overweight phenotypes compared to the lean PCOS population, suggesting a role for obesity in the observed relationship between vitamin D status and IR [22]. Interestingly, all three BMI-based subgroups of women with PCOS demonstrated evidence of vitamin D insufficiency, adding further evidence that PCOS is a state associated with hypovitaminosis D [22]. These findings were reaffirmed in a case control study involving 301 women with PCOS and 113 controls, wherein inverse correlations between HOMA-IR and 25(OH)D levels were observed [21]. The associations in both groups (women with PCOS and controls) showed a negative correlation between 25(OH)D levels and BMI [21]. In another observational study, Kotsa et al. reported significant increase, compared to baseline, in the first phase of insulin secretion during an oral glucose tolerance test (OGTT) following 1 μg/day treatment with alpha-calcidiol over 3 months in 15 obese women with PCOS [25].

In a vitamin D interventional trial in women with PCOS, supplementation of 50,000 IU of cholecalciferol weekly for 8 weeks resulted in increased circulating levels of soluble receptors for advanced glycation end products (rAGEs) and decreased serum VEGF [56, 57]. AGEs are a marker of inflammation and are associated with the development of diabetes in animal models [58]. Increasing rAGEs decreases the inflammatory effects of AGEs. VEGF is overexpressed in ovaries of women with PCOS and is associated with the characteristic hyperthecotic stroma of PCOS and increased risk of ovarian hyperstimulation syndrome [59, 60]. Vitamin D supplementation in non-PCOS controls did not increase rAGEs, specifically highlighting the importance of vitamin D in PCOS inflammatory pathophysiology and downstream metabolic dysfunction of PCOS [56].

More directly, 50,000 IU vitamin D oral supplementation weekly for 8 weeks has been shown to improve clinical indices of insulin resistance, such as HOMA-IR and fasting glucose, in randomized trials in women with PCOS [61–64], although studies looking at different dosages and frequency of vitamin D supplementation have not shown as convincing an effect [65–67]. This concept is expanded on section "Dose and Duration of Vitamin D Supplementation." A meta-analysis looking at vitamin D supplementation's effects on fasting glucose and HOMA-IR in women with PCOS showed improvement in both parameters, even when vitamin D supplementation was less than 4000 IU/day [68].

In conclusion, a growing body of data accrued in women with PCOS suggests a relationship between vitamin D deficiency and IR, whether through inverse correlations between vitamin D and indices of IR [22, 25] or by demonstrating an attenuation through vitamin D supplementation in circulating inflammatory markers that are known to play a role in the pathogenesis of metabolic disease [56, 57, 61–64]. To strengthen the argument for a causal relationship, there is a need for appropriately powered randomized controlled trials to establish if vitamin D supplementation can attenuate the dysmetabolic milieu of PCOS in vitamin D-deficient women.

Vitamin D: Relationship with Hyperandrogenemia of PCOS

Vitamin D status is hypothesized to modulate circulating androgen levels through interactions with sex hormone-binding globulin (SHBG) and PTH. Recognized as a modulator of circulating levels of free sex hormones, hepatic SHBG is the dominant carrier protein that binds circulating androgens including testosterone and androstenedione and, hence, minimizes percentage of free androgen available to act at the target tissue; a decline in SHBG levels, as seen in states of IR, is associated with increase in circulating free androgen levels and, hence, worsening features of hyperandrogenism.

Serum levels of 25(OH)D are shown to correlate positively with SHBG and inversely with circulating androgen levels [37]. In an observational study of 206 women with PCOS, Wehr et al. reported positive correlation of 25(OH)D levels with SHBG; the association, however, was abolished on adjustment for BMI [20]. Hahn et al. reported a positive correlation between 25(OH)D and SHBG levels; statistically nonsignificant inverse correlations were observed between 25(OH)D with total testosterone, androstenedione, and DHEAS levels, and with the free androgen index (FAI) in 120 women with PCOS [22]. Li et al. also described positive correlations between 25(OH)D and SHBG and inverse correlation between 25(OH)D and FAI [18]. The SHBG levels were lower in the severely vitamin D-deficient women with PCOS, but the above relationship was rendered insignificant on BMI adjustment [18]. This was corroborated in an interventional pilot study undertaken by Pal et al. wherein, over a 3-month period of vitamin D and calcium supplementation in 12 overweight women with PCOS, a significant reduction in serum levels of total testosterone and androstenedione was observed [23]. In a randomized study of women with PCOS, Jamilian et al. showed supplementation with 4000 IU of vitamin D daily for 12 weeks decreased FAI and increased SHBG significantly more than in women receiving just 1000 IU of vitamin D or placebo [69]. In another randomized study of overweight women with PCOS, receiving 50,000 IU vitamin D weekly for 12 weeks significantly decreased testosterone and increased SHBG; these changes were not observed in the placebo group [70].

When looking at clinical signs of hyperandrogenemia, Wehr et al. observed a significant negative correlation between serum 25(OH)D levels with hirsutism scores; notably, this relationship remained statistically significant after adjusting for BMI [20]. In a single-arm intervention trial of 6 months of vitamin D and calcium supplementation (50,000 IU vitamin D weekly and 1500 mg of elemental calcium daily) in 13 hyperandrogenic women with PCOS, Thys-Jacobs et al. described clinical improvement in acne, suggesting therapeutic potential of vitamin D supplementation against symptoms of hyperandrogenism [17]. In a randomized controlled trial of 68 PCOS women with vitamin D deficiency, Irani et al. showed a normalization of serum 25(OH)D level and a decrease in hirsutism scores after supplementation with 50,000 IU of vitamin D weekly for 8 weeks; no similar benefit was observed in the placebo group [71]; these findings were corroborated by Al-Bayyari et al. [70]. Similar clinical improvements were shown by Jamilian et al.; women with PCOS randomized to 4000 IU daily of vitamin D supplementation for 12 weeks had

improvements in hirsutism scores when compared to patients only receiving 1000 IU daily or placebo [69].

Serum PTH levels are intimately related to vitamin D status and are known to be higher in obese compared to lean individuals, and this phenomenon is deemed secondary to lower vitamin D levels in the obese population [71–76]. Panidis et al. reported significantly higher PTH levels and lower 25(OH)D levels in obese compared to normal-weight women with and without PCOS; investigators additionally observed significant positive correlation between PTH and total testosterone levels, a relationship that was independent of both age and BMI. These observations are suggestive of mechanistic implications of vitamin D deficiency-related secondary hyperparathyroidism for the hyperandrogenemia of PCOS [21]. In an interventional trial, vitamin D-deficient women with PCOS who were supplemented with 50,000 IU vitamin D weekly for 8–12 weeks had decreased PTH levels after treatment, compared to women who received placebo [70, 71].

To summarize, the existing data consistently show that vitamin D insufficiency is correlated with hyperandrogenemia of PCOS, and normalization of vitamin D leads to improvement of hyperandrogenemic parameters. As discussed in the section on IR, while a "cause-and-effect" nature of the observed associations between vitamin D deficiency and hyperandrogenemia may only be suggested given that the bulk of the data are observational in nature. Appropriately powered randomized controlled trials of vitamin D supplementation are needed to firmly establish if correction of vitamin D deficiency can improve the androgenic milieu and phenotypic burden of PCOS.

Vitamin D: Implications for Ovarian Physiology in PCOS

Ovulatory dysfunction is one of the diagnostic criteria for PCOS. Vitamin D has been implicated in animal and human models to play a role in ovulatory regulation [14–20, 22]. Two translational studies by Xu et al. in nonhuman primates (rhesus macaques) [77, 78] underscore the direct role vitamin D has upon follicular maturation and the promotion of follicular health during folliculogenesis. They demonstrate vitamin D receptor (VDR) immunostaining to be localized predominantly on the oocyte during the early developmental stages of folliculogenesis (i.e., primordial and primary follicles). As follicular growth progresses, VDR immunostaining becomes evident in granulosa cells. In mice, vitamin D has been shown to modulate steroidogenesis in granulosa cells through phosphorylation of AMP-activated kinase [79], though in humans the specific mechanisms whereby vitamin D signaling affects granulosa cell function remain unclear. As an endocrine and paracrine/autocrine factor, vitamin D demonstrates a direct trophic effect upon early and late folliculogenesis promoting survival, growth, and function with steroid and anti-Müllerian hormone (AMH) production prior to blastocyst development via the AMH pathway in a stage- and dose-dependent manner [77, 78].

Several clinical studies establishing a link between vitamin D status, calcium homeostasis, and ovulatory dysfunction in PCOS have been conducted [17, 20, 27].

In a single-arm intervention trial undertaken in 13 anovulatory, oligo-/amenorrheic women with PCOS, Thys-Jacobs et al. reported improvement in menstrual disturbances following vitamin D and calcium supplementation over a 6-month period [17]. All participants were normocalcemic, whereas 5 of the 13 exhibited elevated PTH levels suggestive of secondary hyperparathyroidism. Within 2 months of supplementation with 1500 mg/day of elemental Ca and ergocalciferol (D2) 50,000 IU weekly or biweekly, resumption of normal menstrual cyclicity is described in 7/9 with oligomenorrhea (78%) and improvement in dysfunctional bleeding patterns described in an additional two participants; the authors surmise these phenomenon as being secondary to facilitatory influences of vitamin D and calcium on ovarian physiology [17]. Wehr et al., in an interventional trial, supplemented 46 PCOS women with weekly 20,000 IU cholecalciferol for 24 weeks and reported resolution of menstrual disturbances in 50% of the cohort by week 24 [80]. Rashidi et al. randomized women with PCOS-related infertility to calcium 1000 mg plus vitamin D 400 IU alone (group 1, referent population), calcium 1000 mg plus vitamin D 400 IU plus metformin 1500 mg/day (group 2), and metformin 1500 mg/day alone (group 3), and it showed improved spontaneous ovarian follicular development in the group receiving metformin, calcium, and vitamin D compared to the referent group within 3 months [27]. The authors conclude that addition of vitamin D and calcium to a metformin regimen is more effective in correcting menstrual disorders and follicular growth than either metformin or calcium and vitamin D alone. A second randomized controlled trial looking at supplementation of 50,000 IU vitamin D weekly for 8 weeks in vitamin D-deficient women with PCOS showed a shortening of the intermenstrual interval from 80 days to 60 days [56], compared to no change in women taking placebo.

AMH is produced by granulosa cells of large preantral and small antral ovarian follicles, of which there is an abundance in patients with PCOS [81, 82]. Accordingly, patients with PCOS have been shown to have higher serum AMH than their non-PCOS peers [83–87], and in fact several studies have shown that high AMH can be used to predict PCOS [88, 89]. High AMH inhibits selection (cyclical recruitment) of small antral follicles to become larger antral preovulatory follicles in response to follicle-stimulating hormone, thus suppressing follicular growth, resulting in abnormal or aberrant folliculogenesis (Fig. 24.3) [91, 92]. High AMH is also known to inhibit aromatization of androgens to estradiol in the granulosa cell contributing to greater androgens seen in PCOS. A vitamin D response element has been identified in the promoter region of the AMH gene [93], and this observation underlies the biological nexus between vitamin D and AMH. Vitamin D's precise action on the AMH response element is unclear [94, 95] and may be dependent upon endogenous AMH levels. In the context of PCOS with elevated AMH and vitamin D deficiency, vitamin D supplementation has been shown to lower and normalize AMH in women with PCOS [56, 96–99], perhaps through vitamin D's suppression of the transcription of AMH. However, in a different context of low AMH as present in women with diminished ovarian reserve and low vitamin D, supplementation with vitamin D has been shown to increase AMH levels [98].

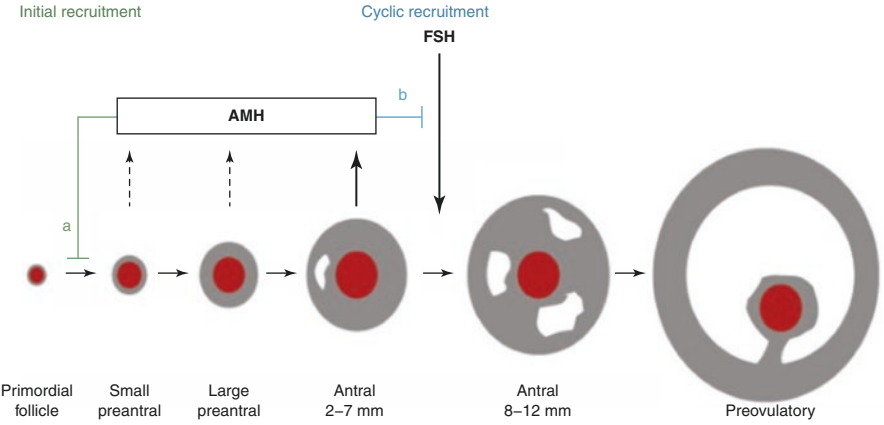

Fig. 24.3 The role of AMH in folliculogenesis. (Adapted from Broekmans et al. [90], with permission)

Vitamin D: Potential Implications for Reproductive Success in Women with PCOS

Animal experimental models have identified vitamin D as essential for procreative success beyond ovarian folliculogenesis, with recognized effects also on spermatogenesis, steroidogenesis, and implantation [30, 100, 101]. Although VDR has been identified in the human ovarian follicle, endometrium, myometrium, cervical, and breast tissues [102], data supportive of relevance of vitamin D signaling for reproductive biology in humans are sparse. Vitamin D is recognized to enhance action of the enzyme aromatase, which is responsible for conversion of androgens to estrogens in the ovarian granulosa cells [103], and interruption of estrogen signaling is suggested as a mechanism for reproductive disturbances in the setting of vitamin D deficiency [103, 104] as well as in the context of elevated AMH often present in PCOS. One could speculate that aromatase inhibition already present in PCOS by elevated AMH is further exacerbated in the context of low levels of vitamin D. Furthermore, by normalizing serum levels of vitamin D with use of oral vitamin D supplementation, elevated AMH would normalize and no longer inhibits aromatase. In studies of infertile women undergoing in vitro fertilization, significantly higher 25(OH)D levels were observed in the ovarian follicular fluid of women who achieved clinical pregnancy following fresh embryo transfer [105].

In the context of PCOS-related infertility, spontaneous conception was achieved by 2/13 women completing a 6-month trial of vitamin D and calcium supplementation undertaken by Thys Jacobs et al. Given an absence of details regarding the proportion of participants who were attempting to conceive in this study, it is difficult to interpret the two spontaneous conceptions as evidence of therapeutic success of vitamin D and calcium in PCOS-related subfertility [17]. Ott et al. [106] reported a prospective cohort study wherein serum 25(OH)D levels were assessed in 91

anovulatory women with PCOS undergoing ovulation induction treatment with clomiphene citrate (CC). Serum 25(OH)D levels were positively predictive of likelihood for achieving a dominant follicle in response to CC treatment ($p = 0.014$) and of successful pregnancy ($p < 0.001$). Conversely, the likelihood for ovarian responsiveness to CC was reduced by 77% (OR 0.33, 95% CI 0.13–0.85) and for CC treatment-related pregnancy was reduced by 76% (OR 0.24, 95% CI 0.07–0.84) in women with evidence of severe vitamin D deficiency (serum 25(OH)D level <25 nmol/L or <10 ng/mL) [106].

In a retrospective cohort approach, Butts et al. reported on vitamin D status of women with PCOS who had participated in the PPCOSII (Pregnancy in PCOS II), a randomized controlled trial that compared the efficacy of CC versus letrozole for managing PCOS related ovulatory infertility. Serum 25(OH)D levels were assayed in stored serum samples in 607 of the original 750 (81%) participants in PPCOS II clinical trial; vitamin D deficient women with PCOS were 18 % less likely to ovulate (AOR 0.82) and 37% less likely to achieve live birth (AOR 0.63) compared to the non-deficient PCOS population [107]. These findings corroborated observations of an earlier study wherein reproductive relevance of vitamin D status was examined in PCOS participants of another seminal randomized controlled trial (Pregnancy in PCOS 1) which had examined the effectiveness of CC versus metformin versus a combination of CC plus metformin for managing PCOS-related ovulatory infertility was examined [108, 109]. Similar to findings from PPCOS II, vitamin D status of women with PCOS was an independent predictor of successful ovulation and live birth in the PPCOS 1 trial [108]. Interestingly, the study by Butts et al. also included a comparison group of women from the Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) trial with unexplained infertility in contrast to infertility attributed to PCOS – these women with unexplained infertility demonstrated no decrease in live birth rate associated with vitamin D deficiency [107, 110]. Thus, this study emphasizes the role vitamin D status plays specifically in PCOS in contrast to other etiologies of infertility. A more recent systematic review and meta-analysis [111] failed to demonstrate any relevance of vitamin D status for outcome of in vitro fertilization (IVF) cycles.

In a randomized placebo-controlled trial, Irani et al. [57] explored the effect of vitamin D replacement on elevated VEGF levels in women with PCOS and found vitamin D to decrease VEGF levels and transforming growth factor-beta availability in PCOS. Thus, it is speculated that by normalizing vitamin D levels in women with PCOS it is likely to decrease the incidence of ovarian hyperstimulation syndrome (OHSS) in women with PCOS receiving ovulation induction for infertility treatments [57]. In another randomized placebo-controlled trial comparing administration of clomid 100 mg for 5 days with vitamin D 10,000 IU twice weekly during the course of ovulation induction versus clomid 100 mg alone found that patients randomized to the vitamin D arm had higher rates of ovulation (92.5% versus 78.5%), but this study did not determine the overall vitamin D status of study participants, and it did not find significant differences in pregnancy rate and live birth [112]. Additional appropriately designed and adequately powered prospective clinical trials are welcomed to firmly establish if infertility treatment

success, especially in women with PCOS, can be further improved through optimization of vitamin D status. Possible ovarian biological mechanisms by which vitamin D contributes to infertility clinical improvement in vitamin D-deficient women with PCOS include beneficial influence upon ovarian folliculogenesis, inflammation, and angiogenesis.

Vitamin D: Potential Implications for Cardiovascular Health in PCOS

Epidemiological data identify vitamin D deficiency as a risk factor for enhanced cardiovascular morbidity and mortality [113–115]. Vitamin D receptors are located in the vascular smooth muscle [116] and endothelium [117], and inflammation, dyslipidemia, hypertension, coronary artery disease, cardiac failure, and accelerated carotid atherosclerosis have been described in association with vitamin D insufficiency across populations [113–115]. Limited data are additionally available on the relationship between vitamin D status and CVD risk in the PCOS population [18, 20, 22]. Li et al. identified 25(OH)D levels to relate inversely with C-reactive protein (CRP), a known risk factor for CVD [18], and multiple studies have identified positive correlations between 25(OH)D and HDL levels [18, 22, 53]. Wehr et al. demonstrated inverse correlations between vitamin D status and serum levels of triglycerides, total cholesterol, and systolic and diastolic blood pressure [20].

In a randomized clinical trial by Irani et al., 8 weeks of vitamin D supplementation in women with PCOS was shown to decrease triglycerides from 138 to 117 mg/dL, a change not seen in the placebo group [71]. This is hypothesized to be related to a vitamin D-mediated decrease in VEGF after vitamin D supplementation [57]. Additionally, vitamin D supplementation in patients with PCOS has been shown to decrease total and LDL cholesterol [61] and CRP [69], which was not shown in patients taking placebo.

In a pilot study, improvements in blood pressure (BP) parameters (systolic blood pressure [SBP], diastolic blood pressure [DBP], and mean arterial blood pressure [MAP]) followed a 3-month period of vitamin D and calcium supplementation undertaken in 12 overweight and vitamin D-deficient women with PCOS. Notably, at baseline, 17% (2/12) met the criteria for hypertension (SBP >140/DBP >80 mmHg) compared to 0% at the completion of the clinical trial ($p < 0.001$) [23]. Reduction in SBP by as little as 2 mm has been shown to reduce CVD-related mortality by 6% [118]. The magnitude of reduction in SBP achieved through vitamin D and calcium supplementation (i.e., by an average of 7 mmHg) thus is clinically meaningful [23], although given that the study design lacked a control arm; this observation merits substantiation in a future randomized controlled trial. These studies look at markers of cardiovascular health with short-interval follow-up, longer-term observation after randomization of vitamin supplementation will allow for discernment of actual cardiovascular benefit in these patients with PCOS.

Vitamin D: Potential Implications for Psychological Well-Being in Women with PCOS

Vitamin D deficiency has been linked to depression in the non-PCOS populations [119, 120]. Women with PCOS carry a substantial psychological burden; although the exact underpinnings remain unclear, altered physical appearance (obesity, acne, alopecia, hirsutism), menstrual irregularity, and difficulties in conceiving are recognized as potential contributors to the prevalent issues of depression, anxiety disorders, body image dissatisfaction, and sexual dysfunction encountered in women with PCOS [121–124]. A prospective observational study identified a negative correlation between serum levels of 25(OH)D with depressive symptoms on a validated screening tool (personal health questionnaire [PHQ]) [125], manifest in women with PCOS [126]. Another cross-sectional study found that depressive symptoms were more common in obese women with vitamin D deficiency as opposed to women with normal vitamin D status, regardless of PCOS diagnosis [127]. The observational study designs and lacking controls do not allow determination of a cause-effect relationship to the observed association between vitamin D deficiency and depressive symptoms in women with PCOS.

Dose and Duration of Vitamin D Supplementation

The link between vitamin D and PCOS has been more widely reported on in recent years, including a proliferation of randomized, placebo-controlled trials. These studies allow us to better understand the role of vitamin D dosing regimen on changes in PCOS serum markers and phenotype. Specifically, a randomized, controlled trial comparing vitamin D 4000 IU daily to vitamin D 1000 IU daily to placebo in women with PCOS found the 4000 IU dose only to lead to significant improvements in HOMA-IR, total testosterone, SHBG, Ferriman-Gallwey scores, and CRP [69].

Comparing results of different randomized controlled trials broadens this theme. In a trial looking at supplementing 3200 IU of vitamin D daily for 12 weeks compared to placebo, there were no differences in CRP, lipid profile, glucose or insulin levels, HOMA-IR, testosterone, and SHBG [66]. A different trial looking at 50,000 IU vitamin D every 20 days for three doses showed no difference in insulin or HOMA-IR [65]. However, a study increasing the dosage frequency to 50,000 IU every other week for 8 weeks showed reductions in insulin, HOMA-IR, and cholesterol [61], and 50,000 IU every week for 8 weeks also showed reductions in cholesterol [71]. These studies suggest that a supplemental dose of at least 3500 IU of vitamin D daily is needed to see benefit in insulin resistance and inflammatory parameters for women with PCOS. Alternatively, there may be such a thing as too high of a dose – one study of 12,000 IU vitamin D supplementation daily in women with PCOS found no change in insulin and HOMA-IR, though it did find significant reductions in blood pressure [67].

Summary

A vast body of literature links low vitamin D status to obesity, insulin resistance, menstrual irregularity, depression, and increased CVD risk (Fig. 24.4). PCOS pathophysiology and its associated decrease in successful reproductive outcomes is being linked to vitamin D insufficiency in emerging research. Most studies connecting vitamin D insufficiency with PCOS are observational, but a growing number are prospective and randomized and hence suggest a cause-and-effect nature to the observed associations. A limited number of interventional clinical trials have demonstrated improvement of insulin sensitivity, menstrual dysfunction, CVD risk parameters such as blood pressure, fertility outcomes, and a reduction in circulating androgens in women with PCOS when vitamin D levels are normalized. Appropriately powered double-blind randomized controlled trials with prolonged follow-up are needed to definitively address if vitamin D insufficiency is a modifiable mechanism in the pathophysiology of PCOS and to what degree normalization of vitamin D status can mitigate the endocrine, metabolic, clinical, and reproductive stigmata of PCOS. With our current understanding, supplementation with vitamin D of either 4000 IU daily or 50,000 IU weekly for up to 12 weeks appears to be a safe intervention that may benefit women with PCOS in the realm of reproductive success, insulin resistance, cardiovascular health, and hyperandrogenic symptoms.

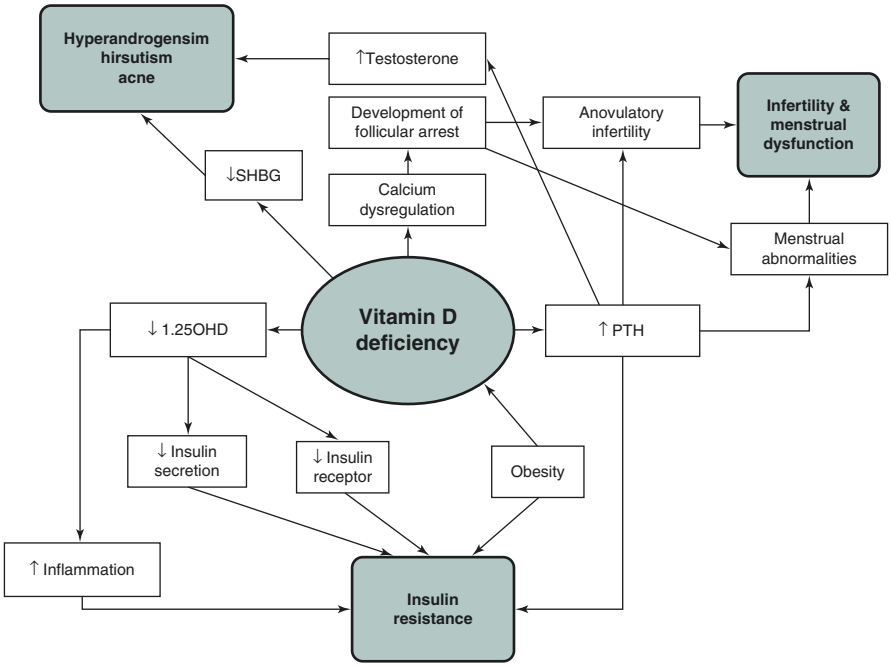

Fig. 24.4 The role of vitamin D deficiency in the pathology of PCOS. (Adapted from Thomson et al. [19], with permission from John Wiley and Sons. © 2012 Blackwell Publishing Ltd)

Acknowledgments The authors and editors wish to acknowledge the significant contributions of Dr. Sanam Lathief towards the original version of this chapter for the first edition of this textbook.

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25 Anti-Mullerian Hormone Signaling: Relevance for Pathophysiology of PCOS and Implications for Novel Therapeutic Approaches to Managing Ovulatory Dysfunction of PCOS

Alice J. Shapiro, Vitaly Kushnir, and David B. Seifer

Key Points

- AMH is a member of the TGFß family and acts via binding of the AMH type II receptor.
- AMH plays a critical role in the sexual differentiation in male fetal development by inducing regression of Mullerian ducts.
- In females, AMH production increases after birth until the mid-twenties and then declines to undetectable levels around menopause.
- In the ovaries, AMH is produced by the granulosa cells of preantral/small antral follicles and inhibits primordial follicle growth.
- Clinically, AMH is used as a measurement of ovarian reserve and correlates with ovarian response during infertility treatments.
- PCOS patients have higher AMH levels due to a higher number of small antral follicles and increased production by granulosa cells.
- Higher AMH levels in PCOS women may contribute to anovulation by inhibiting FSH-mediated effects on follicle selection and growth and by increasing follicular androgen synthesis by inhibiting aromatization.
- AMH levels are positively correlated with VEGF, a key mediator of ovarian hyperstimulation syndrome (OHSS), and contribute to increased OHSS risk in PCOS patients undergoing ovarian stimulation.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_25

- Improved follicular dynamics and ovulation described with insulin sensitizers, laparoscopic ovarian drilling, and vitamin D supplementation may result from a decrease in granulosa cell AMH production.
- AMH structural analogues have shown promise in the treatment of ovarian malignancies of Mullerian origin and endometriosis and are being investigated as a potential novel class of female contraceptives, and AMH antagonists targeting AMH type II receptors are being examined as potential targets for ovulation induction in PCOS patients.
- Due to its association with elevated VEGF levels, the AMH receptor may be another therapeutic target for decreasing the risk of OHSS in the PCOS population.

Background

Anti-Mullerian hormone (AMH), also known as Mullerian-inhibiting substance (MIS), is a member of the transforming growth factor-ß (TGF-ß) family of glycoproteins. This large group of structurally related cell regulatory proteins includes activins, inhibins, and growth and differentiation factors [1]. Similar to other TGF-ß glycoproteins, AMH is a homodimer linked by disulfide bonds with a biologically active component contained in its shorter C-terminal domain which is conserved across the TGF-ß family [2].

AMH signaling occurs through two different serine/threonine kinase receptors referred to as type I (AMHRI) and type II (AMHRII) [3]. AMHRI and AMHRII work together to produce their biological effects. Each AMH receptor is divided into an extracellular portion containing a ligand binding site and an intracellular domain that produces serine/threonine kinase activity connected by a single transmembrane domain [2]. Ligand binding of AMHRII initiates the formation of a receptor complex resulting in the phosphorylation and activation of AMHRI by AMHRII. AMHRI acts as a signal transducer by phosphorylating receptor-specific cytoplasmic proteins, Smads, which are subsequently transported into the nucleus and bind target gene promoter sequences to modulate gene expression [3].

Unlike other members of the TGF-ß family that are widely expressed, AMH is produced by the somatic cells of the gonads and is unique in its important role in both fetal sexual differentiation and reproduction [2]. Early fetal life is characterized by ambisexual development, with both Wolffian and Mullerian ducts coexisting until approximately 8 weeks of gestation [4]. In a male fetus, soon after testicular differentiation at 6–7 weeks gestation, the Sertoli cells begin secreting AMH which induces regression of the Mullerian ducts via AMHRII [2]. AMH is highly expressed by males from the time of fetal testicular differentiation until puberty, after which levels decline [3]. AMH expression by Sertoli cell is regulated by a complex interaction of androgens, gonadotropins, and germ cell maturation [3]. The biological function of AMH in males postnatally has yet to be elucidated [3, 4].

In the absence of AMH production or a defect in the AMH receptor, the fetal Mullerian ducts persist and ultimately give rise to the fallopian tubes, uterus, and

upper vagina [4]. Persistent Mullerian duct syndrome (PMDS) is a recessively inherited disorder caused by mutation of either the AMHRII or the AMH gene, with the affected genetic males exhibiting normal male reproductive organs as well as uterus with fallopian tubes [4]. Although the external genitalia is phenotypically male, further examination may reveal genital abnormalities. These include bilateral cryptorchidism, if the uterus and testes remain in the pelvis, or an inguinal hernia, often containing one or both of the testicles and the ipsilateral fallopian tube – a condition referred to as *hernia uteri inguinalis* [4].

In a female fetus, AMH expression in the ovaries can first be detected near term, at around 36 weeks gestation [5]. AMH production gradually increases after birth until about the mid-twenties and then begins to decline until ultimately reaching undetectable levels near the age of menopause [6] (Fig. 25.1). AMH is produced by the granulosa cells of the preantral and small antral ovarian follicles (Fig. 25.2), with follicles 5–8 mm being responsible for approximately 60% of all AMH production [7]. As follicles grow beyond 8 mm, AMH production drops significantly once a follicle reaches 10 mm in size, corresponding with the selection of a dominant follicle destined for ovulation [7, 8]. The exact mechanism behind this abrupt decline in AMH production yet to be elucidated but increasing intrafollicular estradiol levels may play a role in suppression of AMH gene expression [7]. AMH has also been detected in the follicular fluid of larger follicles after stimulation with exogenous gonadotropins [9].

Current literature suggests that, in the ovary, AMH acts as a gatekeeper of the primordial follicular pool by inhibiting the initiation of primordial follicle growth

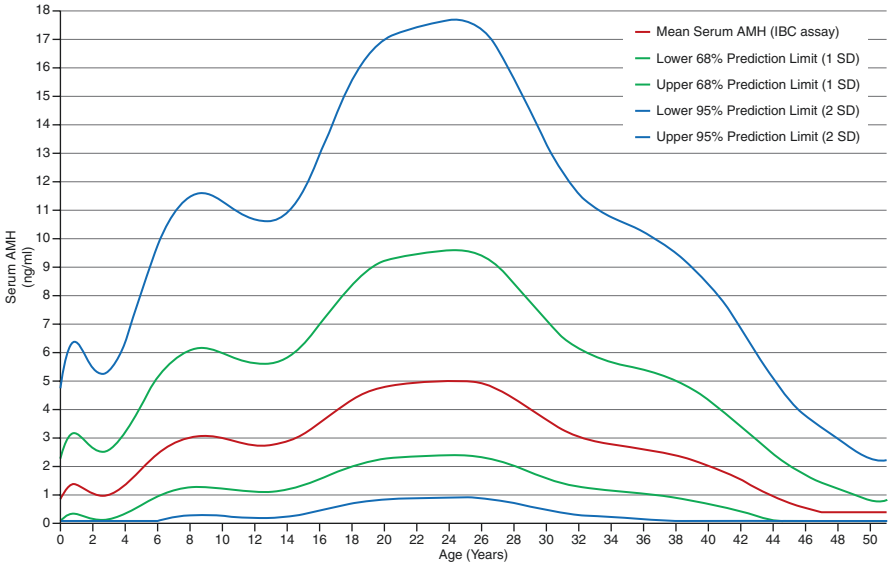

Fig. 25.1 Normal serum AMH levels for girls and women. (Kelsey et al. [6]). With permission from the Public Library of Science (PLOS)

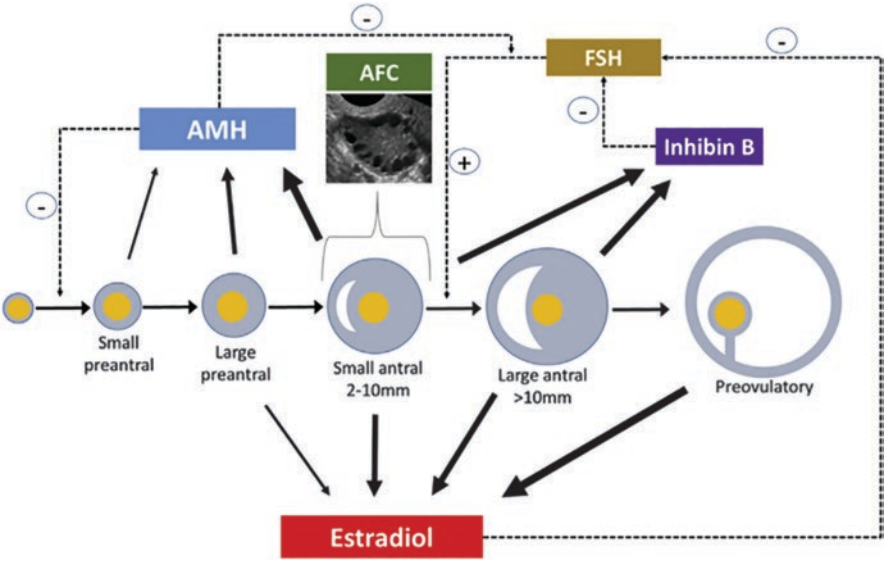

Fig. 25.2 Contribution of various follicular stages to circulating AMH and estradiol levels. (Tal and Seifer [18]). With permission from Elsevier

[10–12]. This inhibitory role was elucidated in a study using AMH gene-deficient mice with intact AMH receptors. The AMH null mice had a similar number of primordial follicles compared to wild type at 25 days of life, but significantly diminished number of primordial follicles at 4 and 13 months of age [10]. Evaluation of the number of small follicles showed 1.5-fold increase in the AMH null mice at 25 days and threefold increase at 4 months but a significantly lower number of small follicles at 13 months [10]. This suggests that although the initial ovarian reserve is similar in AMH null mice when compared to wild type, there is a rapid eventual depletion of primordial follicles resulting in premature exhaustion of the follicular pool [10]. AMH has also been shown to impede oocyte meiosis by inhibiting germinal vesicle breakdown [13, 14]. This inhibitory effect was reversed by the addition of epidermal growth factor (EGF), suggesting a possible mechanism for AMH inhibition of oocyte meiosis via interaction with the EGF tyrosine kinase receptor [13]. AMH may also inhibit granulosa cell proliferation and progesterone production by antagonizing the actions of EGF [15]. Centrally, AMHRII is expressed by hypothalamic GnRH neurons, and it has been postulated that circulating AMH in the serum may cross the blood-brain barrier and access the median eminence through fenestrated capillaries [16]. It has been suggested that AMH can potentially regulate the hypothalamic-pituitary-gonadal axis by increasing luteinizing hormone (LH) secretion through modulation of gonadotropin-releasing hormone (GnRH) pulsatility via AMHRII [16]. Following evidence that the human preovulatory follicles contain AMH [9], serum AMH levels have been associated with the number of retrieved oocytes in women undergoing ovulation stimulation for ART [17].

In current clinical practice, circulating serum AMH levels are measured as a biomarker of ovarian reserve and as a marker of ovarian aging [12, 18]. Ovarian reserve is an estimation of the primordial follicular pool within the ovary which decreases progressively with age. AMH levels demonstrate strong correlation with patient age (Fig. 25.1), antral follicle count (AFC), and follicle-stimulating hormone (FSH) levels (Fig. 25.3) [19]. AMH and AFC demonstrate good positive correlation across consecutive menstrual cycles [20]. Unlike other ovarian reserve markers, such as AFC and FSH levels, AMH can be accurately assessed throughout the menstrual cycle, since the serum levels do not fluctuate significantly [21]. AMH has also been shown to be a useful predictor of ovarian response to injectable gonadotropin medications in patients undergoing in vitro fertilization (IVF) cycles [22, 23]. Higher AMH levels correspond to a greater number of total and mature oocytes retrieved following controlled ovarian hyperstimulation [23]. Furthermore, higher AMH levels relate to a higher number of embryos and more recently have been related to increased clinical pregnancy rates [22–24] and higher cumulative live birth rates [25].

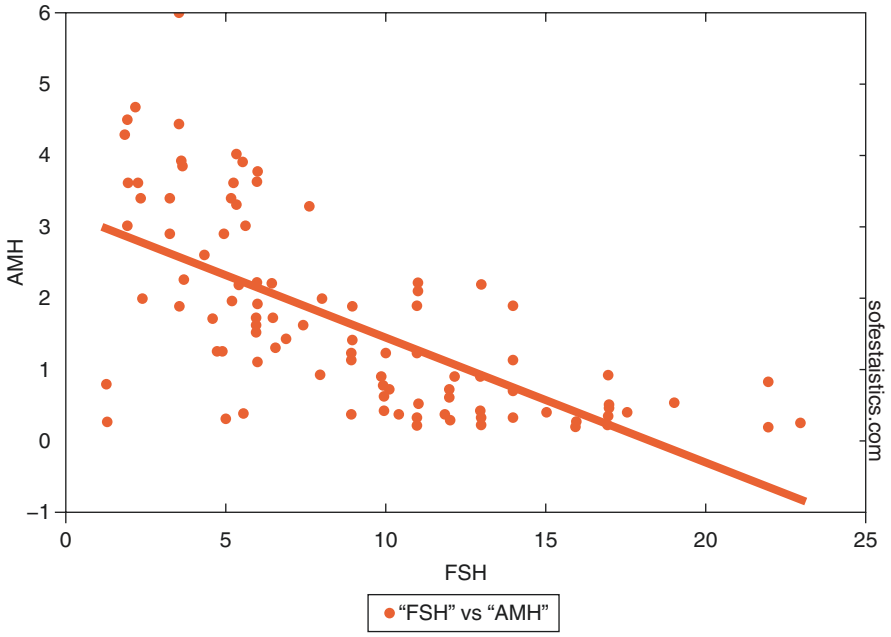

Fig. 25.3 Relationship of AMH to FSH levels. (Moubarak et al. [84]). With permission from the Austin Publishing Group

Role of AMH in the Pathophysiology of PCOS

In addition to its functions in fetal development and in maintenance of the primordial follicular pool, there is mounting evidence that AMH may play an important role in the pathophysiology of polycystic ovary syndrome (PCOS). As covered extensively in other chapters of this textbook, PCOS is the most common endocrine disorder affecting women of reproductive age. Features of oligoovulation, clinical and/or biochemical evidence of hyperandrogenism, and polycystic ovarian morphology (PCOM) are diagnostic hallmarks of this syndrome [26]. According to the Rotterdam criteria, PCOM is defined as either an AFC greater than 12 or an increased ovarian volume of >10 cm^3 [26], although more recent guidelines have recommended increasing the AFC threshold to greater than or equal to 20 due to improvements in ultrasound image resolution [27]. Although not part of the diagnostic criteria of PCOS, elevated serum AMH is a commonly encountered feature of PCOS [8]. The recent approval of a reference reagent by the WHO allowing for comparison of different AMH assays may lead to eventually incorporating AMH into the diagnostic criteria for PCOS [28].

PCOS is often associated with metabolic dysfunction and PCOS patients are at an increased risk of hyperinsulinemia, hypertensive disorders, and cardiovascular disease. The ovaries of PCOS patients frequently exhibit high numbers of small antral follicles, two to three times greater than normal, with most follicles in the 6–9 mm size range [29, 30]. The cyclic selection of a dominant follicle from this pool often fails to occur, resulting in oligoovulation or anovulation [31]. This lack of dominant follicle selection in PCOS patients is often referred to as "follicular arrest" [31]. The increased number of small antral follicles, as well as increased AMH production by their granulosa cells, contributes to elevated serum AMH levels seen in PCOS patients [8, 32, 33]. Although the etiology of increased AMH production by the granulosa cells of ovaries in women with PCOS has been debated, hyperandrogenism is likely a contributing factor [33]. Elevated AMH levels may also contribute to the increased antral follicle count seen in PCOS by preventing atresia of preantral and small antral follicles [34]. Anovulatory PCOS women have been found to have significantly higher AMH levels than ovulatory PCOS women, supporting the notion that elevated AMH contributes to the inhibition of ovulation as well as to its diagnosis [35, 36].

Although the exact mechanism of anovulation in PCOS has yet to be elucidated, studies suggest that elevated AMH levels may affect FSH-stimulated follicular growth – an essential step in dominant follicle selection. Early follicular growth is gonadotropin independent until the 2–5 mm size stage when follicles become responsive to the stimulatory effects of FSH [31]. A negative association has been demonstrated between AMH and FSH serum levels in both PCOS and non-PCOS patients, suggesting that FSH may regulate AMH synthesis in the ovary [31]. The ovaries of AMH knockout mice have a higher number of developing follicles despite lower FSH levels, suggesting that AMH may decrease the sensitivity of granulosa cells to the actions of FSH [10]. AMH has also been shown to inhibit FSH-induced aromatase activity in granulosa cells cultured from mature follicles, resulting in

decreased estradiol production. This suggests a potential mechanism of elevated AMH levels in the interference of dominant follicle selection, and ultimately ovulation, in PCOS patients [37, 38].

Previous studies have shown that AMH may also play an important regulatory role in androgen synthesis [39, 40]. A study using rodent Leydig cells suggests that AMH may inhibit cytochrome P450c17 hydroxylase (CYP17), a key enzyme in the androgen steroidogenesis pathway [39]. Another study using whole genome sequencing identified a number of rare, pathogenic AMH gene mutations in a cohort of PCOS women that decrease both AMH-mediated signaling and AMH-mediated CYP17 inhibition when transfected into a host cell line. These rare AMH variants were identified in 3% of the 700 women with PCOS included in the study and were associated with markedly elevated testosterone levels [40]. The decreased bioactivity of AMH in patients affected by pathologic AMH gene mutations may also account for the significantly elevated serum AMH levels, in addition to providing a biologic mechanism for hyperandrogenism seen in patients with PCOS [40]. These data suggest AMH gene mutations as a possible contributor to PCOS phenotype, as well as a mode of PCOS inheritability for some with hyperandrogenic PCOS phenotypes.

Role of AMH in the Pathophysiology of Ovarian Hyperstimulation Syndrome (OHSS)

Both the diagnosis of PCOS and the presence of elevated AMH levels have been shown to be predictive of an increased risk of ovarian hyperstimulation syndrome (OHSS) in women with PCOS. Spontaneous occurrence of OHSS is rare; however, the risk is real in women with PCOS being treated with exogenous gonadotropins as a strategy for managing anovulatory infertility [41, 42]. Other risk factors for OHSS include age <35 years, high number of small and intermediate size follicles (>35), and elevated serum estradiol levels (>4000 pg/mL) on day of human chorionic gonadotropin (hCG) trigger that is used as a surrogate for the midcycle LH surge to induce final oocyte maturation prior to egg retrieval in women undergoing IVF [41]. A study by Lee et al. demonstrated that serum AMH level is a predictor of OHSS risk in women undergoing controlled ovarian stimulation for IVF, performing similar to estradiol level and AFC [42]. AMH ≥4.7 ng/mL has been shown to be associated with PCOS and therefore predictive of greater risk for developing OHSS during ovulation induction [43]. Although the exact mechanisms are unclear, circulating AMH levels are positively correlated with serum levels of vascular endothelial growth factor (VEGF), an important mediator of both neoangiogenesis and the pathogenesis of OHSS (discussed in detail in Chap. 23) [44]. Elevated serum VEGF concentrations correlate with increased vascularity and ovarian blood flow in unstimulated PCOS patients, which may contribute to the increased rate of OHSS in this population [45]. Use of a GnRH agonist for trigger of final oocyte maturation in antagonist IVF cycles almost eliminates the risk of developing OHSS in high-risk patients due to both a shorter duration of LH activity [46] and lower follicular VEGF production compared to hCG [47], without compromising clinical outcomes [48].

Managing Ovulatory Dysfunction of PCOS: A Potential Role for Modulating AMH Signaling

There are a number of treatment options currently available for ovulation induction in anovulatory PCOS patients, and these are discussed in detail elsewhere in this textbook. The goal of ovulation induction in this patient population is to achieve mono-follicular ovulatory response, thereby minimizing the risks of multiple gestation and of OHSS that women with PCOS are susceptible to.

As discussed in greater detail elsewhere in this textbook, clomiphene citrate (CC) has been the first-line oral ovulation induction agent for the treatment of anovulatory infertility for over 50 years. A selective estrogen receptor modulator, CC acts by binding estrogen receptors at the level of the hypothalamus and blocking the negative feedback of circulating serum estradiol. This increases GnRH pulsatility, which stimulates release of LH and FSH, thereby driving follicular development [49]; successful ovulation rates of between 60% and 80% are attainable with CC, with the risk of multiple gestation ranging from 8% to 13% [50]. Despite the high ovulation rates, less than half of patient who do ovulate with CC will achieve a clinical pregnancy [51], whereas approximately 15–40% will fail to ovulate [52].

Letrozole, an aromatase inhibitor, is increasingly being used as a first-line treatment replacing CC to achieve ovulation induction in PCOS patients. The mechanism of action of aromatase inhibitors involves interruption of androgen to estrogen conversion by the ovarian granulosa cells as well as the adipose tissues resulting in decreased circulating estrogen levels. A consequence is that the negative feedback effect normally mediated by circulating estrogens on the pituitary gonadotropins and the hypothalamus get mitigated with downstream effects, that is, greater release of pituitary gonadotropins, as described with CC [53]. The risk of OHSS is extremely rare with the use of oral ovulation induction agents [54].

Yet another approach to achieve restoration of spontaneous ovulation in PCOS is to target the insulin signaling pathway (also discussed in detail elsewhere in this textbook) [55, 56]. Hyperinsulinemia may impede ovulation in a number of ways: by stimulating intraovarian androgen production, altering gonadotropin secretion [57], or directly affecting follicular development [58]. Multiple studies have demonstrated that use of metformin, an insulin sensitizing agent, alone may be effective in achieving spontaneous ovulation, while essentially eliminating the risk of multiple gestation [55, 56]. Despite this, the use of metformin alone or in combination with CC has demonstrated significantly lower live birth rates when compared to CC [56]. However, pretreatment with metformin before administration of CC in women with PCOS can improve ovulation rates in patients with previous CC resistance [59]. Interestingly, a decrease in AMH levels was described after 4 months of metformin treatment, likely due to recruitment of a smaller follicular cohort after reduction of insulin levels [60].

Myo-inositol, a member of the vitamin B complex group, is another insulin-sensitizer that has shown promise for restoring ovulation in PCOS women. D-chiro-inositol (DCI) phosphoglycan mediates insulin activity and its deficiency has been implicated in the development of insulin resistance in patients with PCOS [61].

When given exogenously, myo-inositol, a precursor of DCI, has the ability to reduce both insulin resistance and androgen production [61]. A number of small studies has demonstrated improvement in ovulatory function and menstrual regularity in PCOS women taking myo-inositol supplementation [61–63]. Despite these promising findings, myo-inositol is not routinely used for ovulation induction during fertility treatment due to lack of consistent in ovarian response. In addition, a meta-analysis did not show that myo-inositol has a significant effect on AMH levels or AFC values [64].

Laparoscopic ovarian "drilling" (LOD) or diathermy is a surgical option for the treatment of anovulation in PCOS patients. This technique uses heat or laser to destroy ovarian tissue and is discussed in detail in Chap. 18 of this textbook. In PCOS patients, LOD results in a significant reduction in serum AMH levels, as well as increased ovulation rates [65, 66]. Although the exact therapeutic mechanism of diathermy and ovarian wedge resection in restoring ovulation are not entirely clear, improved ovulation rates are thought to be due to the decrease in focal androgen and plausibly AMH production with resultant release of folliculogenesis block [67, 68]. Higher preoperative AMH is also associated with decreased likelihood of gaining ovulatory function after LOD [66, 69], with AMH levels of greater than or equal to 7.7 ng/mL associated with a high risk of LOD failure [69]. In PCOS women with very high AMH levels, LOD failure may occur due to inadequate tissue destruction failing to lower AMH levels enough to allow for dominant follicle selection and ultimately ovulation [69].

Vitamin D is a secosteriod hormone that may play a role in AMH signaling, follicular development, and metabolic function [70–72]. Vitamin D deficiency is highly prevalent among women with PCOS and has been associated with increased rates of obesity, hyperandrogenemia, and insulin resistance in this population [71, 72]. Various studies have demonstrated a decrease in hyperandrogenemia [73] and insulin resistance [72], as well as an improvement in menstrual regularity [74] after vitamin D supplementation in vitamin D-deficient women with PCOS. Another study demonstrated that vitamin D supplementation in PCOS women resulted in lower AMH levels, suggesting a potential mechanism by which vitamin D could enhance follicular sensitivity to FSH, decrease intrafollicular androgen production, and improve ovulatory function [75]. Additional research is needed to better define the contribution of vitamin D deficiency to ovulatory dysfunction in PCOS women, as well as potential therapeutic benefits of vitamin D supplementation in this unique population. Relevance of vitamin D for ovulatory response in women with PCOS has been further discussed in Chap. 24 of this textbook.

AMH Analogues: *Potential for Myriad Therapeutic Benefits?*

AMH structural analogues show promise for a variety of therapeutic applications. The unique ability of AMH to induce apoptosis in Mullerian structures during male fetal development has garnered interest in the possibility of its use in the treatment of ovarian adenocarcinomas due to a shared histology originating from coelomic

epithelium, which invaginates to form the Mullerian duct [76–78]. Recombinant AMH has been shown to inhibit the growth of gynecologic cancer cells of Mullerian origin [76, 79]. Both ovarian cancer cell lines and ascites cells isolated from patients with ovarian papillary serous cystadenocarcinoma demonstrated inhibited colony growth after treatment with recombinant AMH via AMHRII, suggesting AMH as a potential therapeutic target for ovarian cancer treatment [76]. AMH analogues may also prove effective for the treatment of other malignancies involving tissues that express AMHRII, such as breast and prostate cancer [79].

Evidence of ovarian cancer suppression with recombinant AMH has led to the investigation of a potential role for AMH in the treatment of endometriosis, a common disorder of ectopic endometrial cells also of coelomic epithelial origin [80, 81]. Endometrial stromal cells express AMHRII, and AMH has been shown to mediate cell cycle arrest and apoptosis in endometrial stromal cells collected from the endometriotic lesions of patients undergoing surgical management of endometriosis [80]. Another study demonstrated that AMH was able to activate both autophagy and apoptosis in an ectopic endometrial cell line [81], suggesting that treatment of endometriosis may be another potential clinical application for AMH analogues.

The use of AMH analogues as contraceptives has also been investigated due to the specific ability of AMH to prevent the primordial follicular recruitment [82]. A recombinant AMH gene therapy vector was shown to prevent early follicular development and menstrual cycling in mice, without affecting future fertility [82]. The same study also demonstrated a decrease in primordial follicle depletion in chemotherapy-treated mice, with a significantly higher ovarian reserve observed after co-treatment with recombinant AMH and carboplatin compared to carboplatin alone [82]. Although more research is needed, this suggests a potential role for AMH analogues not only as a novel type of contraceptive but also in the protection of ovarian reserve during gonadotoxic chemotherapy.

The critical role of AMH in follicular development makes it and its receptor opportune targets for the treatment of infertility. In a mouse model, AMH priming prior to superovulation increases oocytes yield, possibly by creating a larger backlog of small follicles for recruitment after cessation of AMH treatment [83]. Conversely, use of an AMH antagonist and modulation of AMHRII have been proposed as potential targets for ovulation induction in PCOS patients, although this has not yet been substantiated by clinical studies [1]. Blocking the production or action of AMH may increase granulosa cell sensitivity to FSH and remove the AMH-mediated inhibition on FSH-induced aromatase activity, encouraging dominant follicle selection and subsequent ovulation. This could offer the significant advantage of monofollicular development, as the estradiol negative feedback mechanisms remain intact, thereby decreasing the risk of higher-order multiple gestation and OHSS associated with other ovulation induction methods that women with PCOS are susceptible to. Although the exact mechanism is unclear, elevated AMH levels are associated with increased VEGF production, the main mediator of OHSS, making an AMH blockade another potential method for reducing OHSS risk in the PCOS population. This will require the development of high-quality AMH analogues for use in clinical trials.

Although more clinical and translational studies are needed to elucidate the complex role of AMH in the pathophysiology of PCOS, AMH analogues have the potential to revolutionize how we approach the treatment of anovulation and infertility as a whole. Current therapies mostly focus on the last stages of follicular development, while the use of AMH analogues offers the opportunity to improve fertility treatment by targeting the earliest stages of follicle recruitment. This novel class of pharmaceuticals has the potential to treat these disorders with great efficacy, specificity, and safety. Additional research is needed to develop AMH analogues for clinical use.

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Newer Glucose-Lowering Medications and Potential Role in Metabolic Management of PCOS

26

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Abbreviations

CI	Confidence interval
CV	Cardiovascular
CVOTs	Cardiovascular outcome trials
D	Disease
DPP-4	Dipeptidyl peptidase-4 inhibitors
euDKA	Euglycemic diabetic ketoacidosis
FSH	Follicle-stimulating hormone
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1 RAs	GLP-1 receptor agonists
GnRH	Gonadotropin-releasing hormone
HDL	High-density lipoprotein
IGT	Impaired glucose tolerance
LH	Luteinizing hormone
MACE	Major adverse cardiovascular event
PO	Oral
SGLT2	Sodium-glucose cotransporter-2
SHBG	Sex hormone binding globulin
SQ	Subcutaneous
T2DM	Type 2 diabetes mellitus
TZDs	Thiazolidinediones
VLDL	Very-low-density lipoprotein

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_26

Key Points

- Addressing the increased risk of metabolic complications, including type 2 diabetes mellitus, and cardiovascular disease, is important in women with PCOS.
- GLP-1 receptor agonists (GLP-1RAs), dipeptidyl peptidase-4 (DPP-4) inhibitors, and sodium-glucose cotransporter-2 (SGLT2) inhibitors are newer classes of glucose-lowering medications and promising options for women with PCOS.
- As DPP-4 inhibitors improve glycemic control and are weight neutral, they offer potential pharmacologic options in improving metabolism in PCOS, with clinical studies indicating improvements in glucose metabolism, insulin resistance, and hyperandrogenism.
- Since GLP-1 RAs and SGLT2 inhibitors promote weight loss, recent clinical studies examining these newer agents in PCOS women have shown promising results with respect to improvements in metabolic parameters, hyperandrogenism, and reproductive function.
- GLP-1 RAs and SGLT2 inhibitors have been linked to improved CV outcomes, which may provide an additional rationale for use in women with PCOS.

Introduction

A heterogeneous disorder characterized by oligo-anovulation, hyperandrogenism, polycystic ovarian morphology, and metabolic sequelae, polycystic ovary syndrome (PCOS) affects 5–20% of reproductive age women. In our current understanding of the pathophysiology of PCOS, hyperandrogenemia and ovulatory dysregulation occur due to neuroendocrine abnormalities in the hypothalamus and pituitary with altered gonadotropin-releasing hormone (GnRH) pulsatility and increased luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio, leading to ovulatory dysfunction and excessive ovarian androgen production. Elevated adrenal androgen production has also been observed in women with PCOS; the mechanism is unclear, but may be due to hypothalamic-pituitary-adrenal axis dysfunction and generalized adrenocortical hyperresponsivity to ACTH stimulation [1]. Hyperinsulinemia contributes to hyperandrogenemia by augmenting LH-stimulated androgen production by ovarian theca cells and inhibiting sex hormone binding globulin (SHBG) production by the liver, thus increasing total and free testosterone levels [2]. Conventional therapeutic targets for the management of PCOS include hyperandrogenism (e.g., hirsutism, acne, alopecia), irregular menses (and associated risk of endometrial hyperplasia and, potentially, carcinoma), and infertility. The myriad metabolic aberrations associated with PCOS are shown in Fig. 26.1. However, as women with PCOS are at high risk for metabolic sequelae including increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular (CV) disease (D), addressing the metabolic features of PCOS is important. Metformin is commonly used in women with PCOS with T2DM or prediabetes in whom lifestyle modifications are unsuccessful and is a second-line therapy for women with PCOS and irregular menses who are unable to tolerate oral contraceptives. Metformin is also used in the setting of infertility when ovulation induction using clomiphene citrate or aromatase inhibitors

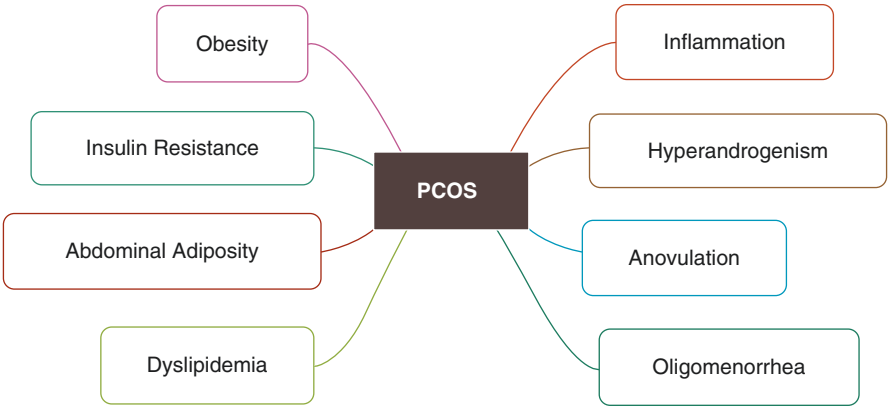

Fig. 26.1 Characterized by androgen excess and oligo-ovulation or anovulation, polycystic ovary syndrome (PCOS) is associated with multiple metabolic aberrations that may predispose to cardiovascular disease

fails. In addition, the insulin-sensitizing thiazolidinediones (TZDs), such as pioglitazone, reduce insulin and fasting blood glucose levels in PCOS, but have not been shown to improve hirsutism or androgen levels. However, TZDs are associated with increases in body weight, which is less desirable in women with PCOS [3]. In this chapter, we will review the evidence for use of newer classes of glucose-lowering medications, dipeptidyl peptidase-4 (DPP-4) inhibitors, GLP-1 receptor agonists (GLP-1 RAs), and sodium-glucose cotransporter-2 (SGLT2) inhibitors which can provide metabolic advantages in the setting of PCOS. Besides improving glycemia, both GLP-1 RAs and SGLT2 inhibitors are associated with weight loss and have been recently shown to improve CV outcomes in older patients with T2DM who are at high CV risk.

Metabolic Consequences of PCOS

Women with PCOS are at increased risk of long-term metabolic consequences, including insulin resistance, T2DM, dyslipidemia, hypertension, endothelial dysfunction, and, ultimately, CVD, compared to age-matched control women [4]. Overweight or obesity in PCOS aggravate several metabolic syndrome features [5]. Insulin resistance appears to play a central role in both the pathogenesis and long-term metabolic complications of PCOS. Up to 70% of women with PCOS have insulin resistance owing to multiple factors, including genetic and environmental contributors, obesity, and aberrations in insulin receptor signaling [6–8]. In addition, insulin resistance and the associated hyperinsulinemia are well-established risk factors for CVD [9, 10]. Increased risk of impaired glucose tolerance (IGT) and T2DM has been reported in women with PCOS. A systematic review of 11 studies revealed an increased prevalence of IGT in women with PCOS vs. controls (odds

ratio [OR] 2.48, 95% CI 1.62–3.77) using either the World Health Organization (WHO) or American Diabetes Association (ADA) definitions for IGT [11]. Similarly, a meta-analysis of 12 studies demonstrated an increased prevalence of T2DM in women with PCOS, with an OR of 4.4 (95% CI 4.06–4.82). BMI-matched studies also showed an increased prevalence of T2DM in PCOS with an OR of 4.0 (95% CI 1.97–8.1) [11]. Furthermore, women with PCOS exhibit dyslipidemia, with increased triglycerides and VLDL and lower HDL levels compared to weight-matched controls [12–15]. In light of the substantial evidence supporting high risk of long-term metabolic complications, addressing insulin resistance and treating modifiable CV risk factors in women with PCOS is warranted.

Incretins

Background and Mechanisms of Action

Initially described in 1964, the incretin effect is defined as the greater amount of insulin secretion that follows oral glucose administration relative to insulin release after the intravenous administration of a comparative amount of glucose [16]. The incretin effect is the result of augmented glucose-stimulated insulin secretion mediated by intestinal hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are released after oral carbohydrate ingestion [17].

GLP-1 and GLP-1 Receptor Agonists (GLP-1-RAs)

Discovered in 1987, GLP-1 is derived from proglucagon in intestinal L cells in the ileum and colon and released into the blood after food intake [18]. GLP-1 is more effective than GIP in stimulating insulin secretion and decreasing peak plasma glucose levels [19]. By inhibiting glucagon release, GLP-1 affects glucose metabolism and is associated with increased glucose uptake and glycogen synthesis in peripheral tissues. GLP-1 also increases satiety and slows gastric emptying [17]. The GLP-1 receptor is expressed by pancreatic islet cells and in the gastrointestinal tract, in addition to peripheral and central nervous system neurons [20–22].

The multiple effects of GLP-1 in target tissues are shown in Fig. 26.2. GLP-1 is rapidly metabolized in the circulation by the protease dipeptidyl peptidase 4 (DPP-4) and has a half-life of only 1–2 minutes in human plasma [23, 24]. To use GLP-1 as the basis of clinically useful glucose-lowering therapy, GLP-1 mimetics with modifications of the original GLP-1 peptide were developed. The half-life of currently available GLP-1 RAs ranges from 3 hours to 7 days, and all are administered via subcutaneous (SQ) injection except for semaglutide, which also has an oral formulation (Table 26.1). GLP-1 as a potential agent for diabetes treatment was demonstrated in the 1990s, when it was shown that intravenous administration of GLP-1 reduced the insulin requirements for a meal in subjects with T2DM [25].

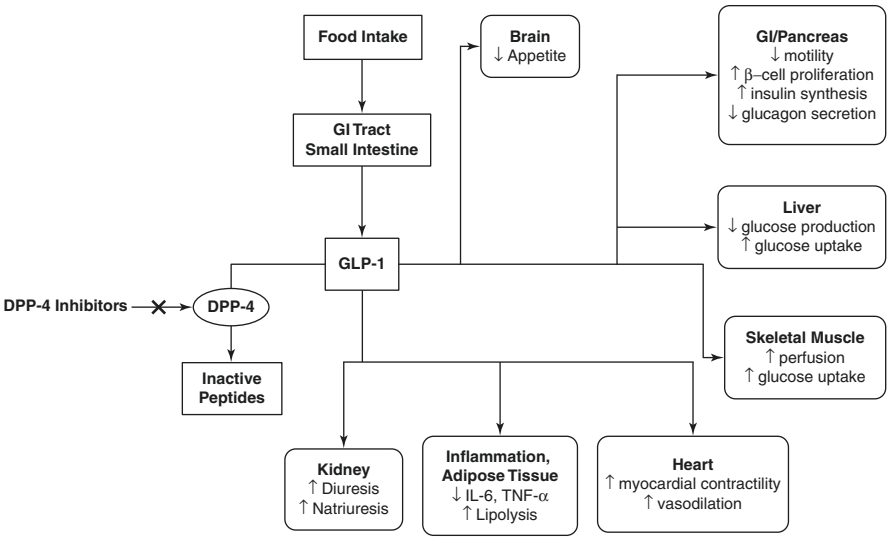

Fig. 26.2 Released from the gut after oral carbohydrate ingestion, GLP-1 has multiple potentially beneficial actions in target tissues. GLP-1 is rapidly metabolized in the circulation by the protease DPP-4. DPP-4 inhibitors inhibit the degradation of GLP-1 by DPP-4

Table 26.1 GLP-1 receptor agonists: Dosing and average weight loss

Drug (brand)	Half-life	Dosing	Weight loss (kg)
Dulaglutide (Trulicity®)	~5 days	1.5 mg SQ weekly	~3
Exenatide (Byetta®)	24 hours	10 mcg SQ twice daily	~2
Exenatide extended-release (Bydureon®)	>7 days	2 mg SQ weekly	~3
Liraglutide (Victoza®[1], Saxenda®[2])	12–14 hours	1.8 mg SQ daily[1] 3 mg SQ daily[2]	~3[1] ~8[2]
Lixisenatide (Adlyxin®)	~3 hours	20 mg SQ daily	~2
Semaglutide (Ozempic®)	~7 days	0.5–1 mg SQ weekly[3] 2.4 mg SQ weekly[4]	~4–4.5[3] ~15[4]
Semaglutide (Rybelsus®)	~7 days	7–14 mg PO daily	~3

Data for weight loss obtained from Refs. [40–42], and a new steady state of body weight is reached within 3–6 months of treatment
SQ subcutaneous injection, *PO* by mouth
[1]1.8 mg SQ dosing of Victoza and that ~3 kg weight loss expected
[2]3 mg SQ dosing of Saxenda and that ~8 kg weight loss expected
[3]0.5-1 mg SQ weekly Ozempic (semaglutide) and 4-4.5 kg weight loss
[4]2.4 mg SQ weekly dosing of semaglutide and ~15 kg weight loss

Current Clinical Use of GLP-1 RAs

GLP-1 RAs have become a cornerstone of glucose-lowering therapy in T2DM since the approval of exenatide in 2005. Over the past 15 years, several GLP-1 RAs have become available, including semaglutide, which is the first GLP-1 RA also available

as an oral formulation with daily dosing. GLP-1 RA class members are listed in Table 26.1. Based on the prevailing recommendations of the American Diabetes Association, metformin remains the first-line therapy for T2DM. The addition of a GLP-1 RA is recommended as one of the second-line therapies if the hemoglobin A1C (HbA1C) level remains above the patient's individualized target (typically <7%). GLP-1 RAs are specifically advisable if there is a compelling need to promote weight loss (or minimize weight gain). Based on recent CV outcome trials, GLP-1 RAs are also indicated in those with T2DM and established or at high risk for atherosclerotic cardiovascular disease (ASCVD). The latter category includes those aged 55 years or more with coronary, carotid, or lower extremity artery stenosis (>50%), left ventricular hypertrophy, an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, or albuminuria [26]. GLP-1 RAs may be combined with other glucose-lowering therapies as well, except for the other incretin-based category, the DPP-4 inhibitors. This includes, in more advanced patients, addition of incretin-based medications to basal insulin in order to delay the use of mealtime insulin and reduce the risk of hypoglycemia and weight gain. A meta-analysis of clinical studies indicated that GLP-1 RA treatment is associated with A1C reductions ranging from −0.42% for exenatide 5 μg SQ BID, −0.50% for lixisenatide 20 μg SQ once daily, −0.71% for liraglutide 1.2 mg SQ once daily, −0.75% for exenatide 10 μg SQ BID, −1.03% for liraglutide 1.8 mg SQ once daily, to −1.09% for exenatide 2 mg SQ weekly and dulaglutide 1.5 mg SQ, compared to placebo [27]. In a head-to-head trial, dulaglutide 1.5 mg SQ once weekly reduced HbA1c by 1.4%, while semaglutide 1 mg SQ weekly was associated with a 1.8% reduction in HbA1c [28].

The most common adverse events with GLP-1 RAs are nausea, vomiting, and diarrhea, which may occur in up to 30, 15, and 15% of patients, respectively, and can lead to discontinuation of treatment. Typically, these adverse effects occur with initial exposure to the GLP-1 RA and are dose dependent, so the amount administered needs to be slowly uptitrated [29]. Warnings regarding the risk of acute pancreatitis are included in the prescribing information for GLP-1 RAs, but thus far, available data has not demonstrated a causal association between the use of GLP-1 RAs and pancreatitis [30]. A meta-analysis of observational studies also did not support an association between use of incretin-based therapies and acute pancreatitis [31]. Also, GLP-1 RAs are contraindicated in patients with personal or family history of medullary thyroid cancer or multiple endocrine neoplasia syndrome, owing to preclinical data in animal models [32].

With the publication in 2008 of diabetes drug development guidelines mandating that all new anti-diabetic drugs rule out excess cardiovascular risk, the FDA ushered in a new age of long-term cardiovascular outcome trials (CVOTs). These CVOTs include patients from high-risk populations, including those with known CVD and renal impairment. Since the first positive report on the LEADER trial examining liraglutide's impact on cardiovascular outcomes in high-risk T2DM patients, there has been accumulating evidence for the potential cardiovascular benefits of GLP-1 RAs. To date, there have been seven large clinical trials investigating the effects of GLP-1 RAs on cardiovascular outcomes in T2DM patients. These trials evaluated cardiovascular death, nonfatal MI, nonfatal stroke, and hospitalization for unstable

angina or heart failure (3- or 4-point major adverse cardiovascular event [MACE]). With the exception of lixisenatide in the ELIXA trial, all other cardiovascular outcomes trials testing GLP-1 RAs showed a mostly consistent pattern with respect to effects on MACE – there was either a significant reduction (by 12–26%) or at least a strong trend towards a reduction in MACE events [33–39]. These data have resulted in specific indications for certain members of this class to reduce CV events (liraglutide, semaglutide, dulaglutide) in high-risk T2DM patients, as reflected in the aforementioned clinical guidelines.

In preclinical studies, GLP-1 administration was found to reduce food intake and increase satiety in animals and humans mediated via peripheral and central mechanisms [40, 41]. All GLP-1 RAs can induce weight loss by interacting with GLP-1 receptors in brain areas involved in the homeostasis of energy intake, energy expenditure, and energy balance [17]. However, the amount of weight loss is different among the numerous GLP-1 RAs. Average weight loss with exenatide, lixisenatide, liraglutide, and dulaglutide is 2–4 kg, with more weight loss observed with semaglutide [42–44]. Semaglutide was associated with significantly greater weight loss (−7 kg) compared to dulaglutide (−3 kg) [44]. In addition, there is considerably more variability in the amount of weight loss between individuals than there is for glycemic control. Some patients treated with GLP-1 RAs do not lose weight, while others can lose up to 25 kg over 6 months [45, 46]. Typically, a plateau of body weight is reached after 3–6 months of treatment. As long as the GLP-1 RA is continued, most of this initial weight loss is maintained. However, if treatment is discontinued, weight will be regained. GLP-1 RAs have additional effects on the cardiovascular system. All GLP-1 RAs lower systolic blood pressure by 2–5 mmHg, with less consistent effects on diastolic blood pressure. At the same time, an average increase in pulse rate of 2–5 beats per min has been observed in patients treated with GLP-1 RAs, but this does not seem to attenuate the cardiovascular benefits. In addition to weight loss and lowering of systolic blood pressure, all GLP-1 RAs slightly lower LDL cholesterol and triglycerides and may have anti-inflammatory effects as well [47, 48].

In light of their glucose-dependent mechanism and beneficial effects of incretin-based therapies on β-cell function in patients with T2DM, studies have examined the potential role of GLP-1 RAs in prediabetes. Liraglutide reduces the risk of progression to T2DM in obese or overweight prediabetic individuals. Three-year treatment with liraglutide (3 mg SQ daily) revealed that the time to onset of T2DM was 2.7 times longer with liraglutide than with placebo (95% CI 1.9–3.9, $p < 0.0001$). Overall, 2% (26 out of 1472) individuals in the liraglutide group were diagnosed with T2DM while on treatment, compared to 6% (46 out of 738) in the placebo group [49]. One recent randomized open-label parallel-group controlled study evaluated the efficacy of exenatide (10–20 μg SQ daily), metformin (1500–2000 mg daily), or combination for prediabetes in PCOS. The remission rate in the exenatide group (56%, 28/50) and combination group (64%, 32/50) was significantly higher than that in the metformin group (32%, 16/50) ($P = .027$ and .003, respectively) [50]. GLP-1 RAs may be promising for diabetes prevention in at-risk populations, but data are needed in women with PCOS for this indication.

Emerging Evidence for GLP-1 RAs in PCOS

Data regarding either fasting or postprandial GLP-1 levels in women with PCOS are mixed. Some studies found similar fasting GLP-1 levels in PCOS compared with age- and BMI-matched controls, whereas others reported either decreased or increased fasting levels in PCOS [51–53]. Similarly, there have not been consistent findings regarding postprandial GLP-1 levels in women with PCOS [52–56]. GLP-1 RAs are an emerging therapy for obese women with PCOS. Of the multiple GLP-1 RAs available, only liraglutide and exenatide have been studied to date in this context. Overall, several studies have demonstrated the expected weight reduction with GLP-1 RAs alone or in combination with metformin and improvement in glucose parameters with variable results regarding impact on clinical stigmata of PCOS. We will delve into clinical studies examining GLP-1 RAs in obese and overweight women with PCOS here, with key studies summarized in Table 26.2.

The first study investigating the use of GLP-1 RA in women with PCOS was published in 2008 and evaluated exenatide alone and in combination with metformin [57]. This 24-week study randomized overweight women with PCOS to metformin (1000 mg PO [oral] twice daily), exenatide (10 μg SQ twice daily), or combination treatment (metformin 1000 mg PO and exenatide 10 μg SQ twice daily). Exenatide alone resulted in a mean weight loss of 3.2 ± 0.1 kg, while combination therapy with exenatide and metformin resulted in a 6 ± 0.5 kg weight loss versus 1.6 ± 0.2 kg with metformin alone ($p = 0.019$ for exenatide and combination therapy, relative to metformin). Insulin sensitivity as assessed by calculation of Matsuda Index from 2-hour oral glucose tolerance testing improved in all treatment groups. Combined therapy was superior to either exenatide alone or metformin alone in decreasing total cholesterol and triglycerides and in improving menstrual frequency and ovulation rate. For example, the ovulation rate was 86% in the combined group compared to 50% in exenatide monotherapy and 29% in metformin only group ($p < 0.01$ for combination therapy compared to either exenatide or metformin alone) [57].

The majority of studies examining use of GLP-1 RAs in women with PCOS describe the use of liraglutide in patients previously or concurrently treated with metformin. In addition, with the approval in 2014 of liraglutide at the 3 mg SQ daily dose for obesity treatment, there has been increasing interest in examining dose response by comparing liraglutide 3 mg daily to liraglutide 1.8 mg daily (which is the maximal dose approved for diabetes management) in PCOS [58].

In 30 obese women with PCOS, a short-term intervention of 12 weeks with metformin plus liraglutide 1.2 mg SQ daily (COMBO) was compared to liraglutide 3 mg SQ daily (LIRA3) alone [59]. A significant weight loss of −3.6 ± 2.5 kg relative to baseline ($p = 0.002$) was observed in the COMBO group and −6.3 ± 3.7 kg weight loss relative to baseline ($p = 0.001$) in LIRA3. However, the amount of weight loss in the COMBO group versus LIRA3 group was not significantly different ($p = 0.062$). BMI and waist circumference reduction in LIRA3 were greater than in COMBO (−2.2 ± 1.3 vs. −1.3 ± 0.9 kg/m^2, $p = 0.05$ and −4.2 ± 3.4 vs. −2.2 ± 6.2 cm, $p = 0.014$, respectively). Notably, improved glucose homeostasis

Table 26.2 Key studies with GLP-1 receptor agonists in women with PCOS

Study	Intervention	Improved insulin resistance	Weight loss (kg)	Fasting glucose	Improved hyperandrogenism	Menstrual pattern/ ovulation
Elkind-Hirsch (2008)	Met 1 g PO BID vs. Exe 10 μg SQ BID vs. Combo (Met + Exe)	Yes	Met: −1.6 ± 0.2 Exe: −3.2 ± 0.1 Combo: −6 ± 0.5	↓	Yes	↑ ovulation
Kahal (2015)	Lira 1.8 mg SQ daily in obese PCOS vs. Lira 1.8 mg SQ daily in control	Yes	PCOS: −3 ± 4 Control: −3.8 ± 3	↓	No	N/A
Jensterle (2015)	Met 1 g PO BID vs. Lira 1.2 mg SQ daily vs. Rof 500 mg PO daily	N/A	Met: −0.8 ± 1 Lira: −3.1 ± 3.5 Rof: −2.1 ± 2	↓	Rof: ↓ testosterone	↑ menstrual frequency
Jensterle (2017)	Met 1 g PO BID + Lira 1.8 mg SQ daily (combo) vs. Lira 3 mg SQ daily	Yes	Combo: −3.6 ± 2.5 Lira3: −6.3 ± 3.7	↓	Combo: ↓ testosterone	N/A
Nylander (2017)	Lira 1.8 mg SQ daily vs. Placebo	↔	Lira: −5.2	↓	Lira: ↑ SHBG, ↓ free testosterone, ↓ ovarian volume, ↔ Ferriman-Gallwey score	↑ bleeding ratio
Frøssing (2018)	Lira 1.8 mg SQ daily Vs. Placebo	↔	Lira: −5.2 ± 0.7 Placebo: +0.2 ± 0.9	↓	Lira: ↑ SHBG, ↓ free testosterone	N/A

Adapted from Cena et al. [117]
BID twice daily, *Lira* liraglutide, *Met* metformin, *Exe* exenatide, *Rof* roflumilast, ↔ no significant change, *N/A* not applicable

parameters were observed in both LIRA3 and COMBO groups with decreases in glucose and insulin levels during oral glucose tolerance test [59]. A meta-analysis of 7 randomized trials assessed the outcomes of weight, BMI, waist circumference, systolic blood pressure, fasting insulin levels, insulin resistance via the HOMA-IR

equation, total testosterone, and SHBG levels with GLP-1 RA intervention in women with PCOS [60]. This meta-analysis excluded data pertaining to the concurrent use of metformin and GLP-1 RAs and only included GLP-1 RA data. Three months of liraglutide (maximal dose 1.8 mg SQ daily) treatment decreased BMI by 1.7 kg/m^2 (95% confidence interval 0.72–2.58, p = 0.0005) in 172 women [60]. Waist circumference decreased to 117 cm from a baseline of 120 cm (95% confidence interval −7.10–0.97 cm, p = 0.142) with liraglutide [60]. Changes in insulin levels and insulin sensitivity were highly heterogeneous among the studies. GLP-1 RA therapy was associated with total testosterone decreasing from 1.89 to 1.6 nmol/L (95% CI −0.44 to −0.13; p = 0.0003). However, no significant difference in SHBG levels after treatment with GLP-1 RAs was found [57, 60–62]. Compared with metformin, GLP-1 RAs were more effective in improving insulin sensitivity as assessed by HOMA-IR (standard mean difference [SMD] −0.40, 95% confidence interval [CI] −0.74 to −0.06, p = 0.02) and in reducing body mass index (SMD −1.02, 95% CI −1.85 to −0.19, p = 0.02) and abdominal girth (SMD −0.45, 95% CI −0.89 to −0.00, p = 0.05) in a meta-analysis of 8 randomized controlled trials of women with PCOS [63]. GLP-1 RAs were associated with a higher incidence of nausea and headache than metformin, but there were no significant differences in other parameters (menses frequency, serum total testosterone, SHBG, LH, triglycerides, Ferriman-Gallwey hirsutism scores). Therefore, compared with metformin, GLP-1 RAs might be a reasonable choice for obese patients with PCOS, especially those with obesity and insulin resistance.

The effect of liraglutide on atherothrombotic risk and body composition has been examined in women with PCOS. The effect of liraglutide (1.8 mg SQ daily) for 6 months on atherothrombotic markers (high-sensitivity C-reactive protein [hsCRP], endothelial adhesion markers, HOMA-IR, and weight) was assessed in obese women with PCOS and age- and weight-matched controls [62]. These data indicate that both obese women with and without PCOS respond equally to liraglutide, with 3–4% weight loss and significant reductions in markers of inflammation, oxidative stress, and improvement in atherothrombotic risk markers (endothelial function, clot structure) [62]. Liraglutide use was associated with a significant decrease in visceral adipose tissue (VAT) area with whole-body composition determined by dual-energy X-ray absorptiometry (DXA) (p = 0.015) [62]. Another study examined the effect of liraglutide on ectopic fat in PCOS; in a double-blind placebo-controlled randomized clinical trial of 72 women with PCOS, 26 weeks of liraglutide (1.8 mg SQ daily) treatment led to reductions in body weight by −5.2 ± 0.7 kg (5.6%), liver fat content by 44%, VAT by 18%, and the prevalence of non-alcoholic fatty liver disease (NAFLD) by two-thirds (all $p < 0.01$) [64]. Overall, existing data identify liraglutide as a promising option to potentially improve markers of atherothrombotic risk and body composition in women with PCOS.

In light of the link between PCOS pathogenesis, obesity, and impaired fertility, a few studies have investigated the effect of GLP-1 RA on reproductive endpoints in women with PCOS. In a double-blind, randomized trial, 72 women with PCOS were allocated to liraglutide (1.8 mg SQ daily) or placebo. Bleeding pattern, circulating levels of anti-Mullerian hormone (AMH), sex hormones, and gonadotrophins

were assessed, and ovarian morphology was evaluated by ultrasound. Six months of liraglutide therapy was associated with a mean 5.2 kg (95% CI 3.0–7.5, $p < 0.0001$) weight loss compared with placebo. Furthermore, liraglutide improved bleeding pattern and was associated with increased SHBG (mean + 7.4 nmol/L, 95% CI 4.1–10.7, $p < 0.001$), decreased free testosterone (mean −0.005 nmol/L, 95% CI −0.25–0.10, p = NS), and reduction in ovarian volume (mean −1.6 mL, 95% CI −3.1 to −0.9, $P < 0.001$) relative to placebo. Overall, liraglutide improved markers of ovarian function in overweight women with PCOS [65]. Although other studies have shown reductions in body weight associated with liraglutide use in women with PCOS, menstrual pattern was unchanged [59–61, 64]. However, these studies have generally included short-term use of liraglutide and small sample sizes with high attrition rates.

GLP-1 RA preconception treatment may improve pregnancy rates in women with PCOS. In a 24-week trial, 176 overweight or obese women with PCOS were randomized to either exenatide (10 μg SQ twice daily) or metformin (1000 mg orally twice daily) for the first 12 weeks, followed by metformin alone for the remaining 12 weeks. During the second 12-week period, the rate of natural pregnancy in the exenatide-treated group was significantly higher than in metformin-treated patients (43.6% vs. 18.7%; $p < 0.05$). Of note, patients in both the exenatide and metformin groups experienced significant improvement in menstrual frequency, but only the exenatide group had decreased total fat mass and greater decreases in HOMA-IR (i.e., more improvement in their insulin sensitivity) and insulin levels compared with metformin alone [66]. Preconception use of GLP-1 RAs has been shown to favorably affect in vitro fertilization pregnancy rates in PCOS. In a randomized, open-label pilot study with 28 women with obesity and PCOS, 12-week preconception treatment with low-dose liraglutide (1.2 mg SQ daily) in combination with metformin was superior to metformin alone in increasing in vitro fertilization pregnancy rates [67]. Pregnancy rate per embryo transfer was significantly higher in the liraglutide plus metformin group compared to metformin alone (85.7% vs. 28.6%, respectively, p = 0.03). Notably, the cumulative pregnancy rate over 12 months was 69% with the combination therapy compared with 36% in the metformin-only group (p = NS) [67].

Summary: Using GLP-1 RAs in Women with PCOS

Weight loss has been demonstrated to improve hyperandrogenism, reproductive function, and metabolic parameters such as hyperlipidemia, glycemic control, and hypertension, in women with PCOS [68–70]. Furthermore, the association between obesity and infertility is well known, and, in particular, obesity and insulin resistance are linked to PCOS pathogenesis. Several studies have demonstrated that GLP-1 RA (mostly liraglutide) administration alone or in combination with metformin leads to significant weight loss. GLP-1 RAs are an emerging component of the arsenal of treatments available to overweight and obese women with PCOS. Additional studies are warranted to examine preconception use of GLP-1 RAs as an adjunct in women with PCOS seeking pregnancy.

Dipeptidyl Peptidase-4 (DPP-4) Inhibitors

Background and Mechanism of Action

DPP-4 inhibitors were first introduced for glycemic management in type 2 diabetes mellitus in 2006. DPP-4 is a multifunctional membrane protein with widespread expression in several tissues, including the liver and pancreas, intestinal and renal brush border membranes, and vascular endothelium. As discussed previously, DPP-4 rapidly degrades GLP-1 and GIP in the circulation. Of note, DPP-4 may also have broader effects on metabolism through proteolytic effects on other regulatory peptides and may affect insulin sensitivity through nonenzymatic interactions with other proteins, and increasing BMI and hyperglycemia have been associated with altered expression of DPP-4 [71]. Orally active small molecules that inhibit DPP-4 by >70–80%, DPP-4 inhibitors result in a 2–3-fold elevation of endogenous GLP-1 levels, leading to a glucose-dependent stimulation of insulin secretion and an inhibition of glucagon secretion [18, 71]. Importantly, the DPP-4 inhibitors do not increase the risk of hypoglycemia because the glucose-lowering effects of DPP-4 inhibitors are self-limited and incretin hormone actions are glucose dependent and only occur in hyperglycemia [72].

Current Clinical Use of DPP-4 Inhibitors

DPP-4 inhibitors are one class of agents recommended by the ADA for add-on therapy and are most frequently used in addition to metformin for type 2 diabetes mellitus in the absence of high risk for or established ASCVD, CKD, or heart failure [26]. DPP-4 inhibitors may also be combined with other oral therapy, i.e., metformin, SGLT2 inhibitors, as well as insulin. However, the combination of DPP-4 inhibitors and GLP-1 RAs should be avoided since both are incretin-based drugs. Notably, in the VERIFY trial, early intervention with metformin and vildagliptin in type 2 diabetes mellitus was associated with a slower decline in glycemic control compared to individuals receiving metformin monotherapy and with vildagliptin added stepwise to metformin [73].

As DPP-4 inhibitors are not associated with increased risk of hypoglycemia, they are a particularly favorable alternative for intensification of anti-hyperglycemic therapy, compared to the insulin secretagogues, sulfonylureas, and glinides. DPP-4 inhibitors are orally administered and have sufficient action with once daily dosing with the exception of vildagliptin which has to be dosed twice daily [74]. A meta-regression analysis of randomized controlled trials including over 20,000 patients evaluating the efficacy of DPP-4 inhibitors on HbA1C levels estimated a mean decrease of −0.74% in HbA1C from baseline [mean baseline HbA1C of 8.03%] [75]. There have been several large, placebo-controlled CV safety outcome trials with the DPP-4 inhibitors in patients with type 2 DM and established CVD (SAVOR-TIMI 53, EXAMINE, TECOS, CARMELINA, and CAROLINA) [76–80]. An increase in the risk of hospitalization for heart failure was observed in the saxagliptin group in the SAVOR-TIMI 53 trial, but overall confirmed the CV safety of saxagliptin. In the CAROLINA trial, linagliptin was noninferior to glimepiride with respect to risk of major CV events over 6 years in

patients with type 2 DM. Overall, the DPP-4 inhibitors have neither increased nor reduced CV events.

DPP-4 inhibitors are generally quite well tolerated and are weight neutral. Common observed adverse effects included nasopharyngitis, headache, and upper respiratory tract infections. The risk of acute pancreatitis is noted on the label of all DPP-4 inhibitors, and similar rates of pancreatitis were reported in the EXAMINE (alogliptin), CARMELINA (linagliptin), SAVOR-TIMI (saxagliptin), and TECOS (sitagliptin) trials [76–78, 80]. There have been conflicting results regarding risk of pancreatitis in meta-analyses, and one meta-analysis demonstrated an increased relative risk of acute pancreatitis (odds ratio 1.79; 95% CI 1.13–2.82) versus placebo, although the absolute risk increase was low (0.13%) [81, 82]. Also, serious skin reactions have been reported with DPP-4 inhibitors in preclinical studies and post-marketing studies. For example, blistering skin diseases such as bullous pemphigoid and Stevens-Johnson syndrome have been reported [83]. In a retrospective analysis of more than 9000 patients treated with DPP-4 inhibitors in Japan, the prevalence of bullous pemphigoid was 0.09% in total, with a trend toward a higher risk associated with vildagliptin use compared to the other DPP-4 inhibitors [84]. DPP-4 inhibitors are effective in individuals with CKD and are particularly helpful in decreasing the risk of hypoglycemia. Dose adjustments are not necessary for linagliptin, which is mainly eliminated through the enterohepatic system. On the other hand, sitagliptin, saxagliptin, alogliptin, and vildagliptin require dose adjustment in patients with CKD [74, 85]. DPP-4 inhibitors may have a mild effect on lipid profiles in T2DM, with reductions in total cholesterol and triglycerides [86].

Evidence for Use of DPP-4 Inhibitors in PCOS

Since DPP-4 inhibitors improve glycemic control and are weight neutral, this class offers potential pharmacologic options in improving metabolism in PCOS. Sitagliptin, saxagliptin, and alogliptin have been studied in women with PCOS. A list of key studies of DPP-4 inhibitors in women with PCOS is provided in Table 26.3. A double-blind, crossover study of 18 overweight or obese women with PCOS (BMI >25 kg/m^2) investigated the effects on glucose levels and body composition after 1 month of either sitagliptin 100 mg PO or placebo daily [87]. Sitagliptin was associated with lower peak glucose after 75 gram glucose load during oral glucose tolerance testing compared to placebo (mean difference, −17.2 mg/dL, 95% CI, −27.7 to −6.6; $p < 0.01$) and decreased glucose over time (mean difference −5.13 mg/dL; 95% CI, −10.26–0.00; $p = 0.05$, relative to placebo). Notably, although sitagliptin enhanced early insulin secretion, as demonstrated by an increase in the insulinogenic index (from 1.9 ± 1.2 to 3.2 ± 3.1; $p = 0.02$) after glucose ingestion, it did not affect peripheral insulin resistance (Matsuda) or hepatic insulin resistance (HOMA-IR) [87]. This study also determined changes in body composition using DXA. Sitagliptin treatment improved body composition, with decreased VAT mass ($p = 0.02$ vs. placebo) and volume ($p = 0.02$ vs. placebo), but did not significantly affect weight or overall percent fat. Also, sitagliptin improved lipid profiles, with reductions in total cholesterol (from 168.8 ± 26.3 at baseline to 162.5 ± 22.2 mg/dL; $p = 0.02$), mainly due to a decrease

Table 26.3 Key studies with DPP-4 inhibitors in women with PCOS

Study	Intervention	Improved insulin resistance	Fasting glucose	Improved hyperandrogenism	Menstrual Pattern/ ovulation
Devin (2020)	Sitagliptin 100 mg PO daily vs. Placebo	↔	↓	↔	N/A
Ferjan (2018)	Sitagliptin 100 mg PO daily vs. Lifestyle intervention	↔	↓	N/A	N/A
Jensterle (2017)	Met 1 g PO BID + alogliptin 25 mg PO daily vs. Met 1 g PO BID + Alogliptin 25 mg PO daily + Pioglitazone 30 mg PO daily	Yes	↓	All groups: ↓ testosterone	N/A
Elkind-Hirsch (2017)	Saxagliptin 5 mg PO daily vs. Met 2 g PO daily vs. Saxagliptin 5 mg PO daily + Met 2 g PO daily	Yes	↓	↔	Yes
Tao (2018)	Saxagliptin 5 mg PO daily vs. Met 2 g PO daily vs. Saxagliptin 5 mg PO daily + Met 2 g PO daily	Yes	↓	All groups: ↓ testosterone	N/A

BID twice daily, *Lira* liraglutide, *Met* metformin, *SQ* subcutaneous, ↔ no significant change, *N/A* not applicable

in LDL (from 101.9 ± 23.8 to 96.2 ± 21.5 mg/dL; $p = 0.06$). Finally, sitagliptin did not affect testosterone or estradiol levels [87]. As previously noted, metformin is often offered to women with PCOS with prediabetes or T2DM; however, many individuals have difficulty tolerating metformin due to common gastrointestinal side effects, including diarrhea, nausea, and abdominal discomfort. A 12-week prospective, open-label study of 30 obese metformin-intolerant women with PCOS randomized to lifestyle intervention plus sitagliptin 100 mg PO daily or lifestyle intervention alone found that sitagliptin improved beta-cell function as assessed by the homeostasis model assessment for beta-cell function index (HOMA-B) of 45.9 ± 35.8 ($p = 0.001$) and modified beta-cell function index (MBCI) of 7.9 ± 7

($p = 0.002$) compared to baseline [88]. These differences in HOMA-B ($p = 0.001$) and MBCI ($p = 0.010$) were also significant in between the sitagliptin and the lifestyle intervention alone groups. Furthermore, women in the lifestyle intervention only group had an increased probability of worsening glycemic control and transition to impaired glucose homeostasis compared to those taking sitagliptin [88].

In line with the use of DPP-4 inhibitors as add-on therapy in T2DM, a few studies have examined the use of DPP-4 inhibitors in combination with metformin as well as other oral medications, such as the insulin-sensitizer pioglitazone, in women with PCOS. One group aimed to evaluate whether the DPP-4 inhibitor alogliptin alone or in combination with pioglitazone improves improved β-cell function and insulin resistance in metformin-treated obese women with PCOS. In this study, obese women with PCOS already taking metformin (1000 mg PO twice daily) were randomized to alogliptin (25 mg PO daily) or alogliptin plus pioglitazone (30 mg PO daily) for 12 weeks [89]. Both metformin-alogliptin and metformin-alogliptin-pioglitazone resulted in significant decreases of HOMA-IR by -1.6 ± 2.3 ($p = 0.039$) and -2.9 ± 3.3 ($p = 0.001$), respectively, compared to baseline (4.82 ± 2.52, mean ± SD) after 2-hour meal tolerance test (containing 44.4 grams carbohydrate, 13.8 grams protein, and 10.8 grams lipid). While the decrease in HOMA-IR on metformin-alogliptin-pioglitazone tended to be greater compared to metformin-alogliptin, the between-treatment differences were not significant. Women treated with metformin-alogliptin lost an average of 1.9 ± 1.7 kg, while those treated with metformin-alogliptin-pioglitazone lost 0.3 ± 3.3 kg (p = ns). In addition, there were small but statistically significant decreases in total testosterone in the metformin-alogliptin (from 1.8 ± 0.9 to 1.2 ± 0.6 nmol/L, $p < 0.01$) and in the metformin-alogliptin-pioglitazone group (from 1.4 ± 0.7 to 1.2 ± 0.7 nmol/L, $p < 0.05$) [89].

Another group aimed to evaluate the efficacy of saxagliptin (5 mg PO daily), metformin-extended release (2000 mg PO daily), and combination therapy over 16 weeks in women with PCOS and impaired glucose tolerance [90]. After 16 weeks of therapy, 19 of 34 patients (56%) had normal glucose tolerance: 3 of 12 patients taking metformin (25%), 6 of 11 patients taking saxagliptin (55%), and 10 of 11 patients taking saxagliptin plus metformin (91%). All 3 treatments significantly reduced fasting blood glucose levels ($p = 0.0001$). Combination therapy with metformin plus saxagliptin was superior to metformin alone in normalizing fasting and post-challenge glucose concentrations ($p = 0.007$). While all 3 treatments improved BMI ($p = 0.035$), waist circumference ($p = 0.004$), free androgen index ($p = 0.001$), and insulin sensitivity, saxagliptin plus metformin therapy was associated with significant improvement in insulin secretion-sensitivity index by oral glucose tolerance test and improved menstrual regularity. All treatments were associated with significant reductions in total cholesterol ($p = 0.014$) and LDL-C ($p = 0.014$); however only triglyceride levels were lowered significantly with saxagliptin plus metformin ($p = 0.004$) and saxagliptin ($p = 0.001$) therapy compared with metformin alone [90].

In an open-label, randomized study, 63 women with recently diagnosed type 2 DM and PCOS were treated with 24 weeks of metformin (2000 mg PO daily), saxagliptin (5 mg PO daily), or combination therapy with metformin plus saxagliptin [91]. There was a significant reduction in HbA1C in the metformin plus saxagliptin

group compared to the monotherapy groups (saxagliptin vs. combination treatment vs. metformin: −1.1 vs. −1.3 vs. −1.1%, $p = 0.016$). In addition, saxagliptin, metformin, and combination therapy significantly improved insulin sensitivity, as indicated by reduced HOMA-IR and Matsuda Index ($p < 0.01$ for all). Saxagliptin and metformin alone also significantly reduced BMI and high-sensitivity C-reactive protein levels ($p < 0.01$ for both). Overall, both saxagliptin and metformin are similarly effective in improving glycemic control and β-cell function and reducing inflammation in women with PCOS and newly diagnosed T2DM [91].

Though DDP-4 inhibitors are generally regarded as weight neutral, one study investigated whether sitagliptin could potentially prevent weight regain compared to metformin alone in obese women with PCOS who had been previously treated with liraglutide. This prospective randomized open-label study included 24 obese women with PCOS who had been previously treated with liraglutide (3 mg SQ daily) for obesity and randomized them to a combination of sitagliptin (100 mg PO daily) plus metformin (1000 mg PO twice daily) or metformin alone [92]. Over 12 weeks of treatment, women treated with metformin alone regained 4.7 ± 2.7 kg ($p = 0.002$) compared with 0.9 ± 2.5 kg in the sitagliptin plus metformin group ($p = 0.147$). Furthermore, BMI increased by 1.7 ± 0.9 kg/m^2 in the metformin group ($p = 0.002$) compared with 0.3 ± 0.8 kg/m^2 increase in the sitagliptin plus metformin group ($p = 0.136$). Of note, the women in the combination therapy group also reported greater ability to resist emotional eating [92]. While sitagliptin in combination with metformin appears to have prevented weight regain in this small study, the preponderance of evidence indicates that DPP-4 inhibitors are essentially weight neutral.

Sodium-Glucose Cotransporter-2 (SGLT2) Inhibitors

Background and Mechanism of Action

SGLT2 inhibitors are the latest class of oral anti-hyperglycemic drugs approved for T2DM. First isolated from the bark of the apple tree in 1835, phlorizin was initially used for its antipyretic effects and was observed to induce glycosuria in 1886. Beginning in the 1950s, studies began to focus on the mechanism of action of phlorizin, and in 1973, the active transport system for the renal reabsorption of glucose was localized to the luminal membrane of renal proximal tubular cells [93]. In addition, phlorizin's binding affinity for the renal glucose transporter (SGLT) proteins was found to be 1000–3000 times the affinity of glucose for these carriers [94]. Expressed in the proximal convoluted tubule of the kidneys, SGLT2 proteins function independently of insulin and account for approximately 90% of the reclamation of filtered glucose. The normal renal threshold for glucose reabsorption corresponds to a plasma glucose concentration of 180 mg/dL. Hyperglycemia in the setting of T2DM can increase this threshold and upregulates SGLT2 expression, leading to further elevations in plasma glucose [95]. SGLT2 inhibitors emerged as a novel approach to management of T2DM since inhibition of SGLT2 decreases the renal glucose threshold to under 70 mg/dL, thus increasing urinary glucose excretion and

improving hyperglycemia [96]. Importantly, SGLT2 inhibitors reduce blood glucose without stimulating insulin release and therefore cannot result in hypoglycemia.

Current Clinical Use of SGLT2 Inhibitors

SGLT2 inhibitors are recommended by the ADA for patients with T2DM as an add-on to metformin and lifestyle changes, particularly in individuals at with established or at high risk for ASCVD, as well as those with heart failure or chronic kidney disease (CKD, eGFR 30–60 ml/min/1.73m^2) [26]. SGLT2 inhibitors are especially useful in T2DM as they lower A1C without hypoglycemia, induce modest weight loss and reduce blood pressure. SGLT2 inhibitors are taken by mouth once daily in the morning as monotherapy or up to twice daily as combination drugs. Of the FDA-approved SGLT2 inhibitors, empagliflozin has the highest selectivity for SGLT2 receptors, while canagliflozin is the least selective [97]. SGLT2 inhibitors and combination drugs are listed in Table 26.4. Monotherapy with SGLT2 inhibitors results in HbA1C reductions of −0.5–0.1%, and in combination with other oral antihyperglycemic medications or insulin, they can boost additional decreases in HbA1C [98].

In multiple large outcomes trials, SGLT2 inhibitors have demonstrated cardiorenal benefits in patients with T2DM and established or high risk for CVD. For example, in addition to reducing the risk of all-cause mortality by 32%, empagliflozin decreased the risk of cardiovascular death by 38% and hospitalization for heart failure by 35% compared to placebo [99, 100]. Potential mechanisms to explain the cardiovascular benefits of SGLT2 inhibitors include improvement in ventricular

Table 26.4 SGLT2 inhibitors: Dosing and average weight loss

Drug (brand)	Dosing	Weight loss (kg)
Canagliflozin (Invokana®) Canagliflozin/metformin (Invokamet®) Extended release (Invokamet XR®)	100–300 mg daily 50/500–300/2000 mg BID 100/1000–300/2000 mg daily	~3
Dapagliflozin (Farxiga®) Dapagliflozin/metformin (Xigduo XR®)	5–10 mg daily 5/500–10/2000 mg daily	~3
Empagliflozin (Jardiance®) Empagliflozin/metformin (Synjardy®) Extended release (Synjardy XR®)	10–25 mg daily 5/500–12.5/1000 mg BID 5/1000–25/2000 mg daily	~2 **in patients with T2DM*
Ertugliflozin (Steglatro®) Ertugliflozin/metformin (Segluromet®)	5–15 mg daily 2.5–500–7.5/1000 mg BID	~3 **in patients with T2DM*

Data for weight loss obtained from Refs. [115, 118–120] and SGLT2 inhibitor-induced weight loss is observed over 3 months of treatment, with a plateau around 6 months of therapy. SGLT2 inhibitors taken alone should be dosed daily and taken in the morning to avoid nocturia. Unless combined with metformin, all (except canagliflozin) may be taken with or without food. When combined with metformin, all should be taken with food

BID twice daily

conditions through a reduction in preload via osmotic diuresis and natriuresis and in afterload via a reduction in blood pressure, improved cardiac metabolism and energetics, decrease in cardiac fibrosis, and alterations in adipokines and cytokine production that decrease inflammation [100]. SGLT2 inhibitors significantly reduce both systolic (1.7–6.9 mmHg) and diastolic (0.9–3.5 mmHg) blood pressure. The reductions in blood pressure are postulated to be due to diuresis, volume depletion, and, potentially, inhibition of the renin-angiotensin system [96].

While some trials have shown no change in lipid parameters, others have demonstrated small but statistically significant increases in both HDL and LDL cholesterol with no effect on triglycerides [96]. In multiple clinical trials and real-world studies, patients treated with SGLT2 inhibitors have experienced weight loss of 1–3 kg. The weight loss associated with SGLT2 inhibitors typically occurs rapidly within the first few weeks of treatment and then becomes more gradual [101, 102]. Mechanisms underlying SGLT2 inhibitor-mediated weight loss include increased urinary glucose excretion leading to some loss of body water, but also chronic calorie losses through the urine, and a metabolic shift towards lipolysis and ketogenesis leading to reduced body fat [102]. Finally, SGLT2 inhibitors are being studied for a potential role in diabetes prevention. A recent subanalysis of the DAPA-HF trial, which was a placebo-controlled international study of dapagliflozin in people with chronic heart failure and a reduced ventricular ejection fraction, found a reduced incidence of T2DM in those who received dapagliflozin compared to placebo. Dapagliflozin decreased new-onset diabetes by 32%, with 4.9% of dapagliflozin patients developing T2DM, compared to 7.1% in the placebo group (HR 0.68 (95% CI, 0.50–0.94; $p = 0.019$) [103]. This is similar to the 31% relative risk reduction in diabetes incidence in individuals taking metformin (850 mg BID) compared to placebo in the Diabetes Prevention Program (DPP). Nonetheless, intensive lifestyle intervention was significantly more effective compared to metformin and led to a 58% relative risk reduction in T2DM incidence [104].

SGLT2 inhibitors have a much lower risk of hypoglycemia compared to sulfonylureas since their mechanism of action is insulin independent. Genital mycotic infection due to glucosuria is a relatively common adverse side effect of this class of agents. Urinary tract infections (UTIs) are possible given the mechanism of action; however, in the large outcomes trials, there appears to be no significant difference in the incidence of UTIs between active therapy and placebo groups. These drugs should still be avoided in those with a prior history of frequent or complicated UTIs or in the setting of indwelling urinary catheters. Since SGLT2 inhibitors lower blood pressure via osmotic diuresis, some individuals, particularly those who are elderly or taking diuretics, may develop dehydration or orthostatic hypotension. Diabetic ketoacidosis (DKA), which may be euglycemic, has been observed with SGLT2 inhibitors, though predominantly during off-label use in type 1 diabetes [105]. DKA can occur in treated patients with T2DM but usually in those on insulin and in the setting of severe concurrent illness, decreased food and fluid intake, perioperative settings, or alcohol use [105]. SGLT2 inhibitor therapy can lead to reductions in insulin doses, while promoting lipolysis and increased glucagon levels, all of which may promote ketogenesis. SGLT2 inhibitors, particularly canagliflozin,

have also been associated with an increased risk of fracture relative to placebo, particularly low trauma fractures [106]. Potential mechanisms may include elevated serum phosphate levels due to increased tubular reabsorption of phosphorus induced by SGLT2 inhibitors, leading to downstream homeostatic changes including elevations in parathyroid hormone with resulting bone resorption and adverse effects on bone, such as decreased bone density [107]. In a double-blind, placebo-controlled clinical trial, patients randomized to two different doses of canagliflozin (100 mg and 300 mg) had placebo-corrected declines in BMD at the total hip of 0.9% and 1.2%, respectively, and at the lumbar spine of 0.3% and 0.7%, respectively, after 2 years of treatment [106]. However, follow-up observational studies have not found an increased risk of fractures in patients exposed to canagliflozin, dapagliflozin, or empagliflozin [108]. Also, canagliflozin was associated with a nearly doubled risk of lower extremity amputation in patients with T2DM (6.3 vs. 3.4 participants per 1000 patient-years; hazard ratio, 1.97; 95% CI, 1.41–2.75) [109]. The mechanism for increased risk of lower extremity amputations is unclear, but hypotheses include diuresis and volume depletion leading to circulatory failure in distal peripheral arterial beds and the presence of underlying peripheral arterial disease [110]. Nonetheless, this adverse event has not been observed with dapagliflozin or empagliflozin [111].

Evidence for Use of SGLT2 Inhibitors in PCOS

Since SGLT2 inhibitors have been shown to promote weight loss and provide cardiovascular benefits in individuals with T2DM, these agents may also be beneficial in PCOS which is associated with obesity and increased risk for T2DM and cardiovascular risk. To date, data for use of SGLT2 inhibitors in PCOS are sparse. An open-label, randomized study of 40 obese women with PCOS compared empagliflozin (25 mg daily) to metformin (1500 mg extended-release daily) for 12 weeks [112]. Women in the empagliflozin group experienced a mean weight loss of 1.5 kg. Percentage changes from baseline showed statistically significant differences in weight in the empagliflozin group (−1.4 ± 3.2%) versus metformin (+1.2 ± 2.3%) ($p = 0.006$). Similarly, although there was no significant change in BMI, percentage change in BMI from baseline was significant between the two groups (empagliflozin, −1.4 ± 3.2% vs. metformin, + 1.1 ± 2.2%; $p = 0.007$). Women who received empagliflozin experienced decreased waist circumference (−1.6 ± 2.8% vs. metformin 0.2 ± 2.1%; $p = 0.029$) and hip circumference (−2.0 ± 3.0% vs. metformin 1.1 ± 1.9%; $p = 0.001$). No changes were seen between the empagliflozin and metformin groups in blood pressure, fasting glucose, insulin levels, or HOMA-IR. However, women who received empagliflozin demonstrated significant increases in SHBG ($p = 0.049$), and estradiol levels ($p = 0.032$), though no differences in androgen levels were seen [112]. There is limited information regarding the use of SGLT2 inhibitors in pregnancy. Due to the results of animal studies suggesting that SGLT2 inhibition may affect renal development and maturation, use of these drugs is not recommended during the second and third trimesters of pregnancy

[113]. Additional trials of longer duration are needed to confirm these beneficial effects of empagliflozin and provide further insights into the effects of empagliflozin on PCOS-related outcomes in women with different PCOS phenotypes and PCOS-related complications.

Summary: Using SGTL2 Inhibitors in Women with PCOS

SGLT2 inhibitors are a promising therapeutic option for women with PCOS as they decrease measures of adiposity including visceral and subcutaneous fat depots and waist and hip circumference, which are important factors reflecting cardiometabolic risk in individuals with T2DM [114, 115]. Since empagliflozin has also been shown to reduce waist and hip circumference in PCOS, it may also portend decreases in cardiometabolic risk in this population. SGLT2 inhibitors may play a role in the management of PCOS as an add-on agent to lifestyle interventions or, potentially, metformin, to promote weight loss and reductions in adiposity. More data are needed to determine whether particular subtypes of PCOS may respond favorably to SGLT2 inhibitors, relative to other drugs.

Future Directions

Although GLP-1 RAs, DPP-4 inhibitors, and SGLT2 inhibitors have not been approved for use in PCOS, the potential role of these drugs in targeting obesity and the increased risk of T2DM should be considered as it is germane to this metabolically vulnerable population. While obesity is associated with an increased risk of adverse maternal and fetal outcomes, medications for weight loss therapy, such as GLP1-RAs, are not recommended at conception or during pregnancy due to lack of potential benefit and possible fetal harm [116]. SGLT2 inhibitors are not recommended in pregnancy either. As a result, women with PCOS intending to achieve pregnancy should discontinue incretin and SGLT2 inhibitor therapy prior to conceiving. Clinical studies investigating GLP-1 RAs, DPP-4 inhibitors, and SGLT2 inhibitors in PCOS women have been limited to 3- to 6-month interventions. Longer-term studies are needed to confirm and determine whether these medications improve metabolic and reproductive endpoints in PCOS. Currently, there is a clinical trial in adolescents with PCOS underway, comparing the use of GLP-1 RA therapy (semaglutide 3 mg and 7 mg PO daily) to a dietary intervention with outcomes including changes in hepatic fat fraction and whole body and adipose tissue insulin sensitivity. A few groups have previously compared the effect of metformin versus metformin in combination with a GLP-1 RA in PCOS, but clinical studies are needed to examine the effect of metformin in combination with SGLT2 inhibitors. As women with PCOS commonly have metabolic syndrome and obesity, future studies investigating combination therapy with GLP-1 RAs or DPP4-inhibitors in combination with SGLT2 inhibitors are needed in this population.

In summary, with the early evidence that GLP-1 RAs, DPP-4 inhibitors, and SGLT2 inhibitors reduce the risk of developing T2DM and improve hyperandrogenism and ovarian function, these drugs are emerging tools in the armamentarium to target prediabetes and metabolic complications in women with PCOS.

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Intergenerational Implications of PCOS

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Molly M. Willging, David H. Abbott, and Daniel A. Dumesic

Introduction

Alterations in maternal physiology can have adverse influences on fetal development and cause permanent changes in both male and female offspring physiology and metabolism after birth to increase the risk for disease in adulthood [1–3]. This chapter, however, will focus on female vulnerability to intergenerational transmission of polycystic ovary syndrome (PCOS). Fetal stress from maternal undernutrition favors genes important for energy conservation, creating metabolic adaptions that are beneficial in times of food scarcity, but predispose to obesity and diabetes when food becomes abundant later in life [2, 4]. In their original description of adult consequences following adverse gestation, Barker and Osmond [5] associated the highest infant mortality rates in early-twentieth-century northern England with subsequent, decades later, highest mortality rates from coronary heart disease. The original report has since been reliably supported by associations between low birth weight and adult development of cardiovascular disease (CVD), hypertension, insulin resistance, and type 2 diabetes mellitus (T2DM) [1, 6], now widely recognized as developmental origins of adult disease (DoHAD).

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5_27

Conversely, maternal overnutrition also can predispose offspring to adult disease, thus revealing a "U-shaped curve" in infant birth weights relative to subsequent childhood and adult obesity, hypertension, and insulin resistance [7]. In addition, the "U-shaped curve" confirms that fetal weight deviation from optimal size, regardless of direction, increases the risk of developing metabolic disease in later life [4]. Consequently, small-for-gestational-age infants are at increased risk of developing metabolic disease as adults from impaired fetal nutrient availability that includes placental insufficiency, while large-for-gestational-age infants exhibit comparable increased metabolic risks, but these are from successful fetal adaptation to maternal nutrient overabundance, i.e., maternal obesity and/or high-fat/high-calorie diets [3, 7].

These different maternal-placental-fetal environments have important developmental implications for daughters born to women with PCOS, a heterogeneous syndrome characterized by luteinizing hormone (LH) hypersecretion, hyperandrogenism, menstrual irregularity, and polyfollicular ovarian morphology with accompanying anti-Mullerian hormone (AMH) hypersecretion [8]. An altered maternal-placental-fetal environment likely programs childhood and adult disease through epigenetic modifications of fetal genetic susceptibility to PCOS that phenotypically manifests after birth [9, 10]. Evidence for this can be shown through clinical studies of PCOS women and their daughters integrated with nonhuman primate (NHP) models that either use discrete experimentally induced gestational testosterone (T) excess to program a permanent PCOS–like phenotype [3, 11] or a naturally occurring PCOS-like phenotype [12].

Key Points

- PCOS is characterized by hyperandrogenism, oligo-anovulation, and insulin resistance.
- Women with classic PCOS have insulin resistance and preferential abdominal fat accumulation that are worsened by obesity.
- PCOS is a heritable syndrome that interacts with risk-increasing environmental factors to fully explain its prevalence.
- Maternal endocrine-metabolic alterations can modify fetal genetic susceptibility to PCOS after birth.
- Maternal AMH overproduction could contribute to intergenerational transmission of PCOS.
- Hyperandrogenism in PCOS may be an ancestral metabolic adaptation that predisposes to lipotoxicity in today's obesogenic environment.
- Nonhuman primates share close genetic homology with humans, along with comparable physiology, reproduction, and development with aging.
- Nonhuman primate models are important to show how intergenerational transmission of a PCOS phenotype can be developmentally programmed.
- Prenatal androgen exposure in nonhuman primates programs a permanent PCOS–like phenotype.
- Naturally occurring hyperandrogenism in adult female nonhuman primates accompanies LH hypersecretion and evidence of previous fetal androgen excess.

Polycystic Ovary Syndrome (PCOS)

As the most common endocrinopathy of women, PCOS is a complex syndrome of hyperandrogenism, oligo-anovulation, and polycystic ovaries [13]. In today's obesogenic environment, most women with PCOS have insulin resistance and abnormal steroid metabolism, combined with preferential abdominal fat accumulation that promotes anovulatory infertility, T2DM, and metabolic syndrome [8, 13, 14]. Furthermore, when pregnancy is achieved, the risks for gestational and T2DM increase relative to non-PCOS women regardless of age and body mass index (BMI) [15].

These endocrine-metabolic abnormalities are particularly evident in women with hyperandrogenism in combination with oligo-anovulation, excluding other endocrinopathies, as defined by 1990 National Institutes of Health (NIH) criteria [16]. With the more recent Rotterdam diagnostic criteria for PCOS, endocrine-metabolic abnormalities often accompany PCOS women with phenotype A (hyperandrogenism + oligo-anovulation + polycystic ovaries) and phenotype B (hyperandrogenism + oligo-anovulation), both now referred to as "classic" PCOS [15, 17]. Women with classic PCOS (previously NIH-defined PCOS) are generally more hyperandrogenic and obese than the other PCOS phenotypes and therefore are at increased risk of developing reproductive and metabolic disorders. Ovulatory PCOS patients (phenotype C) have a lower body mass index [18] and lesser degrees of hyperinsulinemia and hyperandrogenism than women with classic PCOS [18, 19], while women with nonhyperandrogenic PCOS (phenotype D) are least affected regarding metabolic risk without obesity.

Importantly, PCOS phenotypic expression can change with age, since 20- to 30-year-old women with PCOS are more likely to exhibit reproduction-related issues, whereas older women with PCOS are more likely to experience metabolic dysfunction [20]. Consequently, 40% of PCOS women develop glucose intolerance or T2DM by the fourth decade of life [21].

From a developmental prospective, healthy, normal-weight women with classic PCOS also exhibit preferential abdominal fat accumulation, increased intra-abdominal fat mass, and adipose insulin resistance (adipose-IR) in vivo [22, 23], defined by the product of fasting circulating free fatty acid (FFA) and insulin levels [23]. Their subcutaneous (SC) abdominal stem cells (ASCs) also exhibit enhanced lipid accumulation during adipocyte maturation in vitro that positively correlates with serum androgen levels [24] and predicts improved systemic insulin sensitivity in vivo [25], consistent with the function of SC adipose in humans to normally store lipid as protection against insulin resistance, while intra-abdominal adipose has the opposite effect [26]. Hyperandrogenism in PCOS may be part of ancestral metabolic adaptations to enhance SC fat storage that in today's obesogenic environment predisposes to lipotoxicity as excess FFAs deposit in ectopic locations, such as muscle, liver, and pancreas, where increased oxidative stress is linked with insulin resistance and inflammation [27, 28].

Genetic Contributions to PCOS

While a heritable etiology for PCOS is suggested by its peripubertal onset and familial clustering [8], several gene candidates identified by genome-wide association studies (GWAS), including those regulating gonadotropin secretion and action, androgen biosynthesis, metabolism, and follicle development, contribute only a minor effect to PCOS phenotypes [29]. Variants in *DENND1A*, a GWAS-identified PCOS candidate gene regulating androgen biosynthesis, however, together with two family-based whole genome sequence (WGS)-identified PCOS candidate genes (i.e., anti-Mullerian hormone [*AMH*] and its specific receptor *AMHR2* regulating ovarian follicle development and reproductive neuroendocrine function) have been found in ~50% and ~7%, respectively, of women with PCOS [29].

From a metabolic dysfunction perspective, a recent clustering analysis of 893 women with NIH-defined PCOS has shown two distinct PCOS subtypes with different genetic heterogeneity: a "reproductive" group (23% of cases), characterized by higher LH and SHBG levels with relatively low BMI and insulin levels, and a "metabolic" group (37% of cases), characterized by higher BMI, glucose, and insulin levels with lower SHBG and LH levels. These findings suggest that different PCOS subtypes exist and may differ in their developmental origins [30]. In this latter regard, heritability of PCOS interacts with risk-increasing environmental factors, including maternal obesity and gestational diabetes, to fully explain its prevalence. Such genetic environmental interactions likely begin before birth when an altered maternal-placental-fetal environment generates epigenetic modifications in fetal genetic susceptibility to PCOS, which continues after birth into adulthood as metabolic adaptations to enhance fat storage, but predispose to lipotoxicity [31].

The Maternal-Placental Environment in PCOS

Alterations in the maternal-placental-fetal environment can permanently program adult disease through epigenetic modifications of genes that affect fetal susceptibility to disease after birth [3]. In today's obesity epidemic, maternal obesity accompanies gestational diabetes and hypertension, preeclampsia, preterm delivery, stillbirth, early neonatal death, and small- as well as large-for-gestational-age infants [32]. Obese women also are at increased risk of having infants with defects involving the heart, ventral wall, and neural tube, along with other anomalies [32]. That maternal obesity, by itself, increases the risk of premature mortality from a cardiovascular event in their adult offspring provides strong evidence that obesity during pregnancy underlies intergenerational programming of metabolic disease in offspring [33].

Through this perspective, pregnant women with PCOS are at increased risk for metabolic dysfunction versus other pregnant women, with preconception hyperandrogenemia and glucose intolerance in PCOS women predicting several suboptimal maternal and neonatal outcomes, including preeclampsia [15]. Pregnant women with PCOS maintain hyperandrogenemia and higher fasting as well as postprandial insulin values [34–36] than normal women, along with exaggerated dyslipidemia

and elevated circulating inflammatory markers that predict increased risks of gestational diabetes, hypertensive disorders, and adverse obstetrical/neonatal outcome [37, 38]. Consequently, the prevalence of glucose intolerance, gestational diabetes, and T2DM in PCOS women is 3- to 5-fold higher than that of other women, independent of body mass index (BMI) and worsened by obesity [15], with PCOS women exhibiting a 2.5–4-fold increased risk of developing GDM and other pregnancy complications [39].

Pregnant women with PCOS also continue to have elevated ovarian AMH production compared to normal women [34, 36]. Although developmental programming effects of this finding in PCOS remain unclear, gestational exposure of mice to recombinant AMH during a critical time in pregnancy induces maternal neuroendocrine-driven androgen excess while diminishing placental aromatization, causing a PCOS-like phenotype in female offspring and their descendants across three generations [10, 40]. Interestingly, Mullerian anomalies may be more prevalent among women with some clinical features of PCOS [41–44], implicating exaggerated mid-gestation AMH production in utero [45] with Mullerian duct differentiation, since maternal AMH does not cross the placenta [40].

Mid-gestation maternal T levels in women positively correlate with high AMH levels in adolescent daughters, suggesting a link between mid-gestation maternal hyperandrogenism and perturbed ovarian function in daughters [46]. Nevertheless, although reduced aromatase activity in term placenta from PCOS women has been reported [47], thereby potentially increasing androgen exposure to female fetuses, maternal hyperandrogenemia from PCOS may not directly program PCOS in offspring if placental aromatization is normal [48, 49]. More likely, maternal metabolic dysfunction in PCOS mothers alters placental function of a female fetus susceptible to PCOS and thereby induces fetal hyperinsulinemia as a cause for hyperandrogenism and altered ovarian follicular development in utero [3, 11, 50, 51].

The Fetal Environment

Maternal metabolic dysfunction from type 1 diabetes can adversely affect the health of their offspring, who in turn are at increased risks of developing T2DM and obesity in response to the prior metabolic environment in utero. Adolescent offspring of mothers with type 1 diabetes have increased body weight, dyslipidemia, and insulin resistance following previous exposure to hyperinsulinemia in utero [52], while adult offspring of mothers with gestational or type 1 diabetes who were previously exposed to fetal hyperglycemia show epigenetic changes in SC adipose, involving leptin activity, mitochondrial function, and fat storage [53]. Gestational hyperinsulinemia in NHPs induces ectopic pericardial and perirenal fetal lipid accumulation and increased stillbirths [54], potentially predisposing to lipotoxicity in overweight and obese adults.

Similar maternal metabolic abnormalities likely affect the human fetal ovary during the second trimester of development when the primordial ovarian follicular pool and abdominal fat are established and the ovary contains several steroidogenic enzymes, genes encoding steroid-signaling pathways, and receptors to steroids,

insulin, and insulin-like growth factors [8, 55, 56]. Midtrimester human and NHP fetal ovaries can metabolize pregnenolone to dehydroepiandrosterone (DHEA) and androstenedione and also can secrete these androgenic precursors with T in vitro [8, 57, 58]. They are likely less responsive than testes, however, to the transient mid-gestation rise in fetal gonadotropins [59, 60], which causes a transient sex difference in amniotic androgen levels in humans [8, 51, 61] and in the fetal circulation of NHPs [60] that disappears by birth.

Theoretically, the human mid-gestation fetal ovary may produce androgens in response to hyperinsulinemia in utero, particularly in the female fetus with a genetic susceptibility to PCOS. Mid-gestation amniotic fluid T levels are elevated in female fetuses of both diabetic [62] and PCOS [51] mothers, while theca and pancreatic beta cell hyperplasia accompany ovarian theca-lutein cysts in hirsute female stillbirth offspring of diabetic women [63, 64]. Moreover, infant hyperandrogenism [48] and pancreatic beta cell hyperplasia [65] accompany infant hypoglycemia, hyperinsulinemia, and excess weight gain [66] in female NHPs exposed to early-to-mid-gestation T-excess.

As a reliable postnatal biomarker of mid-gestation fetal hyperandrogenism, elongated anogenital distance is present in female infants of PCOS mothers and in women with PCOS [67, 68]. As another ovarian hormone, AMH has been detected in the cord blood of a second-trimester female fetus from a mother with PCOS, occurring earlier than normal ovarian AMH expression in the third-trimester human fetal when developmental susceptibility to endocrine disruption may have closed [69]. Therefore, AMH overproduction in newborn females and girls, as an endocrine antecedent of PCOS in susceptible women [45, 70–73], may coexist with mid-gestation fetal hyperandrogenism during completion of Mullerian duct differentiation [45], thereby contributing to Mullerian abnormalities and mild genital virilization, respectively [74]. However, additional investigation into these complex hormone interrelationships in utero and their effects on human fetal development is warranted to differentiate causal mechanisms from epiphenomenon [75].

Umbilical cord T and androstenedione levels also are elevated in some [76, 77], but not all [45, 78–81], female infants of PCOS mothers. Testosterone levels in the near-term fetal female, however, are affected by several factors including gestational weight gain [82], amniotic fluid cortisol levels [82], labor characteristics [83], gestational age [83], and maternal age [82]. Since the sexual dimorphism of fetal androgen levels is diminished by late gestation [61], term birth is a suboptimal time for obtaining evidence of fetal female hyperandrogenism.

Nonhuman Primate Models for PCOS

Reproduction

To understand causal mechanisms underlying these human maternal-fetal relationships, animal models have employed gestational exposure to androgen excess in NHPs, sheep and rodents to induce metabolic and reproductive abnormalities

resembling PCOS [11, 84]. Gestational T-exposed NHPs and sheep have been essential animal models used to program a permanent PCOS–like phenotype of LH hypersecretion, ovarian/adrenal hyperandrogenism (NHPs) or functional hyperandrogenism (androgen receptor upregulation, sheep), ovulatory dysfunction, and impaired glucose-insulin homeostasis [11]. This is because tissue differentiation in these species is completed during fetal life (i.e., precocial species), as in humans, unlike mice in which only partial differentiation is completed before birth (i.e., altricial species). This chapter will focus on NHP models, alone, because of their close genetic homology with humans, particularly at protein-coding exons, and their highly comparable physiology, reproduction, development, behavior, and aging [85].

Neuroendocrine Dysfunction

Neuroendocrine PCOS-like traits in fetal, infant, and adult gestational T-exposed female NHPs are characterized by LH hypersecretion from reduced hypothalamic sensitivity to steroid negative feedback and enhanced GnRH pulsatility [48, 86]. Comparable neuroendocrine abnormalities are found in adolescent hyperandrogenic girls, likely representing a precursor to adult PCOS [87]. Moreover, PCOS women show a sexually dimorphic pattern of exaggerated early LH responsiveness to GnRH analog that more closely resembles that of men or women with congenital adrenal virilizing disorders (e.g., classical CAH and adrenal virilizing carcinoma) than normal women [88, 89]. These common characteristics between a gestational T-exposed NHP model and PCOS women implicate androgen excess during human fetal development with permanently reduced steroid negative feedback on LH and enhanced GnRH pulsatility that persists into adult life. Consistent with these findings, adult female NHPs with naturally occurring hyperandrogenism also exhibit LH hypersecretion, as well as positive correlations between anogenital distance and circulating androgen levels, suggesting fetal origins of both their hyperandrogenism and neuroendocrine defect [12].

AMH synthesized in the ovary [90] or GnRH neurons [91] is critical for hypothalamic GnRH neuronal survival, maturation, and adult function in mice and humans. GnRH neurons must express AMH and AMH receptor type 2 (AMHR2) in order to survive and migrate from the fetal nose to the hypothalamus during early to mid gestation; in female mice, ~50% of adult hypothalamic GnRH neurons retain expression of AMHR2, while many neurons within the arcuate nucleus of the hypothalamus (the neural site of the kisspeptin-GnRH pulse generator) also express AMHR2. Intracerebroventricular infusion of bioactive recombinant human (rh) AMH_C increases GnRH neuronal activity directly and, by involving activation of GABAergic neurons that synapse onto GnRH neurons, increase episodic LH release [91, 92]. Interestingly, the duration of GnRH neuronal responses to AMH is diminished by preovulatory increases in ovarian estradiol in mice [92], raising speculation that T could induce the opposite effect, potentially implicating gestational T-excess in enhancing fetal AMH-excess-mediated action on fetal neuroendocrine differentiation, as illustrated in Fig. 27.1.

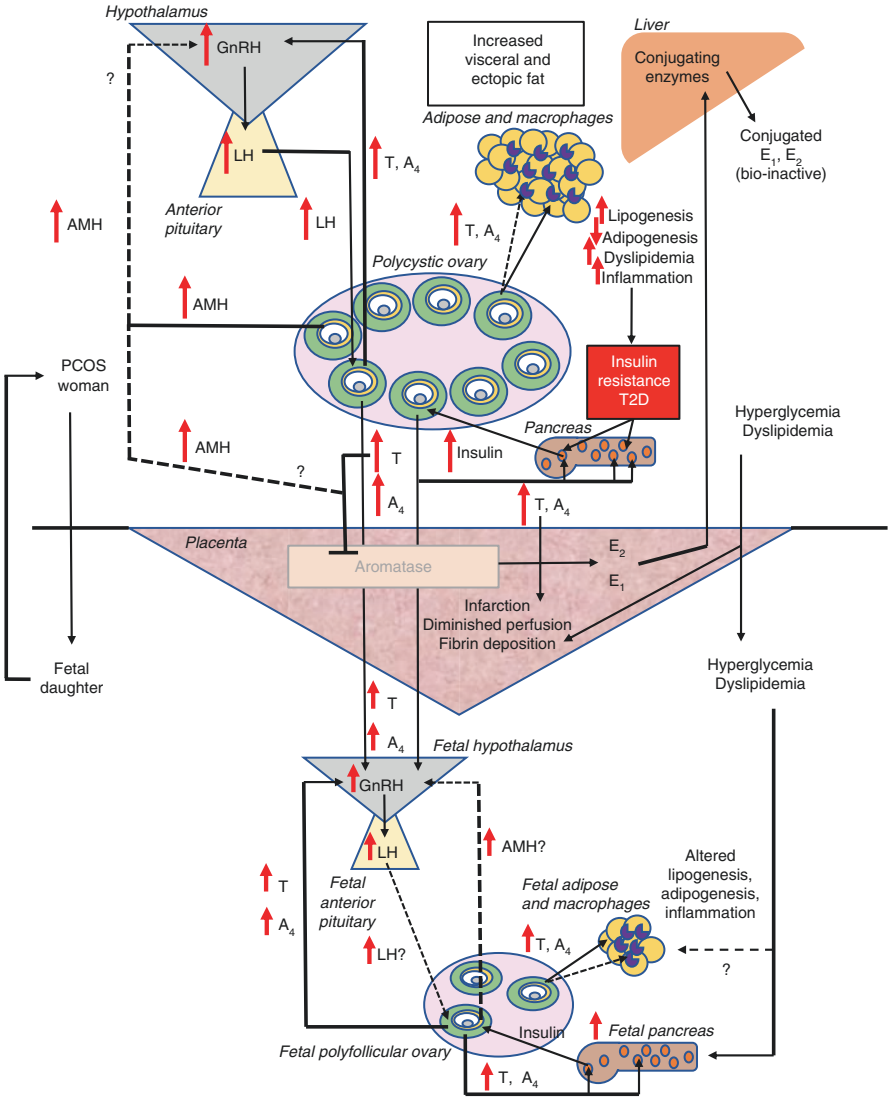
Hypothalamus
GnRH
?
LH
Anterior pituitary
AMH
LH
T, A4
Increased visceral and ectopic fat
Adipose and macrophages
T, A4
Liver
Conjugating enzymes
Conjugated E1, E2 (bio-inactive)
Lipogenesis
Adipogenesis
Dyslipidemia
Inflammation
Polycystic ovary
AMH
Insulin resistance T2D
PCOS woman
AMH
Pancreas
Insulin
T
A4
?
T, A4
Hyperglycemia Dyslipidemia
Placenta
Aromatase
E2
E1
Infarction
Diminished perfusion
Fibrin deposition
Fetal daughter
Hyperglycemia Dyslipidemia
T
A4
Fetal hypothalamus
GnRH
LH
AMH?
T
A4
Fetal anterior pituitary
LH?
Fetal adipose and macrophages
Altered lipogenesis, adipogenesis, inflammation
T, A4
?
Insulin
Fetal pancreas
Fetal polyfollicular ovary
T, A4

Fig. 27.1 Hypothetical feed-forward mechanisms for intergenerational metabolic and hyperandrogenic epigenomic transmission of PCOS in genetically susceptible daughters. Elevated maternal insulin- and LH-enhanced hyperandrogenism, as well as AMH overproduction by polycystic ovaries, contribute to increased hypothalamic GnRH release. Compensatory maternal pancreatic beta cell hyperinsulinemia also occurs in response to insulin resistance in multiple organ systems as a result of hyperandrogenism-induced lipogenic, adipogenic, and inflammatory dysfunction. During gestation, maternal hyperandrogenism can potentially inhibit placental aromatase, and together with maternal metabolic dysfunction and inflammation, disrupt placental structure-function, permitting transplacental access of excess androgens and nutrients from the mother to a female fetus. If placental aromatase activity is sufficient, extensive maternal hepatic conjugation inactivates excess estrogens, while androgen overproduction in the mid-gestation female fetus may result from hyperinsulinemia in utero in response to excess nutrient delivery from the maternal circulation. If placental aromatase activity is impaired, androgen excess in the mid-gestation female fetus may also result from an androgenic contribution from the maternal circulation. Androgen excess in the mid-gestation female fetus could (1) impair hypothalamic steroid negative feedback to promote LH-stimulated hyperandrogenism, (2) induce pancreatic beta cell hyperinsulinism, and (3) alter adipose and macrophage function. Precocious fetal ovarian AMH release could also potentially amplify fetal hyperandrogenism. These collective maternal-placental-fetal events could establish an antecedent susceptibility that favors development of a metabolically and reproductively compromised PCOS phenotype by adolescence. Solid lines and arrows indicate functions identified previously, while dashed arrows and lines are currently hypothetical. Within ovarian antral follicles, green, theca cells; yellow, granulosa cells; gray, oocyte; white, follicular fluid in the antrum. Within pancreatic islets, orange, beta cells. Within adipose and macrophages, beige, white adipocytes; purple, macrophages. GnRH gonadotropin-releasing hormone, LH luteinizing hormone, AMH anti-Mullerian hormone, T testosterone, A4 androstenedione, E1 estrone, E2 estradiol. (Modified from Ref. [140])

Ovarian Dysfunction

Developmental programming of ovarian function resembling that of PCOS has been more controversial. Gestational T-treatment in NHPs induces female subfertility, which includes impaired quality of primate oocytes [93]. Enlarged, polyfollicular ovaries are accompanied by ovarian hyperandrogenism [94]. While an exaggerated age-related decline in serum AMH levels in gestational T-exposed NHPs [95] is also found in some women with PCOS [96], this is atypical of PCOS. AMH levels in gestational T-exposed adult NHPs decline from similar values as controls in contrast to AMH values in women with PCOS in whom the age-related decline begins from values that are higher than those in women without PCOS [96]. NHPs with naturally occurring hyperandrogenism and PCOS-like traits, however, exhibit elevated AMH levels [12]. AMH overproduction is a characteristic of many women with PCOS and likely represents opposing effects of stimulatory reproductive (hyperandrogenism and increased antral follicle number) versus inhibitory metabolic (body fat) factors [97].

Placental Aromatization

Whether maternal T can serve as a source of fetal hyperandrogenism or as a programming mechanism for a PCOS-like phenotype in female offspring remains unclear. Gestational AMH excess in mice, however, can induce LH-mediated maternal T-excess with reduced placental aromatization of maternal androgens, together with placental dysfunction, potentially programming a PCOS-like phenotype in female offspring [10, 40].

Reduced aromatase activity in term placenta from PCOS women also has been reported [47], potentially increasing exposure of female fetuses to androgen excess. PCOS-related enhancement of fetal hyperandrogenism through placental dysfunction agrees with metabolically compromised PCOS placenta exhibiting features related to hypoxia, a condition known to downregulate placental aromatase [98, 99]. Maternal androgens of PCOS mothers, however, may not substantially contribute to programing PCOS in their offspring due to sufficient placental aromatization [48, 49]. More likely, an altered maternal endocrine-metabolic environment accompanying both PCOS and placental compromise [50, 99] probably promotes fetal ovarian hyperandrogenism via hyperinsulinemia acting as a secretagogue in utero, with complex androgen-insulin interactions reprograming sensitive target tissues in susceptible female offspring [3, 11, 85], as illustrated in Fig. 27.1. Nevertheless, pharmacological inhibition of placental aromatase by letrozole administration during late gestation in NHPs induces fetal female hyperandrogenism with subsequent adult insulin resistance in the absence of dyslipidemia and PCOS-like traits [100, 101].

Metabolic Dysfunction

Since NHPs are precocial, gestational T-treated female NHPs provide mechanistic links between endocrine-metabolic dysfunction in pregnancy and its long-term reproductive and metabolic consequences in offspring. In gestational T-treated NHPs, maternal glucose intolerance causes hyperglycemia in their female fetuses, stimulating fetal insulin release, which then potentiates insulin action within the fetus [66], and induces subsequent newborn hypoglycemia and infant hyperinsulinemia, typical of daughters born to women with diabetic pregnancies [102]. These endocrine-metabolic interactions during pregnancy are also influenced by placental function, which further modifies the in utero environment to program offspring in different ways.

Gestational T-treatment of female NHPs programs adipose dysfunction and insulin resistance in adult offspring [11] so that they develop a PCOS-like phenotype characterized by progressive metabolic dysfunction with age, insulin resistance, and preferential abdominal adiposity accompanied by hyperlipidemia [103–105]. Early-to-mid-gestation T-treated female NHPs exhibit increased visceral fat [103], adipose insulin resistance, and impaired insulin secretion [105], while late-gestation T-treated female NHPs have increased total body (non-visceral) fat [104] and

normal insulin sensitivity and pancreatic insulin secretion [94], contributing to an increased incidence of T2D in early-to-mid-gestation T-exposed female NHPs, alone. A TGF-beta signaling pathway involving altered DNA methylation patterns of visceral fat in early-to-mid-gestation T-treated female rhesus monkeys implies an epigenetic basis for reprogramming of adipose [106].

Adipogenic Dysfunction

Subcutaneous adipose normally can increase its capacity to properly store fat through adipocyte enlargement and new adipocyte formation [107]. Prenatally T-treated adult rhesus monkeys have an altered ability to store SC fat relative to BMI [104], suggesting abnormal adipogenesis, whereby multipotent adipose stem cells (ASCs) initially undergo commitment to preadipocytes and then differentiate into newly formed adipocytes [108]. Subcutaneous abdominal adipose of early-to-mid-gestation T-treated females in their late reproductive years shows impaired preadipocyte differentiation into adipocytes (i.e., decreased C/EBPα mRNA expression) accompanying hyperandrogenemia and enhanced earlier ASC commitment to preadipocytes (i.e., increased Zfp423 protein expression), perhaps as a compensatory mechanism for the impaired preadipocyte differentiation [109]. In agreement, androgen inhibits early-stage human SC abdominal adipogenesis in vitro [108], while ZFP423 gene knockout in mice exaggerates diet-induced obesity, ectopic fat deposition, and insulin resistance [110].

In normal human SC abdominal adipose, androgen induces insulin resistance and impairs catecholamine-stimulated lipolysis through reduced protein expression of β_2-adrenergic receptor and hormone-sensitive lipase (HSL) [111, 112]. Relevant to SC abdominal adipose of PCOS women, androgen excess also induces lipolytic catecholamine resistance [113, 114], but with amplification of intra-adipose T generation from androstenedione due to enhanced aldo-keto reductase 1C3 (AKR1C3) that further increases SC fat storage by enhancing lipogenesis (lipid formation) and suppressing lipolysis (lipid breakdown) [115]. Such androgen-related events in vivo likely alter SC fat storage and predispose to lipotoxicity from ectopic lipid accumulation in non-adipose tissues [108], agreeing with findings in normal-weight PCOS women of altered SC abdominal ASC gene expression of adipogenic/angiogenic functions involving T through TGF-β signaling [23].

In the absence of systemic T, however, cultured SC abdominal ASCs from normal-weight PCOS women show accelerated differentiation into adipocytes in vitro that favors insulin sensitivity in vivo [24, 25]. This PCOS-related characteristic of SC abdominal ASCs corresponds with changes in chromatic accessibility [31] and altered gene expression favoring SC abdominal lipid metabolism [116], suggesting an ancestral metabolic adaptation to enhance SC fat storage that predisposes to insulin resistance and lipotoxicity in today's obesogenic environment.

In contrast, human visceral fat normally shows increased lipolytic activity without androgen-induced lipolytic catecholamine resistance compared to SC abdominal fat, despite the presence of androgen receptors [112]. Furthermore, exaggerated

catecholamine-induced lipolysis in visceral adipose of nonobese PCOS women accompanies normal antilipolytic insulin action [111, 114], perhaps from a functional increase in the PKA-HSL complex that could promote insulin resistance from elevated portal free fatty acids [111, 117]. In PCOS women, therefore, such a phenomenon could promote lipolysis for fatty acid oxidation through insulin resistance to curtail fat accretion with age, as shown in nondiabetic Pima Indians with a tendency for excess weight gain [118]. It emphasizes how PCOS as a metabolic adaptation to ancestral environments of caloric restriction [9, 13] predisposes to endocrine-metabolic dysfunction preceding and during pregnancy, leading to the intergenerational transmission of PCOS in today's obesogenic society.

Altered SC Abdominal Adipocyte Size

Within an adipose depot, the size distribution of adipocytes represents a balance between adipocyte enlargement and new adipocyte formation [119]. An increased proportion of small SC abdominal adipocytes occurs in early-to-mid-gestation T-treated adult rhesus monkeys [109]. It also occurs in normal-weight PCOS women [22], preceding the presence of enlarged mature adipocytes in overweight PCOS women [120]. Enhanced small adipocyte formation accompanying *ZFP423* upregulation and epigenetic changes in the *ZFP423* promoter region protects against insulin resistance in humans [121]. That cultured SC abdominal ASCs of normal-weight PCOS show enhanced ZFP423 protein expression inversely related to fasting plasma glucose levels emphasizes how metabolic adaptation to enhance SC fat storage to maintain glucose-insulin homeostasis during caloric restriction predisposes to insulin resistance and lipotoxicity in today's obesogenic environment [24].

Maternal-Fetal Interactions

Large- and small-for-gestational-age infants have been observed in pregnant women with PCOS [37, 38]. Low birth weights in some of these infants can accompany placental abnormalities, such as chronic villitis/intervillositis, impaired decidual trophoblast invasion, and reduced placental size, which limit nutrient delivery to the fetus, with [122] or without [99] reducing the fetal-to-placental-weight ratio. Low infant birth weight also had been reported with precocious puberty and PCOS in northern Spanish [123] and Chilean as well as Iranian [124, 125] women, but not in other ethnic groups [99, 126]. Conversely, macrosomic infants of obese glucose-intolerant mothers result from successful fetal adaptations to maternal nutrient overabundance [127], with birth weight and maternal mid-gestation BMI positively correlated with newborn adiposity [80].

Infants of PCOS mothers can also have normal birth weights [3, 126]. Therefore, without altering infant birth weight, developmental programming of adiposity can still likely occur in the human fetus [56] and increase the risk of excessive postnatal weight gain and onset of PCOS in susceptible offspring.

Gestational Metabolic and Endocrine Dysfunction in Obese Versus T-Excess NHP Models

While maternal obesity per se does not appear to play a primary role in initiating PCOS during gestation, it appears to modify fetal genetic and epigenetic susceptibility to PCOS. Consistent with this, NHP models of maternal metabolic dysfunction, including maternal and offspring high fat diets, have yet to report evidence of female offspring exhibiting PCOS-like reproductive traits. NHP models of maternal metabolic dysfunction, nevertheless, demonstrate widespread gestational pathologies. These include placental infarctions, reduced placental blood flow, altered placental nutrient transport, hypoxia, and inflammation, resulting in female offspring with increased body weight and adiposity (despite diminished calorie intake, increased locomotor activity, but age-typical metabolic rate among offspring), fatty liver, hyperlipidemia, and increased inflammatory cytokines [128], as well as impaired pancreatic islet vascularization and innervation [129], and increased anxiety-like behavior [130]. In contrast, NHP models combining maternal high-fat diet induced obesity with experimentally induced gestational T-excess demonstrate synergism between metabolic dysfunction and hyperandrogenism in exacerbating gestational weight gain, glucose dysregulation, placental compromise, and pregnancy loss [131]. Interestingly, NHP models utilizing gestational T-excess induce maternal glucose dysregulation and increased gestational weight gain that positively correlate with elevated fetal female glucose levels, increased fetal growth, and dyslipidemia, resulting in newborn hypoglycemia, hyperinsulinemic responses to glucose, and increased infant weight gain [66]. Figure 27.1 illustrates a potential complex interaction between maternal metabolic dysfunction and hyperandrogenism theoretically contributing to intergenerational transmission of PCOS.

Endocrine Antecedents of PCOS

Infant girls born to PCOS versus non-PCOS mothers exhibit increased sebum production by the pilosebaceous unit [132] as well as increased anogenital distance [67], both biomarkers of gestational androgen excess. They also exhibit AMH overproduction, as a marker of increased numbers of growing follicles, which persists into prepubertal life [70, 133] and improves, along with exaggerated ovarian responsiveness to GnRH analog administration, when PCOS mothers receive metformin in pregnancy, beginning at or before conception [134]. Consistent with these findings, female children of PCOS women have enlarged ovaries and hyperinsulinemia that precede onset of LH hypersecretion and androgen excess during puberty in some [134, 135], but not all [136] studies.

From a metabolic perspective, adiposity in infants from PCOS women positively correlates with birth weight and maternal mid-gestation BMI [47], the latter of which increases disproportionally in normal-weight and overweight PCOS women compared to other pregnant women [34]. Although use of the insulin sensitizer, metformin, in pregnancy appears to improve some ovarian characteristics in female

offspring of PCOS mothers [134], the additional findings of increased childhood adiposity, insulin resistance, and increased newborn head size following gestational treatment of PCOS women with metformin demonstrate the need to minimize gestational interventions in humans [137]. In addition, interactions between genes of the susceptible fetus and the maternal PCOS endocrine-metabolic environment are likely further altered by postnatal environmental inputs, since widespread epigenomic remodeling occurs throughout human aging, being inherited across generations through nongenetic mechanisms [138, 139].

Conclusion

Epigenetic changes in fetal life likely impact the developmental origins of PCOS, given a critical time interval of fetal susceptibility during early to mid gestation when developmental programming occurs [11, 85]. By inducing permanent PCOS–like reproductive and metabolic phenotypes, NHP models of gestational T-excess support clinical studies of PCOS women by determining when developmental programming occurs during fetal development, how maternal-fetal-placental alterations affect fetal growth, and perhaps why birth weights of PCOS women differ by ethnicity and maternal nutrition. By recognizing PCOS through metabolic adaptations predisposing to lipotoxicity in today's obesogenic environment, improved maternal endocrine-metabolic health of PCOS women to lower their risk of pregnancy-related complications could potentially diminish their risks of maternal-fetal complications, reduce intergenerational susceptibility to PCOS, and optimize the long-term health of their offspring.

Acknowledgments *Financial support:* D.H.A., NIH R01 DK121559, P50 HD102172, P51 OD011106; D.A.D., funded by the Eunice Kennedy Shriver National Institute of Child Health & Human Development, National Institutes of Health under award P50 HD071836.

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L. Pal, D. B. Seifer (eds.), *Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-030-92589-5