Understanding the clinical significance and pathophysiology of polycystic ovaries and polycystic ovary syndrome has evolved over more than 150 years. The first description of enlarged, polycystic ovaries surrounded by a smooth capsule was reported in 1844. This was followed by similar observations, including a description of hyperthecosis in 1897. In the early 1900s, there was a growing awareness that dysfunctional uterine bleeding was associated with multiple cystic follicles of the ovary, which in part, led to the therapeutic recommendation of bilateral ovarian wedge resection. In 1926, it was demonstrated in rodents that gonadotropic extract derived from the urine of pregnant women was capable of inducing multiple ovarian cyst formation. This finding suggested that abnormal secretion of anterior pituitary hormones may be responsible for the morphologic changes in the ovary. Subsequently, in 1935, the classic description of polycystic ovaries that was reported by Stein and Leventhal codified the association with hyperandrogenism, amenorrhea, and infertility as well as established the syndrome named for the authors. As investigators began to study the pathogenesis of this disorder and the number of relevant publications increased, there followed a gradual and distinct terminologic conversion to what has become known as polycystic ovary syndrome (PCOS).

Currently, PCOS refers to a multisystem reproductive–metabolic disorder that has evolved over decades and stands to be further defined. The principal clinical manifestations are hyperandrogenism and irregular menstruation, the latter of which leads to infertility. The ovaries of women with these symptoms are polycystic and conform to a specific anatomic appearance that may be detected on ultrasound imaging, although the ovarian morphology can exist in women without the overt clinical manifestations. The associated metabolic dysfunction includes insulin resistance, dyslipidemia, and in the United States, an apparent increased prevalence of obesity. The intriguing nature of PCOS rests with the underlying mechanisms responsible for the juxtaposed abnormalities of hypothalamic–pituitary–ovarian–adrenal function with those of altered metabolic physiology. All of these factors may contribute to the clinical phenotype, pose increased long-term health risks, and serve as targets for therapeutic intervention in women with PCOS.

Epidemiology

PREVALENCE

It is estimated that the prevalence of PCOS is 4% to 12% of women in their reproductive years, which designates this disorder as the most common reproductive endocrinopathy of women. Estimates may vary among reports, based on the study population and whether ultrasound imaging of the ovary was included in the diagnostic criteria. In a study of 277 unselected black and white women in the southeastern United States, 4.6% were found to have PCOS. As investigators began to study the pathogenesis of this disorder and the number of relevant publications increased, there followed a gradual and distinct terminologic conversion to what has become known as polycystic ovary syndrome (PCOS).

In Greece, the rate has been estimated at 9%, whereas in Spain, the prevalence was 6.5%. Among Caribbean Hispanic women, the prevalence of PCOS was found to be twice that of African-American women. Recently, it has been suggested that PCOS is more prevalent in women of South Asian descent, based on clinical findings in South Asian immigrants and white women in Britain.
Some women with hirsutism unaccompanied by menstrual abnormalities have been shown to exhibit polycystic ovaries by pelvic ultrasonography. In these hirsute women, observed increases in circulating androgen levels may have facilitated the development of abnormal ovarian morphology. This notion is supported by studies in which female-to-male transsexuals treated with high-dose androgen acquire the typical appearance of polycystic ovaries. In the absence of hirsutism, women with anovulation appear to have a high incidence of PCOS. In 206 nonhirsute women, biochemical assessment showed elevated androgens consistent with the diagnosis in 87% of those with oligomenorrhea and 32% of those with amenorrhea.

Although the sonographic demonstration of polycystic ovaries in the presence of typical clinical features is regarded as confirmatory for the syndrome, it has been clearly shown that polycystic ovaries can be found in normal ovulatory women without a history of hyperandrogenism. In 257 volunteer women who considered themselves to have normal menstruation without hyperandrogenic symptoms, 22% had the characteristic image of polycystic ovaries. However, careful reexamination of those with polycystic ovaries showed that 75% experienced some degree of menstrual disturbance and 45% had objective evidence of hirsutism. Despite the likelihood of PCOS in these unsuspecting normal individuals, many of the women with polycystic ovaries did not show evidence of the disorder. In stark contradistinction, some ovulatory women with PCOS have entirely normal follicle morphology. This notion is supported by studies in different parts of the world.

Coexisting conditions that alter the bioactivity of androgens, such as hypothyroidism and obesity, may also give rise to excessive hair growth. These conditions are associated with lowered SHBG, which provides increased availability of free testosterone. Sequence variations within the coding region of separate SHBG alleles have been reported in a heterozygous woman who presented with severe hyperandrogenism during pregnancy. Her serum SHBG
levels were barely detectable, and non–protein-bound testosterone concentrations were markedly higher than the normal reference range. A single-nucleotide polymorphism within an allele encoded a missense mutation that permitted normal steroid hormone binding, but caused abnormal glycosylation and decreased secretion of SHBG.

MENSTRUAL IRREGULARITY

In PCOS, menstrual dysfunction is primarily characterized by irregular, infrequent, or absent menstrual bleeding. Commonly, bleeding is not preceded by premenstrual symptoms, which is typical for anovulatory bleeding and therefore is unpredictable. Typically, this pattern of bleeding is an extension of postmenarchal irregularity, and monthly menstrual cyclicity is never established. In some women, the onset of chronic anovulation emerges beyond adolescence, but this is unusual. The volume of blood loss associated with menstrual irregularity is generally mild, but in women with significant endometrial proliferation, the bleeding can be substantial and may result in anemia with transient orthostatic hypotension. Prolonged heavy bleeding should raise consideration of abnormal endometrial hyperplasia and even endometrial adenocarcinoma. The thickened endometrium is prone to superficial sloughing or tissue breakdown in response to persistent estrogen secretion or spontaneous decreases in circulating estrogen, respectively. The physiologic link between anovulation and irregular menses is related to persistent estrogen production. Within the polycystic ovary, granulosa cells generate very little estrogen, based on a lack of mature follicle development. Rather, chronic unopposed estrogen secretion most likely results from extraglandular conversion of androgen to estrogen. By comparison, a mechanism to account for arrested follicle growth has not been established.

In approximately 20% of women, there is complete absence of menses, whereas 5% to 10% of patients show regular ovulatory function. Recognition of normal ovulation in PCOS is significant in that a history of regular menstrual cycles does not exclude the diagnosis. In late reproductive life, women with PCOS have been observed to experience regular ovulation for unknown reasons.25 Aging women with PCOS with regular menstrual cycles appear to have a smaller follicle cohort, higher serum FSH levels, and lower FSH-induced inhibin release compared with age-matched women with PCOS who have persistent anovulation.26 Notably, in the older ovulatory women with PCOS, serum androgen levels were also significantly lower than those observed in the anovulatory women. Whether changes in the follicle population or alterations in the ovarian endocrine milieu may be responsible for resumption of ovulation in older women with PCOS remains to be determined.

OVARIAN MORPHOLOGY

In the original description of women with PCOS, the ovaries were enlarged, with numerous peripheral small antral follicles and increased central stroma (Fig. 20-2). From this classic appearance, the definition of polycystic ovaries has been modified to include the presence of at least 12 antral follicles per ovary, with no consideration of distribution or stromal area.27 Although the process that leads to excessive antral follicle development in PCOS is not completely understood, it has been proposed that
normal follicular growth appears to occur up to the midantral stage, after which maturation ceases. However, the finding that follicle development becomes arrested at the midantral stage does not necessarily signal the immediate onset of atresia. In a careful study of granulosa cells obtained by aspiration of antral follicles from unstimulated ovaries of women with PCOS, viability measures indicated robust cell survival with substantial steroidogenic potential compared with cells obtained from ovaries of normal women in the early follicular phase of their menstrual cycles. It was concluded that despite the presence of apoptosis among granulosa cells, the majority of antral follicles in PCOS retain ample functional capacity. This would account, at least in part, for the progressive accumulation of follicular fluid that expands the antrum and gives rise to the classic appearance of cystic follicles. Eventually, the loss of granulosa cells is overwhelming and the follicle ceases to be steroidogenically active. Whether the rate and extent of programmed granulosa cell death exceeds that observed in follicles from normal ovaries has not been examined.

The ovarian follicle population in PCOS also is distinctive in that histomorphometric studies have revealed a two- to threefold increase in the numbers of primary, secondary, and tertiary follicles compared with those of the normal ovary. Whether the ovaries are endowed with a greater number of follicles or whether the rate of programmed cell death is decelerated compared with the normal ovary has not been systematically studied. Relevant to this issue, anti-müllerian hormone (AMH) may contribute, at least in part, to the increased growing follicle population in polycystic ovaries. As a member of the transforming growth factor β superfamily, AMH is an exclusive product of granulosa cells, primarily in growing preantral and small antral follicles. Within the ovary, AMH appears to negatively regulate the advancement of follicle growth. Its expression in growing preantral follicles of polycystic ovaries is decreased compared with normal ovaries. Thus, a lack of AMH may permit accelerated entrance and advancement of growing ovarian follicles, consistent with the altered follicular dynamics of polycystic ovaries. Paradoxically, in women with PCOS, circulating levels are elevated two- to threefold compared with those of normal women, which probably reflects the greater number of growing preantral and small antral follicles in their ovaries compared with those of normal women.

Recently, it has been shown that follicles from polycystic ovaries show a decreased rate of atresia in culture, suggesting a mechanism for maintaining a larger follicle pool throughout reproductive life in women with PCOS. These observations are in agreement with the finding of increased granulosa cell proliferation in primary follicles from women with PCOS compared with those of normal women. Thus, it appears that several factors may be involved in the generation of increased ovarian follicle number in women with PCOS.

A mechanism for the morphogenesis of the polycystic ovary has not been established. However, a role for androgen excess in follicle growth and development has been suggested from ovarian morphology in hyperandrogenic women with congenital adrenal hyperplasia and androgen-producing ovarian tumors. In particular, polycystic ovaries have been demonstrated in male-to-female transsexuals receiving long-term androgen treatment. These ovaries contained many follicle cysts and granulosa cells exhibited intense nuclear staining for androgen receptor, which was considerably greater than that found for normal premenopausal women. The underlying basis for androgen-induced follicle formation in nonhuman primates has been explored in studies that showed increased ovarian size and follicle number after subcutaneous placement of silicone (Silastic, Dow Corning, Midland, MI).
capsules containing testosterone. In situ hybridization studies showed that androgen receptor messenger RNA was expressed primarily in healthy small and medium antral follicles compared with large preovulatory follicles. Moreover, androgen receptor messenger RNA was found to colocalize with that of the FSH receptor and testosterone treatment increased the expression of FSH receptor mRNA.

The polycystic ovary has been characterized by ultrasound examination (Fig. 20-3). In 1986, a description was provided to include ovarian enlargement, 10 or more antral follicles ranging from 2 to 10 mm in diameter arranged in a peripheral distribution, and increased central stroma of greater than 25% of the ovarian area. However, the criteria for polycystic ovaries was modified in 2004 to include greater than 12 follicles per ovary or an ovarian volume greater than 10 mL. This rather specific radiologic description of the polycystic ovary should not be confused with the ultrasound appearance of the multifollicular ovary, which may reflect spontaneous ovarian follicular activity in a woman recovering from hypogonadotropic hypogonadism or ovarian stimulation as a result of ovulation induction. The multifollicular ovary has been described as being of normal size or slightly enlarged, containing six or more follicles without peripheral displacement, and having no increase in central stroma.

**OBESITY**

Early studies of the clinical features of PCOS showed that obesity was present in slightly more than 50% of cases. However, of recent note, the rate of obesity associated with PCOS has not been corroborated and there is a growing impression that the incidence may be greater, at least in the United States, than that previously described. Commonly, an increase in the upper body or central distribution of fat gives rise to an increased waist-to-hip ratio compared with obese women without PCOS. This fat distribution pattern has been termed android obesity and can be found in other hyperandrogenic states, diabetes, and hyperlipidemia. Notably, there is a preponderance of visceral fat compared with peripheral fat, not unlike the distribution of adipose tissue in individuals with insulin resistance. In contrast, women with gynecoid obesity generally have an enhanced accumulation of normal fat in the hips, buttocks, and thighs. As a result, the waist-to-hip ratio in these individuals is usually less than 1.

Whether women with PCOS are predisposed to obesity has not been clarified. Obese women with PCOS tend to have great difficulty in achieving significant and permanent weight loss, despite dietary regimens and exercise. It has been shown that postprandial thermogenesis may be decreased in PCOS, thereby contributing, at least in part, to weight gain. However, resting energy expenditure in PCOS appears to be equivalent to that of normal weight-matched control subjects, which suggests a relative disparity of increased caloric intake and decreased total energy expenditure.

Obesity may cause functional abnormalities that ultimately affect the clinical features observed in PCOS. This is particularly true for obesity-induced insulin resistance and resultant hyperinsulinemia, which is independent of PCOS. Serum insulin is inversely correlated to SHBG concentrations, which increases the clinical consequences of hyperandrogenism in affected women. Correspondingly, the effects of chronic unopposed estrogen secretion are also magnified by increased bioavailable estradiol (E2). These reproductive–metabolic alterations may accompany the broader recognized risks of obesity that pose significant long-term health outcomes for these individuals.

**INSULIN RESISTANCE**

It has been well documented that women with PCOS are insulin resistant and have compensatory hyperinsulinemia as a result of their disorder. The prevalence of insulin resistance in PCOS has been reported to range from 20% to 40%. The common occurrence of insulin resistance in obesity may account, in part, for the rather wide prevalence. Nevertheless, independent of obesity, a defect in insulin action in PCOS has been clearly established. Generally, the degree of insulin resistance is mild, although the prevalence of glucose intolerance and subsequent diabetes has been reported to be as high as 31% and 7.5%, respectively. Notwithstanding the increased risk of diabetes, there is indirect evidence to indicate that insulin resistance may worsen the clinical manifestations of PCOS. Administration of insulin-lowering drugs has been shown to improve insulin sensitivity, reduce androgen levels, and restore ovulation in some, but not all, patients with this disorder. Insulin resistance may also contribute to metabolic dysfunction in PCOS, including an increased likelihood of lipid abnormalities. In addition, the association of insulin resistance with visceral fat distribution is underscored by the displacement of central fat to the peripheral compartment, with improvement of insulin sensitivity after administration of insulin-lowering drugs or weight reduction.

The exaggeration of insulin responses in women with PCOS after a glucose load belies a distinct abnormality of beta cell function in this disorder. Since the 1970s, it has been recognized that insulin secretion in PCOS is reduced compared to that in normal women.
resistance in each individual. Consequently, the product of these measures can be calculated (disposition index) and related to the hyperbolic relationship of these measures established in normal women. Other studies have confirmed these findings in obese and nonobese women with PCOS. Beta cell function in PCOS has also been quantitated by assessing the insulin secretory response to graded doses of insulin infusion and the ability of the beta cell to respond to oscillations in the plasma glucose level. Women with a family history of type 2 diabetes exhibit impaired responses compared with those without a family history, particularly when expressed in relation to the degree of insulin resistance.

The discovery and documentation of insulin resistance in PCOS has been achieved by time-consuming and complex procedures conducted in the course of clinical investigation. By comparison, identification of insulin resistance in the clinical setting has been difficult. Most patients exhibit normal fasting blood glucose levels, and increased circulating insulin levels are not common. As a result, effective and convenient screening tests to determine evidence-based therapeutic modalities have been limited.

ACNE AND ACANTHOSIS NIGRICANS

Women with PCOS may experience increased skin oiliness secondary to excessive stimulation of the pilosebaceous unit by increased androgen production. However, increased sebaceous gland activity in PCOS is not associated with acne, nor is acne correlated with increased ovarian androgen production. Therefore, as an isolated symptom, acne should not be considered a sign of PCOS.

Perhaps more common is the finding of acanthosis nigricans, which has been observed in 5% to 50% of hyperandrogenic women and is related to the presence and severity of hyperinsulinemia. A dermatologic condition features symmetrical, darkened, velvety plaques that most commonly appear on the nape of the neck; in the intertriginous areas of the body, such as skinfolds; and on pressure-bearing surfaces, such as knuckles and elbows (Fig. 20-4). In women who are hyperandrogenic and obese, the vulva is commonly affected. Acanthosis nigricans originates from epidermal hyperkeratosis and dermal fibroblast proliferation. There is no evidence of an increased number of melanocytes or melanin deposition, despite apparent increased pigmentation. Whereas acanthosis nigricans is considered a potential marker for insulin resistance and diabetes in adults, a similar etiology in children remains to be established. In PCOS, reduction of hyperinsulinemia is associated with improvement in the darkened skin areas.

INFERTILITY

A significant number of patients have infertility as a presenting feature of PCOS. Clearly, anovulation would appear to be the primary defect responsible for the failure to achieve pregnancy in this disorder. However, other potential considerations may preclude fertility. There is mounting evidence that women with PCOS have a higher incidence of spontaneous pregnancy loss, the mechanism of which remains unclear. Conversely, it has been reported in a small series that the prevalence of polycystic ovaries in women with recurrent miscarriage was 56%. Subsequently, in a much larger study, it was observed that polycystic ovarian morphology was not predictive of pregnancy loss among women with recurrent pregnancy loss. The potential link between insulin resistance and repetitive pregnancy loss in PCOS has been suggested in studies that showed a significant reduction of first-trimester loss in women treated with metformin. Clearly, additional research is necessary to determine the prevalence as well as the underlying mechanism of recurrent pregnancy loss in PCOS.

Clinical Evolution of PCOS

OVARIAN DEVELOPMENT

Early development of the ovary begins at midgestation (see Chapter 8). At this point, the gonad is replete with primitive germ cells, oogonia, and early development of the ovarian vascular network is evident. These oogonia are gradually encircled by pregranulosa cells to form primordial follicles, composed of an immature oocyte surrounded by a layer of flat granulosa cells enclosed in a basement membrane. Subsequent follicle growth occurs in utero, and the fetal ovary becomes endowed with primary, secondary, and antral follicles. Intratumerine follicular development is a dynamic process, and both healthy and atretic follicles are evident on histologic examination. This pattern of follicular activity is maintained as the ovary undergoes progressive growth during the newborn period and childhood. During this interval, the ovary may be occupied by antral follicles of varying size distributed throughout the ovarian cortex. The pattern of antral follicle formation

Figure 20-4. Acanthosis nigricans. Note the darkened, velvety plaque along the nape of the neck, giving the appearance of hyperpigmentation in a patient with severe insulin resistance, hirsutism, and polycystic ovaries.
appears to coincide with that described for adult women with multifollicular ovaries shown on ultrasound imaging.\textsuperscript{96-98} It has been reported that polycystic ovaries may accompany the clinical manifestations of PCOS in adolescent children.\textsuperscript{99-102} However, little information exists as to whether this ovarian morphology occurred before, after, or at the same time as the reproductive–metabolic abnormalities described in these girls.

In adolescent girls with irregular menstrual cycles, the prevalence of polycystic ovaries is significant. In a study of nonobese adolescent girls with oligomenorrhea, polycystic ovaries were found in 45%.\textsuperscript{102} Similarly, in 73 healthy girls with menstrual irregularities, the ultrasound appearance of the ovary was homogeneous in 36%, multifollicular in 23%, and polycystic in 41% of cases.\textsuperscript{98} Subsequent examination performed 2 to 7 years later showed that the percentage of individuals with polycystic ovaries increased despite no change in the rate of anovulation. In contrast, 40% of oligomenorrheic girls with elevated LH levels and enlarged ovaries were shown to spontaneously normalize ovarian function and size during longitudinal follow-up.\textsuperscript{101} These results point out that polycystic ovaries are a common finding in adolescent girls with abnormal menstrual cycles and suggest that the likelihood of spontaneous restoration to normal ovarian morphology is relatively small as long as menstrual irregularity persists.

**ADOLESCENT PCOS**

Characteristically, the symptoms of PCOS emerge insidiously, coincident with changes that accompany normal pubertal development (see Chapter 17). The events of puberty have been well documented and include acceleration of growth in height, breast budding and enlargement, appearance of sexual hair, and menstrual bleeding.\textsuperscript{103} Once the physical changes have commenced, the temporal pattern of subsequent development is predictable. This process is gradual and may require several years to complete. The normal transition into regular menstrual function is marked by irregular bleeding as a result of anovulation, which may persist for 1 to 3 years.\textsuperscript{104} The finding that the emergence of PCOS commonly can be traced to the events of puberty suggests that this disorder may be related to an abnormal expression of those factors that initiate and regulate the process of puberty. Because the duration of menstrual irregularity that accompanies normal puberty may be variable, it is difficult to rely solely on this historical feature as a basis for diagnosis. Moreover, with the recognition that some women with PCOS may exhibit normal ovulatory function, evidence of regular cyclic bleeding does not preclude the disorder in adolescence. Rather, early detection of PCOS in adolescent girls is predicated primarily on hyperandrogenic symptoms, such as hirsutism and acne. In the obese individual, associated metabolic–reproductive abnormalities may create uncertainty as to the mechanism of hyperandrogenism. Reduction in SHBG is directly correlated to obesity, giving rise to increased free testosterone levels. In addition, obese adolescents, particularly those with evidence of acanthosis nigricans, are highly likely to have insulin resistance with compensatory hyperinsulinemia, which is known to suppress SHBG and probably contributes to excess ovarian androgen production. An ultrasound image of polycystic ovaries virtually confirms the diagnosis. However, the utility of ultrasonography is often limited by the necessity of an abdominal versus vaginal approach and the difficulty in securing adequate imaging in obese girls. As a result, there are no studies that have thoroughly examined the morphologic appearance of ovaries through puberty or in girls with PCOS.

It has been well documented that adolescent girls with PCOS have increased levels of circulating androgens as well as elevated LH levels and increased LH/FSH ratios.\textsuperscript{98-102,103,106} Previous studies have shown that hyperandrogenic girls with likely PCOS exhibited changes in gonadotropin secretion patterns that were similar to those found in adults with PCOS.\textsuperscript{98,101,102,107} Increased concentrations of serum LH are accompanied by an increase in pulse frequency and amplitude, which are significantly greater than those of normal control subjects. In addition, mean serum levels of testosterone and androstenedione were elevated. Twenty-four-hour pulsatile LH secretion studies have shown that premenarchal hyperandrogenic girls showed higher LH levels while awake, whereas the sleep-entrained increases were minimal compared with those of developmentally matched control subjects.\textsuperscript{107} In postmenarche, there was greater LH pulse activity during waking hours, whereas pulse frequency was slowed with sleep (Fig. 20-5). By comparison, in postmenarchal normal girls, LH levels were lower and the pulse frequency reduced while awake, whereas during sleep, LH pulse frequency was significantly less than that of hyperandrogenic girls. Thus, the pattern of gonadotropin secretion in the postmenarchal normal control subject resembled that of the younger premenarchal hyperandrogenic girl. These findings suggest that the transition through neuroendocrine puberty may be accelerated in hyperandrogenic girls compared with girls who undergo normal puberty. Moreover, the data pose the question as to whether there is a chronologic difference in the onset of increased LH levels and pulse frequency between hyperandrogenic and normal pubertal girls.

Among postmenarchal girls with irregular bleeding, it has been estimated that approximately 50% of oligomenorrheic adolescent girls have increased levels of serum LH associated with mild elevations of circulating androgens.\textsuperscript{97,105,106,108,109} In addition, these individuals exhibited an increased rate of LH release as determined from frequent sampling studies,\textsuperscript{97} which suggested a diagnosis of PCOS. Notably, in a long-term follow-up study of oligomenorrheic girls, those with normal serum LH values eventually had regular ovulatory function compared with more than half of those with elevated LH levels. In these girls, gonadotropin abnormalities persisted along with hyperandrogenism.\textsuperscript{101,110} These intriguing findings suggest that oligomenorrhea in early adolescence may be associated with an endocrinologic phenotype of PCOS in the absence of overt signs of hyperandrogenism. Whether the transitory nature of elevated LH and androgens represents an extension of normal hypothalamic–pituitary development remained to be determined.
Similar to their adult counterparts, hyperandrogenic girls, with likely adolescent PCOS, exhibit abnormal insulin responses to glucose loading. Correspondingly, assessment of 24-hour patterns of insulin showed greater release in hyperandrogenic girls than that observed for normal girls, whereas a reciprocal relationship was found for insulin-like growth factor binding protein 1 (IGFBP-1) secretion (Fig. 20-6). Pulsatile growth hormone secretion was not altered in adolescent PCOS. Lipid profiles in girls with increased androgen indices show higher ratios of low-density lipoprotein (LDL) cholesterol to high-density lipoprotein (HDL) cholesterol in conjunction with lowered SHBG levels. These data suggest that any risk of long-term health consequences may be established early in reproductive life for these girls.

**PREPUBERTAL DISPOSITION**

Girls with premature pubarche are at increased risk for functional ovarian hyperandrogenism and polycystic ovaries after puberty. This is particularly true of girls with premature pubarche and oligomenorrhea compared with those with regular cycles. Moreover, with subsequent development of hyperandrogenism and hyperinsulinemia, there is a corresponding reduction in birth weight of these individuals. The link between low birth weight and insulin resistance in children appears to be persistent throughout life, as indicated by studies performed in early and late adulthood. This relationship may be of particular relevance to a possible mechanism for PCOS in this population. Low birth weight is commonly associated with hypoplasia of the fetal adrenal and correspondingly low serum DHEAS levels. DHEAS secretion also serves as a marker for adrenarche that is independent of and precedes gonadarche by several years. In pairs of discordant siblings who achieved similar weight in childhood, DHEAS levels were higher in those of low birth weight compared with those of normal birth weight. Thus, if as proposed, fetal growth modulates adrenarche, then increased DHEAS may have reflected an exaggerated adrenarche in these children. The resultant increased androgen pool may set in motion a cycle of altered physiology that is characteristic of PCOS. This notion is further reinforced by the presence of hyperinsulinemia and insulin resistance, which...
may enhance androgen production in adolescent girls at risk for PCOS. In addition, elevated levels of circulating insulin coincide with a reciprocal decline of SHBG, thereby allowing for increased availability of free testosterone. Thus, the detection of hyperinsulinemia in postmenarchal girls with hyperandrogenism relates temporally to physiologic insulin resistance during puberty and may be of critical importance in the genesis of PCOS.

**FETAL PREDISPOSITION**

It has been shown that adult female Rhesus monkeys exposed to testosterone in utero, at concentrations equivalent to those found in males, may exhibit increased LH secretion, impaired insulin secretion, hyperandrogenic anovulation, and enlarged ovaries with multiple cystic follicles. Similar outcomes have been observed in female adult sheep after in utero exposure to high doses of testosterone at various phases of pregnancy. These observations have led to the hypothesis that the clinical phenotype of PCOS may be the result of intrauterine androgen exposure during pregnancy. In human pregnancy, it has been reported that high maternal serum testosterone levels do not confer this clinical consequence in female offspring. This is likely due to increased circulating levels of SHBG as well as the metabolic capacity of placenta aromatase to neutralize maternal androgen production. Rather, an in utero effect of hyperandrogenism may occur in the presence of abnormal steroidogenesis by the fetal ovary or adrenal gland. In support of this concept, it has been reported that in fetal ovaries, P450c17 was highly expressed at concentrations equiva-

**HYPOTHALAMIC–PITUITARY INTERACTION**

In PCOS, LH secretion is characterized by increased pulse frequency and amplitude, elevated 24-hour mean serum concentrations, and greater responses to gonadotropin-releasing hormone (GnRH) compared with normal women (Fig. 20-7). The mechanisms responsible for this increased release of LH are not well understood. A particular characteristic of LH secretion in PCOS is increased pulse frequency, the periodicity of which is approximately 1 hour. This rapid rate of LH release does appear to be altered by experimental manipulation or physiologic change. These observations imply that corresponding pulsatile release of hypothalamic GnRH is increased. In PCOS, the relationship between GnRH pulse frequency and gonadotrope responsiveness may be a key issue relative to inappropriate gonadotropin secretion. Previous studies in rodents have shown a preference for LHβ gene expression in response to rapid rates of GnRH delivery. Not only does GnRH drive the release of LH, but in normal women it also has been shown to self-prime the pituitary. Therefore, it contributes to increased LH sensitivity to subsequent GnRH stimulation. Collectively, these findings have led to the suggestion that the profound abnormality of gonadotropin secretion in PCOS may be a primary consequence of increased hypothalamic GnRH activity.

In humans, progressive increases in the frequency of GnRH stimulation have resulted in corresponding increases in the rate of LH release as well as elevated basal concentrations. In GnRH-deficient women, an increase in the rate of GnRH administration from every 90 minutes to every 60 minutes was not associated with corresponding increases in serum LH or with changes in pulse amplitude, whereas GnRH given at 30-minute intervals resulted in elevated LH levels and reduction of pulse amplitude. Although consistent with the primary of increased hypothalamic GnRH, an LH pulse frequency of 1 hour
may represent a physiologic limit beyond which more frequent pulses in women do not occur. In normal ovulatory women, during the late follicular phase and at midcycle, as well as in postmenopausal women, it has been documented that LH pulse frequency approximates 60 minutes.\(^{147}\) Thus, in women with PCOS, the tempo and, to some degree, the magnitude of pulsatile gonadotropin secretion are probably established by hypothalamic GnRH activity. However, beyond the requisite need for GnRH, LH responsiveness to GnRH and, accordingly, maximal increases in LH pulse amplitude are probably reliant on other factors.

It has been suggested that the positive feedback effects of chronic estrogen secretion associated with this disorder may bring about an increase in LH, either by a direct effect on gonadotrope sensitivity to GnRH or indirectly by facilitating GnRH pulse frequency.\(^{129,148,149}\) In vitro, estrogen has been shown to increase the fraction of individual gonadotropes responding to GnRH, which is consistent with amplification of LH responses to GnRH in normal women receiving E\(_2\) benzoate.\(^{150}\) In PCOS, baseline levels of estrone and E\(_2\) have been correlated with LH responses to GnRH.\(^{120}\) However, prolonged administration of estrone to patients with PCOS did not raise circulating levels of LH beyond baseline values or increase GnRH-stimulated LH responses.\(^{148}\) Alternatively, in animals, it has been demonstrated that estrogen enhances GnRH pulse frequency, and in women with PCOS, serum GnRH levels are increased.\(^{151,152}\) The idea that estrogen may exert an effect on the hypothalamus in PCOS is supported by the strong positive correlation of mean serum E\(_2\) levels and GnRH pulse frequency.

Hyperandrogenemia has also been implicated as a potential cause of increased LH secretion in PCOS. In vitro, it has been shown that androgen administration resulted in increased GnRH pulse generator activity.\(^{153}\) Examination of LH secretion in hyperandrogenic patients with congenital adrenal hyperplasia showed that mean LH levels and LH responses to GnRH were increased and tended to normalize with the onset of treatment and a corresponding lowering of androgen levels.\(^{40}\) By comparison, other studies have not been able to detect an increase in LH after the administration of androgen. Short-term infusion of androgen to both normal women and women with PCOS did not alter basal LH secretion.\(^{154}\) Moreover, high-dose androgen infusion in normal women appeared to result in an acute reduction of serum LH levels.\(^{32,155}\)

Notwithstanding these findings, recent studies have indicated that excess androgen production may have a profound influence on LH pulse frequency in women with PCOS. Previously, it had been shown that the administration of progesterone, either alone or in combination with estrogen (oral contraceptive), suppressed mean LH and LH pulse frequency in both women with PCOS and normal women.\(^{156}\) The observation that suppression of LH release was more pronounced in normal women compared with women with PCOS suggested to the investigators that increased LH pulse frequency may reflect a fundamental
The potential role of hyperinsulinemia on gonadotropin secretion and, in particular, LH release has not been extensively studied. It has been previously shown that rat pituitary cells preincubated with insulin exhibit increased LH responsiveness after the administration of GnRH in a dose-dependent manner compared with those of untreated cells, which suggests a facilitative role for insulin on GnRH-stimulated LH release.\textsuperscript{158-160} No effect of insulin was observed when these studies were performed in serum-supplemented media. Efforts to determine the effect of insulin in women with PCOS have not shown consistent alterations in LH secretion or release after GnRH stimulation.\textsuperscript{49,161} Reduction of hyperinsulinemia by the administration of insulin-lowering drugs to patients with PCOS resulted in decreased mean serum levels of androgens and LH in some cases, whereas in others, an accompanying fall of LH was not found.\textsuperscript{49,63,66,68-72,161-170}

Recently, we explored the role of insulin in gonadotropin secretion in women with PCOS and normal women. Using the hyperinsulinemic-euglycemic clamp technique, we found that episodic gonadotropin secretion and LH response to multidose GnRH stimulation were not altered by insulin infusion over an interval of 12 hours in both groups. In particular, endogenous serum LH levels were unchanged before and immediately after the initiation of the insulin clamp (Fig. 20-9). These findings confirm and clarify previous studies, which have not shown consistent alterations in LH secretion or release after GnRH stimulation and insulin administration in women with PCOS.\textsuperscript{49,161} In addition, our results may explain why changes in serum LH were not observed in women with PCOS who were treated with insulin-lowering drugs, despite significant reductions in circulating androgen levels.\textsuperscript{49,63,66, 68-72, 162, 164,165-170}

Alternatively, interpretation of these clinical trials is potentially confounded by several factors. First, most of the patients studied were obese, and it has been shown recently that obesity is inversely correlated with LH secretion in PCOS (Fig. 20-10).\textsuperscript{162-164,165-170} Second, hyperinsulinemia is positively correlated with BMI in women with this syndrome. Third, the occurrence of ovulation in PCOS is associated with a lowering of LH levels into the normal range. Although additional studies are necessary, the evidence to date has not been able to clearly demonstrate a functional interaction between insulin and LH in this disorder.

Increased LH pulse frequency and other features of PCOS have been described in women with epileptic disorders or women treated with antiepileptic drugs.\textsuperscript{171-175} These observations have led to the intriguing consideration that epilepsy or treatment with antiepileptic drugs, in particular, sodium valproate and PCOS, are causally related. The link between epileptic and postseizure ictal states may involve stimulation of excitatory neurotransmitters, the receptors of which exist in hypothalamic nuclei that influence GnRH release. Thus, epileptic activity may result in increased GnRH activity and simulate the pattern of increased LH secretion in PCOS.\textsuperscript{176,177} In addition to altered LH secretion, polycystic ovaries and hyperandrogenism have been reported in untreated and treated women with epileptic seizures. These findings strengthen the possible association between PCOS and epilepsy. A mechanistic role for antiepileptic medication, including sodium valproate, in the development of excess androgen production or follicle cyst formation in treated subjects has not been elucidated. Unfortunately, the vast majority of reports

\begin{figure}[h]
\centering
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\caption{The change in luteinizing hormone pulse frequency between flutamide treatment alone and after estradiol (E2) and progesterone (P) administration for 7 days during continued flutamide treatment. Data are shown as a function of the mean plasma P level during E2 and P administration with flutamide for control subjects and patients with polycystic ovary syndrome (PCOS). Shaded area shows the range of responses in an identical protocol performed in the absence of flutamide. The slopes for the linear regression analysis are as follows: control subjects (with flutamide), \(-0.53\); control subjects (without flutamide), \(-0.76\); women with PCOS (with flutamide), \(-0.72\); and women with PCOS (without flutamide), \(-0.57\). (From Eagleson CA, Gingrich MB, Pastor CL, et al. Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab 85:4047-4052, 2000.)}
\end{figure}
linking epilepsy or antiepileptic medication to PCOS have been beset by poor experimental design and insufficient rigor to determine causality.

In contrast to pituitary LH, FSH secretion in PCOS is decreased, as indicated by significantly lower serum concentrations compared with those found in normal women during the early follicular phase of the menstrual cycle. In addition, FSH responses to GnRH stimulation are reduced as shown in some, but not all, studies. The precise underlying basis for decreased FSH secretion in PCOS has not been determined, although the negative feedback effect of chronic unopposed estrogen secretion in these women has been implicated as a mechanism. Support for this concept has been demonstrated by a study in which women with PCOS were treated with E₂ benzoate for 2 weeks. Daily measurement of serum gonadotropin levels showed a progressive decline of circulating FSH, whereas serum LH concentrations remained unaltered, resulting in a decrease in the LH/FSH ratio. The reduction in serum FSH may also reflect the activity of hypothalamic GnRH. As mentioned earlier, increased frequency of pulsatile GnRH predisposes to a preference for LHβ gene expression at the expense of the FSHβ gene.

THECA CELL FUNCTION

The most notable clinical feature of PCOS is hirsutism, which is the result of excessive androgen production. Both the ovary and the adrenal glands contribute to the pool of increased circulating androgens. However, in PCOS, the major serum androgens, androstenedione and testosterone, are produced by the ovary, whereas elevated concentrations of DHEAS are derived from the adrenal glands. Within the polycystic ovary, numerous antral follicles are surrounded by hyperplastic theca cells that are the predominant site of androgen overproduction. There is firm evidence to indicate that excess ovarian androgen production is driven by abnormally increased secretion of pituitary LH acting on
Nonetheless, the rather broad range of LH secretion in PCOS suggests that other mechanisms may be instrumental in the excessive production of androgens, including increased theca cell sensitivity to LH and the presence of cogonadotropic growth factors. The former notion is supported by in vitro studies that have established that theca cells from polycystic ovaries in culture produce significantly more androgen after LH stimulation than that generated by theca cells from normal ovaries. In these studies, the rate of androgen production in PCOS was greater than in normal control subjects, but the magnitude of response was similar in both groups, reflecting the higher basal production of androgen in the PCOS group. Clinically, stimulation of theca cell androgen production by a GnRH agonist has shown significant increases in serum 17-hydroxyprogesterone and androstenedione concentrations in PCOS compared with those of normal women (Fig. 20-11). Differences in testosterone responses between the two groups were not found. The pattern of individual steroid hormone responses indicated overexpression of the cytochrome P450c17 gene, which regulates 17-hydroxylase and C17,20-lyase activity. Moreover, these findings suggested that differential androgen production in PCOS may arise de novo within the theca cell.

It has been shown in animals that subtle increases in LH may induce cytochrome P450c17 gene expression, a mechanism that might relate to higher basal levels of androgens in women with PCOS. To further examine this issue, steroid responses to human chorionic gonadotropin (hCG) were examined in women with PCOS and in normal subjects before and after administration of a GnRH agonist that effectively suppressed and eliminated any disparity in basal LH secretion and ovarian androgen production. Serum 17-hydroxyprogesterone responses to hCG were not abrogated by agonist treatment, which provided further evidence that in PCOS the magnitude of androgen responsiveness to LH is due to a primary abnormality of theca cell steroidogenesis. Despite these findings, LH may still influence selective steroid hormone production indirectly through activation of P450c17 because 17-hydroxylase and C17,20-lyase are differentially regulated. In contrast to the stimulatory effects of low-dose LH on 17-hydroxylase, high doses of LH down-regulate the C17,20-lyase component of P450c17, which would account for the greater incremental change of 17-hydroxyprogesterone compared with androstenedione after GnRH agonist stimulation. Additionally, the relative increase in 17-hydroxyprogesterone over androstenedione may also be attributed, at least in part, to the observation that 17-hydroxyprogesterone is not a substrate for C17,20-lyase in the human ovary. Collectively, these data indicate that in PCOS, to a degree, abnormal androgen production by the theca cell is an inherent defect of steroidogenesis, although the...
complexity of the process is evident and remains to be elucidated.

Exclusive of the direct stimulus–response dynamic of ovarian androgen production, the human theca cell is also subject to the effects of co-gonadotropin growth factors, the most notable of which are insulin and insulin-like growth factor (IGF). Receptors for insulin, IGF-I, and IGF-II have been localized to the theca compartment of ovaries from both normal women and patients with PCOS. Accordingly, in vitro studies of normal human theca tissue have shown that these growth factors are capable of enhancing androgen responses to LH as well as independently stimulating androgen production. In vitro, IGF-I has been shown to synergistically augment LH-stimulated androgen production from cultured theca cells. Facilitative action of IGF-I has been attributed to increased LH receptor induction, greater expression of steroidogenic enzymes, and enhanced LH-induced cyclic AMP production. However, because IGF-I mRNA and protein are not expressed in human granulosa cells, it is likely that IGF-II is the principal paracrine influence of these two proteins. In human theca cell tissue, IGF-II stimulated basal androgen release as well as enhanced LH-induced androgen production, the magnitude of which was equivalent to that of IGF-I. In the same study, insulin, either alone or in combination with LH, also amplified theca cell androgen production. These findings were consistent with previously published results that showed that the enhanced effect of insulin was dose-dependent.

In contrast, studies performed on theca cells from hyperandrogenic women, including those with PCOS, have not shown a synergistic effect of LH and insulin. Similarly, IGF-I did not augment LH-induced androgen formation by theca tissue obtained from women with PCOS. In vivo studies performed in Rhesus monkeys showed that IGF-I, in either the absence or presence of human growth hormone, had no effect on gonadotropin-stimulated serum androstenedione levels. The inability to show an effect of insulin on theca cell androgen production may reflect the presence of insulin resistance in the ovary similar to diminished insulin sensitivity in muscle, fat, and liver. However, recent studies have shown that insulin may increase androgen production from theca cells of women with PCOS though an alternative pathway involving insulinotocytokine, a downstream mediator of insulin signaling. When theca cells were incubated with insulin in the presence of antibody to insulinotocytokine, androgen production was inhibited, whereas hCG-stimulated androgen production was unaltered. Thus, it appears that there are at least two separate signaling pathways for insulin and LH.

Clinically, the effect of hyperinsulinemia on ovarian steroidogenesis has been examined using an oral glucose tolerance test or the euglycemic hyperinsulinemic clamp technique. Most studies have not shown significant changes in androgen secretion in women with PCOS or normal women, despite considerable increases in circulating insulin levels. In one study, minimal increases in androstenedione were noted during insulin infusion. Whether prevailing hyperinsulinemia precluded any additional effect of superimposed insulin administration on ovarian androgen production in these patients is not clear. However, these in vivo studies may reflect insulin resistance by theca cell tissues, despite in vitro findings to the contrary. In addition, clinical evidence suggests that intact granulosa cells in women with PCOS show insulin resistance. Nevertheless, a reduction in hyperinsulinemia has been associated with significant decreases in serum androgens without corresponding changes in LH in women with PCOS who are treated with insulin-lowering drugs. This observation indirectly suggests a role for insulin in LH-stimulated androgen synthesis.

**GRANULOSA CELL FUNCTION**

In PCOS, the mechanisms responsible for the persistence of ovulatory failure are not well understood. According to histomorphometric studies, ovarian follicle development is arrested at the midantral stage of growth, and the granulosa cells that line these follicles appear to be in various stages of degeneration. Earlier studies implicated a deficiency of aromatase activity to explain the lack of follicle development because E2 concentrations were low in follicular fluid and granulosa cells contained little measurable aromatase enzyme. However, subsequent in vitro studies have demonstrated that these cells are, in fact, vital and exhibit substantial capacity for steroidogenesis. We have previously shown that cultured granulosa cells obtained from women with PCOS exhibit a significantly greater rise in E2 after FSH stimulation compared with normal granulosa cells, which indicated that the inherent capacity of these cells to respond to FSH was ample. However, the time course of response was characterized by an inability to sustain peak levels, in contrast to the pattern of normal cells, which implied suboptimal granulosa cell function (Fig. 20-12). Our recent clinical studies have corroborated these in vitro findings. In women with PCOS, the capacity for E2 production in response to the stimulatory effects of recombinant human FSH (r-hFSH) was clearly enhanced compared with that of normal women. Notably, this difference was dose-dependent and only manifested at 150 IU, because increases in circulating E2 after 37.5 IU and 75 IU r-hFSH were similar in both women with PCOS and normal women (Fig. 20-13). The amplification of E2 responses beyond an apparent FSH threshold dose range indicated greater in vivo granulosa cell responsiveness in PCOS in the presence of an abundance of aromatase substrate because profiles of circulating FSH levels at each dose were essentially identical in both groups. This heightened E2 response may have reflected greater follicle number in the polycystic ovary compared with the normal ovary. This explanation is consistent with the recent report in which comparative E2 responses to FSH in women with PCOS and those in normal women during ovulation induction were more likely related to the number of stimulated ovarian follicles rather than to differences in the FSH threshold.

Alternatively, PCOS granulosa cells are extremely sensitive to FSH stimulation. We previously showed that in granulosa cells obtained from 4- to 7-mm follicles of polycystic ovaries, FSH induced an approximately fourfold increase in E2 levels compared with baseline. Comparison
of the effective dose 50 for FSH-stimulated E₂ production showed an eightfold higher granulosa cell sensitivity to FSH in PCOS compared with normal cells. Clinically, increased granulosa cell responsiveness to 150 IU FSH in women with PCOS was nearly twofold greater than that observed in normal women. However, lower amounts of r-hFSH stimulation produced E₂ responses that were similar in women with PCOS and normal women, which raised the possibility of a putative aromatase inhibitor within the polycystic ovary microenvironment. In hyperandrogenic women with PCOS, the abundance of aromatase substrate would have been expected to result in greater E₂ production in response to all doses of r-hFSH compared with normal women. In this regard, it is noteworthy that FSH in low pharmacologic amounts uniformly induced aromatase gene expression within 3 hours of administration, which suggests that aromatase inhibition, if present in PCOS, is relatively mild.

Whether these results reflect increased numbers of stimulated follicles, increased granulosa cell sensitivity to FSH, or both, it is evident that women with PCOS are susceptible to ovarian hyperstimulation in response to gonadotropin stimulation. It is unknown whether increased granulosa cell sensitivity arises primarily or secondarily as a result of intra- or extracellular factors. Recent studies have shown that, in granulosa cells obtained by aspiration of follicles from unstimulated anovulatory polycystic ovaries, binding of radiolabeled FSH was significantly higher compared with that detected in cells from ovaries of ovulatory women with PCOS or from normal women (Fig. 20-14). Moreover, accompanying studies showed increased E₂ release from the granulosa cells of polycystic ovaries in response to FSH compared with cells from ovulatory ovaries, which is consistent with results from our lab and others. Together, these findings link granulosa cell sensitivity to increased FSH receptor binding in granulosa cells from polycystic ovaries and provide insight into a possible mechanism for increased E₂ responsiveness to FSH stimulation. Moreover, the observation that high-dose androgen administration to nonhuman female primates increases FSH receptor expression lends credence to the possibility that androgen may facilitate granulosa cell hyperresponsiveness to FSH in women with PCOS. Among the factors that have been shown to affect granulosa cell function, insulin, and IGFs appear to act as co-gonadotropins within the ovary. When PCOS granulosa cells were co-incubated with insulin, FSH-stimulated E₂ release was mildly increased, whereas by comparison,
addition of IGF-I amplified the response beyond that encountered with either FSH or IGF-I alone. These findings suggested that in PCOS the role of insulin in granulosa cell function was minimal or, alternatively, the granulosa cell was resistant to the action of insulin. In contrast, other studies have shown that PCOS granulosa cell function was minimal or, alternatively, the granulosa cell was resistant to the action of insulin. Moreover, this facilitory effect of insulin on PCOS granulosa cells was extremely sensitive to insulin across a wide physiologic dose range, regardless of gonadotropin stimulation. Moreover, this facilitory effect of insulin on PCOS granulosa cells was extremely sensitive to insulin across a wide physiologic dose range, regardless of gonadotropin stimulation. In vivo, this facilitory effect of insulin on PCOS granulosa cells was extremely sensitive to insulin across a wide physiologic dose range, regardless of gonadotropin stimulation.

Figure 20-14. Binding of human recombinant 125I-follicle-stimulating hormone (FSH) to granulosa cells from different groups of patients. FSH binding per cell number was significantly (P < 0.05) increased in anovulatory polycystic ovary syndrome (Anov. PCO). The values represent mean ± standard deviation obtained from different cellular preparations, each assayed at least twice in triplicate. Ov. PCO, ovulatory polycystic ovary syndrome. (From Almahbobi C, Anderiesz C, Hutchinson P, et al. Functional integrity of granulosa cells from polycystic ovaries. Clin Endocrinol 44:571-580, 1996.)

Figure 20-15. Time course of mean (± standard error) 24-hour serum estradiol responses after injection of intravenous recombinant follicle-stimulating hormone (r-hFSH), 75 IU, to women with polycystic ovary syndrome treated with pioglitazone without insulin infusion and 2 hours after injection of low-dose (30 mL/m² min) and high-dose (200 mL/m² min)-hyperinsulinemic-euglycemic clamps administered for 10 hours. The integrated estradiol response, as determined by area under the curve, was significantly greater (P < 0.02) in subjects receiving high-dose insulin infusion compared with those observed in women without insulin or with low-dose insulin infusion (Coffler MS, Patel KS, Dahan MH, et al. Enhanced granulosa cell responsiveness to follicle stimulating hormone during insulin infusion in women with polycystic ovary syndrome treated with pioglitazone. J Clin Endocrinol Metab 88:5624-5631, 2003.)
FSH over time is preferred to an escalation of the daily dose because of follicular hyperresponsiveness to FSH in women with PCOS and an increased risk of ovarian hyperstimulation syndrome. These observations, combined with our previous report describing greater E₂ responsiveness in women with PCOS compared with normal women at an FSH dose of 150 IU, suggest a bidirectional narrowing of the FSH threshold range in this disorder (Fig. 20-16).

The effect of insulin on FSH-stimulated follicle development may also involve the IGF system. Both IGF-I and IGF-II have been shown to enhance the response of granulosa cells to FSH. In PCOS granulosa cells, exposure to both IGF-I and FSH resulted in significantly greater E₂ production compared with incubation with each hormone separately. The actions of IGFs are likely mediated by the receptors, which have been identified on granulosa cells of both PCOS and normal follicles. However, despite in vitro studies in which IGF-1 clearly amplified FSH-induced aromatase activity, most studies have not detected the protein or have shown very little in human granulosa cells. By comparison, IGF-II mRNA has been located in all compartments of the human ovary and is strongly expressed in granulosa cells. A relationship between insulin and IGF-II has previously been shown by in vitro studies of rat adipocytes in which insulin increased the expression of cell surface IGF-II receptors, probably by mobilizing internalized receptors. Consistent with this observation are recent studies of human polycystic ovary tissue that showed that estrogen responses to IGF-II were significantly enhanced in granulosa cells preincubated with insulin compared with those without insulin. Insulin also increased the response of granulosa cells to IGF-1, although the incremental change was smaller. These findings point out the potentially critical relationship between insulin and IGF-II in the regulation of granulosa cell function. In particular, in PCOS, excess insulin secretion may down-regulate insulin receptors, which would prevent translocation of subcellular IGF-II receptors to the plasma membrane and deprive granulosa cells of the stimulatory effect of a potent co-gonadotropin.

In PCOS, it has been reported that circulating IGF-I levels are elevated, although this is not a consistent finding. IGFs are complexed with IGFBPs, which regulate their bioactivity. In PCOS, serum IGFBP-1 is decreased as a result of hyperinsulinemia, which implies an increase in the levels of free IGF-I. This concept is supported by the finding of increased circulating levels of free IGF-I in women with PCOS. IGFBPs may be critical in regulating IGF bioactivity at the level of the ovary. Relevant to this notion, IGFBP-2 and -4 proteases have been shown to be increased in follicular fluid derived from androgenic follicles, including those of patients with PCOS. In addition, IGFBP-4 protease was not detectable in these follicles. These results are in stark distinction to those found in healthy antral follicles of normal women, in which IGFBP-2 and -4 proteases were decreased and IGF-II concentrations were increased in the follicular fluid. The effect of the androgenic environment on follicular fluid IGF bioavailability has been documented in transsexuals treated with high-dose androgens. In the follicular fluid of these individuals, IGF-II levels were decreased, IGFBP levels were increased, and IGFBP-4 protease was nondetectable, thus accounting for decreased bioavailable IGF-II. Whether this IGF-IGFBP profile is a consequence of follicular fluid androgen levels is not clear, although the association is compelling and suggests that hyperandrogenism of PCOS, driven in part by insulin-mediated theca cell androgen production, may be instrumental.

The recognition of abnormal follicle growth and development in women with PCOS has been derived primarily from studies involving follicle responses to gonadotropin stimulation and granulosa cell function. However, the potential role of the oocyte in this disorder has not been
investigated. Recent studies have shown that oocyte-derived growth factors may be important to follicle development and function. In particular, growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15)/GDF-9B appear to have major roles in folliculogenesis and female fertility. In vitro studies have shown that these genes are selectively expressed in developing oocytes during folliculogenesis.239-242 In addition, in GDF-9-deficient female mice, disruption of reproductive function has been associated with arrested follicle growth at the primary stage, decreased granulosa cell proliferation, inappropriate theca cell development, ovarian cyst formation, and infertility.243-245 We have examined mRNA expression of GDF-9 and BMP-15 in ovaries obtained from women with PCOS and found that the GDF-9 signal was decreased in oocytes throughout folliculogenesis compared with GDF-9 message in oocytes from normal ovaries.246 By comparison, there was no difference in BMP-15 mRNA expression between groups. These results suggest that the expression of GDF-9 is delayed in oocytes of developing PCOS follicles. Because of the apparent growing importance of GDF-9 in folliculogenesis and fertility, it is suggested that dysregulation of GDF-9 expression may contribute to aberrant folliculogenesis in women with PCOS.

ADRENAL FUNCTION

Approximately 50% of women with PCOS exhibit increased levels of DHEAS and 11β-hydroxyandrostenedione, indicating excess androgen production by the zona reticularis of the adrenal gland.247-249 By comparison, basal circulating adrenocorticotropic (ACTH) levels in women with PCOS are similar to those of normal women.250 In addition, circadian rhythms of serum dehydroepiandrosterone (DHEA) and cortisol in women with PCOS were not different from those exhibited by normal women.251 These findings suggest that the mechanism for adrenal hyperandrogenemia may arise from either altered adrenal responsiveness to ACTH or abnormal adrenal stimulation by factors other than ACTH. Studies to address this issue have not produced consistent results. Increased 17-hydroxyprogesterone responses to ACTH, after dexamethasone, have been observed in women with PCOS and functional ovarian hyperandrogenism, which suggested dysregulation of P450c17.251,252 However, other studies have not been able to confirm these results.253-255 These latter studies did not employ dexamethasone suppression before ACTH stimulation, which may have accounted for the disparate results. In vitro, IGF-1 and insulin have been found to enhance ACTH-stimulated P450c17 expression and adrenal androgen synthesis.250,256 In women with PCOS, serum 17-hydroxyprogesterone and androstenedione responses to ACTH were significantly greater in those with hyperinsulinemia compared with those with normal insulin levels.258 In the same study, circulating cortisol and DHEA responses were equivalent between groups. Consequently, it was shown that in women with PCOS, ACTH administration during insulin infusion was associated with significantly higher 17-hydroxyprogrenolone and 17-hydroxyprogesterone responses than those measured during saline infusion.259 This facilitory effect of insulin appeared to lower the activity of C17,20-lyase, as shown by higher 17-hydroxyprogrenolone/DHEA and 17-hydroxyprogesterone/androstenedione ratios. Similar results have been described in men undergoing hyperinsulinemic clamp studies.260 These findings are consistent with reports that show a reduction in serum DHEAS in women during administration of insulin infusion or a glucose tolerance test.161,203,261-263 Whether adrenal hyperandrogenemia contributes to the perpetuation of PCOS is not clear. However, the suggestion that an exaggerated adrenal response may be an inciting event in the pathogenesis of PCOS warrants further investigation of adrenal gland function in adolescent girls with hyperandrogenism.

INSULIN RESISTANCE

Women with PCOS exhibit insulin resistance, irrespective of obesity (Fig. 20-17).55,56,58,264 The insulin resistance is characterized by decreased insulin-mediated glucose disposal and, in obese women with PCOS, increased hepatic glucose production.49,265 In addition, insulin secretion by pancreatic β cells is increased as a compensatory mechanism. Increased insulin secretion has also been associated with decreased hepatic clearance in insulin-resistant conditions.266 Although decreased clearance has not been firmly established in women with PCOS, assessment of hepatic extraction using ratios of insulin to C-peptide or model analysis is highly suggestive of impaired hepatic clearance.267,268 Elevated fasting insulin levels may not be detected in women with PCOS, but commonly, insulin responses to a glucose load are significantly greater than those observed in normal women.56 In contrast, first-phase insulin release in response to intravenous glucose is comparable between women with PCOS and weight-matched control subjects.82 Nevertheless, evidence for β-cell dysfunction has been described in a series of studies that showed abnormal entrainment to oscillatory glucose infusions and diminished insulin secretory responses after ingestion of food.81,268 Thus, despite the presence of hyperinsulinemia in women with PCOS, there is a relative defect in β-cell production and release of insulin.

In PCOS, insulin receptor binding and affinity have been shown to be normal. However, studies of adipocytes from women with PCOS have shown decreases in glucose transport and lipolysis, indicating an impairment of insulin signaling (Fig. 20-18).269-272 Evidence of decreased tyrosine phosphorylation and increased insulin-independent serine phosphorylation have been shown in cultured skin fi broblasts removed from women with PCOS.271 Serine phosphorylation inhibits tyrosine kinase activity of the insulin receptor and may be responsible for the observed insulin resistance in PCOS.273-275 Notably, increased serine phosphorylation was observed in only 50% of women with PCOS who were studied. The remainder exhibited insulin-stimulated insulin receptor autophosphorylation, which was similar to that found in normal control women.271 Thus, these findings may be applicable to a subset of women with PCOS, whereas in others, the mechanism remains unknown. Importantly, although a defect in insulin receptor phosphorylation may exist for
some women with PCOS, the potential for abnormalities beyond the receptor is suggested by apparent alterations in other downstream signaling events. Moreover, different pathways may be affected to greater or lesser degrees in various insulin-sensitive tissues, which reflects the complexity of the mechanism of insulin resistance in this disorder.

GENETICS OF PCOS

The familial predisposition to PCOS implies a disorder of inheritance, although the mode of transmission has not been established, despite a number of studies. Much of the difficulty resides with methodologic limitations due to small numbers of families and the inability to establish phenotypes among family members. The latter reflects the variable criteria used to designate individuals with PCOS. Studies of twin sisters have not shown a substantial genetic component for polycystic ovaries, although concordance was found in affected twins relative to biochemical markers, including fasting insulin levels and serum androgen concentrations. Similarly, efforts to detect chromosomal abnormalities among patients with PCOS have not shown alterations in number or structure.

In pursuit of the genetics of PCOS, most studies have focused on identifying candidate genes that are linked to recognized abnormalities of steroid hormone production and action, carbohydrate and fuel metabolism, and gonadotropin secretion. Of the genes involved in steroidogenesis, \textit{CYP17}, \textit{CYP11A}, and \textit{CYP21} have been examined to determine whether an association with PCOS exists. There is evidence to suggest that allelic variants of \textit{CYP11A} may have a potential role in excess androgen production and hirsutism in PCOS, although considerable variation in the expression of these genes has been reported.

\textbf{Figure 20-17.} Mean (± standard error) insulin sensitivity ($S_i$) as determined by the modified rapid intravenous glucose tolerance test and 24-hour mean insulin levels in lean and obese women with polycystic ovary syndrome (LPCO, OPCO) and their respective control groups (LC, OC; $n = 8$ for each group). $a$, $P < 0.05$; $b$, $P < 0.01$; $c$, $P < 0.007$ versus corresponding control group; $d$, $P < 0.001$ versus corresponding lean group. Eight- and 24-hour insulin levels (both log-transformed) were inversely correlated for the groups considered together ($r = -0.75$, $P = 0.00001$). (From Morales AJ, Laughlin CA, Butzow T, et al. Insulin, somatotropic, and LH axes in lean and obese women with polycystic ovary syndrome: common and distinct features. J Clin Endocrinol Metab 81:2854-2864, 1966.)

\textbf{Figure 20-18.} Dose–response curve for insulin stimulation of glucose transport in isolated adipocytes from control subjects (green line) and women with polycystic ovary syndrome (purple line). Cells were incubated with insulin for 60 minutes before measurement of initial rates of 3-O-methylglucose transport. Results (mean ± standard error) are normalized against maximal activity for each subject. Inset, Absolute rates (mean ± standard error) of glucose transport in normal control subjects (green bars) and women with PCOS (purple bars). (From Ciaraldi TP, El-Roeiy A, Madar Z, et al. Cellular mechanisms of insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab 75:577-583, 1992.)
investigation remains. In contrast, examination of CYP17 and CYP21 variants and mutations has not shown associations with a phenotype that would support a role in PCOS.\textsuperscript{288-291} The androgen receptor gene persists as a possible candidate for PCOS in light of the inverse relationship between the number of trinucleotide (CAG) repeats and androgen action.\textsuperscript{292} In addition, the report of a nonsense mutation and a frameshift mutation in the SHBG allele, resulting in hyperandrogenism in a woman during pregnancy as well as four women with PCOS, warrants consideration of a genetic role for this protein.\textsuperscript{24} Recently, it has been reported that, with microarray analysis, a cohort of genes with increased abundance of mRNA in PCOS theca cells was identified that included aldehyde dehydrogenase 6, retinol dehydrogenase 2, and the transcription factor GATA6.\textsuperscript{293} Retinoic acid and GATA6 increased the expression of 17\(\alpha\)-hydroxylase, providing a functional link between altered gene expression and intrinsic abnormalities in PCOS theca cells.

With respect to gonadotropin secretion, few studies have been performed to identify candidate genes for PCOS. An Acc1 polymorphism of the FSH\(\beta\) subunit has been described in women with PCOS,\textsuperscript{294} whereas examination of the FSH receptor did not show sequence variants associated with PCOS.\textsuperscript{295} Because dopamine inhibits GnRH secretion, alterations in dopamine receptor genes might account for increased LH secretion in PCOS. In Hispanic women, homozygosity for allele 2 of the D3 receptor has been associated with PCOS.\textsuperscript{296} In a subsequent study, there was no difference in the distribution of three D3 receptor polymorphisms among non-Hispanic white women with PCOS,\textsuperscript{297} which diminishes the likelihood of a D3 receptor candidate gene. Early screening studies suggested that the follistatin gene was overexpressed in PCOS.\textsuperscript{286} As an activin-binding protein, increased follistatin activity might decrease FSH secretion and explain, at least in part, follicular arrest. In addition, activin inhibits ovarian androgen production and increased binding could lead to excessive androgen synthesis. However, in a large follow-up study, the follistatin gene was not observed to associate with the PCOS phenotype.\textsuperscript{286}

Given the predisposition of women with PCOS to insulin resistance, genes that are related to carbohydrate metabolism have been the subject of considerable investigation. Of particular note is the insulin receptor gene, a mutation of which leads to severe insulin resistance, marked hyperandrogenism, and acanthosis nigricans (type A syndrome of insulin resistance). Despite the failure of the insulin receptor gene sequence to discriminate between women with PCOS with and without insulin resistance, two studies identified the region near the insulin receptor gene locus as being associated with PCOS.\textsuperscript{286,299} As a result, this region assumes importance in that it may contain a candidate gene for PCOS. As for the insulin gene, there are conflicting reports linking this marker to PCOS.\textsuperscript{286,300,301} Variants of the insulin receptor substrate protein genes have been shown to exhibit gene dosage effects related to fasting and postprandial plasma glucose levels in insulin-resistant women.\textsuperscript{302} Whether these findings are manifest in women with PCOS requires further study. It has been shown that variation in the gene encoding the cysteine protease, calpain-10, influences susceptibility to type 2 diabetes.\textsuperscript{303,304} However, two subsequent studies were unable to establish associations with multiple DNA polymorphisms in the calpain-10 gene.\textsuperscript{305,306} The 112/121 haplotype combination in African Americans and whites was associated with a twofold increase in the risk of PCOS that warrants additional investigation. Other candidate genes that have been considered for PCOS include leptin, resistin, and TNF-\(\alpha\), although evidence for an association with the latter two have not been found.\textsuperscript{307,309} Recently, a PCOS susceptibility locus was mapped to chromosome 19p13.2 near the dinucleotide repeat marker D19S884.\textsuperscript{310} Resequencing and family-based association studies were done on a large number of family members to determine the effect on reproductive and metabolic function.\textsuperscript{311} The D19S884 allele 8 of the fibrillin-3 gene had the strongest evidence of association with PCOS. This allele was also linked to higher levels of fasting insulin and homeostatic modeling assessment for insulin resistance in women with PCOS. These results suggested that the D19S884 allele 8 is the PCOS susceptibility locus.

### Pathophysiologic Concept

A clear explanation of the pathogenesis for PCOS remains elusive. Nevertheless, there are some intriguing concepts that warrant consideration. There is a growing body of evidence to suggest that excessive androgen production may be essential in the evolution of this disorder (Fig. 20-19). Notably, administration of androgen to nonhuman primates alters ovarian morphology by increasing the size of the ovary, the thickness of the capsule, and the number of preantral and antral follicles.\textsuperscript{44} These data are consistent with the polycystic ovaries of hyperandrogenic women with 21-hydroxylase deficiency and female-to-male transsexuals administered long-term, high-dose androgen therapy.\textsuperscript{211,223-226,231,232} The mechanism that dictates these morphologic changes has not been elucidated, although in Rhesus monkeys, androgen receptor mRNA is colocalized with that of the FSH receptor in granulosa cells and androgen treatment increases FSH expression.\textsuperscript{46} The presence of increased FSH binding in granulosa cells of antral follicles from women with PCOS compared with normal follicles suggests that local exposure of excess androgen may drive this process and account for increased follicle number and size in this disorder.\textsuperscript{29}

An androgen-induced increase of FSH receptors on granulosa cells of women with PCOS may also explain the robust follicular response to FSH observed in vitro and relate to heightened granulosa cell responsiveness to gonadotropin stimulation in women with this disorder.\textsuperscript{28,229,230} It has been long recognized that androgen treatment in rodents and nonhuman primates enhances estrogen response to FSH.\textsuperscript{37,184-190,312} In women with PCOS or those administered androgen, there is intense nuclear staining for androgen receptor in granulosa cells.\textsuperscript{313} In rat granulosa cell culture, studies have shown that testosterone acts at a site upstream from cyclic AMP, which suggests involvement of the FSH receptor, whereas other studies have been unable to determine whether changes in cyclic AMP are
induced by androgen treatment. Despite these inconsistent findings, it is likely that the effect of androgen on the FSH receptor and subsequent granulosa cell function is mediated through its receptor. However, the precise mechanism for this interaction remains to be defined. Amplification of granulosa cell responsiveness to FSH is not unique to androgens because estrogens have long been known to enhance FSH-stimulated E₂ production. In rodent granulosa cells, synergy between E₂ and FSH has been demonstrated with regard to increased FSH receptor binding, induction of LH receptors, increased aromatase activity, and progesterin synthesis. The mechanism for this synergy has not been completely elucidated, although estrogen-induced granulosa cell proliferation or increased FSH-binding capacity per granulosa cell has been suggested. Alternatively, both processes may amplify FSH receptor number in granulosa cells of developing follicles. In nonhuman primate and human granulosa cells, studies have convincingly shown the presence of both estrogen receptor α and estrogen receptor β in the human ovary. In particular, ERβ appears to be predominant in granulosa cells of antral follicles from both normal and polycystic ovaries. Collectively, these findings strongly suggest that in both normal and PCOS granulosa cells, estrogen may enhance FSH-stimulated E₂ release through a mechanism that involves the FSH receptor.

Increased androgen production may have a significant effect on inappropriate gonadotropin secretion in PCOS. The critical feature of LH secretion in PCOS is increased LH pulse frequency, which remains intact in the face of physiologic and most pharmacologic manipulations. Increased pulsatile LH release implies, but by no means guarantees, increased hypothalamic GnRH activity. Until recently, it was difficult to exclude altered GnRH release as an instigating factor in PCOS. The rate of rapid LH release in women with PCOS may be normalized with administration of physiologic doses of estrogen and progesterone after treatment with an anti-androgen. This finding suggests an inhibitory effect of increased androgen bioactivity on steroid negative feedback. Importantly, these results suggest that hyperandrogenism may be responsible for, or at least, contribute to increased LH pulse frequency in this disorder. The prevention of steroid negative feedback may be a unique effect of androgen because E₂ and insulin, the secretion of which are both abnormal, have not been shown to alter LH pulse frequency. Further studies are necessary to elucidate the primary and secondary mechanisms of increased GnRH pulse generator activity in PCOS.

The apparent central role of androgen excess in PCOS underscores the importance of the process by which hyperandrogenism is achieved. It is undeniable the theca cell is the primary source of hyperandrogenism in PCOS. Furthermore, evidence shows increased theca cell responsiveness to LH stimulation compared with normal theca tissue, which suggests a primary defect in this cell. The precise nature of the abnormality is unclear, but it appears to involve facilitation of 17-hydroxylase, allowing for enhanced conversion of progesterone to 17-hydroxyprogesterone. Additionally, 17-hydroxylase is encoded and regulated by the gene CYP17, which also encodes for 17-20 lyase, the enzyme that advances conversion of 17-hydroxyprogesterone to androstenedione. In immortalized human theca cells, including those from polycystic ovaries, the amount of androstenedione converted from 17-hydroxyprogesterone is small, indicating that Δ7-17 lyase activity is correspondingly low. The production of androgen is driven by pituitary LH, which has a bimodal dose effect on the enzymes regulated by CYP17. At low doses, LH primarily stimulates 17-hydroxylase, whereas at high doses, LH down-regulates the 17-20 lyase component of CYP17, which would account for the greater incremental change of 17-hydroxyprogesterone compared with androstenedione after GnRH agonist stimulation. Thus, increased secretion of LH may be pivotal in amplifying the production of excess androgen.

Notwithstanding the physiologic effect of increased theca cell production of androgens, the bioavailability of testosterone is largely influenced by the production of SHBG, the reduction of which may lead to significant hyperandrogenism. Decreases in circulating SHBG concentrations have been observed in women with hyperinsulinemia, excessive weight gain, or hyperandrogenism, with the clinical outcome resulting in various degrees of hirsutism. In PCOS, all of these conditions may coexist...
simultaneously, thereby amplifying the clinical manifestations of excess androgen production. To illustrate the effects of SHBG, marked elevation of serum free testosterone levels and severe hirsutism recently have been reported during pregnancy in a woman who essentially lacked the capacity to produce bioactive SHBG. The deficiency in SHBG production was caused by a single-nucleotide polymorphism within an allele that encoded a missense mutation.  

In approximately 50% of cases of PCOS, adrenal androgen production is increased. 337 Despite the consistency of this finding and the contribution to the androgen pool, the precise role of adrenal hyperandrogenism has not been well established in this disorder. In women with 21-hydroxylase deficiency, the clinical manifestations may be indistinguishable from those of women with PCOS, which clearly shows the severity of hyperandrogenism in individuals with this enzyme defect. Although a milder form of androgen overproduction by the adrenal may accompany PCOS, if the emergence of adrenal hyperactivity at or just before puberty may be pivotal in the onset of this syndrome. The increase in steroidogenesis with puberty is characterized by an increase in the adrenal androgens DHEA and DHEAS. In girls who experience pubic hair growth before age 8 years, “premature pubarche,” the risk of PCOS is increased. 320 Similarly, it has been hypothesized that exaggerated adrenarche at puberty, marked by increased production of DHEA and DHEAS, leads to abnormal androgen exposure and eventual PCOS. 327 These considerations raise the possibility that the genesis of PCOS may be critically linked to the onset of adrenal androgen production at or shortly before puberty.

The role of insulin resistance and hyperinsulinemia as a primary cause of PCOS has not been established. However, there is clear evidence to suggest that hyperinsulinemia may perpetuate the altered reproductive and metabolic physiology in this syndrome. In vitro studies have shown that insulin enhances LH-induced androgen production from normal theca cells. 338,339,199,328 Moreover, improvement in insulin sensitivity through the administration of insulin-lowering drugs has been accompanied by reduction of serum androgen levels without an effect on circulating LH concentrations. 62,63,65,66,68-72,162,214,329 In the granulosa cell, insulin has been shown to enhance E2 responses to FSH in vitro, whereas there is indirect evidence to suggest that insulin may facilitate FSH-stimulated E2 release. 62,63,65,66,68-72,162,214,329 Despite these findings, our recent studies have suggested that the granulosa cell may be insulin-resistant, as indicated by significantly increased E2 responses to FSH during insulin infusion in women with PCOS treated with pioglitazone compared with responses observed before treatment. 203 Further investigation is necessary to resolve these conflicting results. At the level of gonadotropin secretion, insulin appears to exert little effect on pituitary LH and FSH release, which is in contradistinction to results obtained in vitro in the rat model. In studies employing long-term insulin infusion in normal women and those with PCOS, we were unable to detect changes in mean LH levels, LH pulse frequency and amplitude, or LH responses to GnRH compared with results obtained before insulin administration. 330 Collectively, these data suggest that the role of insulin resistance in PCOS is directed primarily, but not exclusively, at the level of the ovary. Concomitantly, insulin resistance may also affect a reduction of serum SHBG as well as reflect a consequence of obesity. Whether insulin resistance is etiologic is unclear because not all women with this disorder exhibit abnormal insulin secretion. In addition, defining cellular defects in insulin signaling and insulin-related cell function have not been consistent within similar or different tissues of women with PCOS. 331

Aside from the effects of increased LH secretion on theca cell androgen production, it has been suggested in PCOS that ovarian follicles undergo premature luteinization as a result of the atretogenic action of LH on the granulosa cell. This concept has been supported by the finding of increased LH-induced progesterone production from medium-sized follicles (<9 mm diameter) of granulosa cells from polycystic ovaries compared with normal granulosa cells. 332 In addition, PCOS granulosa cells have been documented to express significantly greater LH-receptor mRNA than cells from normal follicles. 333 These findings, together with the increase in LH secretion, provide a compelling argument for such a process. Whether this mechanism can explain the arrest of follicle development at the midluteal stage of growth in women with PCOS remains to be shown. Moreover, it is not clear how pervasive is the effect of increased LH secretion on granulosa cell viability. Most granulosa cells derived from unstimulated follicles of polycystic ovaries are extremely vital and exhibit great capacity for steroidogenesis. 30 It may be the case that only the very terminal granulosa cells become atretic quickly and suffer demise.

With several components of the altered physiology unexplained, PCOS continues to remain a pathophysiologic enigma. Although a unifying concept seems elusive, there is reason to invoke a crucial role for excess androgen exposure in the perpetuation and perhaps the pathogenesis of this disorder. This approach would account for the diversity of clinical situations that may give rise to the PCOS phenotype, including primary and secondary causes of increased androgen production as well as conditions in which androgen bioactivity is increased.

Long-Term Consequences

In PCOS, the reproductive–metabolic alterations that are responsible for the immediate concerns of hirsutism, acne, and anovulatory infertility may also pose significant long-term risks to a woman’s general health and well-being. These concerns have been attributed principally to chronic anovulation, insulin resistance, and obesity, all of which are salient features of the disorder.

CANCER

Beyond the immediate problem of anovulatory infertility in PCOS, persistent stimulation of the endometrium by chronic unopposed estrogen may lead to endometrial hyperplasia or adenocarcinoma in some women. Much of this concern is derived from observations of postmenopausal women receiving estrogen replacement therapy, although untreated young women with PCOS have been known to
have endometrial cancer. In a long-term follow-up study of women with PCOS based on ovarian wedge resection, the odds ratio for endometrial carcinoma was 5.3 (95% confidence interval, 1.55-18.60) compared with control subjects. The relationship of endometrial cancer with PCOS was evident in a case–control study, which showed that elevated androstenedione levels were associated with 3.6- and 2.8-fold increased risks of carcinoma in premenopausal and postmenopausal women, respectively. Conversely, in young women with endometrial cancer, a history of menstrual irregularities consistent with anovulation and the diagnosis of PCOS is common. This problem is compounded by obesity and the associated decreased shift in SHBG, resulting in an increase of circulating free E_2. In PCOS, the histopathology of endometrial hyperplasia is not distinctive from that in women without hyperandrogenism. However, in the relatively few cases of endometrial cancer found in women with PCOS, the lesion is usually well differentiated compared with the poorly differentiated or undifferentiated forms seen in older postmenopausal women. It is not clear whether the histologic pattern of endometrial cancer in young women with PCOS might alter consideration of definitive therapy. However, expression of estrogen and progesterone receptors was more commonly encountered in the endometrial tissues of premenopausal versus postmenopausal women. By contrast, in the same study, p53 was overexpressed by almost fourfold in older women compared with those with ovulatory function.

Studies of the association between PCOS and breast cancer generally have not revealed increased risk. However, many of these studies were limited by confounds of design or subject selection. For instance, increased risk was not found among a cohort of women with chronic anovulation from the Mayo Clinic. It was presumed that these women had PCOS, and the study was retrospective. However, a subgroup analysis of postmenopausal breast cancer showed a significantly increased risk, although the affected cohort was composed of only five subjects. The Cancer and Steroid Hormone Study, involving 4730 women with breast cancer and 4688 control subjects, did not identify an increased risk of breast cancer. Moreover, there was actually a 50% reduced likelihood of breast cancer. Fortunately, the diagnosis of PCOS was self-reported and no laboratory documentation was performed. Similarly, the Iowa Women’s Health Study, which did not show increased risk of breast cancer, also relied on self-reporting of PCOS. In a study in which the diagnosis of PCOS was based on histologic appearance and clinical features, no association could be determined in a cohort of 786 women. Recently, women with PCOS were found to have a statistically significant family history of breast cancer compared with control subjects, suggesting a familial association. This study suffered from a lack of enrolled participants, because there were 41 women with PCOS and 66 control subjects. Thus, of the studies conducted to determine the relationship of PCOS to breast cancer, the vast majority have not been able to define a positive association.

A link between PCOS and ovarian cancer has been suggested from the findings of the Cancer and Steroid Hormone Study, in which women with epithelial ovarian cancer had a significantly higher likelihood of reporting a diagnosis of PCOS compared with control subjects. Moreover, after adjusting for age, parity, oral contraceptive use, infertility, and education, the odds ratio, 2.4 (95% CI, 1.0-5.9), for a history of PCOS remained statistically significant. In contrast, longitudinal follow-up studies have not been able to corroborate an increased risk of ovarian cancer in women with PCOS. The Mayo Clinic cohort of 1270 subjects with presumed PCOS showed only one case in 14,499 woman-years of study. Similarly, in 768 women with histologically identified polycystic ovaries followed for an average of 30 years, the mortality rate from ovarian cancer was 1 compared with an expected rate of 2.6 deaths. In addition, a family history of ovarian cancer was not found in 41 women with PCOS compared with normal women.

**DIABETES MELLITUS**

The discovery that women with PCOS have insulin resistance and are at risk for diabetes mellitus has had an enormous effect on the long-term health implications of this disorder. Although insulin resistance has been described as being relatively mild, it has been estimated that 20% to 40% of these patients will have glucose intolerance or type 2 diabetes mellitus by the fourth decade of life. Conversely, premenopausal women with type 2 diabetes appear to be at increased risk for PCOS. Based on the findings of two small cross-sectional, uncontrolled studies, the prevalence of PCOS ranged from 27% to 52%. These data are supported by a recent study that showed that postmenopausal women with a history of PCOS had a significantly higher rate of type 2 diabetes, 13%, compared with control subjects, who had a rate of 2%. Consistent with an increased likelihood of diabetes, women with PCOS with a family history of type 2 diabetes exhibit decreased insulin secretory responses to glucose loading relative to their degree of insulin resistance. Women with PCOS are at increased risk for impaired glucose tolerance and diabetes, and increased circulating concentrations of insulin have been reported to be common in first-degree relatives. The combined prevalence rates for impaired glucose tolerance and diabetes in mothers and fathers of women with PCOS were 46% and 58%, respectively, considerably greater than rates in families without a history of PCOS. In addition, affected sisters of women with PCOS were shown to have higher insulin-to-glucose ratios than their unaffected female siblings. Collectively, these findings illuminate the trend toward abnormalities of glucose metabolism and diabetes in women with PCOS and their immediate family members.

**DYSLIPIDEMIA**

Lipid abnormalities in PCOS are characterized by significant increases in circulating total cholesterol, LDL cholesterol, and triglycerides compared with normal matched control subjects. Conversely, serum levels of total HDL cholesterol and HDL_2 are significantly lower in women with PCOS than in normal women. Although in PCOS,
this lipid profile may exist independently of several risk factors, the effect of these factors on lipid metabolism may be considerable. Previous studies have clearly shown that obesity and impaired glucose tolerance are associated with adverse lipid profiles. The effect of androgen excess on lipid metabolism is less well understood in PCOS. Compared with age-matched normal women, the dyslipidemia of PCOS appeared to be less distinctive when the data were adjusted for body weight. Moreover, serum concentrations of lipoprotein-a and possibly plasmin activator inhibitor I were reduced by testosterone, whereas DHEA increased insulin sensitivity.

Regardless of the mechanisms that predispose patients to lipid abnormalities in PCOS, these patients should be at risk for plaque generation in coronary vessels. Through the action of hepatic triglyceride lipase, very low-density lipoprotein and intermediate-density lipoprotein are converted to LDL cholesterol. LDL cholesterol appears to be the most atherogenic lipoprotein. Another lipoprotein, lipoprotein-a, is also atherogenic and has the most pronounced effect when LDL cholesterol is elevated. Hepatic triglyceride lipase is also responsible for converting HDL₃, which is rich in cholesterol, to cholesterol-poor HDL₂. Although the atherogenic properties of LDL cholesterol are well established, there is evidence to suggest that low HDL cholesterol and high triglycerides may be more predictive of coronary artery disease in women than in men.

CARDIOVASCULAR DISEASE
It is generally believed that women with PCOS are at increased risk for cardiovascular disease. This is based on the presence of several risk factors that predispose to heart disease. These include impaired glucose tolerance, android obesity, hyperandrogenism, dyslipidemia, and hypertension. Whether PCOS itself constitutes a risk factor, independent of known risk factors, is not clear. Evidence to support this association was provided by a case–control study in which 206 women with PCOS and corresponding age- and race-matched control subjects were compared. The PCOS group was found to have significantly higher levels of total cholesterol, LDL cholesterol, and triglycerides than normal control subjects. With adjustment for confounding variables, such as BMI, fasting insulin, exogenous hormones, oral contraceptives, and age, by multiple regression analysis, the lipid abnormalities in women with PCOS remained highly significant. In addition, the dose–response relationship between insulin and PCOS was examined within individual cases and only approximately 20% of the variance could be attributed to insulin. Of note, androgens also did not contribute to the variance in lipids. These findings suggest that dyslipidemia in PCOS involves pathways separate from those of insulin and androgens. To determine whether the risk of cardiovascular disease may be demonstrated clinically in PCOS, a comparison of carotid ultrasonography was performed in patients with PCOS and their matched control subjects. The intima media thickness, which has been directly correlated with cardiovascular disease, was significantly greater in women with PCOS than in control subjects. In addition, atherosclerotic plaque formation was found to be twofold greater in the PCOS group.

Retrospective studies have suggested that women with PCOS or at least the stigmata of PCOS have increased arterial lesions in their coronary vessels. In premenopausal and postmenopausal women undergoing cardiac catheterization, those with coronary artery disease were more likely to have hirsutism, diabetes, and hypertension in addition to previous coronary artery disease. In another analysis that used a statistical risk factor model, women with PCOS had a significantly increased risk of myocardial infarction compared with control subjects. Unfortunately, these studies were limited by their retrospective design, lack of control subjects for obesity, and insufficient categorization of patient groups. Nevertheless, the current literature clearly indicates that women with PCOS cluster risk factors for premature morbidity and mortality as a result of heart disease. Despite the suggestive evidence of greater cardiovascular risk, measurement of actual death from myocardial injury has yet to be established. In two studies that examined the long-term health consequences in 786 women with PCOS, increased risk of coronary heart disease was identified, but mortality and morbidity rates did not differ from those in the age-matched control group.

HYPERTENSION
In a retrospective study, postmenopausal women with a history of PCOS, confirmed pathologically, had an approximately fourfold increase in the rate of hypertension compared with control subjects. In addition, women with PCOS tended to exhibit elevated blood pressure compared with age-matched control subjects. However, increases in blood pressure in both obese and nonobese patients with PCOS do not appear to be greater than those in control subjects, after adjusting for weight and body composition. Thus, despite the presence of insulin resistance in PCOS, particularly in obese women, hypertension may not be common in affected patients. Currently, the relationship of hypertension to PCOS is under investigation.

Differential Diagnosis
The lack of a specific diagnostic test for PCOS, combined with the broad clinical spectrum resulting from anovulation and hyperandrogenism, warrants consideration of related conditions with similar presentations. These include both functional and neoplastic processes. Among the functional disorders are ovarian hyperthecosis, congenital adrenal hyperplasia, and Cushing disease. Included in the neoplastic group are androgen-producing tumors of the ovary and adrenal gland.

OVARIAN HYPERTHECOSIS
Hyperthecosis refers to an unusual proliferative condition in which the ovary contains nests of luteinized theca cells scattered throughout the stroma. The extent of theca cell involvement may vary from minimal to extensive.
Severe hyperthecosis may be accompanied by extensive and dense fibroblast growth that results in an enlarged ovary of extremely firm texture, findings that are clearly distinct from those found in PCOS. Interestingly, the degree of hyperthecotic transformation in the ovary is not correlated to the severity of disease. This observation would suggest that the hyperthecotic tissue may be hypersensitive to gonadotropin stimulation because serum LH levels are commonly in the normal range. Because of markedly high serum androgen concentrations, these individuals have severe hirsutism and a significant percentage of patients exhibit virilizing signs, such as clitoromegaly, temporal balding, a male body habitus, and deepening of the voice. Androgen production may be resistant to conventional forms of long-term ovarian suppression, such as oral contraceptive therapy, although administration of gonadotropin-releasing hormone agonists have been shown to dramatically decrease androgen production. There usually is marked insulin resistance, with substantially elevated circulating insulin levels. In addition, these patients are often obese and exhibit acanthosis nigricans.

CONGENITAL ADRENAL HYPERPLASIA

Among the several enzymatic defects that comprise congenital adrenal hyperplasia (CAH), the incomplete form of 21-hydroxylase deficiency best simulates PCOS (see Chapters 16 and 17). This deficiency is manifested by an accumulation of 17-hydroxyprogesterone, which leads to abnormal elevations of the hormone compared with circulating values found in the follicular phase of the menstrual cycle. Because 17-hydroxyprogesterone is an androgen precursor, expression of this defect is associated with increased production of androstenedione and testosterone, with resultant hyperandrogenism. Notably, the clinical presentation may be indistinguishable from that of PCOS. However, there are several aspects of CAH that may suggest the diagnosis. These include severe hirsutism, clitoromegaly, familial tendency, and short stature. The condition is transmitted by an autosomal recessive inheritance pattern, whereas an explanation for short stature is unknown. Morphologically, the ovaries have been reported to appear similar to those of women with PCOS. The capsule generally is dense and thickened, although peripheral cystic follicles have been an inconsistent finding. The second most common enzyme deficiency is 11β-hydroxylase, which gives rise to a mild hirsutism due to increases in 17-hydroxyprogesterone as well as 11-deoxycortisol, the immediate precursor for this enzyme. The accompanying hypertension often distinguishes this disorder from the 21-hydroxylase form of CAH.

CUSHING SYNDROME

The clinical features of Cushing syndrome primarily result from excessive cortisol production by an adrenal neoplasm or from excessive ACTH production. In most cases, ACTH overproduction is due to a pituitary tumor, although rarely, ectopic sources of ACTH may be encountered, as in adenocarcinoma of the lung. The preponderant findings are obesity, hirsutism, acne, and menstrual irregularity. These suggest the diagnosis of PCOS. However, additional evidence of moon-like facies, buffalo hump, hypertension, muscle wasting, abdominal striae, and osteoporosis indicate a primary problem of cortisol excess. Although circulating androgen levels are elevated, there is also abnormal cortisol secretion characterized by increased basal levels, loss of circadian rhythmicity, and failure of suppression in response to dexamethasone. In contrast to CAH, careful examination of the ovaries does not show changes typical of PCOS in most cases.

ANDROGEN-PRODUCING NEOPLASMS

Androgen-producing tumors may arise from the ovary and the adrenal gland. In contrast to the gradually evolving clinical presentation associated with functional hyperandrogenism, the neoplastic process can be quite dramatic. Within a matter of months, these lesions may induce severe hirsutism, a male body habitus, and virilization marked by clitoromegaly. In addition, there may be acne and lowering of the voice. Despite the severity of androgenic manifestations, in the early stages of development, these tumors can mimic PCOS or other functional hyperandrogenic syndromes. Occasionally, the hormone production may be mixed to include excess production of cortisol and progesterone. Disruption of menstrual cyclicity varies from irregular bleeding to amenorrhea. The rapid onset of symptoms provides an important clue to the diagnosis. In some instances, a pelvic or abdominal mass can be palpated, which suggests an ovarian tumor.

Evaluation

LABORATORY EVALUATION

Laboratory assessment of suspected PCOS is based on clinical evidence of a functional hyperandrogenic disorder compared with a neoplastic process. In the presence of gradual and progressive hirsutism accompanied by irregular menstrual bleeding, the minimum endocrine evaluation includes serum total testosterone or free testosterone and 17-hydroxyprogesterone levels (see Chapter 32). These measurements should be performed in the morning because of diurnal variation. The rapid onset of excessive and severe hair growth, usually within months, should raise consideration of an androgen-producing tumor. In this situation, both serum total testosterone and DHEAS should be obtained; these values may suggest the source of androgen production. Threshold values beyond which a neoplasm should be considered are 200 ng/dL for testosterone and 7000 ng/dL for DHEAS. If circulating concentrations exceed these levels, then imaging studies, such as ultrasound and magnetic resonance imaging, are warranted to determine whether an ovarian or adrenal tumor exists. Occasionally, high circulating androgen levels may not be associated with a distinct lesion, but rather with bilateral multicystic ovarian enlargement. If accompanied by gradual onset of symptoms, this presentation would suggest the diagnosis of hyperthecosis. Commonly, these
patients exhibit severe insulin resistance and acanthosis nigricans.

Determination of 17-hydroxyprogesterone is useful as a screening test for CAH due to 21-hydroxylase deficiency. It has been proposed that a threshold concentration of 3 ng/mL for basal 17-hydroxyprogesterone provides the maximal cost–benefit. A circulating level of less than 3 ng/mL obtained in random anovulatory individuals or during the follicular phase in women with regular menstrual cycle excludes the diagnosis. Values in excess of 3 ng/mL warrant further evaluation with an ACTH stimulation test. After an overnight fast, 250 μg ACTH (1-24) is injected intravenously. Blood is obtained before and 1 hour afterward for measurement of serum 17-hydroxyprogesterone. Nomogram plots of baseline versus stimulated 17-hydroxyprogesterone concentrations result in three distinguishable groups: classic, nonclassic, and an overlap of hydrocortisone and genetically unaffected (see Chapter 32).

Women with Cushing syndrome may also present with a clinical picture consistent with PCOS. The optimal screening test is 24-hour urinary free cortisol, for which the normal value is less than 100 μg/24 hour. Abnormal values require further testing to determine the mechanism and site of excess cortisol production. These include low-dose and high-dose dexamethasone suppression tests as well as imaging studies to identify adrenal hyperplasia, Cushing disease, adrenal adenoma, or ectopic ACTH production.

As part of the assessment of oligomenorrhea due to anovulation, measurements of prolactin and TSH may be desirable. In PCOS, serum elevations of prolactin have been reported to range from 20% to 40% and probably relate to lactotrope stimulation by chronic estrogen exposure. Coexistence of a prolactinoma and PCOS is uncommon. Disorders of thyroid secretion have been associated with irregular menstrual bleeding, although there usually are other accompanying clinical features that suggest the diagnosis.

Some comment is warranted regarding the measurement of serum gonadotropin levels. Despite the widespread practice of measuring serum LH and FSH, circulating levels of these glycoproteins really do not contribute significantly to the diagnosis of PCOS. Increased pituitary LH secretion cannot always be determined by measurement of the serum concentration, because approximately one third of patients have circulating levels of LH in the normal range. Circulating endogenous LH levels are positively correlated with BMI, which suggests that normal LH levels are not uncommon in obese women with the disorder.

The observation that women with PCOS are insulin-resistant and have compensatory hyperinsulinemia raises the question of whether assessment of glucose metabolism and insulin secretion should be evaluated in these patients. Unfortunately, the ability to determine insulin resistance is limited by tests that lack sensitivity or are impractical for implementation. Based on fasting levels of glucose and insulin, a variety of indices have been designed to establish insulin resistance. Although a reasonable correlation exists between each model and provocative glucose tolerance tests, normal values do not preclude insulin resistance (Table 20-1). However, the fasting level of glucose may be used to distinguish glucose intolerance or diabetes and an elevated fasting insulin level will confer insulin resistance. An oral glucose tolerance test also provides valuable information; particularly if glucose measurements are accompanied by circulating insulin values. However, a reliable and reproducible assay for insulin is mandatory. It is unlikely that determination of insulin resistance is essential to the diagnosis of PCOS. Nevertheless, with the availability of insulin-lowering drugs, ascertainment of insulin resistance is warranted, particularly in high-risk individuals.

**IMAGING STUDIES**

In women with PCOS, ultrasound imaging of the ovaries shows bilateral enlargement, an increased number of peripheral cysts, and an increased percentage of central stroma. Because this appearance is unique to the syndrome, ultrasound evidence of polycystic ovaries virtually confirms the diagnosis in women with anovulation and hyperandrogenism. However, because most cases of PCOS may be determined solely from clinical symptoms and polycystic ovaries may be found in normal women, routine use of pelvic ultrasound for the diagnosis is optional. Considerable information about the endometrial response to chronic estrogen exposure may be obtained with ultrasound.

**Treatment**

**ORAL CONTRACEPTIVES**

Notwithstanding infertility, the most problematic issue for women with PCOS is excessive hair growth. Thus, one of the primary goals of treatment includes ameliorating the clinical effects of hyperandrogenism, which may

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<td>Hyperinsulinemic clamp</td>
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<td>Homeostasis model assessment of insulin resistance (HOMA IR)</td>
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<td>Glucose-to-insulin ratio</td>
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<td>Quantitative Insulin Sensitivity Check Index (QUICKI)</td>
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<td>Fasting insulin</td>
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*Normal values may vary depending on the insulin assay used.
be achieved by suppression of ovarian steroidogenesis, interruption of androgen action at the target tissue, and reduction of hyperinsulinemia. Administration of an oral contraceptive containing combination estrogen–progestin has proven to be an effective treatment for hirsutism, although the range of response is variable, depending on the severity of hair growth at the time of treatment. In addition to suppression of ovarian androgen production, oral contraceptives increase SHBG and facilitate metabolic clearance of testosterone. This modality of treatment also has the advantage of instituting regular cyclic withdrawal bleeding and providing sufficient progestin to prevent excessive endometrial proliferation and hyperplasia.

**ANTI-ANDROGENS**

In many instances, anti-androgenic agents have been used in conjunction with oral contraceptives to maximize clinical benefit. Spironolactone is an aldosterone antagonist that, along with its major metabolite, canrenone, competes for testosterone-binding sites, thereby exerting a direct anti-androgenic effect at the pilosebaceous unit. In addition, spironolactone appears to interfere with cytochrome P450, thereby inhibiting steroid enzyme action and resultant androgen production. In the past, it was used to treat mild hypertension and may exert a mild diuretic effect. Because this medication opposes the action of aldosterone, serum potassium levels may increase and therefore should be monitored. Other anti-androgens include flutamide and finasteride. Flutamide competes for the androgen receptor, whereas finasteride inhibits 5α-reductase. Both agents have been shown to be effective for the treatment of hirsutism. In a few cases, flutamide has been associated with liver toxicity; thus, caution should be exercised before recommending this drug.

Clinical studies have determined that these compounds exhibit comparable effectiveness in reducing hair growth. Anti-androgenic agents can also be used in conjunction with oral contraceptives to maximize clinical benefit. For example, spironolactone is often used to prevent excessive endometrial proliferation and hyperplasia.

**INSULIN-LOWERING DRUGS**

Insulin-lowering drugs have been shown to improve insulin sensitivity in women with PCOS who have insulin resistance, which warrants their consideration in the management of this disorder. Most studies have detected a significant decrease in serum testosterone levels. However, others that included patients with severe obesity did not show a similar effect. Metformin, a biguanide, increases insulin sensitivity in the liver to reduce gluconeogenesis and hyperinsulinemia. Clinical studies have shown that administration of metformin to women with PCOS resulted in decreased androgen levels, increased rates of spontaneous ovulation, and enhanced ovulatory response to clomiphene. Despite these findings, the efficacy of metformin for the treatment of hirsutism remains to be established. Recent studies have shown that metformin may have a direct effect on ovarian steroidogenesis, independent of insulin action. Incubation of human ovarian theca-like tumor cells with metformin inhibited mRNA expression of steroidogenic regulatory protein and 17α-hydroxylase, whereas no effect was detected for 3β-hydroxysteroid dehydrogenase (3βHSD) or cholesterol side-chain cleavage. In contrast, metformin was not associated with changes in 17α-hydroxylase or 3βHSD in studies of yeast cells. The disparity between results may reflect differences in the cell systems used. Side effects of metformin include dose-related gastrointestinal symptoms that tend to resolve after several weeks. A rare adverse effect of metformin therapy is lactic acidosis. Therefore, metformin should not be prescribed to patients with renal, hepatic, or major cardiovascular disease, or hypoxia because these patients have a predisposition to elevated lactate levels. Precautionary temporal withdrawal of metformin is advised in patients undergoing radiologic procedures involving intravascular iodinated contrast materials and surgery.

Thiazolidinediones are another group of insulin-lowering drugs that include rosiglitazone and pioglitazone. These drugs act by binding to the peroxisome proliferation activator receptor γ, which forms a heterodimer with retinoic acid receptor and binds to a promoter to increase the expression of genes that regulate glucose homeostasis. It has been well documented that thiazolidinediones decrease androgen levels in women. Similar to metformin, thiazolidinediones have not been shown to significantly lessen hirsutism in hyperandrogenemic women. In addition, in a large multicenter clinical trial, it was shown that improved insulin sensitivity was associated with resumption of ovulation after long-term treatment with troglitazone. This effect was dose-dependent, as determined by the rate of ovulation and the time required to achieve ovulation. Similar to metformin, thiazolidinediones have also been shown to have a direct effect on steroidogenesis. In studies using yeast, the steroidogenic enzymes 17α-hydroxylase and 3βHSD were inhibited by troglitazone and, to a lesser extent, rosiglitazone and pioglitazone. Similar results have been achieved in human granulosa cells. However, studies to determine whether troglitazone influences aromatase enzyme have not produced consistent results in human granulosa cells. Liver toxicity was associated with first-generation drugs of this class of compounds. However, both rosiglitazone and pioglitazone have been virtually devoid of liver effects. Nevertheless, thiazolidinediones should not be initiated in patients with evidence of liver disease.

**OVULATION INDUCTION**

See Chapters 21, 28, and 29 for a discussion of ovulation induction.

The complete reference list can be found on the companion Expert Consult Web site at www.expertconsultbook.com.

**Suggested Readings**
