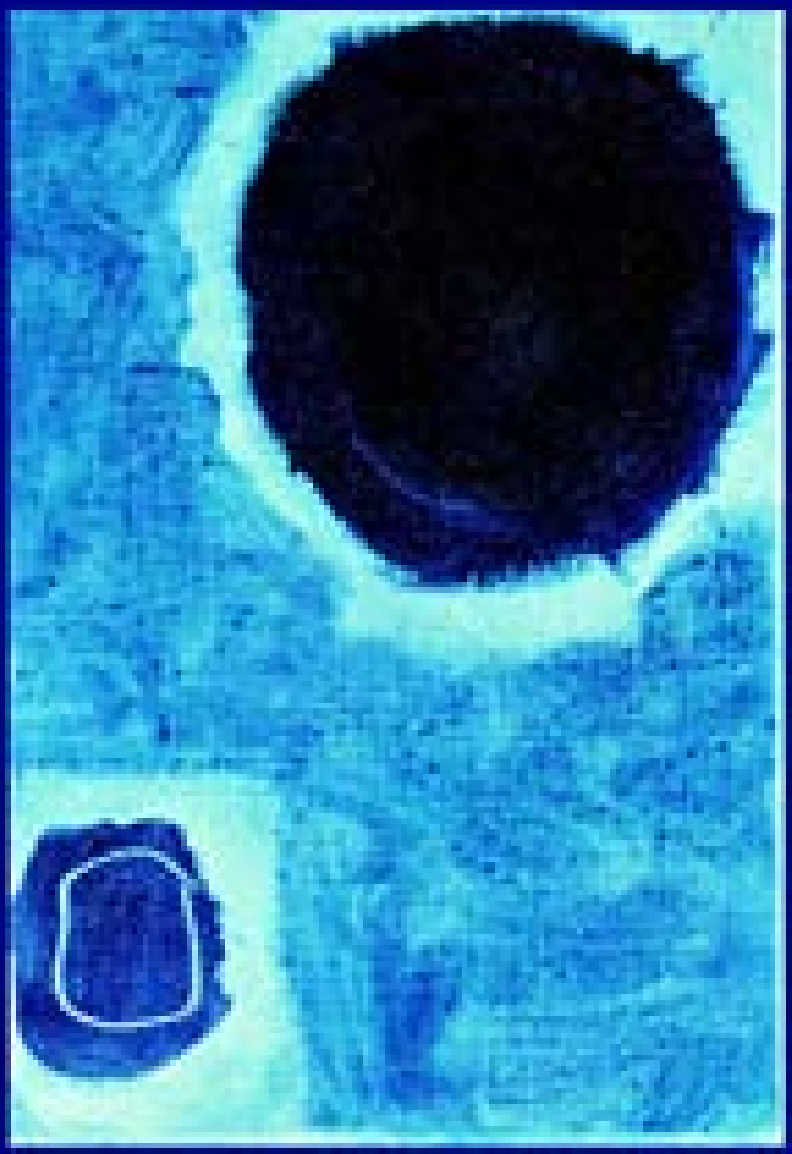
Polycystic Ovary Syndrome

SECOND EDITION

Edited by
Gabor Kovacs and
Robert Norman



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Polycystic Ovary Syndrome

Second Edition

Polycystic ovary syndrome (PCOS) is one of the most common reproductive health problems of women. Despite this, its effective treatment remains a significant challenge to the medical profession. This new edition of a highly successful and well-reviewed book is a thorough update on the syndrome, its etiology, pathology, impact on infertility, and effective medical management. Every chapter has been extensively referenced and completely revised and updated. New chapters cover: hyperinsulinemic insulin resistance; new treatments including in vitro maturation; pediatric origins, including the Barker hypothesis; adrenocortal dysfunction; polycystic ovary syndrome in non-Western societies; surgical treatment of obesity associated with polycystic ovaries; and treatment with vitamins and minerals.

The book is a reference text for all clinicians with an interest in reproductive endocrinology, including gynecologists, IVF specialists, and obstetricians.

Gabor T. Kovacs is Professor of Obstetrics and Gynaecology at Box Hill Medical School, Monash University.

Robert Norman is Director of the Centre for Reproductive Health at The University of Adelaide.

From reviews of the first edition:

". . . clearly written, beautifully laid out and offers concise information on the background of the disease, its diagnosis, clinical manifestations and treatments. . . . I would recommend the book to any practitioner who regularly encounters this condition."

Hospital Doctor

"Each chapter is readable and authoritative. This book could be dipped into by someone wanting information about a particular aspect of PCOS, but equally could be read over the course of one or two days by anyone wanting a comprehensive overview or in-depth introduction to PCOS . . . an excellent reference source for any gynaecologist with an interest in reproductive medicine . . . there is certainly no better book available."

Human Fertility

"The book covers all the major aspects of PCOS and will therefore be of interest to gynaecologists and endocrinologists. It would also make fascinating reading for cardiologists, dermatologists and public health physicians . . .."

Journal of Obstetrics and Gynaecology

"This book, with its impressive range of international experts, attempts and succeeds in reviewing our understanding of PCOS to date . . . concise and well referenced . . . the book should appeal to a variety of clinicians, including endocrinologists, general gynaecologists and fertility experts."

The Obstetrician and Gynaecologist

". . . this book is extremely readable, well presented, and not at all daunting for the generalist, while also providing enough detail for the specialist."

Medical Journal of Australia

". . . trying to distill the breadth of PCOD into 200 pages is a not-inconsequential task, and I am happy to report that Dr. Kovacs has succeeded admirably. This little book will occupy a valued place on my bookshelf, and I recommend it highly to others seeking an entrance to the world of PCOD."

Fertility and Sterility

Polycystic Ovary Syndrome

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Introduction: Polycystic ovary syndrome is an intergenerational problem

Gabor T. Kovacs and Robert Norman

The polycystic ovary syndrome (originally called the Stein–Leventhal syndrome), was popularized by the two Americans whose names have been attached to the condition for 70 years (Stein and Leventhal 1935), and was considered as a problem of anovulation and infertility. They described their treatment of anovulation using wedge resection with remarkable success. However as medical treatment became available with the utilization of clomiphene citrate (Greenblatt 1961), and subsequently the use of follicle stimulating hormone of pituitary (HPG) (Kovacs *et al.* 1989) and urinary source (Wang and Gemzell 1980), surgical treatment became less often used. Interestingly, surgical treatment of resistant anovulation has had a resurgence with the laparoscopic approach initially described by French gynecologists, but popularized by Gjoanness (1984). The history and current status of surgical treatment are discussed in Chapter 11.

It was the use of ultrasound that transformed visualization of the ovaries (Swanson *et al.* 1981). (The use of imaging techniques is described in detail in Chapter 5.) It then became apparent that there were two different clinical spectrums. Almost one quarter of the population had the appearance of polycystic ovaries when examined ultrasonically, but more than half of these had no clinical symptoms whatsoever (Lowe *et al.* 1995, Balen and Michelmore 2002). These women are referred to as having polycystic ovaries (PCO). If the ultrasonic appearance is accompanied by other symptoms, such as hyperandrogenism, the term used is polycystic ovary syndrome (PCOS).

Although the exact definition of PCO/PCOS has had different parameters when described by various experts, following a Consensus Conference held in Rotterdam in 2003, an internationally accepted definition has been adopted by the European Society for Human Reproduction and Embryology and the American

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Society for Reproductive Medicine, known as the ESHRE/ASRM Rotterdam consensus (Rotterdam consensus). This is described in detail throughout this book; its full text is given in The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004).

The etiology of PCO/PCOS is still puzzling. It is now accepted that it is multifactorial, partly genetic, but although a number of candidate genes have been postulated, the mode of inheritance and the responsible gene remain aloof. The other important point that has been made is that the mainstay of treatment is "diet and exercise" (Clark *et al.* 1998) and that greater emphasis needs to be placed on lifestyle factors when consulting these women. The obesity epidemic in the West may unmask more women with PCOS.

There is still no firm clinical evidence that PCO is a health hazard, although there is strong circumstantial evidence that cardiovascular disease risk factors are all increased if we look at surrogate markers in PCOS. Diabetes mellitus is clearly more common.

As the primary biochemical abnormality is insulin resistance, and metformin can restore menstrual regularity (Velazquez *et al.* 1994), there have been a number of advocates for the use of insulin sensitizing agents, not only to restore ovulation but to facilitate weight loss, counteract androgenic symptoms, prevent long-term complications, decrease the risk of early pregnancy loss, decrease the risk of ovarian hyperstimulation syndrome, and even improve the outcome of in vitro fertilization (IVF) therapy. The role of insulin sensitizing agents is reviewed in Chapter 13.

In this second edition of *Polycystic Ovary Syndrome*, we have decided to be more holistic, and we have included chapters on the role of vitamins and nutrients (Chapter 20), as well as the role of bariatric surgery (Chapter 19).

We believe that this book is an up-to-date comprehensive reference manual for all aspects of this fascinating condition.

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Introduction and history of polycystic ovary syndrome

Cindy Farquhar

Since the classical observation of Stein and Leventhal in 1935 (Stein and Leventhal 1935), interest in polycystic ovaries (PCO) and its associated syndrome (PCOS) has evolved from a "gynaecological curiosity to a multisystem endocrinopathy" (Homburg 1996). It is probably the most common endocrine disorder in women, accounting for the majority of cases of hirsutism, of menstrual disturbance, and anovulatory infertility. It is also one of the most poorly defined endocrinological conditions with a complex pathophysiology that has produced considerable scientific debate. Evidence of the ongoing interest in this disorder is not difficult to find; an electronic search on MEDLINE from 1966 to 2005 using the search term "polycystic ovary syndrome" produces 5112 citations; 934 are review articles, and 200 are randomized controlled trials (Fig. 2.1), and the majority of publications occur after 1985.

Recognition

Although Stein and Leventhal were first in the modern medicine era to describe this condition, an earlier description dating back to 1721 reads: "Young married peasant women, moderately obese and infertile, with two larger than normal ovaries, bumpy, shiny and whitish, just like pigeon eggs." (Vallisneri 1721; translated from Italian.) There was further recognition in the nineteenth century when sclerocystic changes in the ovary were described (Chereau 1844), but it was not until Stein and Leventhal first presented their paper at the Central Association of Obstetricians and Gynecologists in 1935 that the syndrome was more comprehensively described. They reported on seven women who had amenorrhea, hirsutism, and enlarged ovaries, with multiple small cysts and thickened tunica (Fig. 2.2). While there had been reports of menometrorrhagia in women with microcystic disease

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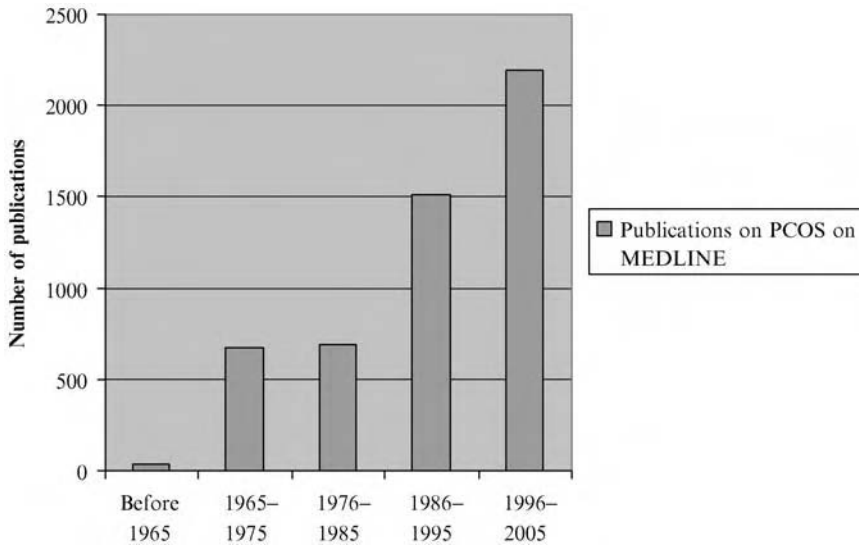

Fig. 2.1 The medical literature on polycystic ovarian syndrome.

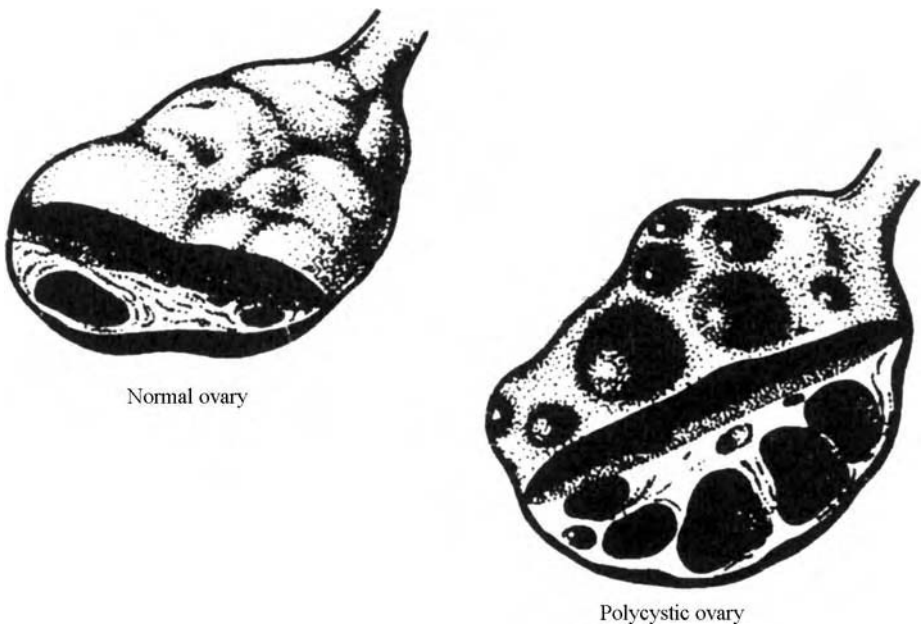

Fig. 2.2 The polycystic ovary compared to the normal ovary.

of the ovary, amenorrhea had not been recognized or reported in such cases until Stein and Leventhal's report. Stein and Leventhal had also performed ovarian wedge resection which resulted in a return of ovulatory cycles. Of the seven patients who underwent wedge biopsy, all returned to regular menstruation and two conceived. It is not clear whether other cases of the disorder were observed that did not fit this particular pattern. Stein subsequently reported on 75 women who underwent bilateral wedge resection reporting that nearly 90% began to have spontaneous menstrual cycles and 65% seeking fertility conceived (Stein *et al.* 1948).

The diagnosis of polycystic ovary syndrome

The advances that have taken place in the past century with regard to diagnosis of this condition have been considerable. Stein and Leventhal's method of diagnosis rested primarily on observing enlarged sclerocystic ovaries at either pneumoroentgenography or at laparotomy in women who were either anovulatory or hirsute or both (Stein and Leventhal 1935). Prior to this there was little choice but to perform repeated vaginal and rectal examinations which did not always reveal the presence of polycystic ovaries. At pneumoroentgenography air was admitted into the peritoneum by an abdominal incision and when the ovaries were three quarters as large as the uterine shadow on x-ray then polycystic ovaries were confirmed. Several examples of this technique are given in Stein and Leventhal's original publication. They often used Lipiodol instillations at the same time to outline the fallopian tubes. However this technique did not really gain popularity and eventually laparotomy and wedge biopsy became the mainstay of both diagnosis and treatment (Goldzieher and Green 1962).

With the development of radioimmunoassay techniques in the 1970s and the introduction of clomiphene citrate, laparotomy and biopsy were largely abandoned as a diagnostic method. In 1958 McArthur, Ingersoll, and Worcester first described elevated urinary levels of luteinizing hormone (LH) in women with bilateral PCO (McArthur *et al.* 1958). Throughout the 1970s and 1980s, elevated serum concentrations of LH and testosterone (T) were considered an essential prerequisite for diagnosis (Yen *et al.* 1970, Rebar *et al.* 1976). For example, Yen (1980) stated that "true PCOS" had typical abnormalities of gonadotropin and androgen secretion. There have been a number of interesting evolutions in the search for diagnostic criteria. Not only was an elevation in the LH level felt to be necessary but in time the LH:follicle stimulating hormone (FSH) ratio was also required to be elevated. Initially it was 2 : 1, then 3 : 1 and even 2.5 : 1 (Yen 1980, Lobo *et al.* 1981, Shoupe *et al.* 1983, Chang *et al.* 1983). Eventually the concept of a ratio was abandoned and the absolute values were relied on for diagnosis (Fox *et al.* 1991, Robinson *et al.* 1992). However, by only defining PCOS in the

presence of elevation of LH concentrations, then obviously all patients will have the condition (Waldstreicher *et al.* 1988, Fauser *et al.* 1991, 1992) and LH becomes a sine qua non for the diagnosis (Franks 1995, Homburg 1996). Elevations in androgens are similarly unhelpful in defining the syndrome as the levels are modestly and inconsistently elevated (Gadir *et al.* 1990). Other limitations of the biochemical diagnosis of PCOS included the variable and imprecise nature of the assays and the dynamic nature of hormonal steroidal release from the ovary (Fauser *et al.* 1991, 1992). LH is secreted in a pulsatile manner and the difference between the peak and nadir of each pulse can be substantial (Santon and Bardin 1973), and therefore measuring the hormone levels only once may be misleading (Franks 1989). Furthermore, there were still many women who were noted to have the clinical symptoms but whose LH and T levels did not fall within the diagnostic criteria (Adams *et al.* 1985). There was a need for a diagnostic test that could observe the ovary without damaging the surface of the ovary and potentially reducing fertility, but that did not just "take a snapshot" of the endocrine state of a patient as a single serum concentration of ovarian hormones does.

Fortunately, real-time ultrasound was developing into a useful diagnostic tool. Ultrasound examination of the ovary has many advantages over observation at laparoscopy or laparotomy; it is non-invasive, simple, and allows careful repeatable measurements, and it is possible to clearly see the follicular structures just below the surface of the ovary as well as demonstrate the dense and frequently increased stroma. Swanson *et al.* (1981) first reported on the ultrasound description of polycystic ovaries. The cysts ranged from 2 to 6 mm and were either peripherally distributed or throughout the parenchyma. Ultrasound descriptions have been shown to correlate with both laparoscopic findings and histological findings (Eden *et al.* 1989, Saxton *et al.* 1990). In the study by Eden *et al.* (1989), direct laparoscopic inspection of the ovaries was considered the reference test for the diagnosis of PCO, and the sensitivity (97%) and specificity (100%) with ultrasound was very good. In the study by Saxton *et al.* (1990) women who were undergoing open hysterectomy and bilateral oophorectomy had an ultrasound within 24 h of surgery where careful measurements and morphological descriptions were made. The measurements were repeated the following day in theater and again in the histopathology laboratory by independent observers with no prior knowledge of the ultrasound findings. There was 100% sensitivity and specificity in the 28 ovaries (of 14 women) that were studied.

The diagnostic criteria described by Adams *et al.* (1985) are frequently cited and although there are ongoing discussions about the number of follicles and the size of the ovary (Fox *et al.* 1991) there has been little change to these criteria (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). The ultrasound diagnostic criteria rest on the observation of more than 12

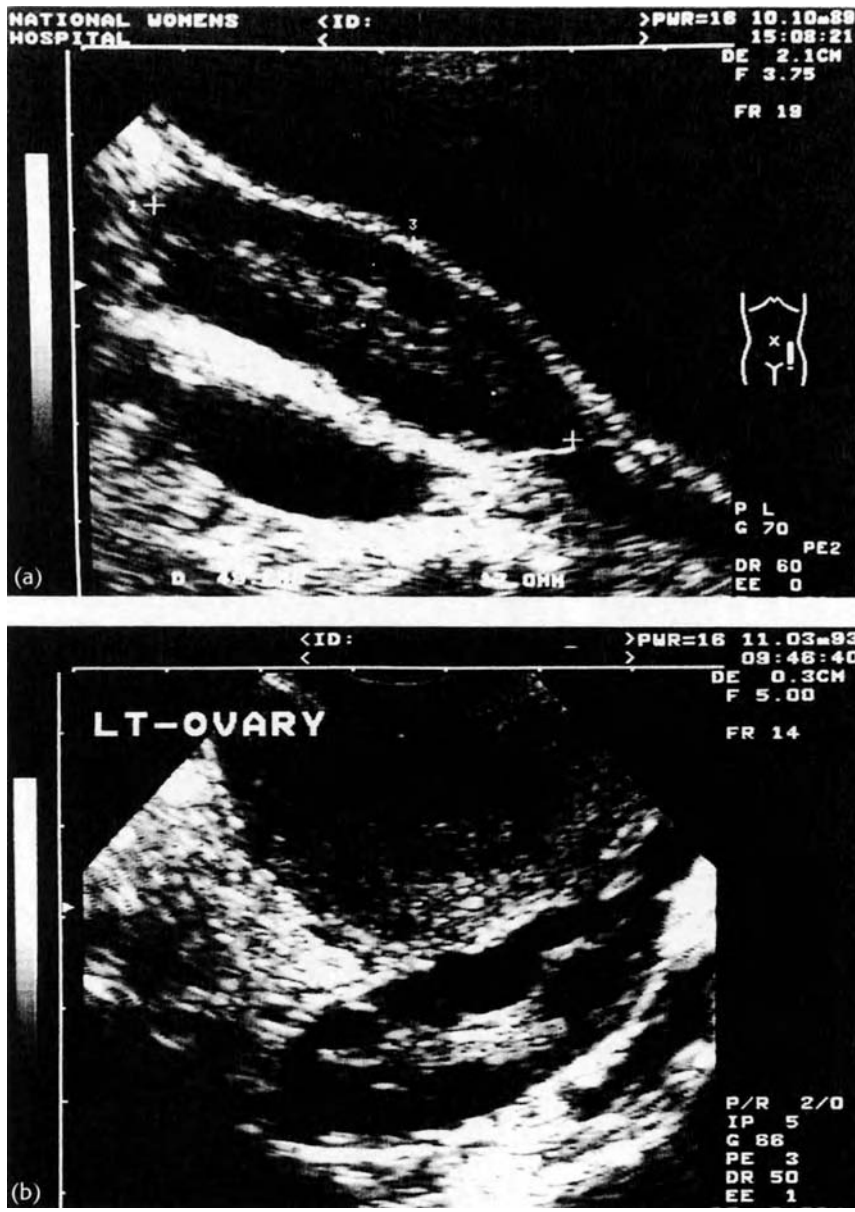

Fig. 2.3 Ultrasound view of polycystic ovary: (a) transabdominal, (b) transvaginal.

discrete follicles of <10 mm, usually peripherally arranged around an enlarged, hyperechogenic, central stroma at either transabdominal or transvaginal ultrasound (Fig. 2.3). The upper limit for ovarian volumes has decreased from >10 cm^2, to as low as >5.5 cm^2 (Orsini *et al.* 1985, El Tabbakh *et al.* 1986, Polson *et al.* 1988, Ardaens *et al.* 1991, Farquhar *et al.* 1994a, Dewailly 1997). A comparison of

transvaginal (TVS) ultrasound and transabdominal (TA) ultrasound by Fox *et al.* 1991 suggested that TA ultrasound failed to detect 30% of PCO compared to an almost 100% detection rate with TVS. However other studies reported similar detection rates for TA and TVS (Farquhar *et al.* 1994b) although TVS has many practical advantages. Recent advances in ultrasound include an objective and quantitative method of measuring the ovarian stroma using a computerized ultrasonic technique (Dewailly 1997) which has demonstrated that women with PCO have a greater stroma than women with normal ovaries. They conclude that an increased ovarian stroma is the most valuable diagnostic factor for PCOS. However, the absence of stroma does not exclude the diagnosis.

Ongoing problems with the diagnostic definitions of PCOS and the variation in diagnostic criteria across research groups and countries led to a new set of definitions. In 1990 the first international conference on polycystic ovary syndrome was held at the National Institutes of Health in the USA. The meeting did not lead to consensus although a questionnaire eventually led to diagnostic criteria being developed. In 2003, a further consensus meeting was held in Rotterdam and it was agreed that two of three of the following criteria were sufficient to diagnose the syndrome: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

Prevalence studies

When discussing the prevalence in the population it is important to be clear on the difference between the definitions that are commonly used. Polycystic ovaries should not be confused with the polycystic ovary syndrome. Polycystic ovaries may be diagnosed in the absence of any clinical syndrome (Polson *et al.* 1988). The polycystic ovary syndrome refers to the presence of polycystic ovaries in a woman with a particular cluster of symptoms which usually includes amenorrhea, oligoamenorrhea, hirsutism, anovulation, and other signs of androgen excess such as acne and crown pattern baldness (Franks 1995, Homburg 1996, Jacobs 1996). However, some women may be diagnosed with polycystic ovaries at the time of having an ultrasound examination for other reasons, who have none of these symptoms. Once ultrasound became commonly used in the 1980s it was recognized that polycystic ovaries were frequently reported in asymptomatic women and this was one of the reasons that prevalence studies were undertaken. The first prevalence study was reported in a group of patients from a population of volunteers who were predominantly hospital workers and medical students (Polson *et al.* 1988). This group reported the prevalence of polycystic ovaries of 23% but the large majority of these women had clinical manifestations of the

Table 2.1. Summary of prevalence studies of polycystic ovaries (PCO) and polycystic ovarian syndrome (PCOS)

Author(s)	Setting	*n*	PCO (%)	PCOS[a] (%)
Polson *et al.* 1988	Volunteers, London, UK	258	23	76
Clayton *et al.* 1992	GP practice, London, UK	190	22	30[b]
Farquhar *et al.* 1994a	Electoral roll, Auckland, New Zealand	183	21	59
Botsis 1995	Women volunteers undergoing PAP smears, Athens, Greece	1078	17%	>80
Cresswell *et al.* 1997	Hospital patients, UK	235	21%	>41
Michelmore *et al.* 1998	GP practice volunteers, Oxford, UK	224	34	65
Lowe *et al.* 2005	Partners of azoospermic men undergoing IVF, Melbourne, Australia	100	23	55

Notes:

[a] Defined as either hirsutism or irregular cycles or both amongst the women diagnosed with PCO on ultrasound.

[b] Irregular or very irregular cycles (does not include hirsutism).

syndrome, namely hirsutism or oligoamenorrhea. Several other prevalence studies have been undertaken (Clayton *et al.* 1992, Farquhar *et al.* 1994a, Michelmore *et al.* 1998, Lowe *et al.* 2005), and a prevalence rate of between 16% and 33% was reported. With the exception of Clayton *et al.*'s study, the other three prevalence studies found that women with PCO were also more likely to have symptoms suggestive of the PCOS, namely hirsutism or menstrual disturbances. The findings of these prevalence studies are summarized in Table 2.1.

Concept of a spectrum

The prevalence studies have led to a greater understanding of this condition. It is now widely recognized that there is a continuum or spectrum of clinical presentations (Balen *et al.* 1995). At one end of the spectrum are the women who ovulate and who have no dermatological manifestations such as acne or hirsutism. These women may have had an ultrasound scan for some other completely unrelated reason. At the other end of the spectrum there may be women with menstrual disturbances; oligoamenorrhea, increased hair growth, acne, crown pattern baldness, evidence of insulin resistance. The patients described by Stein and Leventhal in 1935 probably represented one extreme of the clinical spectrum. The presence of a woman in this continuum is likely to be predetermined by genetic factors but the position on the continuum is likely to be related to

lifestyle and in particular, body mass index (BMI). Although the exact "trigger" that "causes" the expression of the syndrome is unknown, it seems likely that BMI is involved (Balen *et al.* 1995), and women at the PCO end of the spectrum (without PCOS) may move to the other end of the spectrum if they have an increase in body weight (Homburg 1996). Weight reduction in a woman with PCOS will often return her to the other end of the spectrum with ovulatory cycles and improved hirsutism (Kiddy *et al.* 1992, Clark *et al.* 1995). In clinical practice there is a tendency for only women at the severely affected end of the scale (with PCOS) to be referred to infertility or endocrine services. An asymptomatic non-obese woman who is diagnosed with PCO on ultrasound should be counseled about the advisability of maintaining a normal BMI in the future.

Clinical manifestations

Polycystic ovary syndrome appears to be a heterogenous condition with a wide variety of clinical presentations. The more severe the biochemical disturbance the more likely the woman is to have a clinical presentation (Conway *et al.* 1989, Balen *et al.* 1995). The majority of women will present with only one or two of the clinical manifestations. It is unclear why some women may present with anovulation with no hyperandrogenic manifestations, while others may present with severe androgenic symptoms but remain ovulatory.

Menstrual disturbances

Oligoamenorrhea, amenorrhea, and prolonged erratic menstrual bleeding are all aspects of the menstrual disturbances that occur in PCOS. Nearly 90% of women with oligoamenorrhea have PCO on ultrasound, while they are only present in 30% of women with amenorrhea (Adams *et al.* 1986, Franks 1995). Seventy-five percent of women with anovulatory infertility will have PCOS although weight-related amenorrhoea and hyperprolactinemia need to be considered as part of the differential diagnosis (Hull 1987).

Hirsutism, acne, and alopecia

Increased facial and body hair is one of the most commonly presenting symptoms and 92% of women have PCO on ultrasound (Adams *et al.* 1986). Acne has also only recently been acknowledged as an endocrine disorder (Bunker *et al.* 1991) and approximately three-quarters of women who present with acne have PCO on ultrasound (Eden 1991). Alopecia and more specifically crown pattern baldness have been less commonly reported in women with PCOS. All these clinical symptoms reflect the mild androgenic stimulations of the pilosebacious unit. Severe signs of virilization such as clitoromegaly or deepening of the voice, as well as rapid onset of hirsutism,

are rarely manifestations of PCOS and an androgen producing tumor should be excluded (Ehrmann *et al.* 1994). Similarly if the testosterone concentration is >4.8 nmol/l then further investigations need to be undertaken (Balen *et al.* 1995).

Recurrent miscarriage

Polycystic ovaries have been identified as being associated with recurrent miscarriage and early pregnancy following in vitro fertilization (IVF) cycles (Homburg *et al.* 1988, Sagle *et al.* 1988, Regan *et al.* 1990, Rai *et al.* 1996). Hypersecretion of LH was proposed as the underlying cause of the reproductive loss (Homburg *et al.* 1988). There have, however, been conflicting reports which have resulted in much debate. For example, Thomas *et al.* (1989) found no association with LH levels and outcome following IVF while others have reported no increase in LH in women with recurrent miscarriage and PCO (Tulppala *et al.* 1993, Liddell *et al.* 1997). Some authors have advocated lowering elevated LH levels in women trying to conceive by suppression with gonadotropin releasing hormone (GnRH) analogs (Balen *et al.* 1995). Although one trial of 106 women with elevated LH levels and PCO who were given GnRH analogs did not show an improvement in pregnancy outcomes (Clifford *et al.* 1996), further research is needed to evaluate this approach.

Metabolic symptomatology

The metabolic aspects of PCOS are obesity (present in 30–50% of women with PCOS) and insulin resistance, both of which are common. The resulting hyperinsulinemia is found in about 80% of obese women and 30–40% of women of normal weight with PCOS (Dunaif *et al.* 1989). The distribution of fat in women with PCO results in an increased waist : hip ratio (Bringer *et al.* 1993) and is frequently associated with greater insulin resistance than if fat is distributed predominantly in the lower body segment (Pasquali *et al.* 1994). Some women may also present with acanthosis nigricans (a feathering pigmented area of tissue in the neck and axillary regions); this is now recognized as a non-specific marker of moderate to severe insulin resistance (Dunaif 1992a). Hypersecretion of insulin results in ovarian secretion of androgens, leading to hirsutism and menstrual disturbance (Conway *et al.* 1993). There is increasing evidence that women with PCOS are at increased risk for the development of type 2 diabetes mellitus (Ehrmann 1997) and myocardial infarction (Guzick *et al.* 1996, Birdsall *et al.* 1997, Wild 1997, Rajkhowa *et al.* 2000).

Etiology

Although uncertainty exists regarding the etiology of PCOS, genetic factors are strongly implicated. For example, a high percentage of siblings and mothers of women with PCOS have the same morphological appearances at ultrasound

(Hague *et al.* 1988). There is also evidence of an autosomal transmission of the responsible genetic sequences. It is possible that a gene (or series of genes) may render the ovary susceptible to insulin stimulation of androgen secretion while blocking follicular maturation (Nestler 1997). In men this genetic predisposition may be expressed as premature balding (Carey *et al.* 1993). The symptoms frequently begin at puberty although in many women the syndrome is not fully expressed until later in their reproductive years (Lunde *et al.* 1989, Dewailly *et al.* 1994). This topic will be expanded in later chapters.

Pathophysiology

It is beyond the scope of this introductory chapter to discuss in detail the pathophysiological processes that lead to the development of PCOS. It has certainly resulted in vigorous international debate. Fortunately there are some areas of agreement. First, establishing the source of the ovarian androgen production is important to understanding the etiology. Second, insulin resistance probably contributes to the overall androgen levels. The increased ovarian androgen production seen in PCOS is the result of a series of complex biochemical processes which begins with disordered activity in the enzyme cytochrome P450c 17α, which catalyzes 17-hydroxylase and 17/20 lyase activities (Rosenfield *et al.* 1990), the rate-limiting step in androgen biosynthesis (Barnes *et al.* 1989). Ovulatory women with PCOS may also demonstrate this disordered activity (Franks and White 1993). Persistently high levels of LH will produce excessive amounts of androstenedione by causing increased cytochrome P450 activity. Unfortunately this does not explain increased androgen activity in women who have normal LH levels. Various explanations include failed downgrading of the LH receptor midcycle (Rosenfield *et al.* 1990), and increased number of LH receptors in women with PCOS. Insulin-like growth factor 1 (IGF-1) potentiates the expression of LH receptors (Adashi *et al.* 1985) and stimulates LH-induced androgen production and the accumulation of androgens in the ovary (Barbieri *et al.* 1986, Cara and Rosenfield 1988). Although there is no increase in the levels of IGF-1 in women with PCOS (Homburg *et al.* 1992) there is evidence of increased biological activity. Insulin-like growth factor 1 actively induces insulin resistance and also increases androgen secretion. It is likely that IGF-1 stimulates 17β-estradiol production by a combination of granulosa cell proliferation and stimulation of the aromatase complex (Adashi *et al.* 1985). It may also act as an amplifier of the action of FSH by interacting with FSH transduction signal at multiple sites (Adashi *et al.* 1988).

Evidence of insulin resistance and consequent hyperinsulinemia in women with PCOS is plentiful (Khan *et al.* 1976, Burghen *et al.* 1980, Dunaif 1997). Insulin

resistance is most evident in women with a high BMI (Pasquali *et al.* 1994, Dunaif 1997). It occurs in 30–60% of women with PCOS (Dunaif 1992b). In spite of insulin resistance at peripheral sites, e.g., adipose tissue, the ovary remains sensitive to insulin and other stimulatory peptides (e.g., IGF-1) (Bergh *et al.* 1993, Willis and Franks 1995). This phenomenon has been described in women with PCOS with both normal and high BMIs (Plymate *et al.* 1981, Dunaif 1992b). The action of insulin on the liver leads to a decrease in the production of sex hormone binding globulin and IGF-1 binding protein which results in an increase in unbound testosterone. Thus, although the ovary is the major site of increased androgen production in PCOS, insulin resistance may contribute to the overall androgen levels.

Advances in management of PCOS

The initial management of diagnosed PCOS will depend upon the clinical problem – anovulation or hirsutism. Other issues that need to be considered in the future are the avoidance of long-term sequelae of the syndrome.

Hirsutism

The mainstay of management in the first half of the last century depended on hair removal techniques. In moderate cases this may still be the choice of treatment especially if fertility is sought. Antiandrogen therapy was introduced in the 1960s. Spironolactone, cyproterone acetate, and more recently flutamide are successful treatments for symptoms of hyperandrogenism, including acne. Antibiotic therapy is also useful for the management of acne. Androgen-dependent alopecia is generally irreversible. Antiandrogens are usually prescribed with a low-dose oral contraceptive in order to induce regular withdrawal bleeding and provide contraceptive cover.

Anovulation

The first human pituitary FSH was used successfully to induce ovulation in anovulatory women in 1958 (Gemzell *et al.* 1958). However, the initial enthusiasm was somewhat dampened by the high fetal and maternal complication rate, mostly resulting from multiple ovulations and pregnancies. In 1961, Greenblatt and associates reported successful induction of ovulation using the compound clomiphene citrate (Greenblatt 1961). Clomiphene citrate is chemically related to the non-steroidal estrogen chlorotrianisene. The advantages of clomiphene citrate were obvious; it was inexpensive, had low toxicity, and had few side effects. Ovulation occurred in 70–80% of cases and pregnancy resulted in 30–40% (Cudmore and Tupper 1966, Kistner 1966, MacLeod *et al.* 1970). Clomiphene is thought to act essentially through competition with estrogen at hypothalamic

receptor sites, stimulating the release of GnRH and thus gonadotrophins by negative feedback (Ginsburg *et al.* 1975). Following the favorable outcomes with clomiphene, the indications for gonadotrophins (with the exception of assisted reproductive technology) in anovulatory woman with PCOS became limited to those who did not respond to clomiphene citrate (usually at 150 mg/day).

Pituitary gonadotropins were replaced with urinary derived human menopausal gonadotropins in the early 1960s (Thompson and Hansen 1970). The initial regimens resulted in pregnancy and miscarriage rates of less than 30%, high multiple pregnancy rates (30%), and high ovarian hyperstimulation syndrome rates (Wang and Gemzell 1980). The development of a low-dose schedule of gonadotropins has now reduced the sequelae of multiple follicles (Polson *et al.* 1987, Hamilton-Fairley *et al.* 1991, 1992, Sagle *et al.* 1991, Shoham *et al.* 1991). By aiming for a single preovulatory follicle, multiple pregnancy rates of 5–7% with few multiples greater than two have resulted. In the 1980s the urinary product was purified successfully to produce a "pure" FSH (Seibel *et al.* 1984). As there was concern that LH was detrimental to a successful outcome there were high hopes for better ovulation and pregnancy rates with a pure FSH product. This hope of improved pregnancy outcomes, however, was not borne out by the clinical trials although the incidence of ovarian hyperstimulation syndrome (OHSS) was reduced (Hughes *et al.* 1999a). Further developments in the 1990s include the production of a recombinantly derived FSH (Recombinant Human FSH Study Group 1995, Shoham and Insler 1996) which in the future may eliminate the need for urinary products which are time-consuming and inconvenient to collect and prepare.

The introduction of GnRH analogs (GnRHa) also seemed to offer hope for better treatment regimens and outcomes when used in combination with gonadotropins (Yen 1983, Insler *et al.* 1988). Once again this initial enthusiasm has not been borne out by evidence from clinical trials as neither the pregnancy rates nor the OHSS rates are improved (Hughes *et al.* 1999b). There is hope that antagonists may improve pregnancy rates and lower the OHSS rates and clinical trials are awaited (Homburg 2003).

The other medical treatment that is increasingly used for ovulation induction is metformin, an insulin sensitizing agent. Metformin is an oral biguanide, the most commonly prescribed oral medication for hyperglycemia, that does not cause hypoglycemia in normoglycemic patients. A decrease in insulin levels results and, as a consequence, a lowering of circulating total and free androgen levels with a resulting improvement of the clinical sequelae of hyperandrogenism. A systematic review of metformin for ovulation induction has suggested benefit for ovulation although few studies reported on pregnancy rates (Lord *et al.* 2003).

With the advent of laparoscopic surgery in the 1980s, surgical management of PCOS once again regained some favor (Gjoanness 1984). Diathermy or "drilling" of the ovarian stroma at laparoscopy has been shown to restore ovulatory cycles in women with clomiphene-resistant PCOS. Cohort studies report ovulatory rates of 70–90% and pregnancy rates of 40–70% (Donesky and Adashi 1995, Li *et al.* 1998). A systematic review of all the randomized controlled trials comparing laparoscopic ovarian surgery with gonadotropins includes only four studies (Farquhar *et al.* 2005). In all trials the included patients had previously failed to ovulate on clomiphene treatment, usually at doses higher than 150 mg/day. The four trials compared either ovarian electrocautery or CO_2 laser vaporization with either human menopausal gonadotropin or pure FSH (Lazovic *et al.* 1998, Vegetti *et al.* 1998, Farquhar *et al.* 2002, Bayram *et al.* 2004). Additional clomiphene citrate was given in one trial if no ovulation was detected by 8 weeks and if there was still no ovulation by 6 months then gonadotropin therapy was added (Bayram *et al.* 2004). The possibility of adhesions is of concern. However, as the pregnancy rates are reported to be >40% then the impact of adhesions is not likely to be great. Furthermore, Greenblatt and Casper (1993) conducted repeat laparoscopy 6 months after ovarian diathermy and showed that the adhesions were often minimal. The duration of effect is also poorly studied but appears to be 12–18 months. The clinical trials do not report a difference in pregnancy or live birth rate in women receiving either gonadotropins or laparoscopic ovarian drilling but there is a marked reduction in multiple pregnancy rate in women who undergo surgery as well as the possibility of ongoing ovulation. There is concern about premature menopause which will require long-term follow-up studies. Treating women with clomiphene-resistant PCOS with laparoscopic ovarian diathermy resulted in reduced direct and indirect costs (Bayram *et al.* 2004, Farquhar *et al.* 2004, van Wely *et al.* 2004). The reduction in multiple pregnancies makes the alternative of surgery particularly attractive.

Weight loss

The increasing proportions of obesity in modern society will mean that more women will present with the symptoms of PCOS as an excess of body fat accentuates insulin resistance and its associated clinical sequelae. Obese women with PCOS almost inevitably have the stigmata of hyperandrogenism and irregular or absent ovulation. Insulin stimulates LH and ovarian androgen secretion and decreases sex hormone binding globulin concentrations. In addition, being overweight makes treatment less effective and less efficient (Dale *et al.* 1998, Homburg 1998). Obese women being treated with low-dose gonadotropin therapy have inferior pregnancy and miscarriage rates (Hamilton-Fairley *et al.* 1992). Weight loss will improve ovarian function and reverse some of the associated

hormonal abnormalities (Pasquali *et al.* 1989, Kiddy *et al.* 1992, Clark *et al.* 1995). For these reasons, weight loss should be the first line of treatment in women with PCOS who are overweight and wishing to conceive.

Summary

PCOS is a subject that continues to debated amongst the medical and scientific community. Over the past 60 years tremendous advances have been made in diagnosis and management. It is one of the most common endocrine disorders and in the future the focus on management is likely to be the prevention of the long-term sequelae associated with insulin resistance.

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3

Phenotype and genotype in polycystic ovary syndrome

Richard S. Legro

Introduction

Polycystic ovary syndrome (PCOS) is the most common but least understood endocrinopathy. Although a major genetic contribution is suspected, there have been no clear genes or family of genes identified that cause or contribute to PCOS. This may be due to both the difficulty in phenotyping as well as the limited genotyping studies that have been performed to date. The diagnosis of PCOS has traditionally been based on a history of *oligomenorrhea* and/or *hyperandrogenism*, either clinical, i.e., most commonly hirsutism, or biochemical, i.e., elevated circulating total or bioavailable androgens; and/or *polycystic ovaries*. The criteria that emerged from the 1990 National Institute of Child and Human Development (NIH-NICHD) conference identified PCOS as unexplained hyperandrogenic chronic anovulation, making it in essence a diagnosis of exclusion (Zawadski and Dunaif 1992). The "consensus" definition did not include the polycystic ovary morphology, most commonly today found on ultrasound consisting of multiple 2–8 mm subcapsular preantral follicles and increased ovarian volume (Balen *et al.* 2003). These ultrasound criteria were recently incorporated in the revised 2003 Rotterdam criteria which require two out of the three above cardinal stigmata for PCOS: oligomenorrhea, hyperandrogenism, and/or polycystic ovaries (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

None of these expert-generated definitions include insulin resistance, a common but not inevitable finding in PCOS. This diagnostic dilemma has hampered clinical and genetic studies of PCOS. The larger the number of distinct phenotypes within the affected category, the more complex the genetic analysis and the greater the likelihood that investigators using different diagnostic criteria will

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arrive at different conclusions. Other factors that contribute to difficulty in performing genetic studies in PCOS include the associated infertility and low fecundity (? selection bias against the transmission of PCOS genes). Thus, it is rare to find large pedigrees with multiple affected women with which to perform linkage analysis.

Another difficulty is assigning phenotypes to premenarchal girls and postmenopausal women, which also lacks consensus criteria, a problem that also limits the utilization of pedigrees. Although a male phenotype has been postulated, there are no rigorously established clinical or biochemical features that can be used to identify "PCOS males." This makes formal segregation analysis as well as genetic linkage studies more difficult. Finally, the lack of animals that spontaneously develop a PCOS-like phenotype, especially mice, precludes the use of powerful tools of genetic mapping.

The idea that PCOS is a heritable disease is supported by studies documenting ethnic predisposition, familial aggregation, concordant twin studies, and association with other mendelian disorders. This chapter will review the evidence that PCOS is a genetic disease and briefly overview the largely *negative* genetic marker data that have emerged in the last decade.

Ethnic predisposition

There have unfortunately only been limited studies of the prevalence of PCOS and their ethnic influences. Several studies have examined the prevalence of stigmata of PCOS in unselected populations from developed countries and found a similar prevalence of the disorder ranging from 5% to 8%. These include screening a group of women who have presented as prospective employees at a university hospital in the United States (Azziz *et al.* 2004), a group of women from an isolated Greek island (Diamanti-Kandarakis *et al.* 1999), and a group of Spanish women (Escobar-Morreale *et al.* 2000). Despite similar rates of affliction, there appear to be ethnic differences in prevalence and severity of the phenotype.

PCOS may be more common among African Americans than Caucasians, based on a prevalence study among 400 US women (Azziz *et al.* 2004), (8% vs. 4.8%), although it did not in this sample size reach statistical significance. In regards to ethnic variation, Asian women with PCOS may have similar circulating levels of androgens as other ethnic groups, but little hirsutism (Carmina *et al.* 1992). This may be due to differing peripheral tissue specific expression of genes that modify androgen action (such as decreased 5-α reductase expression, and therefore lower intracellular levels of potent non-aromatizable androgens). Insulin resistance appears more common among

certain ethnic groups, for instance those of Latino (Kauffman *et al.* 2002), Caribbean (Dunaif *et al.* 1993), or South Asian origin (Norman *et al.* 1995, Wijeyaratne *et al.* 2002) compared to Caucasians. These ethnic differences in phenotype suggest a link to inherited alleles that influence the expression of the trait.

Familial aggregation

The foundation of genetic studies is the evidence that disease clusters in families. Although familial clustering may also occasionally have environmental causes (selective exposure to the putative agent differentially affects family members), there has been no environmental endocrine disruptor identified to date that causes PCOS. None of the existing family studies of PCOS convincingly establishes a mode of inheritance because the number of families studied was too small; the parental phenotypes could not be firmly established; and the male phenotype is uncertain. Despite the heterogeneity in study design and the inability to obtain comprehensive phenotype information to permit a formal segregation analysis, collectively the existing literature strongly suggests the clustering of PCOS in families with a mode of inheritance consistent with an autosomal dominant pattern (Table 3.1). This suggests a publication bias as subsequent analyses of genetic markers utilizing larger sample sizes often utilize a non-Mendelian model mode of genetic analysis (see below).

These studies need to be critically judged based on their strengths and weaknesses. Diagnostic criteria used to assign affected status differed among the studies as did the methods with which the status of first- and second-degree relatives were ascertained (Table 3.1). By and large, ovarian morphology determined from tissue biopsy, direct visualization or diagnostic imaging, in association with menstrual disturbances and evidence for hyperandrogenism have been used in most studies as the criteria for diagnosing PCOS in probands. Earlier studies tended to characterize relatives on the basis of questionnaires, whereas later studies have focused on more intensive phenotyping.

These studies strongly support the clustering of reproductive abnormalities such as clinical and biochemical hyperandrogenism, polycystic ovaries, and to a lesser degree oligomenorrhea in first-degree relatives. Similarly there appears to be an associated increased prevalence of biochemical insulin resistance and/or hyperinsulinemia, in first-degree relatives. In one study 50% of sisters of women diagnosed with PCOS had elevated total or bioavailable testosterone levels, suggesting that hyperandrogenemia is a common trait, and a bimodal distribution suggesting a dominant form of inheritance (Legro *et al.* 1998) (Fig. 3.1).

Table 3.1. Summary of diagnostic criteria for the proband in familial studies of PCOS and proposed mode of inheritance

Author	Diagnostic criteria for PCOS	Number studied	Mode of inheritance/familial cluster
Cooper *et al.* (1968)	Oligomenorrhea, hirsutism, and polycystic ovaries (by culdoscopy, gynecography or wedge resection)	18 PCOS women and their first degree relatives and a control group	Autosomal dominant with reduced penetrance
Wilroy *et al.* (1975)	Oligomenorrhea, hirsutism, and polycystic ovaries (examination and surgery)	3 multi-generation kindreds	(?X-linked) dominant
Ferriman and Purdie (1979)	Hirsutism and/or oligomenorrhea, 60% with polycystic ovaries (by air contrast gynecography)	381 PCOS women and relatives and a control group	Modified dominant
Hague *et al.* (1988)	Clinical symptoms (menstrual dysfunction, hyperandrogenism, obesity, and infertility) and polycystic ovaries by transabdominal ultrasound	50 PCOS women and 17 women with congenital adrenal hyperplasia and a control group	Segregation ratios exceeded autosomal dominant pattern
Lunde *et al.* (1989)	Clinical symptoms (menstrual irregularities, hirsutism, infertility, and obesity) and multicystic ovaries on wedge resection	132 PCOS women and first- and second-degree relatives and a control group	Unclear, most consistent with autosomal dominant
Carey *et al.* (1993)	Polycystic ovaries (by transabdominal ultrasound)	10 kindreds and 62 relatives	Autosomal dominant with 90% penetrance
Norman *et al.* (1996)	Elevated androgens, decreased sex hormone binding globulin (SHBG), and polycystic ovaries on ultrasound	5 families with 24 females and 8 males	Not stated; metabolic abnormalities associated with insulin resistance in families

Table 3.1. (cont.)

Author	Diagnostic criteria for PCOS	Number studied	Mode of inheritance/familial cluster
Legro *et al.* (1998)	Elevated testosterone levels combined with oligomenorrhea (≤6 menses/yr)	80 PCOS probands and 115 sisters	Hyperandrogenemia consistent with an autosomal dominant trait
Govind *et al.* (1999)	Polycystic ovary morphology on ultrasonograpy	29 families with 53 sisters and 18 brothers	Autosomal dominant
Kahsar-Millar *et al.* (2001)	Oligomenorrhea and either hirsutism or elevated testosterone levels	90 PCOS probands, 50 sisters, and 78 mothers	Increased prevalence of symptoms in first-degree relatives suggesting genetic trait
Legro *et al.* (2002a)	Elevated testosterone levels combined with oligomenorrhea (≤6 menses/yr)	346 PCOS probands, 307 sisters	Increased prevalence of hyperinsulinemia in sisters segregating with hyperandrogenemia
Legro *et al.* (2002b)	Elevated testosterone levels combined with oligomenorrhea (≤6 menses/yr)	87 PCOS probands, 119 brothers	Increased levels of dehydroepiandrosterone sulfate (DHEAS) in brothers
Sir-Petermann *et al.* (2002)	Hyperandrogenism (elevated free androgen index or hirsutism) and oligomenorrhea	106 PCOS probands, 200 parents	Increased diabetes (about twofold) and insulin resistance among PCOS parents
Yildiz *et al.* (2003)	Elevated testosterone levels and oligomenorrhea	52 PCOS probands, 102 first-degree relatives	Elevated testosterone levels in sisters and mothers, increased rates of glucose intolerance in first-degree family members
Kaushal *et al.* (2004)	Polycystic ovary morphology on ultrasonograpy	20 PCO probands and 20 brothers	Increased prevalence of insulin resistance and endothelial dysfunction in brothers

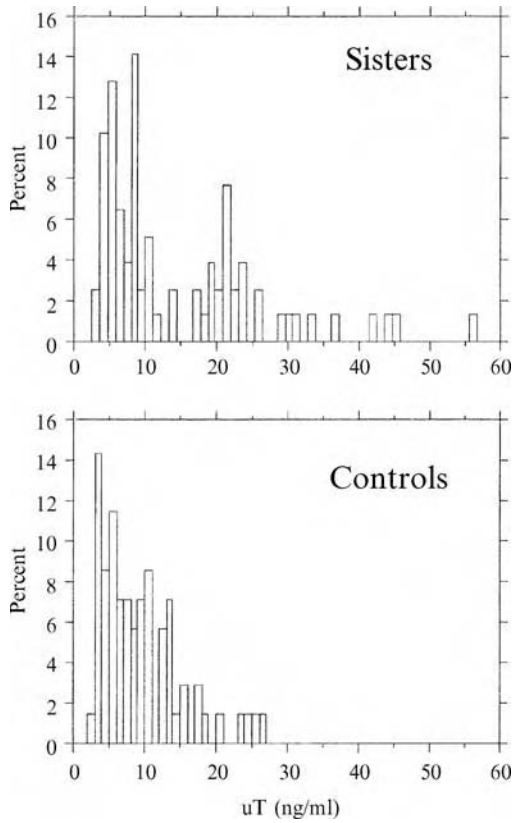

Fig. 3.1 Frequency distribution of testosterone levels (uT = unbound testosterone) in sisters of PCOS probands ($n = 78$; upper panel) and control women ($n = 70$; lower panel). The distribution of the sisters appears to be bimodal. Adapted from Legro *et al.* (1998).

Twin studies

There has been a paucity of studies examining twins affected with PCOS. Twin studies offer a method for examining the relative contributions of genes and environment by comparing the prevalence and penetrance of traits in monozygotic (MZ) to that in dizygotic (DZ) twins. A higher prevalence and more complete penetrance in MZ compared to DZ twins is strongly supportive of a trait under genetic influence. A larger twin study from Australia by Jahanfar *et al.* (1995) reported on twins, both MZ and DZ, who were studied with ultrasound as well as clinical and biochemical parameters. Ethnicity of twins and controls was not discussed. From a starting population of 500 female–female twins who were contacted to participate, eventually only 34 pairs were analyzed. There was

also an unusually high incidence of polycystic ovaries on ultrasound with 50% of the study population affected. The initial study of Jahanfar *et al.* (1995) however noted a high degree of discordance among the twins for polycystic ovaries on ultrasound. This study suggested that PCOS may have a more complex inheritance pattern than autosomal dominant, perhaps X-linked or polygenic. It also suggested that environmental factors may play a significant role. There also appeared to be a significant genetic component to the fasting insulin level, further supporting the familial studies noted above suggesting insulin resistance is a familial trait. Another subsequent twin study that included other features of the syndrome (hyperandrogenism and chronic anovulation) found an overall prevalence of PCOS of 16% among a larger sample of twins, much lower than the prevalence of polycystic ovaries (Jahanfar *et al.* 2004).

Associated mendelian disorders

There are other mendelian disorders that can have polycystic ovaries, hyperandrogenism, and chronic anovulation. The best example of this is non-classical congenital adrenal hyperplasia (NCAH), one of the diagnoses to exclude before arriving at PCOS. NCAH, also referred to as late-onset congenital adrenal hyperplasia, is a homozygous recessive disorder most commonly due to mutations in the *CYP21* gene which results in an abnormal (or absent) activity. This leads to a relative decrease in cortisol and a shift towards the production of androgens. Overall, between 1% and 8% of women with androgen excess have CYP21-deficient NCAH depending on ethnicity, with the highest rates reported in Ashkenazi Jewish populations (Azziz *et al.* 1994). This disorder can present post-menarche in a similar indolent fashion as PCOS. Patients with NCAH may present only with persistent acne or may have moderate degrees of hirsutism and oligoamenorrhea, although frank virilization or even severe hirsutism is relatively rare (Moran *et al.* 2000).

Another disorder that may (but not inevitably) have these features, as well as marked insulin resistance and compensatory hyperinsulinemia, is partial lipodystrophy (Garg 2004). Patients with this disorder have a normal fat distribution in early childhood, but with the onset of puberty, marked compartmental shifts occur. Subcutaneous adipose tissue gradually disappears from the extremities and the gluteal and truncal regions, resulting in a muscular appearance, more striking in females. Simultaneously, adipose tissue may accumulate in other regions, such as on the face and neck, causing a double chin, fat neck, or cushingoid appearance. Adipose tissue may also accumulate in the axillae, back, labia majora, and intra-abdominal region. Affected patients may develop acanthosis nigricans, glucose intolerance and diabetes mellitus, and dyslipidemia consistent with

insulin resistance (decreased high-density lipoprotein cholesterol (HDL-C) and increased triglycerides (TTG) levels). A variety of genes have been found to cause this phenotype including mutations in the peroxisome proliferators-activated receptor (PPAR-γ) gene (Hegele 2005), and in the lamin (nuclear matrix protein) A/C gene (Vigouroux and Capeau 2005). Recently a high prevalence of PCOS was found in one family with pyloric stenosis, endometriosis, and breast cancer (Liede *et al.* 2000).

Modes of genetic analysis utilized in PCOS studies

Association studies

Association studies are case control studies that test whether a particular allele occurs at a higher frequency among affected rather than unaffected individuals. They involve correlation within a population, and not the inheritance of alleles within a family (Lander and Schork 1994), and therefore family members are not required to participate. Association studies are most commonly performed with alleles of a gene thought to have biological significance to the etiology of the complex trait or where familial based linkage studies have shown linkage disequilibrium. This does not preclude their use in studying random alleles of seemingly unrelated genes. The analysis is usually straightforward involving a 2×2 table, and chi square values can be obtained. Each association test may be considered independent of another and statistical correction is necessary to control for this (Lander and Schork 1994). To achieve statistical significance if one were testing 100 markers with six alleles each, the p value with this correction would have to be less than 0.000001. Moreover, post-hoc division of phenotypes to better fit tentative genetic association is not appropriate.

Association studies have been criticized by a number of researchers for their tendency to highlight positive results leading to a publication bias, and the tendency to over-interpret these results as identifying etiologic genes (Lander and Schork 1994, Cardon and Bell 2001). This has certainly been the case with PCOS where innumerable negative and positive associations have been reported (Legro and Strauss 2002). Association studies have provided a number of potential loci with genetic variants that may create or add to a PCOS phenotype (many of these borrowed from similar studies in families with type 2 diabetes), including to mention a few such genes as *Calpain 10* (Ehrmann *et al.* 2003), *IRS-1* and *-2* (El Mkadem *et al.* 2001), and *SHBG* (Cousin *et al.* 2004). Positive associations between a candidate allele and a complex disease are subject to many potential errors (Table 3.2). One of the most common is that smaller sample sizes are more likely to yield positive relationships which do not hold up when the sample size increases, as we have seen in PCOS with a *CYP17* (which encodes a key enzyme

Table 3.2. Common flaws in genetic association studies

1	Small sample size
2	Subgroup analysis and multiple comparisons
3	Poorly matched control group
4	Failure to attempt study replication
5	Failure to detect linkage disequilibrium with adjacent loci

Source: Adapted from Lander and Schork 1994.

involved in androgen biosynthesis) polymorphism (Carey *et al.* 1993), which are not replicated even by the same group in independent data sets (Gharani *et al.* 1996). Population stratification between cases and controls is another problem. Failure to match cases with controls on the basis of racial background can lead to spurious results based on racial differences in allele frequencies unrelated to disease status. To control for multiple comparisons and post-hoc justification of phenotypes, clear a priori phenotypes should be established. Finally an association may exist, but not because the disease allele is the chosen candidate gene, but because the allele is in linkage disequilibrium with a nearby gene. Thus the association is real, only the gene of interest is wrong.

Family-based association studies

To control for genetic mismatching in case control studies, Spielman and Ewens (1996) have suggested further assessment of positive associations with a transmission disequilibrium test (TDT). This requires the genotyping of both parents for, preferably, heterozygous alleles as well as having affected and unaffected offspring. The parent who is heterozygous for this allele should more often transmit the disease allele to the affected offspring than the other allele(s). Chi-square testing can document increased transmission of putative disease markers. Family-based tests, such as this one, provide for an appropriate "internal control" with the use of unaffected siblings, whose environment and overall genetic background (i.e., same ethnic and family group) are as closely similar to the proband as possible.

Linkage analysis

Finally there are modes of linkage analysis, which use DNA sequence polymorphisms (normal variants) that are near or within a gene of interest to track within a family the inheritance of a disease-causing mutation in that gene. These have classically been considered the most powerful means of identifying disease genes, and have the most complex forms of analyses. Parametric methods assume

a specific model of inheritance (i.e., autosomal recessive, etc.), whereas non-parametric methods do not. The non-model based affected relative pair (ARP) analyses (sib pairs, cousin pairs, grandparent–child etc.) have the advantage of not specifying a genetic model and of eliminating the problem of misclassifying young or "non-penetrant" relatives, who are clinically unaffected but may carry the trait allele. Model-based analyses have the advantage of perhaps being more powerful and of allowing for the incorporation of variables such as the estimate of heterogeneity. The difficulty in assigning phenotypes to men as well as premenopausal girls, postmenopausal women, and women of reproductive age on confounding medications or who have undergone oophorectomy or hysterectomy resulting in a lack of solid segregation analyses in PCOS families has hindered parametric analyses. Non-model-based analyses, such as affected sib pair (ASP) analysis, are particularly appealing in PCOS kindreds. This examines the inheritance of disease alleles by identity by descent (IBD). The mathematical analyses in ASP focus on sharing of inherited alleles.

Pitfalls of genetic analyses in PCOS

Studies to date seeking genetic markers of PCOS have been characterized by the identification of candidate genes or gene regions through one of the methods discussed above that appear initially to be associated with the PCOS phenotype (i.e., statistically significant), but that upon further study of independent data sets by the same group or replication studies by other groups lose their significance. This is a common occurrence with complex mendelian disorders. Some of the pitfalls with specific genes will be illustrated by the following examples.

Candidate genes involved in gonadotropin action and regulation

Abnormalities in gonadotropin secretion, particularly LH, are characteristic of PCOS. Since LH plays a permissive role in driving thecal androgen production, there has been interest in exploring genes related to the regulation of LH secretion, LH bioactivity, and LH action.

Follistatin

Follistatin, an activin binding protein, is widely expressed, as is activin, and could play an important role in PCOS. Activin stimulates pituitary follicle stimulating hormone (FSH) secretion and acts directly on the ovary to promote follicular maturation. Neutralization of activin would be expected to cause reductions in FSH levels and arrested follicular maturation as occurs in transgenic mice overexpressing follistatin. In addition, activin inhibits thecal androgen biosynthesis

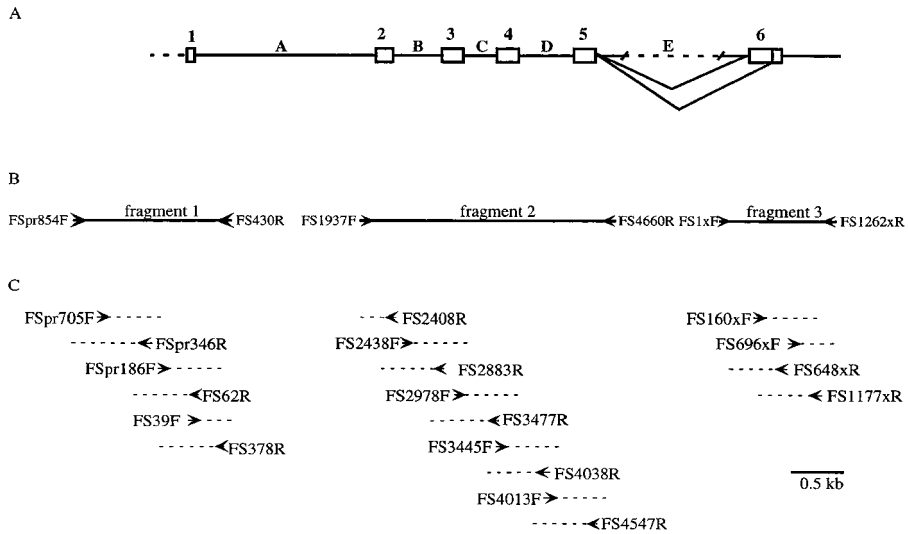

Fig. 3.2 Sequencing strategy for follistatin gene which revealed no follistatin mutations in PCOS. (A) Diagram of follistatin gene. Exons are shown with open boxes. Intronic sequences are indicated with a solid line, and previously unpublished sequences are indicated with a dashed line. Exons are labeled with Arabic numerals, and introns are labeled with uppercase letters. (B) Sequencing templates. The primers used to generate sequencing templates from genomic DNA are indicated by arrows; and the expected polymerase chain reaction (PCR) products by a solid line. (C) Sequencing primers. The approximate annealing position and orientation of each primer are indicated. The expected amount of sequence obtained from each primer is shown with a dashed line. Adapted from Urbanek *et al.* (2000).

and its removal by binding to follistatin might lead to unrestrained theca androgen synthesis driven by luteinizing hormone (LH). The follistatin locus emerged as a promising candidate from the study of 39 affected sib pairs (Urbanek *et al.* 1999), although the strength of the link faded in a larger follow-up series that included detailed sequence analysis of the follistatin gene (Urbanek *et al.* 2000) (Fig. 3.2). The follistatin gene has also not been found to be linked to PCOS in studies by other investigators.

Candidate genes involved in steroid hormone synthesis and action

Abnormalities in ovarian steroidogenesis, particularly androgen production, are a prominent feature of PCOS. As noted above, there has been intensive interest in the regulation of the gene encoding the gateway to androgen synthesis, 17α-hydroxylase/17,20-lyase, encoded by the *CYP17* gene. However, studies on isolated thecal cells from ovaries of women with PCOS revealed alterations in

steroidogenesis that extend beyond 17α-hydroxylase/17,20-lyase activity and include increased progesterone production suggesting other defects or a more global defect (Gilling-Smith *et al.* 1994, Nelson *et al.* 1999).

The *CYP11A gene*

The rate-limiting step in sex steroid biosynthesis is determined by the activity of side-chain cleavage encoded by $P450_{scc}$ encoded by the *CYP11A* gene. Gharani *et al.* (1997) examined 20 families with multiple affected women and found evidence for weak linkage to the *CYP11A* locus. An association study conducted on 97 PCOS women and matched controls revealed significant association of a pentanucleotide repeat (TTTTA)*n* polymorphism at position 528 from the ATG initiation codon in the 5′ region of the *CYP11A* gene which encodes the cholesterol side-chain cleavage enzyme and total serum testosterone levels (Diamanti-Kandarakis *et al.* 2000). Although no regulatory role has been assigned to this polymorphism in terms of *CYP11A* gene transcription, the investigators suggested that allelic variants of the *CYP11A* gene have a role in the hyperandrogenemia of PCOS. This conclusion was supported by another study that found an association between at least four repeats and PCOS, and within the PCOS group an association of this allele group with a lower testosterone level (San Millan *et al.* 2001). In a study with a larger sample size, Urbanek *et al.* (1999) found modestly increased sharing of alleles at the *CYP11A* locus among affected sib pairs, but the sharing was not statistically significant. Most recently the original group in a larger replication study, using both case control and family-based association methods, report no association with the *CYP11A* gene (Gaasenbeek *et al.* 2004).

Candidate genes involved in insulin action

The common occurrence of insulin resistance and pancreatic beta cell dysfunction in association with PCOS and the increased risk for development of type 2 diabetes mellitus is now well recognized. This has led investigators to focus on insulin resistance as a potential central abnormality in PCOS, especially since this defect through a variety of mechanisms can contribute to hyperandrogenism and chronic anovulation. A number of genes have been explored in this area, none particularly fruitful to date.

Insulin gene

Waterworth *et al.* (1997) examined the role of a variable number tandem repeat (VNTR) in the 5′ region of the insulin gene in PCOS. This locus has a bimodal distribution of repeats which have been divided into class I alleles (averaging 40 repeats) and class III alleles with a larger number of repeats (average 157).

Linkage of PCOS to this locus was examined in 17 families in which there were multiple individuals affected with polycystic ovaries on ultrasound scan or premature male balding (before age 30) and the association of these alleles with polycystic ovaries in two additional populations. The authors found that the class III allele was preferentially transmitted from heterozygous fathers but not from mothers to affected individuals. Linkage analysis suggested increased sharing of the locus in affected sibs. The authors concluded that they had discovered strong linkage and association between alleles at the insulin gene 5′ VNTR and PCOS. However, others reviewing the study raised concerns regarding data analysis and use of the program to identify linkage (McKeigue and Wild 1997). Moreover, Urbanek *et al.* found no evidence for linkage of the insulin gene in their analysis or of association between the class III alleles of the insulin VNTR and hyperandrogenemia. Most recently the original group in another larger replication study found no association between this VNTR and PCOS (Powell *et al.* 2005).

Insulin receptor

Mutations in the insulin receptor gene cause severe insulin resistance (the type A syndrome) associated with acanthosis nigricans and PCOS. Yet, a number of studies have examined the insulin receptor gene sequence in PCOS women with and without insulin resistance with negative results (Sorbara *et al.* 1994, Talbot *et al.* 1996). However, genes nearby the insulin receptor may contribute to PCOS. Two studies have found linkage or association between a marker (*D19S884* at 19p13.3) that is located 2 megabases centromeric from the insulin receptor and PCOS (Urbanek *et al.* 1999, Tucci *et al.* 2001). Because two independent studies suggested that this region near the insulin receptor is associated with PCOS, this locus emerges as a strong candidate region deserving of detailed investigation for the identification of the putative PCOS gene.

Summary

Phenotype confusion has characterized genetic studies of PCOS. Although several loci have been proposed as PCOS genes including *CYP11A*, the insulin gene, the follistatin gene, and a region near the insulin receptor, the evidence supporting linkage is not overwhelming. The strongest case can be made for the region near the insulin receptor gene, as it has been identified in two separate studies, and perhaps most importantly has not yet been refuted by larger studies. However, the responsible gene at chromosome 19p13.3 remains to be identified. Because they are rare, and their full impact on the phenotype incompletely understood, routine screening of women with PCOS or stigmata of PCOS for these genetic variants is not indicated at this time.

Thus there have been many tantalizing starts, but no finishes identifying genes associated with PCOS. This may be partially due to the fact that the majority of genes studied have been candidate genes based on current understanding of the pathophysiology of PCOS. Given that the pathophysiology is poorly understood, it is not surprising that these candidates have yielded little. No one to date has performed a genome-wide scan on an adequate sample size to discover new genes and regions unrelated to the expert's best guesses, although these studies are now in progress. Thus the search for PCOS genes is still in its infancy and it is premature to close or open the door on the genetics of PCOS.

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4

The pathology of the polycystic ovary syndrome

Andrew G. Östör

Introduction

Although the condition of polycystic ovaries has been known for a long time (see Chapter 2) it achieved prominence by the seminal article of Stein and Leventhal in 1935 and for many years was referred to as the Stein–Leventhal syndrome. Given the efflux of time and a concerted effort to discourage eponyms (Östör and Phillips 1999), the condition became known as polycystic ovary disease (PCO).

PCO is a clinicopathological syndrome characterized by anovulation or infrequent ovulation, obesity, hirsutism, and numerous follicular cysts in both ovaries, which are usually enlarged (Yen 1980, Scully *et al.* 1998). The finding of polycystic ovaries, however, does not, per se, warrant such a diagnosis. Polycystic ovaries are, in fact, more common in otherwise normal women (unpublished observation). This contention is supported by ultrasonographic studies which have revealed an overlap between women with PCO and overt clinical manifestations of the syndrome and those with multiple follicular cysts associated with menstrual irregularity or evidence of hyperandrogenism so minor that they considered themselves normal (Adams *et al.* 1986). In other studies of "normal" women, more than 20% had polycystic ovaries on ultrasound (Polson *et al.* 1988, Clayton *et al.* 1992). Thus, the boundary between the clinical syndrome associated with PCO and normality is blurred.

Macroscopic features

Typically both ovaries, rarely one (Futterweit 1985), are rounded and enlarged. In one study the ovarian volume was three times that of controls (Delahunt *et al.* 1975, Lunde *et al.* 1988). Occasionally, they are of normal size (Smith *et al.* 1965).

Polycystic Ovary Syndrome, 2nd edn, ed. Gabor T. Kovacs and Robert Norman. Published by Cambridge University Press.

Fig. 4.1 Intraoperative photograph showing enlarged rounded ovaries with an oyster-white appearance.

Classically, the surface of the ovaries is oyster-white, giving the appearance of a true capsule (Fig. 4.1). Small cysts are visible under the surface where they appear as sago-like bodies. Sectioning reveals a thickened, white, superficial cortex and the presence of numerous cystic follicles, usually less than 10 mm in size (Fig. 4.2), near the surface, while the central portion consists of homogeneous stroma with only rare or no stigmata of ovulation, e.g., corpora lutea or albicantes (Green and Goldzieher 1965, Biggs 1981).

Microscopic features

The thickened cortex is hypocellular, fibrotic, and sclerosed, and contains primordial follicles which appear "entrapped." The latter, however, are normal in number and morphology (Fig. 4.3). Thick-walled blood vessels may also be seen (Goldzieher and Green 1962, Hughesdon 1982). Tongues of similarly fibrotic stroma may extend into the deeper cortex. The cystic follicles are typically lined by several layers of non-luteinized granulosa cells (Fig. 4.4) that may have focally exfoliated. An outer layer of luteinized theca interna cells is sometimes referred to

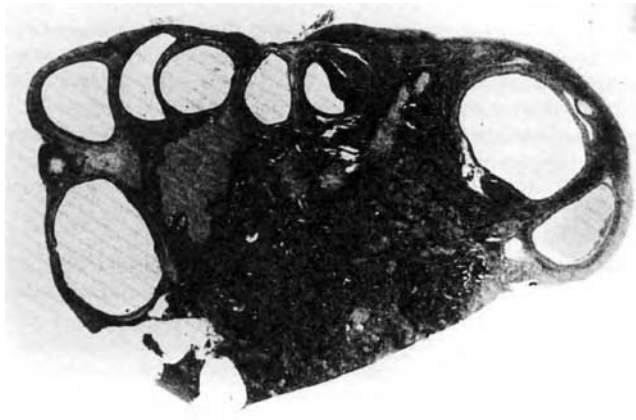

Fig. 4.2 Whole mount section of a polycystic ovary showing numerous follicular cysts. Note absence of a corpus luteum or corpora albicantes.

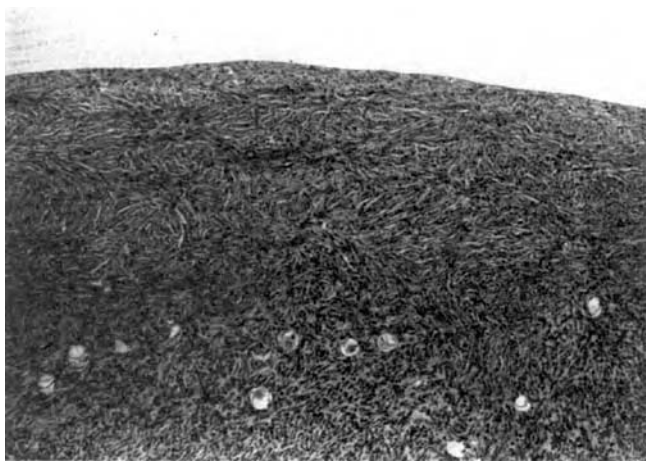

Fig. 4.3 Microphotograph showing a thickened and sclerosed cortical stroma containing "entrapped" primordial follicles.

as "follicular hyperthecosis." It should be stressed that cystic follicles in patients with PCO differ from those in normal women only in their increased number (Green and Goldzieher 1965, Lunde *et al.* 1988). In fact a doubling of ripening follicles and subsequent atretic follicles is generally seen. It may be appropriate to define the various forms of follicular development at this point. The terms *primordial*, *primary*, *secondary*, and *tertiary follicle* refer to follicles with a single layer of flat granulosa cells, a single layer of cuboidal granulosa cells, two or more layers of granulosa cells without an antrum, and the same with an antrum,

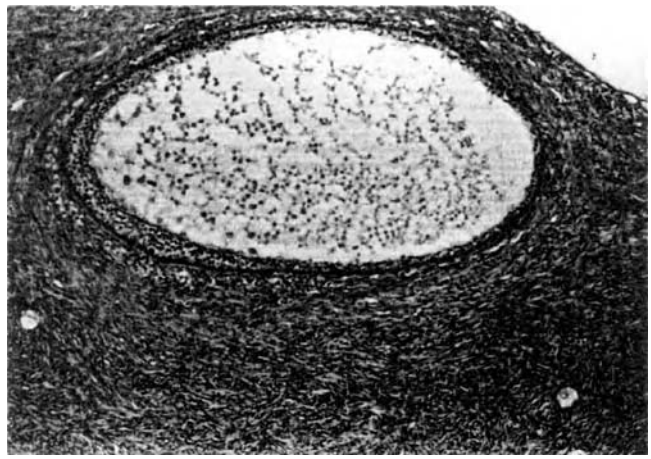

Fig. 4.4 Microphotograph of the lining of a follicular cyst. Note that the wall is composed of an inner layer of non-luteinized granulosa cells and an outer layer of luteinized theca cells.

regardless of size, respectively. *Tertiary*, *antral*, and *graafian* follicles are used interchangeably whilst a *cystic follicle* means their macroscopically visible forms without implying abnormality (Hughesdon 1982). The deeper cortical and medullary stroma may have up to a fivefold increase in volume and may contain luteinized stromal cells and foci of smooth muscle. Nests of hilus (Leydig) cells may be more numerous in patients with PCO than in age-matched controls (Hughesdon 1982). As noted, stigmata of prior ovulation are typically absent but corpora lutea have been reported in up to 30% of cases in some series (Green and Goldzieher 1965, Hughesdon 1982). Examination of only a few sections of wedge biopsies often fails to reveal diagnostic features.

The endometrium in patients with PCO may show weakly proliferative features, simple hyperplasia, atypical hyperplasia, or in less than 5% of cases, adenocarinoma, which is almost always low grade (Massachusetts General Hospital 1966, Coulam *et al.* 1983).

Differential diagnosis

Polycystic ovaries may be encountered in prepubertal children, in otherwise normal girls in the first few years after the onset of puberty (Merrill 1963), in girls in the second decade with primary hypothyroidism (Lindsay *et al.* 1980), adrenal hyperplasia (Benedict *et al.* 1962, Goldzieher 1981, Chrousos *et al.* 1982), autoimmune oophoritis (Bannatyne *et al.* 1990), after long-term use of oral contraceptives (Plate 1967), in association with periovarian adhesions (Quan *et al.* 1963), and after long-term androgen therapy in female-to-male transsexuals

(Pache *et al.* 1991). It seems likely, however, that these conditions are coincidental.

Another condition to be considered in the differential diagnosis is stromal hyperthecosis, which some authorities feel represents one end of the spectrum of PCO (Givens 1977). In this disorder luteinized cells are scattered singly and in small nests or nodules throughout a typically hyperplastic ovarian stroma (Scully *et al.* 1998). Although there is a fair degree of overlap clinically between the two, the pathological features are sufficiently different not to pose a major diagnostic difficulty.

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5

Imaging polycystic ovaries

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Introduction

The need for a calibrated imaging of polycystic ovaries (PCO) is now stronger than ever since the recent consensus conference held in Rotterdam in 2003. Indeed, the subjective criteria that were proposed 20 years ago and still used until recently by the vast majority of authors are now replaced by a stringent definition using objective criteria (Balen *et al.* 2003, The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

Imaging PCO is not an easy procedure. It requires a thorough technical and medical background. The goal of this chapter is to provide the reader with the main issues ensuring a well-controlled imaging for the diagnosis of PCO. Two-dimensional (2D) ultrasonography will be first and extensively addressed since it remains the standard for imaging PCO. Other techniques such as Doppler, three-dimensional ultrasonography, and magnetic resonance imaging (MRI) will be then more briefly described.

Two-dimensional (2D) ultrasonography: technical aspects and recommendations

The transabdominal route should always be the first step of pelvic sonographic examination, followed by the transvaginal route, except in virgin or refusing patients. Of course, a full bladder is required for visualization of the ovaries. However, one should be cautious that an overfilled bladder can compress the ovaries, yielding a falsely increased length. The main advantage of the abdominal route is that it offers a panoramic view of the pelvic cavity. Therefore, it allows

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excluding associated uterine or ovarian abnormalities with an abdominal component. Indeed, lesions with cranial growth could be missed by using the transvaginal approach exclusively.

With the transvaginal route, high-frequency probes (>6 MHz) with a better spatial resolution but a less examination depth can be used because the ovaries are close to the vagina and/or the uterus and because the presence of fatty tissue is usually less disturbing (except when very abundant). With this technique, not only the size and the shape of ovaries can be visualized but also their internal structure, namely follicles and stroma. It is now possible to get pictures that have a definition close to anatomical cuts. However, evaluation of the ovarian size via the transvaginal approach is difficult. To maximize accuracy, one must choose meticulously the picture in which the ovary appears the longest and the widest. This picture must then be frozen. Two means can be proposed for calculating the ovarian area: either fitting an ellipse to the ovary from which the area is given by the machine, or outlining by hand the ovary with automatic calculation of the outlined area. This last technique must be preferred in cases of non-ellipsoid ovaries, as sometimes observed. The volume is the most complete approach. Traditionally, it can be estimated from the measurement of the length (L), width (W), and thickness (T) and the use of the classical formula for a prolate ellipsoid: $L \times W \times T \times 0.523$ (Sample *et al.* 1977, Adams *et al.* 1985, Orsini *et al.* 1985). However, the ovaries have to be studied in three orthogonal planes, a condition that is not always respected. Three-dimensional (3D) ultrasonography is an attractive alternative for the accurate assessment of ovarian volume but this technique is not commonly available (see below).

In order to count the total number of "cysts" (in fact, follicles) and to evaluate their size and position, each ovary should be scanned in longitudinal and/or transversal cross-section from the inner to outer margins.

The consensual definition of PCO

According to the literature review dealing with all available imaging systems and to the discussion at the joint ASRM/ESHRE Consensus Meeting on PCOS held in Rotterdam in May 2003, the current consensus definition of PCO is the following: either 12 or more follicles measuring 2–9 mm in diameter and/or increased ovarian volume (>10cm^3) (Table 5.1).

Priority was given to ovarian volume and to follicle number because both have the advantage of being physical entities that can be measured in real-time conditions and because both are still considered as the key and consistent features of PCO.

Table 5.1. Ultrasound assessment of the polycystic ovary (PCO): international consensus definitions

1. The PCO should have at least one of the following: either 12 or more follicles measuring 2–9 mm in diameter or increased ovarian volume (>10 cm^3). If there is evidence of a dominant follicle (>10 mm) or a corpus luteum, the scan should be repeated during the next cycle.
2. The subjective appearance of PCOs should not be substituted for this definition. The follicle distribution should be omitted as well as the increase in stromal echogenicity and/or volume. Although the latter is specific to PCO, it has been shown that measurement of the ovarian volume is a good surrogate for the quantification of the stroma in clinical practice.
3. Only one ovary fitting this definition or a single occurrence of one of the above criteria is sufficient to define the PCO. If there is evidence of a dominant follicle (>10 mm) or corpus luteum, the scan should be repeated next cycle. The presence of an abnormal cyst or ovarian asymmetry, which may suggest a homogeneous cyst, necessitates further investigation.
4. This definition does not apply to women taking the oral contraceptive pill, as ovarian size is reduced, even though the "polycystic" appearance may persist.
5. A woman having PCO in the absence of an ovulation disorder or hyperandrogenism ("asymptomatic PCO") should not be considered as having PCOS, until more is known about this situation.
6. In addition to its role in the definition of PCO, ultrasound is helpful to predict fertility outcome in patients with PCOS (response to clomiphene citrate, risk for ovarian hyperstimulation syndrome (OHSS), decision for in vitro maturation of oocytes). It is recognized that the appearance of polycystic ovaries may be seen in women undergoing ovarian stimulation for in vitro fertilization (IVF) in the absence of overt signs of the polycystic ovary syndrome. Ultrasound also provides the opportunity to screen for endometrial hyperplasia.
7. The following technical recommendations should be respected:
   - State-of-the-art equipment is required and should be operated by appropriately trained personnel.
   - Whenever possible, the transvaginal approach should be preferred, particularly in obese patients.
   - Regularly menstruating women should be scanned in the early follicular phase (days 3–5). Oligo-/amenorrhoeic women should be scanned either at random or between days 3–5 after a progestogen-induced bleed.
   - If there is evidence of a dominant follicle (>10 mm) or a corpus luteum, the scan should be repeated the next cycle.
   - Calculation of ovarian volume is performed using the simplified formula for a prolate ellipsoid (0.5 × length × width × thickness).
   - Follicle number should be estimated both in longitudinal, transverse and antero-posterior cross-sections of the ovaries. Follicle size should be expressed as the mean of the diameters measured in the three sections.

The usefulness of 3D ultrasound, Doppler or MRI for the definition of PCO has not been sufficiently ascertained to date and should be confined to research studies.

Source: Reproduced from The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004), with permission.

Increased ovarian volume

Many studies have reported an increased mean ovarian volume in a series of patients with polycystic ovary syndrome (PCOS) (Table 5.2). However, the upper normal limit of the ovarian volume suffers from some variability in the literature (from 8 to 15.6 cm^3) (Table 5.2). Such variability may be explained by:

- the small number of controls in some studies;
- and/or differences in inclusion or exclusion criteria for control women;
- and/or operator-dependent technical reasons: it is difficult indeed to obtain strictly longitudinal ovarian cuts, which is an absolute condition for accurate measurement of the ovarian axes (length, width, thickness).

The consensual volume threshold to discriminate a normal ovary from a PCO is 10 cm^3 (Balen *et al.* 2003). It has been empirically retained by the expert panel for the Rotterdam Consensus, as being the best compromise between the most complete studies (Yeh *et al.* 1987, Van Santbrink *et al.* 1997) (Table 5.2). Indeed, no study published so far has used an appropriate statistical appraisal of sensitivity and specificity of the volume threshold. This prompted us recently to revisit this issue through a prospective study including 154 women with PCOS compared to 57 women with normal ovaries. The receiver operating characteristic (ROC) curves indicated that a threshold at 10 cm^3 yielded a good specificity (98.2%) but a bad sensitivity (39%). Setting the threshold at 7 cm^3 offered the best compromise between specificity (94.7%) and sensitivity (68.8%) (Jonard *et al.* 2004). Thus, in our opinion, the threshold at 10 cm^3 should be lowered in order to increase the sensitivity of the PCO definition.

Increased follicle number

The polyfollicular pattern (i. e., excessive number of small echoless regions less than 10 mm in diameter) is strongly suggestive, since it is in perfect accordance with the label of the syndrome (i. e., "polycystic"). It is now broadly accepted that most of these cysts are in fact healthy oocyte-containing follicles and are not atretic.

The consensus definition for a PCO is one that contains 12 or more follicles of 2–9 mm diameter. Again, the expert panel for the Rotterdam Consensus considered this threshold as being the best compromise between the most complete studies, including the one in which we compared 214 patients with PCOS to 112 women with normal ovaries (Jonard *et al.* 2003). By ROC analysis, a follicle number per ovary (FNPO) of $\geq$12 follicles of 2–9 mm diameter appeared as the threshold yielding the best compromise between sensitivity (75%) and specificity (99%) for the diagnosis of PCO (Table 5.3).

The Rotterdam Consensus did not address the difficult issue about the presence of multifollicular ovaries (MFO) in other situations than PCOS. Again, the

Table 5.2. Results of some ultrasound studies described in the literature concerning ovarian volume

Reference	Ultrasound examination route[a]	Volume's threshold indicative of PCOS (cm^3)	Percent of patients with clinical PCOS having the volume criterion	Percent of controls having the volume criterion	Number of patients studied	Number of controls studied
Adams *et al.* (1985)	TA	>15	33	0	76	17
Yeh *et al.* (1987)	TA	>10	70	0	108	25
Pache *et al.* (1992)	TV	>8	About 70	0	52	29
Van Santbrink *et al.* (1997)	TV	>10.7	41	0	330	48
Atiomo *et al.* (2000)	TV	>9	About 70	About 45	32	40
Fulghesu *et al.* (2001)	TV	>13.2	21	5	53	30
Jonard *et al.* (2004)	TV	>7	70	5	154	57

Note:

[a] TA, transabdominal; TV, transvaginal.

Table 5.3. Receiver operating characteristic (ROC) curve data for the assessment of PCO

Follicle number per ovary (FNPO)	Area under the ROC curve	Threshold	Sensitivity (%)	Specificity (%)
2–5 mm	0.924	10	65	97
		12	57	99
		15	42	100
6–9 mm	0.502	3	42	69
		4	32.5	80
		5	24	89
2–9 mm	0.937	10	86	90
		12	75	99
		15	58	100

Source: Jonard *et al.* (2003).

terminology might be better annotated as multifollicular rather than multicystic. There is no consensual definition for MFO, although they have been described as ovaries in which there are multiple ($\geq$6) follicles, usually 4–10 mm in diameter, with normal stromal echogenicity (Adams *et al.* 1985). No histological data on MFO are available. Multifollicular ovaries are characteristically seen during puberty and in women recovering from hypothalamic amenorrhea – both situations being associated with follicular growth without consistent recruitment of a dominant follicle (Venturoli *et al.* 1983, Stanhope *et al.* 1985). Although the clinical pictures are theoretically different, there may be some overlap, however, hence the confusion between PCO and MFO by inexperienced ultrasonographers. This stresses the need for considering carefully the other clinical and/or biological components of the consensual definition for PCOS. We recently revisited the ovarian follicular pattern in a group of women with hypothalamic amenorrhea. About one-third had an FNPO higher than 12 (authors' unpublished data). Since they were an- or oligo-ovulatory, they could be considered as having PCOS if one applied the Rotterdam definition too inflexibly. This might be true for some of them, of whom we do believe that they had truly a PCOS whose clinical and biological expression had been modified by the chronically suppressed luteinizing hormone (LH) levels due to their secondary hypothalamic dysfunction (Reyss *et al.* 2003). In the others however, such an overlap in the FNPO emphasizes the need for a wise and careful utilization of the Rotterdam criteria as well as for considering other ultrasound criteria for PCO in difficult situations.

Other criteria and other definitions

External morphological signs of PCO

At the beginning of the technique in the 1970s, the weak resolution of ultrasonic abdominal probes allowed to detect exclusively the external morphological ovarian features that were used as the first criteria for defining PCO:

- the length, for which the upper limit is 4 cm, is the simplest criterion, but this unidimensional approach may lead to false positive results when a full bladder compresses the ovary (with the transabdominal route) or false negative results when the ovaries are spherical, with a relatively short length
- because of the increased ovarian size and the normal uterine width, the uterine width/ovarian length (U/O) ratio is decreased (<1) in PCO
- PCO often display a spherical shape in contrast to normal ovaries which are ellipsoid; this morphological change can be evaluated by the sphericity index (ovarian width/ovarian length), which is higher than 0.7 in PCO.

These parameters are less used nowadays because of their poor sensitivity (Ardaens *et al.* 1991).

Ovarian area

Ovarian area is less used than the volume and was not retained in the consensus definition but, in our recent study revisiting the ovarian volume (see above) the diagnostic value of the ovarian area (assessed by the ROC curves) was similar to that of ovarian volume (sensitivity 77.6%, specificity 94.7%; authors' unpublished data). We also observed that the measured ovarian area (obtained by outlining the ovary by hand or by fitting an ellipse to the ovary) was more informative than the calculated ovarian area (by using the formula for an ellipse: $L \times W \times \pi/4$). Indeed, ovaries are not strictly ellipsoid and this can explain why the diagnostic value of the former was better than the latter. We previously reported that the sum of the area of both ovaries was less than 11 cm^2 in a large group of normal women (Dewailly *et al.* 1994, Robert *et al.* 1995). Beyond this threshold, the diagnosis of PCO can be suggested.

Increased stroma

Stromal hypertrophy is characterized by an increased component of the ovarian central part, which seems to be rather hyperechoic (Fig. 5.2). In the opinion of ourselves (Ardaens *et al.* 1991, Dewailly *et al.* 1994) and of others (Pache *et al.* 1992), stromal hypertrophy and hyperechogenicity help to distinguish between PCO and MFO, since these features are specific to the former. However, the estimation of hyperechogenicity is considered as highly subjective, mainly because it depends on the settings of the ultrasound machine. Likewise, in the absence of a precise quantification, stromal hypertrophy is also a subjective sign.

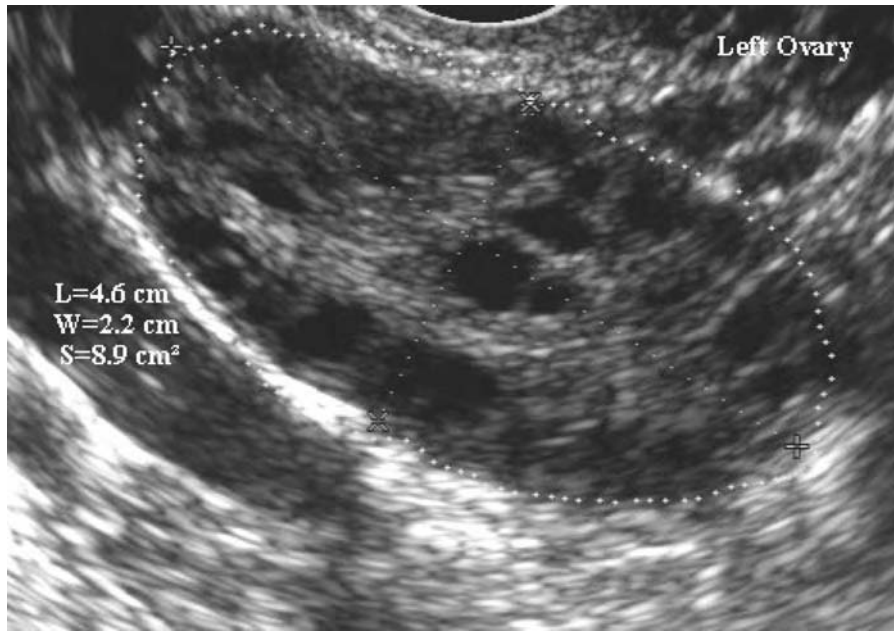

Fig. 5.1 Polycystic ovary. The ovarian length (4.6 cm) and width (2.2 cm) are increased as well as the ovarian area (8.9 cm^2). The number of follicles with a diameter between 2 and 5 mm is more than 10. The distribution within the ovary is mainly peripheral but some follicles can be seen in the central part of the ovary.

For standardizing the assessment of stromal hypertrophy, we designed a computerized quantification of ovarian stroma, allowing selective calculation of the stromal area by subtraction of the cyst area from the total ovarian area on a longitudinal ovarian cut (Dewailly *et al.* 1994, Robert *et al.* 1995). By this means, we were able to set the upper normal limit of the stromal area (i. e., 95th percentile of a large control group of 48 normal women) at 380 mm^2 per ovary. However, given a precise outline of the ovarian shape on a strictly longitudinal cut of the ovaries, the diagnostic value of the total ovarian area equaled the one of stromal area since both were highly correlated.

Fulghesu *et al.* (2001) proposed the ovarian stroma/total area ratio as a good criterion for the diagnosis of PCOS. The ovarian stromal area was evaluated by outlining with the caliper the peripheral profile of the stroma, identified by a central area slightly hyperechoic with respect to the other ovarian area. However, this evaluation seems to be not easy to reproduce in routine practice.

Stromal echogenicity has been described in a semi-quantative manner with a score for normal (1), moderately increased (2), or frankly increased (3) (Pache *et al.* 1991). Echogenicity has been quantified by Al-Took *et al.* (1999) as the sum of the product of each intensity level (ranging from 0 to 63 on the scanner) and

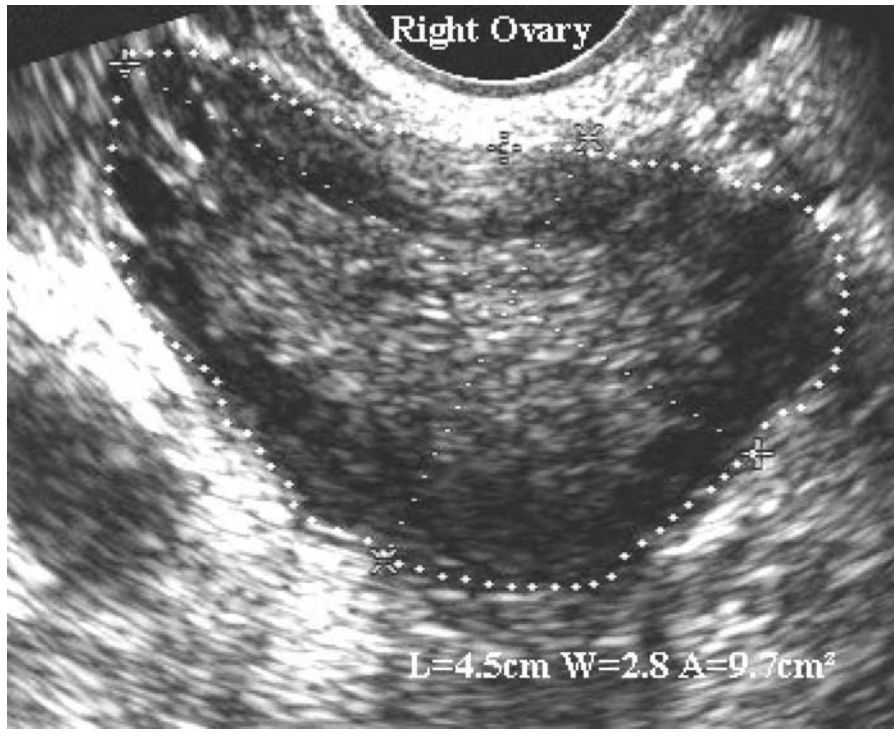

Fig. 5.2 Polycystic ovary. The ovarian length (4.5 cm) and width (2.8 cm) and the outlined area (9.7 cm^2) are increased. The small follicles (2–4 mm) display a typical peripheral pattern, around the hyperechoic stroma.

the number of pixels for that intensity level divided by the total number of pixels in the measured area. Buckett *et al.* (1999) used this same formula but no difference of the stromal echogenicity was found between women with PCOS and women with normal ovaries. The conclusion is that the subjective impression of increased stromal echogenicity is due both to increased stromal volume and to reduced echogenicity of the multiple follicles.

In summary, ovarian volume or area correlates well with ovarian function and is both more easily and reliably measured in routine practice than ovarian stroma. Thus, in order to define the polycystic ovary, neither qualitative or quantitative assessment of the ovarian stroma is required.

Follicle distribution

In PCO, the follicle distribution is predominantly peripheral, with typically an echoless peripheral array, as initially described by Adams *et al.* (1985) (Fig. 5.1 and 5.2). For some authors (Battaglia *et al.* 1998), younger patients display this peripheral distribution more often while a more generalized pattern, with small

cysts in the central part of the ovary, is noticed in older women. At the Rotterdam meeting, this subjective criterion was judged to be too inconstant and subjective to be retained for the consensus definition of PCO (Balen *et al.* 2003).

The contribution of ultrasonography to the understanding of PCO

Ultrasonography is not only a useful diagnostic tool. It can also serve as an informative technique for pathophysiological studies. There is evidence suggesting that the mechanism of PCOS involves a primary ovarian dysfunction (Jacobs 1987, Webber *et al.* 2003). One important line of evidence is the observation by Hughesdon (1982) that PCOS ovaries contain two- to threefold the normal number of follicles, from the time when they start growing to a size of 2–5 mm (antral follicles). Recently, Webber *et al.* (2003) revisited these data by examining ovarian cortical biopsies from normal and PCOS women. They found an even greater (sixfold) increase in the number of primary growing follicles in PCO from anovulatory women, in comparison to normal ovaries. At last, Maciel *et al.* (2004) confirmed that PCOS is associated with a dramatic increase in the number of growing follicles, particularly at the primary follicle stage. The number of primordial follicles was not different from that of the normal ovaries. Another line of evidence is that PCOS follicles stop growing and developing when they reach 4–7 mm in diameter. Therefore, the follicular problem of PCOS seems to be twofold (for review, see Jonard and Dewailly 2004). First, early follicular growth is excessive which is presumably the consequence of intraovarian hyperandrogenism. Second, the selection of one follicle from the increased pool and its further maturation to a dominant follicle does not occur (follicular arrest), due to an impaired action of follicle stimulating hormone (FSH) and/or a premature LH action.

This twofold follicular abnormality can be addressed by ultrasonography (Jonard *et al.* 2003). In this study, two different categories of follicle size were analyzed separately (2–5 and 6–9 mm), using a 7-MHz transvaginal ultrasound scan. In PCO, the mean FNPO in the 6–9 mm range was similar to normal ovaries, but the FNPO in the 2–5 mm range was significantly higher. Therefore, we confirmed that it is the accumulation of 2–5 mm but not 6–9 mm follicles that gave the typical aspect of multifollicular ovaries at ultrasonography in women with PCO. We also showed that this follicle excess was in close relationship with the androgen serum level, in agreement with morphological (Takayama *et al.* 1996) and experimental studies. In another study (Pigny *et al.* 2003), we showed that the FNPO in this diameter range was in very close relationship to the serum anti-mullerian hormone (AMH) level, whose excess might be involved in the follicular arrest (Jonard and Dewailly 2004). Indeed, the discrepancy between

FNPO in the 2–5 and 6–9 mm ranges might reflect the follicular arrest of PCO. The fact that the 6–9 mm FNPO was negatively related to overweight and/or hyperinsulinism (Jonard *et al.* 2003) is in line with the well-known worsening effect of these parameters on the anovulation of PCOS.

Other techniques for imaging PCO

Three-dimensional ultrasound

To avoid the difficulties and pitfalls in outlining or measuring the ovarian shape, three-dimensional (3D) ultrasound has been proposed using a dedicated volumic probe or a manual survey of the ovary (Wu *et al.* 1998, Kyei-Mensah *et al.* 1996a, b). From the stored data, the scanned ovarian volume is displayed on the screen in three adjustable orthogonal planes, allowing the three dimensions and subsequently the volume to be more accurately evaluated. In a study of Kyei-Mensah *et al.* (1998), three groups of patients were defined: (1) those with normal ovaries, (2) those with asymptomatic PCO, and (3) those with PCOS. The ovarian and stromal volumes were similar in groups 2 and 3 and both greater than group 1. Stromal volume was positively correlated with serum androstenedione concentrations in group 3 only. The mean total volume of the follicles was similar in all groups, indicating that increased stromal volume is the main cause of ovarian enlargement in PCO.

Nardo *et al.* (2003) found good correlations between 2D and 3D ultrasound measurements of ovarian volume and polycystic ovary morphology. However, in this prospective study, total ovarian volume, ovarian stromal volume, follicular volume, and follicle number did not correlate with testosterone concentration.

Since 3D ultrasound requires expensive equipment, intensive training, and a long time for storage and data analysis, its superiority over 2D ultrasound for imaging PCO in clinical practice is not evident.

Doppler ultrasonography

The assessment of uterine arteries will not be addressed in this chapter exclusively devoted to PCO imaging. Color (or power) Doppler allows detection of the vascularization network within the ovarian stroma. Power Doppler is more sensitive to the slow flows and shows more vascular signals within the ovaries, but it does not discriminate between arteries and veins (Fig. 5.3). Moreover, the sensitivity of the machines differs from one to another. Pulsed Doppler focuses on the hilum or internal ovarian arteries and offers a more objective approach. Because of the slow flows, the pulse repetition frequency (PRF) is at minimum (400 Hz) with the lowest frequency filter (50 Hz) (Fig. 5.4).

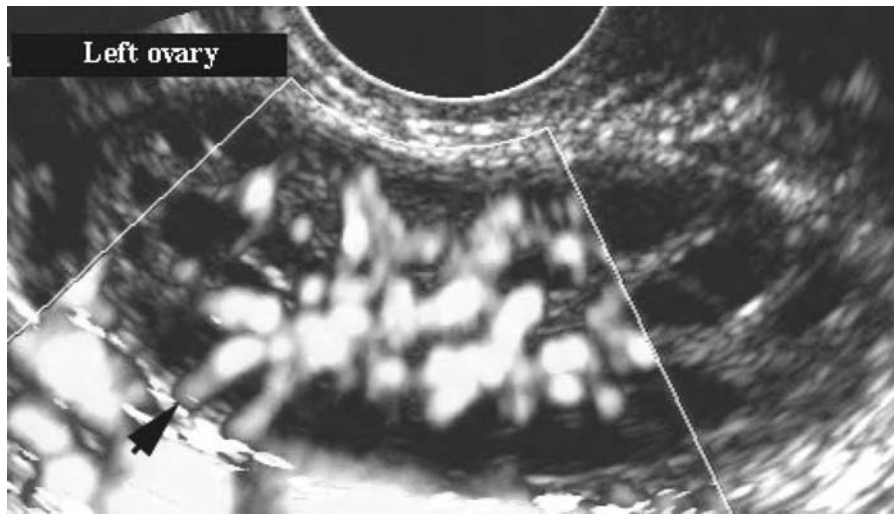

Fig. 5.3 Polycystic ovary, as imaged by power Doppler. The high sensitivity of power Doppler allows the depiction of flow in small vessels, magnifying the increased vascularization in the stroma. In this case, a radiating pattern was observed, with vessels between the small follicles.

The study of the ovarian vascularization by these techniques is still highly subjective. The blood flow is more frequently visualized in PCOS (88%) than in normal patients (50%) in early follicular phase and seems to be increased (Battaglia *et al.* 1996). No significant difference was found between obese and lean women with PCO, but the stroma was less vascularized in patients displaying a general cystic pattern than in those with peripherical cysts. In the latter, the pulsatility index (PI) values were significantly lower and inversely correlated to the FSH/LH ratio (Battaglia *et al.* 1999). In another study (Aleem and Predanic 1996), the resistive index (RI) and PI were significantly lower in PCOS ($RI = 0.55 + 0.01$ and $PI = 0.89 + 0.04$) than in normal patients ($RI = 0.78 + 0.06$ and $PI = 1.87 + 0.38$) and the peak systolic velocity was greater in PCOS ($11.9 + 3.2$ cm/s) than in normal women ($9.6 + 2.1$ cm/s). No correlation was found with the number of follicles and the ovarian volume but there was a positive correlation between LH levels and increased peak systolic velocity. In the study by Zaidi *et al.* (1995), no significant difference in PI values was found between the normal and PCOS groups, while the ovarian flow, as reflected by the peak systolic velocity, was increased in the former. Some data indicate that Doppler blood flow may have some value in predicting the risk for ovarian hyperstimulation during gonadotropin therapy (Agrawal *et al.* 1998). Increased stromal blood flow has also been suggested as a more relevant predictor of ovarian response to hormonal stimulation than parameters such as ovarian or stromal volume (Buckett *et al.* 1999, Engmann *et al.* 1999).

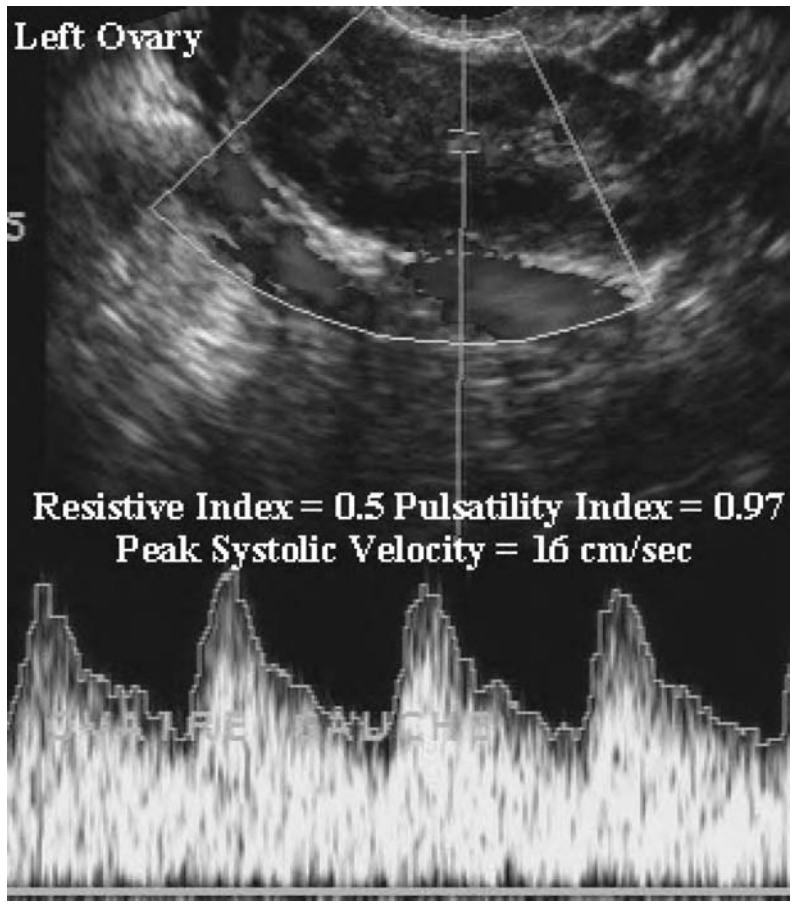

Fig. 5.4 Polycystic ovary, as imaged by color and pulsed Doppler. The arterial flow is more easily depicted in PCOS than in the normal ovary, displaying low resistance indexes.

To summarize, the increased stroma component in PCO seems to be accompanied by an increased peak systolic velocity and a decreased PI at the ovarian Doppler study. However, in all studies, values in patients with PCO overlapped widely the ones of the normal patients. No data so far support any diagnostic usefulness of Doppler in PCO.

Magnetic resonance imaging

Data about MRI for PCO are still scarce in the literature (Maubon *et al.* 1993, Kimura *et al.* 1996, Woodward and Gilfeather 1998). This technique allows a multiplanar approach of the pelvic cavity, which helps to localize the ovaries. Imaging quality is improved by the use of pelvic dedicated phased-array coil receiver. The most useful planes are the transversal and coronal views. The

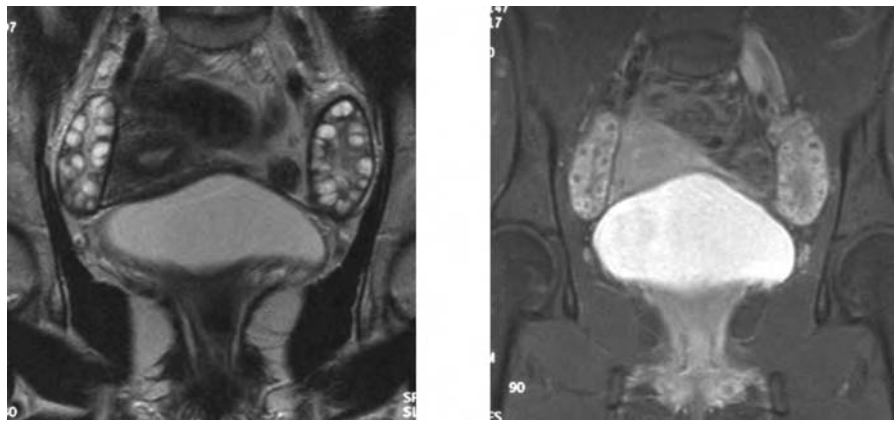

Fig. 5.5 PCOS: magnetic resonance image after gadolinium injection.

T2-weighted sequence is best suited to the ovarian morphology. With this sequence, the follicular fluid displays an hypersignal (white) and the solid component (stroma) a low signal (black). T1-weighted sequences offer less information, but the gadolinium injection allows the stromal vascularization to be studied. The fat saturation technique increases the contrast obtained after the medium uptake by the vascularized areas.

The external signs of PCO (see above) are easy to analyze on MRI transversal sections (Fig. 5.5). In addition, the T2-weighted sequence displays the excessive number of follicles, but their detection and numbering is less easy than with ultrasound, because of the poor spatial resolution of MRI, unless high magnetic fields are used (1 to 1.5 T). As with ultrasound, stromal hypertrophy remains a subjective observation, although obvious in many cases. After gadolinium injection, there is a high uptake by the stroma, suggesting that it is highly vascularized in PCO.

In most cases in practice, MRI does not afford more information than ultrasonography for imaging PCO (Kimura *et al.* 1996). It is only helpful in difficult situations such as a severe hyperandrogenism, when ultrasonography is not possible or not contributive (virgin or obese patients, respectively). Its main role is to exclude a virilizing ovarian tumor which should be suspected when the ovarian volume is not symmetrical and/or when there is a circumscribed signal abnormality, either before or after gadolinium injection. PCO associated with an ovarian tumor might be a pitfall.

Conclusions

The ultrasonographic study of PCO has now left its era of artistic haziness. It must be viewed as a diagnostic tool which requires the same quality controls as a

biological one, such as the plasma LH assay. This supposes that its results are expressed as quantitative variables rather than purely descriptive data. Lastly, it can be used by the clinician only if the ultrasonographer is sufficiently trained and reproducible in his/her results. By its sensitivity (providing that sufficient specificity is guaranteed), ultrasonography has widened the clinical spectrum of PCOS, this has led to a reduction in the numbers of cases diagnosed with "idiopathic hirsutism" and "idiopathic anovulation."

The establishment of an international consensus definition for PCO was essential. However, one should keep in mind that endovaginal ultrasonography is an improving technique and becomes more and more accurate with time. Therefore, the thresholds of the currently used criteria are liable to change and new criteria defining PCO will probably appear in the future. Sooner or later, some new consensus will probably be needed.

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6

Insulin sensitizers in the treatment of polycystic ovary syndrome

Helena Teede

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 6–10% of reproductive-age women (Azziz *et al.* 2004). The definition has evolved over time. The National Institute of Health diagnostic criteria, released in 1990 (Table 6.1) (Zawadzki and Dunaif 1992), highlighted the clinical features of PCOS including ovarian dysfunction (anovulation, irregular cycles, infertility) and hyperandrogenism (hirsutism and acne). The recent and more controversial Rotterdam criteria broaden the condition to include ovarian ultrasound features (Table 6.1) (Zawadski and Dunaif 1992, Group TREAC 2004).

Despite recognition of the important role that insulin resistance plays in PCOS, diagnostic criteria for PCOS do not currently encompass insulin resistance. The difficulties surrounding the diagnosis of PCOS are likely to reflect multiple factors. These include the lack of clarity on the etiology of PCOS, the lack of simple, accurate methods to measure insulin resistance, and the heterogeneity of clinical syndrome that is PCOS today. With greater understanding of PCOS, subcategories are likely to emerge in what is essentially a heterogeneous syndrome (Norman *et al.* 1995). This is important to consider when evaluating results of clinical trials, as effects of therapy may vary across the heterogeneous spectrum that is labeled as PCOS today.

The pathogenesis of PCOS is not well understood. A combination of genetic and environmental factors contributes, with potential etiological factors including insulin resistance, ovarian dysfunction, hyperandrogenism, and hypothalamic pituitary abnormalities. Increasingly it is recognized that in the majority of women with PCOS, insulin resistance leading to hyperinsulinemia plays a central role

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Table 6.1. Diagnostic criteria for polycystic ovary syndrome (PCOS)

1990 Criteria (both 1 and 2)
1 Chronic anovulation
2 Clinical and/or biochemical signs of hyperandrogenism and exclusion of other etiologies
Revised 2003 criteria (2 out of 3)
1 Oligo- or anovulation
2 Clinical and/or biochemical signs of hyperandrogenism
3 Polycystic ovaries and exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome)

(Dunaif 1997). The role of insulin resistance in PCOS is reviewed in some detail in Chapter 13. Essentially, insulin resistance has a complex genetic and environmental etiology. Both lean and obese women have been shown to have insulin resistance, compared to controls (Nestler and Jakubowicz 1997). Insulin resistance appears to be selective with impaired glucose uptake, whilst other intracellular actions of insulin are preserved. Insulin production then increases to overcome this resistance. The resultant hyperinsulinemia upregulates insulin stimulated lipogenesis and androgen production, whilst carbohydrate metabolism remains impaired. This hyperinsulinemic hormonal milieu therefore contributes to the hyperandrogenism, metabolic syndrome, and subsequent ovarian dysfunction in the majority of women with PCOS.

In the setting of insulin resistance and hyperinsulinemia, it has now been established that women with PCOS have both short-term symptoms and long-term sequelae. These include a high risk of gestational diabetes, impaired glucose intolerance (IGT) and type 2 diabetes (DM2) and potentially an increased risk of stroke and cardiovascular disease (De Leo *et al.* 2003).Traditionally, interventions in PCOS targeted short-term symptoms alone, either without addressing or even potentially exacerbating long-term risks. These included the oral contraceptive pill (OCP) and infertility therapies. However, there is now recognition that amelioration of the underlying insulin resistance not only effectively treats short-term symptoms, but may also prevent long-term sequelae of PCOS (De Leo *et al.* 2003).

Amelioration of insulin resistance occurs with lifestyle modification and weight loss resulting in improved ovarian function, cycle regulation, pregnancy rates, and metabolic features in PCOS (Holte *et al.* 1995, Moran *et al.* 2003). Trials with specific dietary modification suggest targets for weight reduction of 5–10% of body weight rather than specific dietary composition (Moran *et al.* 2003). This approach remains the mainstay of treatment in the majority of women with PCOS, but has practical limitations (National Health and Medical Research Council 2003). Weight loss through lifestyle modification is elusive and

even with intensive effort, motivated women with PCOS average around 4 kg weight loss over 6 months. These programs have only been trialed short-term with feasibility and sustainability remaining valid concerns (National Health and Medical Research Council 2003). Yet, if sustainable weight loss can be achieved it is likely to prevent long-term complications including DM2. This has been observed in populations with IGT, where modest weight loss (5–11 kg) leads to a 50% decrease in relative risk of developing DM2 (Chan *et al.* 1994).

Medical therapy is often required in addition to lifestyle change. The OCP has been first line *medical* therapy in women with PCOS who do not desire fertility. It regulates menstrual cycles, provides endometrial protection, decreases bioavailable androgens, and controls clinical features of hyperandrogenism. However, these benefits may occur at the expense of exacerbating underlying insulin resistance, potentially having adverse long-term consequences. In both PCOS and non-PCOS populations, OCP use has been associated with increased insulin resistance, although not consistently (Godsland *et al.* 1990, Creatas *et al.* 2000, Escobar-Morreale *et al.* 2000, Morin-Papunen *et al.* 2000). Evidence suggests the estrogen effect may be dose-related (Godsland *et al.* 1990). Given the insulin resistance, metabolic syndrome, obesity, diabetes risk, and the suggestion of early cardiovascular disease in women with PCOS (Meyer and Teede 2005), any exacerbation of insulin resistance by the OCP is likely to be clinically relevant. Further research is definitely needed in this area, but it is arguable that this should be taken into consideration when using the OCP in women with PCOS.

In this setting, insulin sensitizers including metformin and thiozolidendiones have been trialed with encouraging results. This chapter reviews the role of insulin sensitizers, their safety profile, and potential benefits both in the general population and in PCOS. The effects of insulin sensitizers on reproductive outcomes (ovulation, cycle regulation, pregnancy, birth rates), hyperandrogenism, and hirsutism and the features of the metabolic syndrome (insulin resistance, lipids, hypertension, and obesity) as well as adverse effects are reviewed. It is immediately notable that although encouraging, the studies on insulin sensitizers in PCOS have some limitations. These include the heterogeneity of the underlying condition and a preponderance of short-term, uncontrolled trials often with small sample sizes. Doses vary and end-points studied are not always consistent (Lord *et al.* 2003). These factors arguably somewhat limit the clinical applicability of the data and emphasize the need for further research.

Metformin

Metformin has been used to treat diabetes since 1957. It effectively treats insulin resistance and lowers insulin levels. Mechanisms of action include direct intracellular

effects on the insulin signaling pathway with reduction in hepatic gluconeogenesis, improved non-oxidative glucose metabolism, and improved glucose utilization. Resultant falls in insulin levels reduce lipogenesis and free fatty acid levels, and dyslipidemia improves. Importantly, in the clinical setting, metformin reduces progression from IGT to diabetes by 31% in longitudinal studies (Knowler *et al.* 2002). Clinically, metformin has well-established efficacy in the treatment of DM2, reducing glucose and insulin levels without causing hypoglycemia. Also in established DM2 it reduces mortality rates and reduces cardiovascular complications compared with other hypoglycemic treatments.

Metformin use, given its beneficial effects on insulin resistance, was first reported in PCOS in 1994 (Velazquez *et al.* 1994). Metformin induced symptomatic benefits in PCOS outlined in detail below. Enticingly metformin therapy in PCOS may also translate to reductions in long-term sequelae, akin to benefits observed in other insulin resistant states (IGT and DM2).

Menstrual regulation, ovulation, pregnancy, and birth rates

Menstrual irregularity secondary to ovulatory disturbance is a significant acute clinical problem in PCOS and if untreated, chronic anovulation is associated with an increased risk for endometrial carcinoma (Zawadzki and Dunaif 1992). Currently there is no consensus as to the type, dose, duration, or frequency of medical treatment required to reduce this risk. Standard clinical practice is to induce a withdrawal bleed every 2–3 months. This can be achieved with metformin in the majority of lean and obese women with PCOS as it restores ovulation and regulates menstrual cycles in controlled clinical trials (Lord *et al.* 2003, Palomba *et al.* 2005). Theoretically, metformin may offer additional protection, by reducing insulin resistance, which has been associated with an increased risk of endometrial carcinoma.

Metformin has also been reasonably well trialed in ovulation induction for infertility and is effective with ovulation rates increasing by eight- to tenfold. Metformin has proven efficacy alone and in combination with clomiphene, gonadotrophins and in vitro fertilization (IVF) therapy (De Leo *et al.* 2003). This is now supported by a wealth of literature including a recent Cochrane review (Moghetti *et al.* 2000, Morin-Papunen *et al.* 2000, Vandermolen *et al.* 2001, De Leo *et al.* 2003, Lord *et al.* 2003). The review focused on randomized controlled trials of insulin sensitizing drugs compared to ovulation induction agents (Lord *et al.* 2003). Fifteen trials were included, involving 543 participants. The Cochrane meta-analysis demonstrated that metformin induced ovulation in PCOS with an odds ratio (OR) of 3.88 (confidence interval (CI) 2.25 to 6.69) for metformin versus placebo and 4.41 (CI 2.37 to 8.22) for metformin + clomiphene versus clomiphene alone (Lord *et al.* 2003). Metformin was shown to be an effective

treatment for anovulation in PCOS, with the use of metformin as a first-line agent appearing reasonable (Lord *et al.* 2003), especially, as in contrast to conventional infertility therapies, metformin does not induce ovarian hyperstimulation nor increase multiple pregnancies. In addition a recent review compared metformin with direct ovarian drilling with metformin having equivalent efficacy with fewer complications (Pirwany and Tulandi 2003).

Until recently, there were inadequate data on metformin and birth rates. A recent trial by Palomba *et al.* (2005) made an important contribution to this literature. This was the first head-to-head randomized comparative trial of clomiphene citrate (CC) and metformin with pregnancy outcomes. One hundred non-obese women aged 20–34yrs received metformin + placebo or CC + placebo for 6 months. The cumulative ovulation rates were similar with metformin (63%) and CC (67%). The pregnancy rate per ovulatory cycle was 15% with metformin and 7% with CC ($p < 0.009$). Miscarriage rates were 10% with metformin and 37% with CC ($p < 0.05$). Live birth rates from the study extension period were 84% for metformin and 56% for CC ($p = 0.07$). Failure to ovulate occurred in 7% of metformin group and 34% of the CC group ($p < 0.05$) (20). The number needed to treat to achieve pregnancy in this non-obese group was three women over 6 months. Few side effects were observed.

The use of metformin throughout pregnancy is perhaps the most controversial area of its use in PCOS at present. Potential advantages include lower miscarriage rates noted in the Palomba study and supported by observational data (Glueck *et al.* 2002, 2003a). Studies also suggest that metformin use in pregnancy lowers rates of gestational diabetes in pregnant women with PCOS (Palomba *et al.* 2005). A prospective study assessed incidence of gestational diabetes in pregnancy. It also recorded growth and motor–social development during the first 18 months of life in 126 live births in women with PCOS, all of whom conceived on and continued metformin (1.5–2.55 g/day) through pregnancy. Encouragingly, metformin reduced gestational diabetes to less than controls. The concerns on metformin use in pregnancy center around the risk of teratogenicity. In these 126 live births, metformin was not teratogenic and did not adversely affect birth length and weight, growth, or motor–social development in the first 18 months of life (Glueck *et al.* 2004), yet further research is needed.

Androgen status

Hyperandrogenism is a key feature of PCOS with elevations of ovarian androgens, testosterone, and androstendione (Carmina 2002). Sex hormone binding globulin (SHBG) is generally low in PCOS, primarily due to obesity, leading to higher free testosterone levels. Clinical features of hyperandrogenism are commonly the presenting feature of PCOS and often warrant medical therapy (Carmina 2002).

Whilst the efficacy of the standard OCP (Elter *et al.* 2002, Ganie *et al.* 2004, Yildiz 2004) and anti-androgen therapies in the reduction of androgen levels and the treatment of hirsutism are well established (Elter *et al.* 2002, Ganie *et al.* 2004) the efficacy of metformin is less well studied (Carmina 2002). To date, testosterone levels appear to fall with metformin, although results are variable. This is in part due to the poor quality of the data with uncontrolled, underpowered, and short-term trials. In four separate placebo controlled studies in PCOS from 6 weeks to 6 months involving between 23 and 32 PCOS women, testosterone levels decreased significantly in all with metformin, compared to placebo (Nestler and Jakubowicz 1996, Moghetti *et al.* 2000, Yarali *et al.* 2002, Chou *et al.* 2003). Androgen responses to gonadotropin releasing hormone agonist stimulation also improved with metformin. Metformin has also been shown to reduce androgen levels, when combined with lifestyle in a placebo controlled trial in 20 obese PCOS women over 6 months (Pasquali *et al.* 2000). Metformin appears to reduce androgens via a reduction in insulin resistance (De Leo *et al.* 2003), although other mechanisms may be involved.

Even fewer trials have evaluated the effects of metformin on clinical hyperandrogenism. Biochemical hyperandrogenism is not consistently associated with clinical hyperandrogenism, with other factors including ethnicity impacting on clinical expression. We have recently demonstrated that metformin has comparative efficacy to the OCP in improving clinical hyperandrogenism (Ferriman–Gallway hirsutism scores) in a randomized controlled trial of 110 overweight PCOS subjects over 6 months, despite differential effects on androgen levels (author's unpublished data). Other mechanisms for metformin mediated effects on hirsutism include reduction in insulin levels and insulin-like growth factor 1 (IGF-1) activity, known to stimulate hair growth (Harborne *et al.* 2003). Other controlled trials have reported amelioration of hirsutism with metformin, some but not all noting biochemical reductions in androgens (Pasquali *et al.* 2000). Data are inconsistent though, with another controlled trial in 32 women over 6 months noting no improvement in hirsutism despite reductions in serum testosterone (Morin-Papunen *et al.* 2000). Overall, the limited controlled trials that have reported hirsutism, suggest benefit from metformin. In the Cochrane review, combined results showed significant improvement in hirsutism with metformin (Lord *et al.* 2003).

Body weight

A body mass index (BMI) >27 kg/m^2 is associated with increased insulin resistance in Caucasian populations (Bray 2004). Evidence suggests that even BMIs >23 kg/m^2 are associated with increased insulin resistance in some ethnic groups. Greater visceral fat deposition has been demonstrated in these groups. Obesity, especially increased visceral fat, worsens insulin resistance, exacerbating the clinical

manifestations of PCOS. Trials examining the effect of metformin on BMI and waist/hip ratio (WHR) in overweight women with PCOS have produced inconsistent results. Reductions in BMI and WHR with metformin have been reported and have been attributed to appetite suppressive effects of metformin (Morin-Papunen *et al.* 2000, Pasquali *et al.* 2000, Fleming *et al.* 2002). A controlled trial over 14 weeks in 94 obese women with metformin or placebo noted decreased weight (Fleming *et al.* 2002). Others have noted reductions in insulin resistance with no decrease in adiposity (Diamanti-Kandarakis *et al.* 1998, Moghetti *et al.* 2000). The recent Cochrane review noted inadequate evidence that metformin altered body weight, BMI, or WHR (Lord *et al.* 2003). Although further research is needed, the effect of metformin on weight if any is likely to be small.

PCOS, metabolic syndrome, and cardiovascular disease

Hypertension

Studies of hypertension in PCOS have found variable results with most demonstrating blood pressure (BP) within the normal range in young women with PCOS (Sampson *et al.* 1996). One study suggested an increase in ambulatory 24-h BP, even after adjustment for BMI, body fat distribution, and insulin resistance (Holte *et al.* 1996). Yet, studies by our group suggest that obese PCOS subjects have normal 24-h BP profiles (Meyer *et al.* 2005), which are similar to age and weight matched controls (Meyer and Teede 2005). Despite established links between insulin resistance and blood pressure, metformin appears not to affect BP in PCOS (Hacihanefioglu *et al.* 2002).

Insulin resistance

Insulin resistance is multifactorial in PCOS. The observed clinical sequelae of insulin resistance include increased rates of gestational diabetes. Impaired glucose tolerance has been noted in 30% and DM2 in 15% of obese American postmenopausal women with a history of PCOS (Legro *et al.* 1999). Longitudinally, significant progression in insulin resistance has been documented with 9% of baseline normoglycemic women with PCOS developing IGT and 8% DM2 over 6 years. In the same study, baseline IGT progressed to DM2 in 54% of PCOS women (Norman *et al.* 2001). Diabetes is a potent risk factor for cardiovascular disease, increasing the risk three to fivefold in women. It is likely that women with PCOS have increased cardiovascular risk, but this is yet to be resolved (Pierpoint *et al.* 1998, Wild *et al.* 2000). Any intervention that reduced insulin resistance and prevented the onset of diabetes would be preferable.

Whilst in non-PCOS populations metformin induced reduction in insulin resistance is well established, in most studies the effects of metformin on insulin

resistance in PCOS have utilized non-clamp methods. In PCOS studies, the majority have suggested beneficial effects on insulin resistance with some heterogeneity (Lord *et al.* 2003). It is likely this is related to methodological issues with primarily non-clamp methodology used, small sample sizes, and heterogeneity within the PCOS population. If we limit review to clamp studies, considered the "gold standard" (Katz *et al.* 2000, Radziuk 2000), in 23 overweight women with PCOS, 6 months of metformin compared to placebo reduced hyperinsulinemia independently of changes in body weight (Moghetti *et al.* 2000). This finding is generally supported by most but not all uncontrolled clamp studies (Ehrmann *et al.* 1997, Diamanti-Kandarakis *et al.* 1998, Arslanian *et al.* 2002, De Leo *et al.* 2003).

Finally, metformin induced reduction in insulin resistance has been shown to reduce progression to DM2 by 31% in non-PCOS populations. Metformin has also been shown to reduce overall mortality in subjects with DM2. No equivalent studies exist in PCOS and these long-term outcome studies would be important to complete. It is likely that this observation would apply to insulin resistance PCOS subjects, also especially given that metformin does appear to reduce the risk of gestational diabetes in women with PCOS (Glueck *et al.* 2003a).

Lipids

The dyslipidemia characteristic of the metabolic syndrome (MS) has been documented in women with PCOS with the majority of studies showing reduced high-density lipoprotein (HDL) and increased triglyceride levels compared to weight matched control women (Wild *et al.* 1985, Wild 1995, Talbot *et al.* 1998, Meyer and Teede 2005, Meyer *et al.* 2005). Insulin resistance through impaired insulin dependent suppression of lipolysis and altered expression of lipoprotein lipase and hepatic lipase appears to have a pivotal role in the pathogenesis of the dyslipidemia (Wild *et al.* 1985). The effect of metformin on the dyslipidemia in PCOS is generally neutral or shows small beneficial changes. Randomized placebo controlled studies in obese women over 3 months include a trial in 94 subjects showed improved HDL (Fleming *et al.* 2002) and a trial in 30 women showed improved cholesterol with metformin (Chou *et al.* 2003). The Cochrane review on metformin in PCOS, noted a reduction in low-density lipoprotein (LDL) when data was combined (Lord *et al.* 2003). It is likely that the majority of the trials completed to date are underpowered to detect effects on the mild dyslipidemia seen in PCOS and further research in this area is warranted.

Side effects of metformin

Side effects of metformin are primarily mild and gastrointestinal, including nausea and diarrhea, and are often transient (Pasquali *et al.* 2000). These are dose related and are less severe if doses are started low and increased slowly. The

author titrates from 250 mg daily up to 850 mg bd over 4–8 weeks. With over 50 years of use, there appear to be no adverse effects of long-term therapy, although long-term trials in young women are not yet completed.

The main concerns with metformin use are teratogenicity in pregnancy and the rare but serious adverse effect of lactic acidosis. Metformin has been used during pregnancy for the treatment of women with DM2 and for those who develop gestational diabetes. To date animal studies have shown teratogenicity only with levels never achieved in vivo. There is currently no evidence that human fetal abnormalities are increased; however, further research is needed before safe therapeutic use in pregnancy could be advocated (Bedaiwwy *et al.* 2001).

Lactic acidosis remains a rare side effect limited to older individuals with comorbidities. In a comprehensive review of the adverse effects of metformin in all populations, all comparative or observational studies (1959–2002) evaluating metformin, alone or in combination, for >1 month were included. The incidence of lactic acidosis from 194 studies revealed no lactic acidosis in 36 893 patient–years in the metformin group or in 30 109 patient–years in the non-metformin groups. The probable upper limit for the true incidence of lactic acidosis in the metformin and non-metformin groups was calculated as 8.1 and 9.9 cases per 100 000 patient–years, respectively. Lactate levels also showed no difference with metformin compared with placebo. Overall, even in trials with older subjects with greater comorbidities, there is no evidence that metformin increases the risk of lactic acidosis or increases lactate levels compared with other antihyperglycemic treatments if prescribed appropriately (Salpeter *et al.* 2003). Lactic acidosis primarily occurs with severe underlying illnesses combined with advancing age. There has never been a case of lactic acidosis reported in studies in women with PCOS. The PCOS population are generally young and well with no other major illnesses placing them at very low risk of this rare side effect (De Leo *et al.* 2003).

Thiozolidinediones

The peroxisome proliferator activated receptors (PPARs) are members of the membrane nuclear receptor superfamily and regulate gene expression. Thiozolidinediones are selective ligands for the nuclear transcription factor PPAR-γ (Yki-Jarvinen 2004). Troglitazone, the first Food and Drug Administration (FDA) approved ligand for PPAR-γ was approved in 1997, has been withdrawn because of hepatotoxicity. Currently rosiglitazone and pioglitazone are approved by the FDA and the Therapeutic Goods Administration for use in DM2. These agents improve insulin sensitivity through direct and indirect effects in both the

liver and the periphery. Their hypoglycaemic efficacy appears to be less than metformin (Yki-Jarvinen 2004). Effects of thiozolidinediones on cardiovascular risk factors in non-PCOS populations are variable with increases in HDL, but paradoxically an increase in LDL levels. Insulin resistance and free fatty acids fall but akin to metformin, and there is no discernible effect on BP.

Although these agents potentially provide cardiovascular protection, ultimately, trials with hard clinical end-points are needed to establish if thiozolidinediones reduce cardiovascular events and these are currently under way in subjects with DM2.

Thiozolidinediones are only approved for use in DM2, although they have been used experimentally in other insulin resistance conditions including PCOS (Yki-Jarvinen 2004). The largest randomized placebo controlled trial of insulin sensitizers in women with PCOS was with troglitazone in 410 women over 44 weeks. Improvement in ovulatory function, menstrual cyclicity, pregnancy rates, hirsutism, androgen levels, and insulin resistance was noted compared to placebo, with few adverse effects (Azziz *et al.* 2001). Troglitazone also may directly inhibit steroidogenesis in the ovary, may improve endothelial function (Paradisi *et al.* 2003), and decrease plasminogen activating inhibitor-1 (PAI-1). However troglitazone has since been withdrawn from the market. Smaller limited trials exist for rosiglitazone and pioglitazone in PCOS. Rosiglitazone has been shown to improve ovulation and insulin resistance with reductions in androgen levels but results are not consistent (Romualdi *et al.* 2003, Sepilian and Nagamani 2005). In a 6-month study of metformin, rosiglitazone, both combined or placebo in 100 non-obese PCOS women, testosterone fell in all active treatment groups compared to placebo. Insulin resistance improved significantly after metformin or combination therapies but not after rosiglitazone alone (Baillargeon *et al.* 2004). A randomized, double-blind, controlled trial in 40 women over 3 months using pioglitazone noted decreased insulin resistance and hyperandrogenism and improved ovulation rates in women with PCOS (Brettenthaler *et al.* 2004). In an observational study of 13 women resistant to diet plus metformin, pioglitazone was added for 10 months insulin, glucose, insulin resistance and dehydroepiandrosterone (DHEAS) fell, HDL, cholesterol, and SHBG rose, and menstrual regularity improved, without adverse side effects (Glueck *et al.* 2003b).

Overall, limitations on the use of thiozolidinediones in PCOS include inadequate efficacy data, no long-term safety data in any population, and the side effects including weight gain. There are also concerns over their use in pregnancy, where they are class C drugs with documented increase in fetal abnormalities in animal models. Adverse effects include fluid retention and weight gain. Hepatotoxicity, an idiosyncratic effect of troglitazone, does not appear to be a class effect.

Other drugs

Whilst other insulin sensitizers including diazoxide, somatostatin, and D-chiro-inositol are available, data with these medications are very limited and their role remains in the realm of research into underlying mechanism involved in PCOS and its metabolic consequences.

Conclusions

Lifestyle modification is the primary therapy for women with PCOS who are overweight or have insulin resistance. The feasibility and sustainability of long-term lifestyle change remains a challenge and medical therapy is often required. Insulin sensitizers should be considered an adjunct to, not a replacement for lifestyle change. Of these, the evidence is strongest for metformin. Whilst the literature on metformin has its limitations, efficacy in PCOS is well established in randomized trials, supported by a Cochrane review. Metformin has established benefit in ovulation induction, regulation of cycles, improved fertility, modest improvement in androgens and hirsutism, and metabolic advantages. It also has the potential to reduce progression to diabetes and reduce the risk of cardiovascular disease.

It is increasingly argued that metformin is appropriate first-line medical therapy in women with PCOS. In infertility, this appears reasonably justified given the recent comparative data on metformin versus clomiphene citrate in PCOS related infertility (Palomba *et al.* 2005). In women with significant insulin resistance including a family history of diabetes, impaired glucose tolerance, or diabetes, it would appear that metformin is also appropriate first-line therapy. This case is strengthened by the emerging literature on the OCP related increase in insulin resistance and potential to increase DM2 in women with PCOS.

Metformin appears safe in the otherwise healthy PCOS woman, based on long-term use in DM2 (>50 years) and on trials to date in PCOS, although safety data are not absolutely complete and further trials on the long-term impact of metformin use in younger women and on effects in pregnancy are needed. The use of metformin in PCOS has been endorsed by the Endocrine Society of Australia. Guidelines state that metformin should be considered as part of the management of PCOS in women with period irregularities and anovulation, commenting that women on this therapy should remain under the care of a specialist. The next step is to seek approval from the relevant regulatory authorities to improve access to and use of this effective medication in PCOS.

In contrast, data on glitazone therapy is more limited with further research on efficacy and safety, especially in pregnancy required before advocating clinical use in PCOS subjects. Established side effects, including weight gain, are also of concern.

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7

Long-term health consequences of polycystic ovary syndrome

Eleni Kousta and Stephen Franks

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder to affect women of reproductive age (Franks 1995). Although its first description occurred almost 70 years ago (Stein and Leventhal 1935), there has been no universal agreement about its definition. Recently, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) achieved a new consensus regarding the definition of PCOS (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). This is now defined as the presence of any two of the following three criteria: (1) polycystic ovaries; (2) oligo- and/or anovulation; and (3) clinical or biochemical evidence of hyperandrogenism. This revised definition provides an international framework for the clinical assessment of PCOS and for future research and collaboration.

The clinical and biochemical features of the syndrome are heterogeneous and the combination and degree of expression of these features vary between individuals. In the last few years, it has become clear that PCOS is not simply a combination of hyperandrogenemia and anovulation, but has important long-term health implications (Franks 1995, Dunaif 1997, The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). Although disagreement about diagnostic criteria has, up to now, made it difficult to compare epidemiological studies of long-term health risks, it is now well accepted that PCOS has been associated with metabolic disorders (hyperinsulinemia and insulin resistance, impaired pancreatic beta cell function and increased risk of type 2 diabetes, obesity, hyperlipidemia) and increased risk factors for cardiovascular disease. In addition, chronic anovulation in women with PCOS is thought to carry an increased risk of endometrial cancer (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

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Diagnostic criteria for the metabolic syndrome among women with PCOS have recently been implemented. The definition requires three out of the following five criteria: central obesity, hypertriglyceridemia, low high-density lipoprotein (HDL-C), increased blood pressure, and hyperglycemia (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). These metabolic abnormalities, clustered together, are related to insulin resistance and are important as risk factors for cardiovascular disease and type 2 diabetes among women with PCOS.

In this review the long-term health consequences for PCOS are discussed. In order to assess the impact of each individual factor in long-term health, risk factors – metabolic and cardiovascular – are discussed separately.

Hyperinsulinemia and insulin resistance

Insulin resistance is a metabolic state in which physiological concentrations of insulin produce subnormal effects on glucose homeostasis and utilization (Reaven 1988) so that higher than normal concentrations of circulating insulin are required to maintain normal blood glucose levels. Resistance to insulin mediated glucose disposal and compensatory hyperinsulinemia are common in the general, normoglycemic population. Insulin resistance varies according to body mass index (BMI) from 10% among lean subjects to 26% or higher among obese subjects, and hypersecretion of insulin is more prevalent than insulin resistance in the obese population as a whole (38%) (Ferrannini *et al.* 1997). Although insulin resistance is not a disease, its presence in both obese and non-obese subjects is associated with increased risk of cardiovascular morbidity and mortality and type 2 diabetes (Reaven 2002).

There are several reports assessing insulin action among women with PCOS suggesting that women with PCOS have a greater frequency and degree of both hyperinsulinemia and insulin resistance than age and weight matched controls (Burghen *et al.* 1980, Shoupe *et al.* 1983, Dunaif *et al.* 1987, Conway *et al.* 1990, Robinson *et al.* 1992). Insulin resistance is independent of the effect of obesity; both lean and obese women with PCOS were found to be hyperinsulinemic and insulin resistant compared to weight matched control subjects, but insulin resistance is most pronounced among obese women with PCOS (Conway *et al.* 1990, Dunaif 1997, Mather *et al.* 2000a, Toprak *et al.* 2001). Insulin resistance among PCOS women was associated with upper body fat distribution (Holte *et al.* 1994) and weight reduction in obese PCOS women, especially reduction in abdominal adiposity, resulted in normalization of insulin sensitivity, suggesting that body fat distribution is an important determinant of insulin resistance in PCOS (Holte *et al.* 1995).

Interestingly, insulin resistance is not a feature of all women with PCOS. There are significant differences in insulin sensitivity between ovulatory and anovulatory

women with PCOS. Anovulatory women with PCOS display insulin resistance whereas those with a regular menstrual cycle (but who present with symptoms of hyperandrogenism) do not demonstrate insulin resistance (Dunaif *et al.* 1989, Robinson *et al.* 1993). These observations suggest that there is a strong association between menstrual irregularity and insulin resistance among women with PCOS. Hyperinsulinemia itself may contribute to the mechanism of anovulation and the expression of PCOS (Abbott *et al.* 2002). There is no resistance to the steroidogenic action of insulin in the ovaries although there is recent evidence that there is a selective impairment of insulin mediated glucose metabolism in the ovary (Rice *et al.* 2005). The ovary is therefore exposed and responsive (at least in terms of steroid biosynthesis) to the high circulating levels of insulin. Insulin acts synergistically with luteinizing hormone (LH) to stimulate steroidogenesis and to induce premature arrest of the follicle development (Willis *et al.* 1998).

Insulin resistance in PCOS has been associated with increased androgen levels, particularly free testosterone (by some but not all authors), and the interaction between hyperandrogenemia and the precise etiology of insulin resistance remains unresolved (Toscano *et al.* 1992, Holte *et al.* 1995, Dunaif 1997). However, hirsute women with PCOS and hyperandrogenemia who have regular menstrual cycles have serum insulin concentrations at fasting and after glucose stimulation that are indistinguishable from those in weight matched control subjects and have similar insulin sensitivity to control subjects (Conway *et al.* 1990, Robinson *et al.* 1993). Hyperandrogenemia does not seem to cause insulin resistance; on the contrary, insulin may affect the expression of androgen by suppressing sex hormone binding globulin (SHBG) and augmenting androgen response to LH (Dunaif 1997). These observations suggest that hyperandrogenemia is not a major determinant of insulin sensitivity in PCOS.

The cause of hyperinsulinemia among women with PCOS also remains unknown. It may be related to an intrinsic abnormality of the post receptor insulin signaling pathway and/or abnormal insulin secretion (Holte *et al.* 1995, Dunaif 1997). It may be that metabolic abnormalities in PCOS start very early in life, during the prenatal or prepubertal period, and an early exposure to androgens during development may affect body fat distribution and insulin action (Abbott *et al.* 2002, Eisner *et al.* 2002). The clinical implication of these observations is that women with PCOS are likely to have insulin resistance and/or hyperinsulinemia, particularly when they are anovulatory and obese with central fat distribution. Given that insulin resistance is an independent risk factor for metabolic abnormalities, the presence of insulin resistance among PCOS women suggests future risk of cardiovascular disease and type 2 diabetes. Furthermore, using a cardiovascular risk score, insulin resistance in PCOS was found to be an important determinant of cardiovascular risk among women with PCOS independently of obesity (Mather *et al.* 2000a).

Impaired pancreatic beta cell function and increased risk of type 2 diabetes

Even though the majority of women with PCOS have hyperinsulinemia and insulin resistance and display increased insulin responses to a glucose load, studies that carefully assessed beta cell function demonstrate defects in insulin secretion in addition to insulin resistance (O'Meara *et al.* 1993, Ehrmann *et al.* 1995, Dunaif and Finegood 1996). Insulin secretory responses to mixed meals in insulin resistant women with PCOS are reduced relative to basal insulin secretion, an abnormality characteristic of type 2 diabetes (O'Meara *et al.* 1993). Given that when insulin resistance occurs the beta cell compensates by increasing the secretion of insulin, it is essential to take into account the degree of insulin sensitivity when assessing beta cell function. By using the intravenous glucose tolerance test (IVGTT) beta cell function can be assessed by measuring the acute insulin response to glucose (AIRG) and the disposition index (insulin sensitivity × acute insulin response to glucose), which allows beta cell function to be interpreted in the light of prevailing insulin resistance (Kahn *et al.* 1993). Decreased AIRG and/or disposition index in comparison to a control population are suggestive of impaired beta cell secretory capacity. Using the IVGTT both lean and obese women with PCOS had beta cell dysfunction in addition to insulin resistance (Dunaif and Finegood 1996). Defects in beta cell function are more evident among PCOS women who have first-degree relatives of type 2 diabetes as demonstrated by reduced insulin secretory responses either to boluses (IVGTT) or graded infusions of intravenous glucose and by impairment in the ability to entrain endogenous insulin secretion with glucose (Ehrmann *et al.* 1995). These observations suggest that women with PCOS, even when they are normoglycemic, may demonstrate impaired beta cell function on detailed testing. This may be an early metabolic defect contributing to the development of type 2 diabetes later in life. Similar early metabolic changes have also been reported in other groups at high future risk of type 2 diabetes, such as normoglycemic relatives of subjects with type 2 diabetes, and/or women with a history of gestational diabetes (Fernandez-Castaner *et al.* 1996, Kousta *et al.* 2003).

The prevalence of type 2 diabetes has been reported as 3–4% in the general population, increasing to 10–18% in old age (Harris *et al.* 1987, King and Rewers 1993). Several groups have assessed glucose tolerance among PCOS women and the overall risk of developing type 2 diabetes was found to be increased three to seven times (Dunaif *et al.* 1987, Dahlgren *et al.* 1992a, Ehrmann *et al.* 1999, Legro *et al.* 1999, Wild *et al.* 2000). The figures for prevalence rates of glucose intolerance among women with PCOS are much higher than expected compared with reference populations of women of similar age: 31–35% had impaired glucose tolerance and 7.5–10% had type 2 diabetes (Harris *et al.* 1987, Ehrmann *et al.*

1999, Legro *et al.* 1999). Although obesity and age substantially increase the risk, impaired glucose tolerance and diabetes are frequent even among non-obese PCOS women (10% and 1.5% respectively) (Legro *et al.* 1999). Apart from obesity and age, waist/hip ratio (Legro *et al.* 1999), family history of type 2 diabetes (Ehrmann *et al.* 1999, Legro *et al.* 1999, Gambineri *et al.* 2004), and androgen concentrations (Ehrmann *et al.* 1999, Gambineri *et al.* 2004) were also associated with glucose intolerance. Oligomenorrhea was found to be a risk factor for the development of type 2 diabetes and this risk was accentuated but not totally explained by obesity (Solomon *et al.* 2001).

The risk of glucose intolerance among PCOS women appears to be equally increased in mixed ethnicities of the US population and Asian PCOS groups (Ehrmann *et al.* 1999, Legro *et al.* 1999, Weerakiet *et al.* 2001). However, in a young Mediterranean population with PCOS the prevalence of glucose intolerance was lower than the previous reports (2.5% type 2 diabetes and 16% impaired glucose tolerance), but there were no data for a control group of similar age and ethnicity (Gambineri *et al.* 2004). In that study environmental factors explaining the lower prevalences of glucose intolerance such as dietary habits should be taken into account. In the same study lower birthweight and earlier age of menarche were associated with increased risk for glucose intolerance (Gambineri *et al.* 2004).

Furthermore, the onset of glucose intolerance in PCOS women seems to occur at an early age, typically in the third to fourth decade of life, which is earlier than in the normal population (Harris *et al.* 1987, King and Rewers 1993, Ehrmann *et al.* 1999, Legro *et al.* 1999). Abnormalities in both insulin action and secretion and glucose intolerance have been observed even in adolescents with PCOS (Arslanian *et al.* 2001, Lewy *et al.* 2001), and in young girls with premature adrenarche (who commonly go on to develop PCOS in adolescence (Ibañez *et al.* 2000)). The prevalence of glucose intolerance has been reported to be as high as 30–40% among both lean and obese adolescents with PCOS (Palmert *et al.* 2002).

An association between gestational diabetes and PCOS has been observed. The prevalence of polycystic ovary (PCO) morphology in women with previous gestational diabetes is significantly higher than control women (41–52%) (Anttila *et al.* 1998, Holte *et al.* 1998, Kousta *et al.* 2000), suggesting that women with PCO are at risk of developing gestational diabetes. Given that gestational diabetes is a forerunner of type 2 diabetes and that the majority of the women with gestational diabetes will develop type 2 diabetes in later life (O'Sullivan 1995), these observations reinforce the increased risk of diabetes in women with PCOS.

A significant percentage of first-degree relatives of the general population of women with PCOS have defects in insulin action and secretion or type 2 diabetes. In a recent study a strong family history of type 2 diabetes was noted among

parents of women with PCOS: 46% of the mothers and 58% of the fathers of women with PCOS had impaired glucose tolerance or type 2 diabetes and female family members with normoglycemia had insulin resistance (Yildiz *et al.* 2003). Hyperinsulinemia was found to be common among female and male first-degree relatives of women with PCOS (Norman *et al.* 1996). Evidence for a familial correlation of beta cell dysfunction (strong sibling correlation for the acute insulin secretion and the disposition index) but not insulin resistance was noted among families of women with PCOS (Colilla *et al.* 2001). In another study 25% of the sisters of women with PCOS were affected (having menstrual irregularities and/or hyperandrogenemia) and had increased insulin levels and decreased glucose/insulin ratios suggestive of insulin resistance compared to control women (Legro *et al.* 2002). These observations suggest that abnormalities of glucose regulation and type 2 diabetes are common among relatives of women with PCOS and that there is a strong association between the two diseases.

Women with PCOS have a greater chance of developing glucose intolerance, at an earlier age than the general population. The risk is further emphasized from the recent epidemiological observations noting increased mortality from the complications of diabetes among women with PCOS (Pierpoint *et al.* 1998). Central obesity and a family history of type 2 diabetes are parameters influencing the risk as in the general population. Menstrual irregularity is probably an additional risk factor. Ethnicity does not appear to influence the risk, suggesting that PCOS is a more important risk factor.

Obesity

Obesity is a known independent risk factor for the development of type 2 diabetes and cardiovascular disease. The prevalence of obesity is rising in societies who have adopted a Westernized lifestyle and is already a major health issue (Kuczmarski *et al.* 1994). In the general population, hyperinsulinemia seems to be the main metabolic feature among normoglycemic normotensive obese subjects, whereas insulin resistance is not as prevalent as previously thought (Ferrannini *et al.* 1997). Most importantly, cardiovascular morbidity and mortality are increased in obese women independently of other risk factors (Manson *et al.* 1990).

The association between PCOS and obesity has been observed since the early studies on PCOS. The prevalence of obesity among women with PCOS was noted to be as high as 41% when the diagnosis was based on histological features after wedge resection (Goldzieher and Green 1962). This prevalence varies between countries depending on the population studied and the criteria used to define PCOS. In the UK studies the prevalence of obesity among PCO women is 35–38%

(Kiddy *et al.* 1990, Balen *et al.* 1995), 36% in a black and white population in the USA (Knochenhauer *et al.* 1998), 10–38% in Mediterranean countries (Diamanti-Kandarakis *et al.* 1999, Asunción *et al.* 2000), and as high as 63% in Australia when the diagnosis was based on stricter criteria (Norman *et al.* 1995). Women with PCOS tend to have a central distribution of adiposity (Talbott *et al.* 1995, Taponen *et al.* 2003). Obesity in adolescence and weight gain after adolescence were associated to PCOS symptoms among women of reproductive age (Laitinen *et al.* 2003).

Obesity in women with PCOS not only has long-term implications for the general health but also has additional important reproductive impacts. Obese women with PCOS have a higher prevalence of menstrual disorders and infertility (Kiddy *et al.* 1990), are more likely to be hirsute, and have lower SHBG levels, leading to higher serum concentrations of free testosterone (Conway *et al.* 1990, Kiddy *et al.* 1990) than lean women with PCOS. Obese women with PCOS are less likely to respond to induction of ovulation treatment (Hamilton-Fairley *et al.* 1992, White *et al.* 1996, Kousta *et al.* 1997), require larger doses of gonadotropin to achieve ovulation and pregnancy (Hamilton-Fairley *et al.* 1992, White *et al.* 1996) and are more likely to miscarry (Sagle *et al.* 1988, Hamilton-Fairley *et al.* 1992, Wang *et al.* 2001). Furthermore, obesity in the overall population is associated with a higher prevalence of fetal abnormalities and more pregnancy complications including a higher risk for gestational diabetes (Norman and Clark 1998).

Weight loss may (at least partially) reverse the biochemical abnormalities (including insulin resistance, hyperlipidemia, and dyslipidemia) and improve reproductive function and hirsutism in obese women with PCOS (Kiddy *et al.* 1992, Andersen *et al.* 1995, Holte *et al.* 1995). Weight reduction in obese women with PCOS is encouraged before considering therapy to induce ovulation. Modifying additional lifestyle factors, including alcohol consumption, psychosocial stressors, and smoking, are also crucial in the long-term outcome of PCOS (Norman *et al.* 2002).

The factors other than diet that contribute to obesity among women with PCOS are unclear. An abnormality in energy expenditure in women with PCOS, notably in postprandial thermogenesis, has been implicated. Postprandial thermogenesis was significantly reduced in women with PCOS and there was a direct correlation of postprandial thermogenesis with insulin resistance (Robinson *et al.* 1992). It was calculated that if the difference in energy balance were maintained long term (as seems likely) over a year women with PCOS would have an excess of 1.9 kg of fat. It may be that this represented an evolutionary advantage in periods of calorie restriction, but when calorie intake is excessive it may contribute to obesity.

In summary, a significant proportion of women with PCOS are affected by obesity. The cause of obesity among women with PCOS is unknown, but the presence of obesity in PCOS women has important long-term metabolic and reproductive consequences including increased risk for type 2 diabetes, cardiovascular disease, anovulation, infertility, and miscarriage. Diet and lifestyle modification may improve reproductive function and fertility.

Hyperlipidemia

It is well known that lipid abnormalities are associated with risk for cardiovascular disease. Levels of HDL-C (Wilson *et al.* 1988, Bass *et al.* 1993) and triglyceride are strong independent predictors of cardiovascular death in women and low-density lipoprotein (LDL-C) and total cholesterol levels were poorer predictors of cardiovascular mortality (Bass *et al.* 1993).

Dyslipidemia is common among women with PCOS. There are several studies reporting higher total cholesterol, LDL-C (Talbott *et al.* 1995, 1998, Wild *et al.* 2000, Legro *et al.* 2001), very low density lipoprotein (VLDL)–cholesterol (Wild *et al.* 1985, Pirwany *et al.* 2001), triglycerides (Talbott *et al.* 1995, 1998, Wild *et al.* 2000, Pirwany *et al.* 2001) and lower HDL–cholesterol levels (Wild *et al.* 1985, Talbott *et al.* 1995, 1998, Robinson *et al.* 1996) among women with PCOS compared with control women. Even after controlling for obesity, lipid abnormalities still persist between PCOS and control women (Talbott *et al.* 1995, 1998, Mather *et al.* 2000a, Vrbikova *et al.* 2003). In a large study of young white women with PCOS increased LDL-C levels were the predominant lipid abnormality independent form of obesity (Legro *et al.* 2001). Furthermore, women with PCOS have raised concentrations of small dense LDL (LDL III) subfractions, considered to be more atherogenic (Austin *et al.* 1988) and increased hepatic lipase activity compared to weight matched control women (Pirwany *et al.* 2001). The difference in lipid profile is stronger at earlier ages and little difference is noted over the age of 40 years between PCOS and control women, suggesting a higher risk for atherosclerosis at an earlier age (Talbott *et al.* 1995, 1998). This may be due to an earlier onset of hormonal disturbances, obesity and intra-abdominal fat distribution among PCOS women or may reflect the LDL-C increase with age among controls. Lipid abnormalities in PCOS appear to be related to central adiposity (Pirwany *et al.* 2001) and hyperinsulinemia (Mather *et al.* 2000a, Pirwany *et al.* 2001) in some but not in all studies (Legro *et al.* 2001). Androgen levels were associated with triglyceride levels but not with other lipids (Legro *et al.* 2001), whereas others found no association between androgen levels and dyslipidemia (Pirwany *et al.* 2001). Interestingly, it was observed that women with hirsutism and regular cycles do not have dyslipidemia compared to control

women whereas those with both hirsutism and oligomenorrhea had lower HDL-C and higher triglycerides (Taponen *et al.* 2004), suggesting an association between menstrual irregularity and dyslipidemia.

Paradoxically, in a large study of young white women with PCOS, HDL-C levels were significantly higher even among obese subjects; however, when this difference was not significant after adjusting for age, BMI, fasting insulin, and other variables (Legro *et al.* 2001). It is unknown whether this increased HDL-C levels confer some protection against cardiovascular disease in young women with PCOS.

The cause of dyslipidemia among women with PCOS remains unknown. Dyslipidemia is more pronounced among obese subjects but cannot totally be attributed to obesity. Dyslipidemia seems to affect women with PCOS at a younger age, increasing therefore the risk for atherosclerosis. Known risk factors of dyslipidemia such as central adiposity and insulin resistance may play a significant role, but the role of androgens is still unclear. Women with menstrual irregularities are likely to be those exhibiting more pronounced dyslipidemia.

Prevalence of other cardiovascular risk factors

Hypertension

Data on blood pressure on women with PCOS are controversial. Blood pressure has been reported to be raised among PCOS women compared with control women, even after adjusting for BMI (Wild *et al.* 2000, Vrbikova *et al.* 2003) by some but not all investigators (Mather *et al.* 2000a). Obese women with PCOS were found to have increased systolic but not diastolic BP compared to weight matched control women, but there was no difference in blood pressure levels among the non-obese group (Legro *et al.* 2001). Women with oligomenorrhea and hirsutism had raised both systolic and diastolic blood pressure compared to control women (Taponen *et al.* 2004). Existing data however need to be interpreted with caution, and it needs to be noted that even small differences in blood pressure may be of clinical relevance, since at a group level a difference of 1.5–2 mm Hg could have a large impact on population cardiovascular risk (Rose 1981).

Endothelial dysfunction, chronic inflammation, and altered vascular function

Endothelial dysfunction is an early event in the process of arterial lesion formation and precedes atherosclerosis. Assessment of endothelial function may be a useful prognostic tool for cardiovascular disease (Verma *et al.* 2003). Endothelial function can be assessed by measuring peripheral circulation (flow mediated vasodilatation) or, indirectly, by the use of inflammatory markers.

An initial study on a small number of obese women with PCOS and age matched control women reported no difference in endothelial function (assessed

as endothelium dependent and independent vascular function by brachial artery ultrasound) between the two groups, despite the presence of insulin resistance and hyperandrogenism among PCOS subjects (Mather *et al.* 2000b). The authors commented that their findings could be attributed to the protective effects of estrogen exposure and/or HDL cholesterol, which did not differ between the two groups. However, subsequent studies reported decreased endothelial function among women with PCOS (Paradisi *et al.* 2001, Orio *et al.* 2004, Tarkun *et al.* 2004). These findings were present even among young, normotensive, non-obese, and non-dyslipidemic women with PCOS, as assessed by flow mediated dilatation on the branchial arteries (Orio *et al.* 2004). Resistance to the vasodilating action of insulin was noted and endothelial dysfunction was associated with obesity, insulin resistance, and androgen levels in women with PCOS (Paradisi *et al.* 2001). In this study, leg blood flow responses to intrafemoral artery infusions of the endothelium dependent vasodilator methacholine chloride and to euglycemic hyperinsulinemia were assessed. Abnormal vascular compliance suggesting vascular stiffness (assessed by recording pulse wave velocity across the aorta and brachial artery) and functional defect in the vascular action of insulin (studied by wire myography, by measuring the concentration response curve to norepinephrine before and after incubation with insulin) was shown in obese women with PCOS compared to weight matched control subjects (Kelly *et al.* 2002). These findings are consistent with early vascular changes associated with insulin resistance.

Endothelin-1 is a marker for abnormal vascular reactivity, which is postulated to contribute to the atherosclerotic process (Lerman *et al.* 1991). Insulin has a stimulating effect on endothelin-1 and it was hypothesized that that interaction could play a significant role in the development of atherosclerotic lesions in hyperinsulinemic conditions (Ferri *et al.* 1995). Endothelin-1 is elevated in patients with atherosclerosis (Lerman *et al.* 1991), diabetes, and obesity (Mather *et al.* 2002). Endothelin-1 was found to be raised in women with PCOS, independently of obesity (Diamanti-Kandarakis *et al.* 2001, Orio *et al.* 2004), suggesting an early vascular impairment. Chronic inflammation results in endothelial dysfunction and facilitates the initiation of an atherosclerotic process. Several studies suggest that low grade inflammation, reflected by elevated C-reactive protein (CRP) can contribute to the development of arteriosclerosis (Kuller *et al.* 1996, Ridker *et al.* 2000). C-reactive protein is considered not only an inflammatory marker of atherosclerosis but also a mediator of the disease because it contributes to the pathogenesis of lesion formation by interacting with the endothelium, and therefore CRP can be seen as a measure of endothelial function (Verma *et al.* 2003); CRP can independently predict type 2 diabetes (Freeman *et al.* 2002) and has been linked to insulin resistance (Festa *et al.* 2000). However, the role of

inflammation in the etiology of cardiovascular and other metabolic diseases is still disputed and is not generally accepted (Redberg *et al.* 2000, Blackburn *et al.* 2001, Folsom *et al.* 2001). Concentrations of CRP were significantly increased in women with PCOS compared to BMI matched control women, correlated with the degree of obesity and inversely with insulin sensitivity (Kelly *et al.* 2001, Tarkun *et al.* 2004), although not with total testosterone concentrations (Kelly *et al.* 2001). In another study, CRP levels were higher among the women with oligomenorrhea and/or hirsutism than control women, but after stratification by BMI, no differences persisted between the CRP levels of the cases and the controls (Taponen *et al.* 2004).

Premature atherosclerosis

Greater carotid intima media thickness (IMT), suggestive of an increased risk for atherosclerosis, was shown in a small number of women with PCOS over 40 years of age, but no significant difference in the prevalence of carotid plaque between cases and controls (Guzick *et al.* 1996). When the study was extended to a larger group of white women, the difference in mean carotid IMT between PCOS cases and controls, after adjustment for age and BMI, was only noted in women $\geq$45 years, but not in the younger group (aged 30–44 years) (Talbott *et al.* 2000). Central obesity was associated with a greater extent of carotid IMT among PCOS. Furthermore, the overall group of PCOS women had an increased prevalence of carotid plaque compared to control women (Talbott *et al.* 2000). Subsequent studies confirmed the increase in IMT among PCOS women compared to age and BMI matched control subjects (Orio *et al.* 2004).

Recently, obese premenopausal PCOS women (aged 30–45 years) were shown to have a greater prevalence and extent than expected of coronary artery calcium, a marker for coronary atherosclerosis, measured by electron beam computed tomography (Christian *et al.* 2003). However, it may be that central obesity and dyslipidemia, present in that group of women with PCOS, contribute to the increased risk for atherosclerosis (Christian *et al.* 2003). Also, the presence of PCO (on ultrasound scan) was associated with more extensive coronary artery disease among a mixed population of pre- and postmenopausal women (all younger than 60 years, with a mean age of 54 and 52 years among women with normal and PCO ovaries, respectively) who had coronary angiography (Birdsall *et al.* 1997).

These observations suggest that women with PCOS display altered endothelial function, low-grade chronic vascular inflammation, and increased vascular thickness. These abnormalities are indicative of the early stages of atherosclerosis and therefore suggest an increased risk of atherosclerosis at an earlier age than expected. It is not known whether the cause of these abnormalities can be attributed to PCOS per se or to the metabolic disturbances of the syndrome.

Cardiovascular events

It has been predicted based on a risk factor model – taking into account risk factor variables and comparing them to a reference population – that the risk of developing myocardial infarction is considerably increased (relative risk of 7.4) for women with PCOS compared to age matched control women (Dahlgren *et al.* 1992b).

Although cardiovascular risk factors are increased among women with PCOS, increased prevalence of cardiovascular events has not been confirmed. In a large retrospective study of 786 women with PCOS in the UK, mortality or morbidity rates from cardiovascular disease are not higher than expected (Pierpoint *et al.* 1998). The diagnosis of PCOS was made on ovarian histopathology; the majority of these women had undergone wedge resection about 30 years before the follow-up study. Observed death rates were compared with expected deaths using standardized mortality ratios. There was an increased number of deaths where type 2 diabetes was a complicating factor (Pierpoint *et al.* 1998). A more detailed subsequent study by the same research group showed that cardiovascular morbidity and mortality in women with PCOS were similar to those in the general population (Wild *et al.* 2000). Although the history of coronary heart disease was not more common among women with PCOS, the history of cerebrovascular disease was increased. Women with PCOS had higher levels of several cardiovascular and metabolic risk factors such as diabetes, hypertension, hypercholesterolemia, hypertriglyceridemia, and increased waist/hip, and after adjusting for BMI the differences for diabetes, hypertension, and hypercholesterolemia remained significant. An increased prevalence of family history of type 2 diabetes was also noted (Wild *et al.* 2000).

Given the increased prevalence of cardiovascular and metabolic risk factors among women with PCOS, it is perhaps surprising that there was no significant increase in the prevalence of cardiovascular events. Subjects studied were middle-aged at the time of observation (the mean age in the Wild *et al.* (2000) study was 57 years), and the prevalence of cardiovascular events in both study and reference groups was low. It may be that, as the cohort ages, there will be a divergence between PCO and control groups in the incidence of coronary heart disease. Also, these subjects were recruited mostly through their recorded treatment by ovarian wedge resection. This treatment is known to have short-term benefits for PCOS, but, although unlikely, long-term benefits in terms of cardiovascular health in those women cannot be excluded. On the other hand those women with PCOS who underwent wedge resection are likely to be those with the more severe symptoms of the syndrome. But, irrespective of any effect of surgical intervention, the presence of a protective factor against cardiovascular disease related to PCOS cannot be excluded. Further long-term epidemiological studies are needed before definite conclusions can be drawn. In the meantime, it seems sensible to

counsel patients "at risk" (i.e., particularly obese, anovulatory women with PCOS) about possible long-term risk of cardiovascular disease and to implement dietary and lifestyle changes where appropriate (Franks 2001, The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

Endometrial cancer

An association between PCOS and endometrial carcinoma was first suggested in 1949, 14 years after the original description of the syndrome (Speert 1949). Since then several studies have been published that seem to support this association. Women with PCOS have been thought to be at increased risk for endometrial cancer through chronic anovulation with unopposed estrogen exposure to endometrium. However, the evidence for such an association in epidemiological studies is incomplete and contradictory (Hardiman *et al.* 2003). Many of the studies providing evidence of an increased risk of endometrial cancer in women with PCOS do not provide an estimate of the relative risk compared with the general population or when the observations were compared to control women the numbers of the subjects were small (Hardiman *et al.* 2003).

The risk of developing endometrial cancer has been shown to be influenced by a number of factors including long-term exposure to unopposed estrogen, obesity, nulliparity, and infertility (Dahlgren *et al.* 1991). Women with anovulatory infertility were found to be at risk of developing endometrial hyperplasia, and in some cases this will be atypical and therefore premalignant (Coulam *et al.* 1983, Dahlgren *et al.* 1991, Escobedo *et al.* 1991). In one of these studies it was noted that the risk for endometrial carcinoma was increased only among the obese subgroup of anovulatory women (Coulam *et al.* 1983). Although most, but not all, of the women with chronic anovulation have PCOS, and additionally not all women with PCOS have anovulation, the risk for endometrial carcinoma based on anovulatory subjects cannot be applied to all women with PCOS.

Obese women in the general population are at increased risk of endometrial cancer compared to normal weight women, and hypertension and relative hyperglycemia were found to be significant markers of risk (Weiderpass *et al.* 2000, Furberg and Thune 2003). Women with types 1 and 2 diabetes mellitus have been also shown to be at increased risk for endometrial cancer (Weiderpass *et al.* 2000). It needs to be borne in mind that the apparent association between PCOS and endometrial carcinoma may be related to metabolic abnormalities arising as a result of the syndrome. In a retrospective study of 345 women with PCOS, analyzing all cause morbidity and mortality, a significant risk of endometrial carcinoma was observed among PCOS women (odds ratio (OR) 5.3), but obesity could be a confounding factor (Wild *et al.* 2000).

There are no prospective studies demonstrating increased risk for endometrial carcinoma among women with PCOS. The syndrome is associated with risk factors for endometrial cancer, but it has not yet been clarified whether the incidence or mortality from endometrial cancer among PCOS is increased. It is likely that obese women with PCOS and oligomenorrhea or amenorrhea are at greater risk. Due to limited data, universal screening or preventative strategies for these women cannot be applied at present but in order to prevent endometrial hyperplasia it has been considered important to shed the endometrium by inducing withdrawal bleed at least every 3 months among women with PCOS who have oligomenorrhea or amenorrhea (Balen 2001).

Summary

The overall risk of developing type 2 diabetes among women with PCOS was found to be increased three to seven times. Women with PCOS have several risk factors for developing type 2 diabetes including (central) obesity, abnormalities in insulin action and secretion, and a family history of type 2 diabetes. Menstrual irregularity may be an additional risk factor. Furthermore mortality from the complications of diabetes is increased among women with PCOS.

Women with PCOS have increased levels of cardiovascular risk factors: insulin resistance, obesity, dyslipidemia, hypertension, and markers of abnormal vascular function. This adverse cardiovascular risk profile may start at an early age and may lead to premature atherosclerosis. However, the level of risk of cardiovascular disease is uncertain. The limited epidemiological data available to date have shown no increase in cardiovascular events although the incidence of cerebrovascular events was increased.

Women with PCOS are predisposed to obesity. Obesity is an important independent risk factor for cardiovascular disease and type 2 diabetes. The presence of obesity in PCOS women has not only long-term metabolic health consequences but also important reproductive consequences including anovulation, infertility, and miscarriage. Diet and lifestyle modification may improve reproductive function and fertility.

Women with PCOS have been thought to have increased risk for endometrial carcinoma, through anovulation and increased exposure to unopposed estrogen; however, epidemiological data to support this hypothesis are limited. Among the overall group of women with PCOS those who have the greater prevalence of cardiovascular risk factors and are at higher risk for type 2 diabetes and endometrial carcinoma are those who are anovulatory and obese.

Importantly, there are, as yet, no large long-term studies of well-characterized women with PCOS that will allow clear assessment of the risk of disease in later

life. Such epidemiological studies are needed to assess the risk of long-term health consequences, to identify the subgroups among PCOS women which need to be targeted, and determine the timing and nature of measures for intervention and prevention.

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8

Skin manifestations of polycystic ovary syndrome

Rodney Sinclair and Jack Green

Introduction

The pilosebaceous unit consists of the hair follicle and associated sebaceous and apocrine glands. Hair follicles and sebaceous glands have cellular androgen receptors and react to circulating androgens. Physiological androgen levels induce secondary sexual hair development at puberty, and mild acne is a near universal accompaniment. Androgens also induce pattern hair loss the prevalence and severity increasing with age. Polycystic ovary syndrome (PCOS) is associated with androgen excess and may induce hirsutism and seborrhea and accentuate androgenetic alopecia and acne (Table 8.1). Acanthosis nigricans is a cutaneous marker of insulin resistance that is also associated with PCOS.

Physiology of the sebaceous gland

Sebaceous glands occur on all parts of the skin except on the glabrous skin of the palms and soles. They are most numerous on the face, scalp, and back occurring at a concentration of between 400 and 900 glands per square centimeter. Each of the several lobes of the gland has a duct lined with keratinizing squamous epithelium, and these join to form a main duct that enters the follicular canal. Glandular cells, which divide at the periphery, move towards the center of the glands and become increasingly filled with sebaceous material. During this process, cells undergo complete dissolution and discharge all cellular contents into the sebaceous duct. The lipid composition of sebum differs from epidermal lipid in that it contains wax esters and squalene that the former does not, although there are a similar percentage of glycerides (Cunliffe and Simpson 1998). Sebum secretion is determined by the size of the sebaceous gland, which in turn is regulated by androgens.

Polycystic Ovary Syndrome, 2nd edn, ed. Gabor T. Kovacs and Robert Norman. Published by Cambridge University Press.

Table 8.1. Dermatological features of PCOS diagnosed by direct ovarian visualization, ultrasound, or histology

	Balen *et al.* (1995)	Conway *et al.* (1989)	Franks (1988)	Goldzieher and Axelrod (1963)
Number of patients	1741 (ultrasound diagnosis)	556	300	1079 (histological diagnosis)
Hirsutism %	66.2	61	64	69
Acne %	34.7	24	27	
Alopecia %		8	3	
Acanthosis nigricans %	2.5	2	1	

Source: Modified from Simpson and Barth (1997).

Acne

Definition

Acne is a chronic inflammatory disorder of the pilosebaceous units.

Incidence

Acne is usually first evident in the early adolescent years and has a peak incidence in the later teenage years affecting up to 80% of schoolchildren. Although the incidence decreases in the third and subsequent decades, a minority of patients has persistence of lesions into middle age.

Pathogenesis

Pathogenic factors involved in acne include increased sebum production, abnormal keratinization of the pilosebaceous duct, abnormal microbial flora, in particular relating to increased *Priopionibacterium acnes*, and inflammation (Cunliffe and Simpson 1998). The presumed sequence of events is that androgens enlarge sebaceous glands that are themselves responsible for converting androgens into more active metabolites causing further sebaceous gland enlargement. These enlarged glands subsequently produce more sebum, which promotes increased *P. acnes* colonies within the hair follicle as these bacteria thrive in a sebaceous environment. The *P. acnes* digest the sebum, making it more viscous as well as producing inflammatory by-products. Subsequent hyperkeratosis in response to the inflammation, as well as the increased sebum viscosity, lead to follicular plugging and subsequent formation of comedones, the initial lesion of acne. If the follicular structure is then disrupted the release of inflammatory mediators into the surrounding dermis will lead to papule, pustule, and cyst formation.

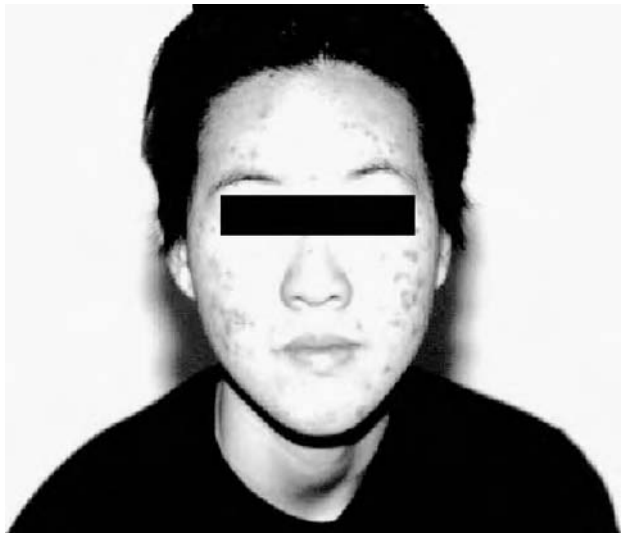

Fig. 8.1 Moderately severe acne.

Association with PCOS

Acne is seen in approximately one-third of PCOS patients (Franks 1988, Conway *et al.* 1989, Balen *et al.* 1995) and conversely a majority of women with severe or resistant acne have PCOS (Betti *et al.* 1990, Eden 1991). It is increased sebaceous secretion (Burton and Shuster 1971, Holmes *et al.* 1972) that is principally associated with PCOS, and this correlates with the severity of acne (Burton and Shuster 1971). While some studies have demonstrated an association between acne incidence and androgen levels (Darley *et al.* 1982, Held *et al.* 1984, Lucky *et al.* 1994), or acne severity and androgen levels (Marynick *et al.* 1983), others have demonstrated no such relationship (Levell *et al.* 1989).

Clinical features

Acne primarily affects the face and less often the back and chest. Lesions comprise open comedones (blackheads), closed comedones (whiteheads), papules, pustules, cysts, and nodules. Inflammatory lesions are usually tender (Fig. 8.1).

The lesions of acne are not static and comedones may evolve into pustules and pustules into modules. A significant proportion of lesions heal with scarring, but disfiguring scarring is only seen in a minority of acne patients. Ice-pick-like depressed scars occur most often on the cheeks while hypertrophic scars occur most commonly on the trunk.

Treatment

Mild disease can be treated topically. Topical therapies include keratolytics such as salicylic acid; retinoids; antiseptics such as benzyl peroxide; and antibiotics such as clindamycin 1% lotion and erythromycin 2% gel. Topical retinoids are most effective for comedones.

Moderate to severe disease generally requires oral therapy. Available agents include antibiotics such as the tetracyclines, erythromycin, and trimethoprim; antiandrogens such as spironolactone and cyproterone acetate (only indicated in women); and oral retinoids such as isotretinoin.

Oral antiandrogens are useful for women who experience premenstrual accentuation of acne or who have other signs of cutaneous virilization. The choice of an oral contraceptive in women with acne is also important as some can cause an exacerbation. In general oral contraceptives that are higher in estrogen and lower in progestogen are preferred as induction of sex hormone binding globulin (SHBG) reduces the free androgen index. There is also a preference for combinations containing cyproterone acetate, desogestrel, or norethisterone as the progestogen Yasmin.

Isotretinoin is effective in all forms of acne and produces long-term remission in more than 70% of patients. Isotretinoin is teratogenic and adequate contraceptive measures need to be taken during treatment and for 1 month afterwards. For this reason, and the expense of therapy, isotretinoin use is generally reserved for patients with severe cystic acne, acne unresponsive to other therapy, or acne associated with scarring (Ryan and Sinclair 2003).

Physiology of the hair follicle

The average person has approximately 2 million hair follicles of which only 100 000 are on the scalp (Messenger *et al.* 2004). Most of these follicles produce vellus hairs. Vellus hairs are fine, lightly pigmented, cosmetically imperceptible hairs produced by smaller hair follicles. Terminal hairs are longer, pigmented, easily visible hairs that are found on the scalp, eyebrows, and eyelashes prior to puberty. Some children also have terminal hairs on their forearms and shins. Around puberty, vellus hairs convert into terminal hairs in the axillae and pubic area and terminal hairs become more abundant on the forearms and legs. In men, terminal hairs also appear on the beard, chest, shoulders, and back.

Human hair growth is cyclical. The cycle consists of three phases. The growth phase, called anagen, generally lasts several months on the body and between 2 and 5 years on the scalp. As terminal hair growth rate is relatively constant (around 1 cm per month), the duration of anagen is the main determinant of hair length. Anagen is followed by catagen, lasting around 2 weeks. During catagen

hair growth stops and the lower two-thirds of the hair follicle undergoes apoptosis. The third phase is telogen, a resting phase, which lasts approximately 3 months.

At the conclusion of telogen/onset of anagen, stem cells located at the insertion of the arrector pili muscle regenerate an entirely new hair bulb, which produces an entirely new hair. In contrast to all other mammals where synchronized hair growth produces seasonal molting, human hair growth is not synchronized and at any given time, adjacent follicles will be at different stages of the growth cycle.

Pubertal androgens stimulate hair growth in some sites such as the axillae, pubic area, and male beard area, but inhibit growth on the scalp in genetically predisposed men and women. Androgens transform vellus hairs into terminal hairs in the through stimulation of androgen receptors in dermal papilla cells and production of insulin-like growth factor 1 (IGF-1). On the scalp androgen activation of dermal papilla cells induces transforming growth factor-β1 (TGF-β1), a diffusible keratinocyte growth suppressive factor, which transforms terminal into vellus hairs and androgenetic alopecia (Inui *et al.* 2003).

Hirsutism

Definition

Hirsutism may be defined as the growth of terminal hair on the body of the woman in the same patterns and sequence as that which develops in the normal postpubertal male (Dawber and Sinclair 2001) As the number of hair follicles on both scalp and body remains relatively constant from birth until death (Sinclair *et al.* 2004a) hirsutism is not characterized by an increase in the number of hairs but rather it is the quality, size, and degree of pigmentation as well as the length of the hairs produced by the individual follicles that defines hirsutism.

Incidence

Cultural norms and fashion determine the amount of hair that is seen as acceptable on the female face and body. Deviations from these norms, real or perceived, can be a source of anxiety and low self-esteem in susceptible individuals. Allowing for these cultural and personal influences on the definition of hirsutism approximately 9% of the young female population are considered to be hirsute (McKnight 1964).

Pathogenesis

Dihydrotestosterone (DHT) is thought to be the predominant androgen involved in the development of hirsutism. The dermal papilla has been shown to express androgen receptors and interactions of dihydrotestosterone with the androgen

receptors in the dermal papilla are thought to directly influence the size of the hair follicle and, consequently, the hair produced by that follicle.

Association with polycystic ovaries

Polycystic ovary syndrome is the most commonly diagnosed ovarian cause of hirsutism (Bailey-Pridham and Sanfilippo 1989, Sperling and Heimer 1993). Even in the context of regular menstrual cycles ultrasonic investigation will demonstrate a high rate of polycystic ovaries in hirsute patients (Adams *et al.* 1986, O'Driscoll *et al.* 1994)

The incidence of hirsutism in women diagnosed with PCOS is of the order of 60% to 70% amongst Caucasians (Goldzieher and Axelrod 1963, Franks 1988, Conway *et al.* 1989, Balen *et al.* 1995) and approximately 30% amongst Japanese women (Lobo 1991). The Japanese women with PCOS are less likely to develop hirsutism than their counterparts from America or Italy, even when controlled for differences in androgen status and insulin resistance, which suggests that there is also a racial influence on the prevalence of hirsutism.

Clinical features

Clinically one sees variable amounts of terminal hairs on sites associated with male secondary sexual development. The Ferriman and Gallwey grading system can be used to score the extent and severity of this terminal hair growth (Ferriman and Gallwey 1961) (Fig. 8.2). The nine "hormonal sites" are scored on a scale of 1 to 4 according to the degree of terminal hair growth. The scores are then added and used for comparison. Other methods that assess the degree of terminal hair growth on the upper lip, chin, lower abdomen, and thighs have also been described and used in clinical trials (Derksen *et al.* 1993), but the Ferriman and Gallwey scale remains the most popular.

The main differential diagnosis is hypertrichosis that tends to have an earlier onset and is not responsive to anti-androgen medication (Dawber and Sinclair 2001).

Treatment

Psychological

This aspect of treatment is easily overlooked but is important given the significant psychological morbidity that may accompany hirsutism. A simple explanation of the problem and education regarding attainable goals of treatment are important (Conn and Jacobs 1998) as is awareness of when psychiatric referral is required.

Physical methods of hair removal

There are several physical methods that are employed in the removal of unwanted hair. These include bleaching, shaving, plucking, depilatory creams, electrolysis, and laser.

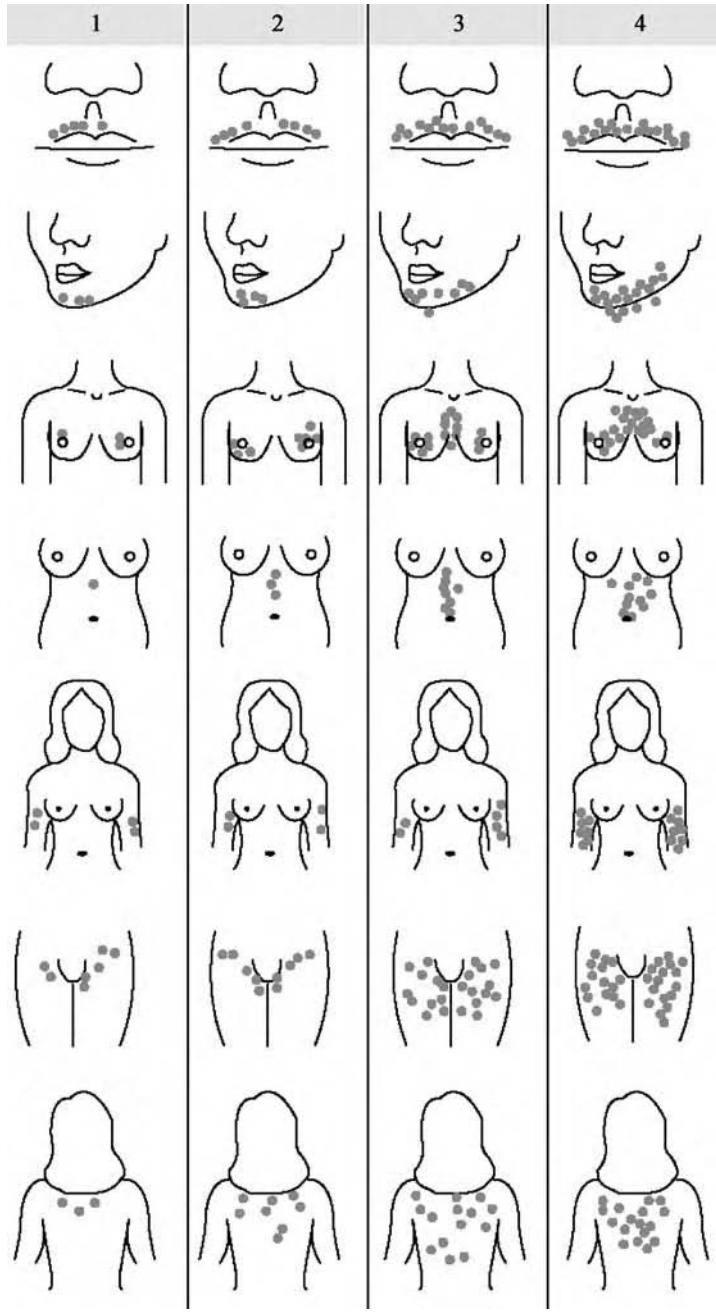

Fig. 8.2 Clinical grading scale for hirsutism. (Adapted from Ferriman D. and Gallwey J. (1961) Clinical assessment of body hair growth in women. *J. Clin. Endocrinol. 21*: 1440–1447.)

Bleaching of the hair with hydrogen peroxide is used to disguise pigmented facial hairs. For many women the cosmetic result is sufficient for this to be used first. Occasionally bleaching leads to discoloration of the skin or an artificial yellow hue to the hair.

Shaving is a common method for controlling unwanted hair and, contrary to common belief, does not affect the rate or duration of the anagen (growing phase) of the hair cycle, nor the hair diameter (Trotter 1923). Side effects include irritation and pseudofolliculitis. Some women find it unacceptable to shave their facial hair as the act of shaving itself reinforces feelings of masculinity (Conn and Jacobs 1998).

Epilation is simple and perhaps the most commonly used method of hair removal. Repetitive plucking may lead to permanent hair follicle matrix damage leading to finer, thinner hairs (Hamilton and Potten 1974). Side effects include postinflammatory hyperpigmentation, folliculitis, pseudofolliculitis, and, rarely, scarring (Olsen, 1999).

Chemical depilatories are usually thioglycates that disrupt the disulfide bonds of the hair shaft. They are spread on an area of unwanted hair for up to 15 minutes and then wiped away with the destroyed hair. Skin irritation is common and whilst useful for bikini lines they are less well tolerated in the axilla and on the face.

Electrolysis is a proven method of permanent hair removal (Dawber and Sinclair 2001). Two forms exist, galvanic (direct current electrolysis) and thermolysis (alternate current electrolysis). It works by cauterizing the dermal papilla of the hair follicle. Several treatments may be necessary. The procedure is very operator dependent. Poor results are common and folliculitis, hyperpigmentation, and scarring are not uncommon sequelae.

Lasers produce monochromatic light that is absorbed by specific chromophores in the skin and converted into thermal energy. This leads to vaporization of the target tissue. Lasers currently in use for hair removal include the ruby laser, the alexandrite laser, and the semiconductor diode laser, which all target melanin in the hair bulb, the YAG laser, which works via absorbance of carbon particles in a topical paste that is massaged into the skin prior to its use and thus falls into the follicular tracts. The Epilight, which is an intense pulsed light system, is not a laser because the light generated is not monochromatic, is also used for hair removal (Olsen 1999). Side effects of these treatments include erythema, hyper- and hypopigmentation, blistering, and pain. Most often, several treatments are required to improve efficacy but none of the presently utilized lasers has been proven to permanently destroy hair (Olsen 1999).

Weight loss

There is an increased frequency of hirsutism in obese as compared with thin women with PCOS (Kiddy *et al.* 1990). This is associated with a fall in the

concentration of SHBG and a rise in concentration of free testosterone. Weight loss by obese hirsute women both with and without polycystic ovaries usually leads to a reduction in body hair growth (Ruutiainen *et al.* 1988, Kiddy *et al.* 1992, Piacquadio *et al.* 1994)

Pharmacological methods of hair removal

Prior to the commencement of treatment, patients should understand that any effect on hair growth will take several months to become apparent and in general only partial improvement may be expected. The drugs currently available are only suppressive and will need to be taken long term (Dawber and Sinclair 2001). As there is the potential to feminize a male fetus women should be advised not to fall pregnant whilst on treatment.

Oral contraceptives

Oral contraceptives work by suppression of ovarian androgen production and elevation of SHBG, thereby reducing free testosterone. They can be used alone or in combination with other antiandrogens such a cyproterone acetate or spironolactone. Diane35ED (Falsetti and Galbignani 1990) containing 2 mg cyproterone acetate and 35 μg of ethinyl oestradiol and Marvelon containing desogestrel have been shown to be effective (Ruutiainen 1986) in the treatment of hirsutism. Certain oral contraceptive pills in particular those containing increased doses of norethisterone and levonorgesterol will exacerbate hirsutism and should be avoided.

Cyproterone acetate

Cyproterone acetate has a dual mode of action. It antagonizes the androgen receptor in the skin and also acts as a weak progestogen that inhibits gonadotropin secretion thereby decreasing ovarian androgen production (Fig. 8.3).

It is normally administered with cyclical estrogen to minimize menstrual irregularity and for contraception. Cyproterone is teratogenic. Dosage recommendations are 50–100 mg on days 5–15 of the menstrual cycle. While one dose-ranging study found no difference in efficacy with different doses (Barth *et al.* 1991), many clinicians find higher doses more effective (Messenger *et al.* 2004).

Potential side effects include: loss of libido, weight gain, fatigue, breast tenderness, gastrointestinal upset, headaches, and lowered mood, as well as the teratogenesis to male fetuses. As cyproterone acetate is lipophilic, conception is best avoided for 3 months from when the drug is ceased.

Spironolactone

Spironolactone is an oral aldosterone antagonist with antiandrogenic properties. It complexes with the intracellular androgen receptor, forming a biologically

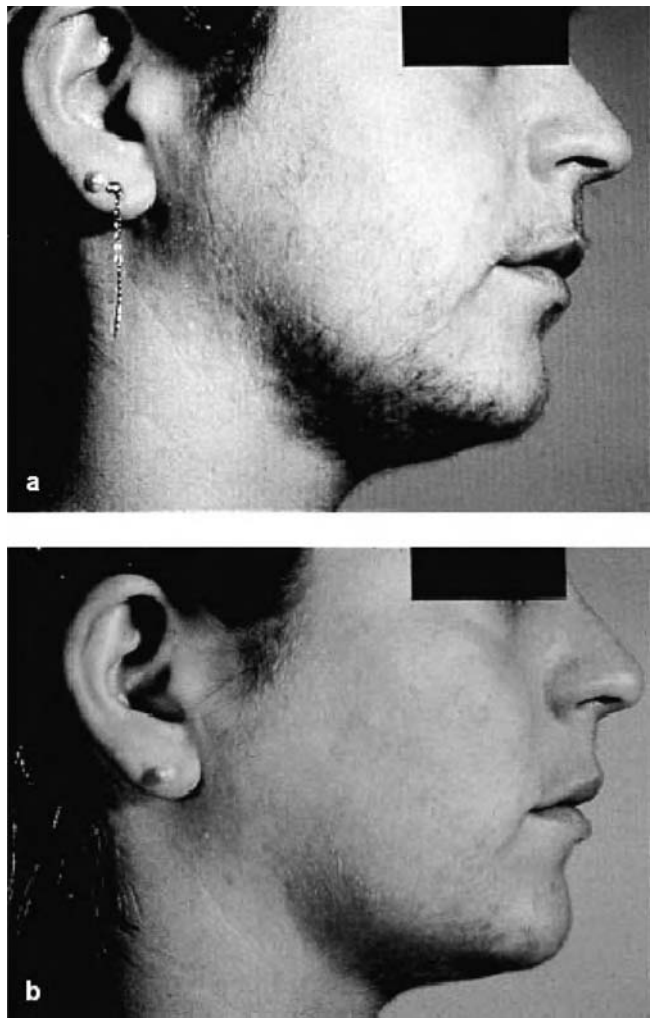

Fig. 8.3 Facial hirsutism, before (a) and after (b) treatment with cyproterone acetate. (Reproduced with permission from Oxford Clinical Communications.)

inactive compound. It also reduces the bioavailability of testosterone by interfering with its production, increasing its metabolic clearance and reducing cutaneous 5α-reductase activity. The usual dose is 100–200 mg daily.

Potential side effects include menstrual irregularities, breast tenderness, gastrointestinal disturbance, headache, and dizziness. Due to its potassium-sparing diuretic effect hyperkalemia is a risk if it is combined with potassium-sparing diuretics, angiotensin converting enzyme inhibitors, or certain non-steroidal anti-inflammatory drugs, or if used in patients with renal failure. Electrolytes should be checked whilst on this medication. Oral contraception is not required

in conjunction with this medication (except for menstrual control) but adequate contraceptive measures should be taken whilst taking spironolactone.

Flutemide

Flutemide is a non-steroidal pure antiandrogen. It has no glucocorticoid, progestational, androgenic, or estrogenic activity. It works by binding to androgen receptors but has no effect on gonadotropin secretion. Studies have demonstrated its efficacy and tolerability (Cusan *et al.* 1990, Falsetti *et al.* 1997). Side effects include: dry skin, menstrual disturbance, fatigue, lowered libido, and gastric upset; hepatotoxicity is an infrequent but significant adverse reaction that develops in the first few weeks of therapy, and as a result flutemide is rarely if ever used for hirsutism.

Finasteride

Finasteride is a type II 5α-reductase inhibitor which has been demonstrated to reduce hirsutism with minimal side effects in patients with PCOS (Castello *et al.* 1996, Tolino *et al.* 1996). However, due to the potential risk of emasculation of the fetus, and its long biological half-life, it is best avoided in women of childbearing potential.

Androgenetic alopecia (female pattern hair loss)

Definition

Androgenetic alopecia is a progressive, non-scarring, patterned loss of scalp terminal hairs. It requires both a hereditary predisposition and sufficient androgens to realize this genetic potential (Messenger *et al.* 2004).

Incidence

Using a 5-point visual analog scale (Fig. 8.4), where stage 1 is the normal prepubertal hair pattern, hair loss can be seen with increasing frequency with advancing age (Table 8.2). While only 10% of women aged 20–29 experience hair loss, fewer than 50% of women who reach the age of 70 do so with a full head of hair. Twenty-five percent will develop mild hair loss and 30% moderate to severe hair loss (Gan and Sinclair 2004).

Pathogenesis

While most women with androgenetic alopecia have normal levels of circulating androgens, women with PCOS are more likely to develop female pattern hair loss. In males the normal levels of circulating androgens are sufficient for full expression of a genetic predisposition to baldness, while in females the degree of alopecia is in part, related to circulating androgen levels (Miller *et al.* 1982,

Table 8.2. Hair patterns in female subjects

Age	Hair thickness					Total	Total less stage 1
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5		
5–9	72 (100%)					72	–
20–29	50 (88%)	5 (9%)	1 (2%)	1 (2%)		57	7 (12.3%)
30–39	73 (83%)	14 (16%)		1 (1%)		88	15 (17.0%)
40–49	91 (75%)	28 (23%)		3 (2%)		122	31 (25.4%)
50–59	106 (72%)	29 (20%)	11 (7%)	1 (1%)		147	41 (27.9%)
60–69	73 (59%)	37 (30%)	11 (9%)	2 (2%)	1 (1%)	124	51 (41.1%)
70–79	58 (46%)	35 (28%)	24 (19%)	6 (5%)	2 (2%)	125	67 (53.6%)
>= 80	23 (43%)	15 (28%)	8 (15%)	8 (15%)		54	35 (57.4%)
Total	474(66%)	163 (23%)	55 (8%)	22 (3%)	3 (0.4%)	717	247 (32.2%[a])

Note:

[a] Adjusted to age.

Fig. 8.4 Sinclair scale for clinical diagnosis of female pattern hair loss. Stage 1 is the normal pattern found in prepubertal girls. Stage 2 indicates widening of the central part line. Stages 3 indicates widening of the central part line and in addition translucency at the edge of the part. Stage 4 indicates development of a bald patch and stage 5 indicates advanced hair loss. Stages 3, 4, and 5 have a positive predictive value for androgenetic alopecia of 97%.

De Villez and Dunn 1986). Both adrenal and ovarian dihydroepiandosterone (DHEA) and testosterone are implicated in the pathogenesis of female balding (De Villez and Dunn 1986). In particular, DHT, a reduction metabolite of testosterone, is thought to be the principal androgen responsible for androgenetic alopecia.

Association with PCOS

A number of women with PCOS suffer with baldness (Franks 1988, Conway *et al.* 1989). Reliable numbers are hard to find due to the difficulty diagnosing early androgenetic alopecia and the population bias in reported studies (Messenger *et al.* 2004).

Fig. 8.5 Ludwig stages I, II, and III of androgenetic alopecia.

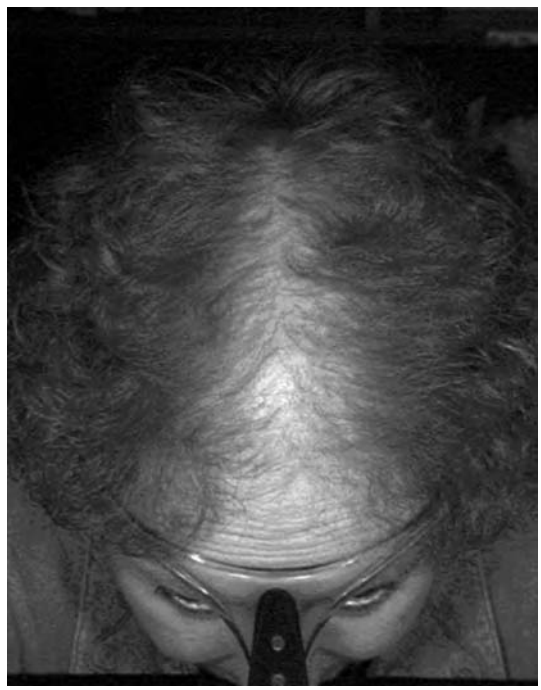

Fig. 8.6 Diffuse alopecia predominantly over the vertex of the scalp.

Clinical features

Unlike men, women never develop vertex balding. The predominant form of hair loss experienced by women with androgenetic alopecia is from the mid-frontal scalp. This pattern of hair loss was first described by Ludwig (1977) (Fig. 8.5), who stated that women experience diffuse hair loss over the crown with preservation of the frontal hair line (Fig. 8.6). This was subsequently shown to be incorrect as many women also experience bitemporal recession (Venning and Dawber 1988).

Bitemporal recession may be seen in up to 13% of premenopausal and 37% of postmenopausal women, and occurs as an age-related event, independent of mid-frontal hair loss (Birch and Messenger 2003, Gan and Sinclair 2004).

Widening of the mid-line hair part is an early sign of androgenetic alopecia. Some women present with increased hair shedding before there is any visible widening of the central part line. Scalp biopsy in these women will demonstrate androgenetic alopecia in over 60%. However while a decrease in the ratio of terminal to vellus hairs to $<4:1$ on scalp biopsy is specific for androgenetic alopecia, it lacks sensitivity, as scalp biopsy fails to demonstrate androgenetic alopecia in around 40% of women with stage 2 hair density (Sinclair *et al.* 2004b).

Many women, and in particular those who do not experience increased hair shedding, either fail to notice their hair loss or underestimate its severity (Biondo *et al.* 2004). Those who are aware of hair loss find it distressing and hair loss has a profound negative impact on quality of life for those women (Biondo *et al.* 2004).

Treatment

Non-pharmacological treatment

As for hirsutism, it is important to address psychological sequelae of alopecia as well as educating patients regarding both the cause of the hair loss and realistic goals regarding treatment outcomes. Some studies have shown that weight loss benefits patients with androgenetic alopecia possibly by being associated with decreased androgen production (Piacquadio *et al.* 1994).

Appropriate hair styling and camouflage can greatly improve the appearance and diminish morbidity in androgenetic alopecia. Wigs are appropriate for women with more extensive hair loss. Hair transplantation may also be appropriate for some women. The diffuse hair loss experienced by women often extends into the potential donor site and limits the potential for transplantation.

Pharmacological treatments

Minoxidil

Minoxidil is a piperidinopyrimidine derivative that causes vasodilatation and prolongs anagen. It is used topically on the scalp and is available as a 2% and a 5% solution. A dose of 1 ml of the 2% solution should be applied twice daily; however, many women find once daily application of the 5% solution sufficient. It should be applied across the entire mid-frontal scalp and the hair should not be wetted for an hour after application. Several months of treatment are needed before reduction in hair shedding or regrowth is observed. Regrowth may be delayed for up to 12 months with the 2% solution. Common side effects include mild hypertrichosis on the forehead and temples as well as erythema, pruritus, and scaling of the scalp.

Cyproterone acetate, spironolactone, and finasteride

Cyproterone acetate, as well as being useful in hirsutism, is used to treat androgenetic alopecia. The dose used is the same as that for hirsutism, 50–100 mg daily for 10 days of each cycle. A contraceptive pill should also concomitantly be used. Progression of hair loss is arrested in up to 90% while fewer than 40% experience regrowth. No effect is usually noticed for a period of 3–6 months. Hair shedding can be diminished, as soon as 2–3 month in some cases, but it may also continue despite visible hair regrowth. Fluctuation in hair shedding is not a reliable measure of response to therapy (Sinclair *et al.* 2004c).

Spironolactone is also used to treat androgenetic alopecia at the same doses as for hirsutism of 100–200 mg daily. An oral contraceptive is not required but renal function should be checked as there is a risk of hyperkalemia. Again a period of at least 3–6 months is required before the patient will notice any response to therapy (Sinclair *et al.* 2004c).

Finasteride is only infrequently prescribed for androgenetic alopecia in females and usually only in post menopausal women (Shum *et al.* 2002, Thai and Sinclair 2002). More studies are required to demonstrate its efficacy in female androgenetic alopecia and to determine the optimal dose.

A major problem in treatment of androgenetic alopecia is assessing treatment response. This is compounded by the slow but inexorable progression of androgenetic alopecia. Despite successfully arresting hair loss, many women abandon therapy prematurely as they are unconvinced of the benefits of therapy. Serial scalp photography helps overcome this and increases patient adherence to treatment.

Acanthosis nigricans

Acanthosis nigricans (AN) is characterized by hyperkeratosis, papillomatosis, and increased pigmentation. It occurs in up to 5% of women with PCOS (Dunaif *et al.* 1985, Franks 1988, Conway *et al.* 1989, Balen *et al.* 1995). The papillomatosis gives the skin a velvety contour (Fig. 8.7). Plaques most often occur in the axillae, the nape of the neck, under the breasts, and in the flexures (Schwartz 1994). Various clinical subtypes of AN have been described. The variety associated with PCOS is the benign acanthosis nigricans. When women with this benign subtype have had their ovarian morphology identified polycystic ovaries have been an almost universal finding (Rendon *et al.* 1989, Conway and Jacobs 1990).

The term HAIR–AN syndrome has been coined to describe the constellation of symptoms of hyperandrogenism, insulin resistance and acanthosis nigricans (Dunaif *et al.* 1985, Corenblum and Baylis 1990, Simpson and Barth 1997).

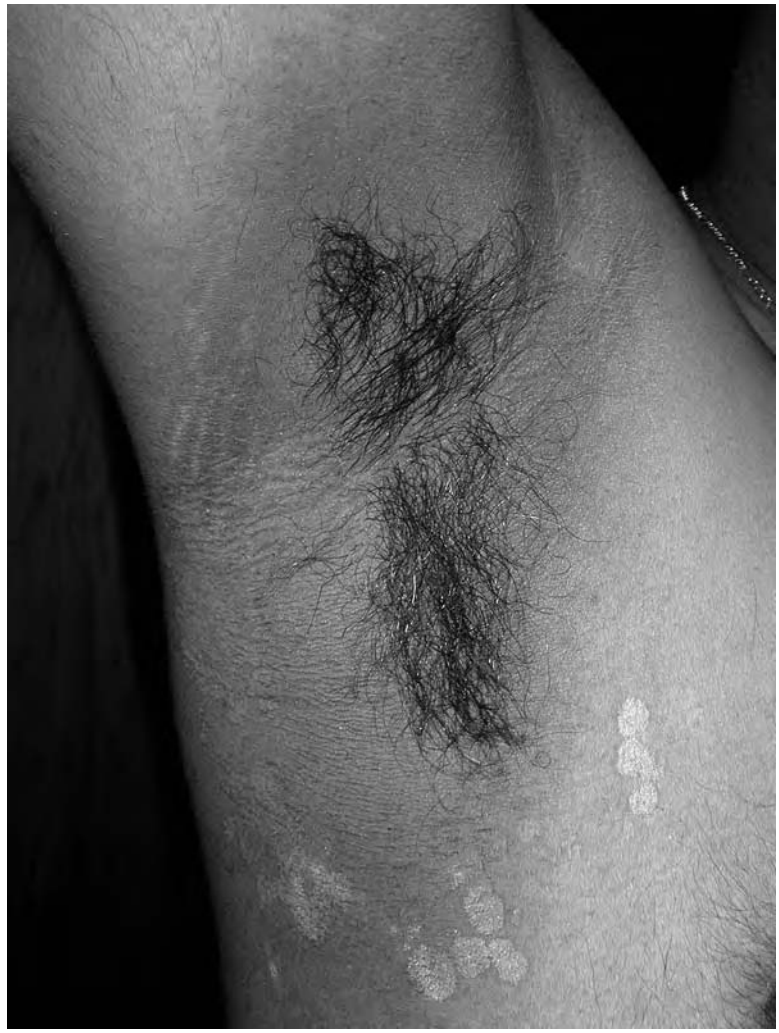

Fig. 8.7 Acanthosis nigricans of the axilla.

Conclusion

Dermatological manifestations are common in women with PCOS. Approximately 30% get acne, 60–70% get hirsutism, and many also develop androgenetic alopecia. Acanthosis nigricans is less common.

Hormonal manipulation will control many of those conditions; however, there are many adjunct treatments that are also beneficial and which may be used in conjunction or as alternatives. When the skin problems are severe or causing concern to the patient a combined approach between the gynecologist, dermatologist, and general practitioner is appropriate.

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9

Lifestyle factors in the etiology and management of polycystic ovary syndrome

Robert Norman and Lisa Moran

Introduction

Polycystic ovary syndrome (PCOS) is thought to arise from a combination of familial and environmental factors that interact to cause the characteristic menstrual and metabolic disturbances. It is our contention that alteration of the environmental components of this condition is fundamental to the management of the condition and that pharmaceutical treatment (including clomiphene citrate, gonadotropins, and insulin sensitizing agents) should only be used after adequate counseling and action relating to lifestyle alterations. Attention to weight loss, altered diet, and exercise are important aspects to discuss with the patient as well as stopping smoking and improving psychological attitudes. Because of the importance of overweight in the majority of women with this condition, much of this chapter will concentrate on obesity in PCOS.

Obesity and disease

Obesity is a costly and increasingly prevalent condition in Western society. In the USA 50% of Americans are overweight with women (34%), blacks (49%), and Hispanics (47%) showing the highest rates of obesity. In Australia 40% of the population are overweight or obese according to recent Australian Bureau of Statistics data. The prevalence of overweight/obesity increases in Australian women as they age with 34% of all women between 20–69 years having a body mass index (BMI) (weight in kg/height in m^2) over 25 and 12% with a BMI over 30. In the Repromed Centre at the University of Adelaide, 40% of women have BMI over 25 and 20% a BMI over 30 based on over 5000 patients presenting between 1991 and 1997.

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Obesity is associated in women with an increased risk of diabetes mellitus, osteoarthritis, cardiovascular disease, sleep apnea, breast and uterine cancer, and reproductive disorders. While women have increased body fat as an essential requirement for reproductive efficiency in pregnancy (Frisch 1985, 1987), fat in excess of the normal can lead to menstrual abnormality, infertility, miscarriage, and difficulties in performing assisted reproduction. Observational and theoretical considerations indicate that body weight has an inverted "U" effect on reproduction whereby low and high body mass contribute to infertility, menstrual disorders, and poor reproductive outcome (Correa and Jacoby 1978).

The commonest used index of obesity is the BMI, which correlates reasonably well with body fat although at height extremes there is less association. Total body fat has been assessed by physical methods, such as skinfold thickness, underwater weighing, dexa densitometry, magnetic resonance imaging, and infrared spectroscopy (Pasquali and Casimirri 1993). The distribution of fat is also thought to be important and can be assessed by these methods although the waist/hip ratio (WHR) or, simply, the waist circumference is the easiest clinical method. The reproductive literature is largely based on weight or BMI with little data available on body composition or fat distribution. However, the general medical literature does contain evidence that even peripheral (non-central) obesity is highly significant in poor health outcomes and the absence of information on fat distribution relating to reproductive factors may not be essential to an understanding of the importance of obesity.

Women have greater fat reserves than men but fat distribution is more likely to be peripheral (gynecoid) than abdominal (android). Obese and overweight women are overrepresented in gynecological and reproductive medicine clinics. Ranges for BMI are usually defined as follows: underweight (<19), normal weight (19.1–24.9), overweight (25–29.9) and obese (>30). Central or peripheral obesity is usually divided at WHR of 0.82–0.85.

Metabolic activity of fat tissue

Adipose tissue is an important site of active steroid production and metabolism (Pasquali and Casimirri 1993). It is able to convert androgens to estrogens (aromatase activity) and estradiol to estrone and dehydroepiandrosterone to androstenediol (17β HSD activity). Aromatase is found in bone, the hypothalamus, liver, muscle, kidney, and adipose tissue in the breast, abdomen, omentum, and marrow. Within adipose tissue, aromatase activity has been identified in the cells of periadipocyte fibrovascular stroma and may be different between various depots of adipose tissue. While in "simple" obesity, blood levels of androgens do not appear to differ from those of non-obese controls, production and clearance

rates are significantly different (Pasquali and Casimirri 1993). Abdominal fat distribution also significantly influences androgen and estrogen metabolism. In hyperandrogenic obesity, such as PCOS, increased production rates of androgens are associated with menstrual irregularities. The amount of androstenedione converted to estrone varies depending on the total body weight with women in the following groups showing conversion rates in brackets: 49–63 kg (1.0%), 63.1–91 kg (1.5%), >91kg (2.3%). Other mechanisms that influence the adipose tissue as an endocrine organ are: (1) metabolism of estrogens to 2-hydroxy estrogen (relatively inactive) or to 16-hydroxylated estrogen (active in obesity); (2) the storage of steroid hormones in fat; and (3) the effects of adiposity on insulin secretion from the pancreas and hence the levels of sex hormone binding globulin (SHBG).

Leptin is the product of the *ob* gene, a protein produced in fat cells that signals the magnitude of the energy stores to the brain and has significant effects on the reproductive system of rodents. Absence of the full-length *ob* gene, or its receptor, leads to obesity and reproductive dysfunction. Replacement of leptin in *ob*/*ob* mice restores fertility. Administration of recombinant leptin to rodents induces puberty earlier than in control animals indicating important effects, directly or indirectly, on ovarian function. Zachow and Magoffin (1997) have shown that leptin directly affects insulin-like growth factor (IGF) induced estradiol production in the rodent ovary in the presence of follicle stimulating hormone (FSH).

Initial reports suggested that leptin was increased in a significant proportion of women with anovulation, specifically with PCOS. Subsequently, this has not been confirmed (Chapman *et al.* 1997) and leptin does not alter in women who are put on insulin sensitizing agents such as troglitazone (Mantzoros *et al.* 1997) or gonadotropin reducing drugs such as the oral contraceptive pill (Nader *et al.* 1997). The true role of leptin in influencing ovarian function therefore remains unclear. This hormone may have an affect on ovarian function directly via actions on the ovary or alternatively through the hypothalmic–pituitary–ovarian axis. While the exact role of leptin remains unclear in PCOS, current techniques for intervention by weight loss or drugs have little impact on concentrations of leptin other than by reduction of body fat. Other hormones such as adiponectin and resistin are produced by cells associated with fat tissue and may play a role in metabolism in PCOS. We and others have also shown that appetite regulation may be disturbed in PCOS and that this may be regulated through a gut hormone named ghrelin (Moran *et al.* 2004).

Obesity, PCOS, and menstruation

The original description of what is now known as PCOS associated obesity and anovulation with infertility. Classical studies by Mitchell and Rogers (1953) and

Table 9.1. Impact of obesity on reproduction

Condition	Associated risks
Menstruation	↑ risk of menstrual dysfunction: amenorrhea, oligomenorrhea, and menorrhagia
Infertility	↑ risk of ovulatory and anovulatory infertility: anovulation, poor response to fertility drugs
Miscarriage	↑ risk of miscarriage, spontaneously and after infertility treatment
Glucose intolerance	↑ risk of impaired glucose tolerance and type 2 diabetes mellitus
Infertility treatment	↑ requirement for clomiphene citrate/gonadotropin ovulation induction. ↓ success rate for IVF/ICI/GIFT pregnancies
Pregnancy	↑ prevalence of pregnancy induced hypertension, gestational diabetes, Cesarean section, and Down's syndrome

Abbreviations: GIFT, gamete intrafallopian transfer; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

Hartz *et al.* (1979) confirmed these findings in much larger groups of women. The former group reported that obesity was present at a four times higher rate in women with menstrual disturbances than in women with normal cycles (Table 9.1). Forty-five percent of amenorrheic women were obese while only 9–13% of women with normal periods were overweight. Hartz *et al.* (1979) studied 26 638 women by questionnaire and noted that anovulation was strongly associated with obesity. Grossly obese women had a rate of menstrual disturbance 3.1 times more frequent than women in the normal weight range. In that study, teenage obesity was positively correlated with menstrual irregularity later in life and obesity was correlated with abnormal and long cycles, heavy flow, and hirsutism. This study was however selective in that volunteers were recruited from a weight control organization, data were self-reported, and only one-third of all subjects were suitable for analysis. Lake and colleagues (1997) studied nearly 5800 women who were born in 1958 and seen at ages 7, 11, 16, 23, and 33 years. Obesity in childhood and the early 20s increased the risk of menstrual problems (odds ratio (OR) 1.75 and 1.59 respectively). Women who were overweight at 23 years (BMI 23.9–28.6) were 1.32 times more likely and if obese (>28.6) 1.75 times more likely to have menstrual difficulties. Interestingly, in view of the association between overweight and early menarche, girls with menarche at 9, 10, or 11 were more likely to have menstrual problems at 16.5 years (OR 1.45 for mild and 1.94 for severe menstrual abnormality) but this was not reflected at age 33 years. Balen and colleagues (1995) in London have also shown a close relationship between weight and menstrual disorders. Of 1741 subjects with PCOS, 70% had menstrual

disturbances and only 22% had normal menstrual function if their BMI was over 30. Similar results were reported by Kiddy *et al.* (1990): obese subjects with PCOS had a 88% chance of menstrual disturbance compared to 72% in non-obese subjects with PCOS.

It is likely that obesity and overweight do contribute to a significant proportion of menstrual dysfunction. There is little in the literature to separate predisposing or associated features such as PCOS from so-called "simple" obesity, although there are suggestions that women with polycystic ovaries suffer more from weight-related menstrual dysfunction than those with normal ovaries (Hartz *et al.* 1979).

Obesity, PCOS, and infertility

Many multiparous women are obese and indeed most obese women are able to get pregnant readily. Initial study of the literature suggests that several excellent investigators have not been able to confirm the adverse effect of weight on reproductive performance. The Oxford Family Planning Study (Howe *et al.* 1985) did not show any relationship between conception rates and weight or BMI in women stopping contraception, but those women were a selected group of largely parous subjects. Similar criticism applies to a well-conducted prospective study on fecundity in volunteer women who were followed for 6 months to examine the effect of environmental agents on reproduction.

Hartz *et al.* (1979) found in a very large study that obesity in the teenage years was more common among married women who never became pregnant than for married women who did become pregnant. In the Nurses' Health Study studying 2527 married infertile nurses, the risk of ovulatory infertility increased from a RR of 1.3 (1.2–1.6) in the group with a BMI as low as 24 to a rate of 2.7 (2.0–3.7) in women with a BMI over 32 (Rich Edwards *et al.* 1994). In the same year, Grodstein and colleagues (1994) showed that anovulatory infertility in 1880 infertile women and 4023 controls was higher in those with a BMI of >26.9 (RR 3.1, 2.2–4.4) with a smaller non-significant risk of 1.2 (0.8–1.9) in those with BMI of 25–26.9. So even high normal to slightly overweight levels may have an effect on fertility.

Lake *et al.* (1997) studied a cohort of women born in 1958 and followed up at 7, 11, 16, 23, and 33 years. They showed that weight during childhood did not predict subsequent fecundity but weight at 23 years did predict fecundity if the woman was obese (OR 0.69, 0.56–0.87). Results relating BMI at 33 years were weaker but consistent with those for 23 years. Obese women at 23 years were less likely to become pregnant within 12 months than women of normal weight (infertility rates = obese 33.6% vs. normal weight 18.6%). Zaadstra *et al.* (1993)

found that the upper quartile of BMI (33.1) in a group of apparently normal women who were undergoing donor insemination had a reduced chance of pregnancy (OR 0.43). This was a particularly significant study because few of the women required medication to stimulate ovulation. Balen *et al.* (1995) in the UK also found that obese women had higher infertility rates. In 204 North American women studied by Green *et al.* (1988) there was a reduced fertility rate among women with more than 20% of ideal body weight (OR 2.1) – this did not apply to women who had previously been pregnant.

The literature is therefore quite clear in associating increased body mass with a higher incidence of infertility. Most of the studies do not clearly classify women as having PCOS or normal ovaries but a large percentage of the obese female population are likely to have this condition.

Obesity, PCOS, and miscarriage

Weight excess is associated with an increased risk of miscarriage. In a study of over 13 000 women seeking their first spontaneous pregnancy (Hamilton-Fairley *et al.* 1992), 11% of women with a BMI 19–24.9, 14% with BMI 25–27.9, and 15% of those with BMI >28 miscarried (OR 1, 1.26, and 1.37 respectively). Women weighing over 82 kg are more likely to miscarry than thinner women after ovulation induction (Bohrer and Kemmann 1987) (OR 2.7 for 82–95 kg and 3.4 for >95 kg) while even a mild increase in BMI (25–28) leads to a significant risk of pregnancy loss (OR 1.37, 1.18–1.60) in some series (Hamilton-Fairley *et al.* 1992, Pettigrew and Hamilton Fairley 1997) but not in others (McClure *et al.* 1992). It has been generally claimed that women with PCOS are more likely to miscarry than those without PCOS. This has been attributed, at least in part, to higher luteinizing hormone (LH) concentrations in PCOS that may lead to impaired oocyte and embryo quality.

Obesity, PCOS, and pregnancy

Since the 1940s, there have been many articles on the effect of obesity on pregnancy and obstetric outcome. Some of the American studies detail results on massively obese women indicating much higher health risks and increased costs to the health system. Studies from Europe (Galtier Dereure *et al.* 1995) confirm that high pre-pregnancy weight is associated with an increased risk in pregnancy of hypertension, toxemia, gestational diabetes, urinary infection, macrosomia, Cesarean section, and increased hospitalization and cost. Despite this, overall neonatal outcome appears to be satisfactory.

Obesity, PCOS, and glucose intolerance

There is abundant evidence associating increasing BMI with diabetes mellitus. Subjects with PCOS have a substantial added risk of glucose intolerance. In a study from Adelaide, 18% of all women over BMI 30 in their 20s and 30s had impairment of glucose metabolism while 15% of women with PCOS who had normal glucose tolerance when initially studied showed conversion to impaired glucose tolerance or frank diabetes when restudied 5–7 years later (Norman *et al.* 2001, Wang and Norman 2004). Conway *et al.* (1992) showed that 8% of lean and 11% of obese women with PCOS had abnormal glucose tolerance. In a recent prospective study, we have shown that women with PCOS convert from initially normal glucose tolerance to impaired glucose tolerance or diabetes mellitus at a rate of approximately 3% per year. Almost all of this change can be associated with increasing obesity (Norman *et al.* 2001) and prevention of weight gain would be expected to be beneficial in the minimization of abnormal glucose tolerance.

There is controversial evidence that women with PCOS are more likely to exhibit gestational diabetes when pregnant and that many women with glucose intolerance in pregnancy have features of PCOS. This makes it even more important that potential complications from PCOS should be sorted out before embarking on treatment to induce pregnancy.

Obesity, PCOS, and response to infertility treatment

Most studies show conclusive evidence that increasing BMI is associated with an increased requirement for clomiphene citrate. In several of these, large doses of clomiphene (up to 200 mg/day) were required to ensure ovulation in the heaviest women. If this drug is considered have some association with ovarian cancer, it is probably undesirable for so much to be used per cycle of treatment. There does not, however, appear to be an association of body weight with poor conception rates in cycles of oral antiestrogens. In a study of 2841 cycles of clomiphene citrate, there was no association between body weight and pregnancy rate (Dickey *et al.* 1997) as previously suggested by Friedman and Kim (1985). Doses of gonadotropins required to induce ovulation are also higher in anovulatory women and those requiring ovarian stimulation for any reason (McClure *et al.* 1992).

Recently we have shown that the procedure of intrauterine insemination with gonadotropins in women with normal menstrual cycles and unexplained infertility gives similar pregnancy rates up to BMI 30 (Fuh *et al.* 1997). Although women with a BMI >35 have a lower pregnancy result, paradoxically this treatment was statistically more successful in women with BMI between 30 and 34.9 suggesting a subtle endocrine abnormality in this group of women.

The data relating to in vitro fertilization (IVF) and other assisted reproduction is less certain. While Clark *et al.* (1995) found a poor success rate in IVF pregnancies for the very obese, other studies have not shown any difference for women with moderate to severe obesity (Lewis *et al.* 1990). However, more recent studies from our group (Wang *et al.* 2002) and others confirm the negative association between IVF outcome and weight status.

Fat distribution, PCOS, and reproduction

Obesity can be central or peripheral in distribution and there is a lot of data suggesting different hormonal and metabolic responses depending upon the distribution of fat. Women with central fat have high levels of LH, androstenedione, estrone, insulin, triglycerides, very low density lipoproteins, and apolipoprotein B, and lower levels of high density lipoprotein (Pettigrew and Hamilton-Fairley 1997). Gynecological effects of central adiposity are also significant. Zaadstra *et al.* (1993) showed that a high WHR (central adiposity) was associated with a markedly lower conception rate in a donor insemination program. The Iowa Women's Health Study also indicated that high WHRs were associated with more menstrual abnormalities and higher prevalence of infertility (Kaye *et al.* 1990). Norman *et al.* (1995a, b) showed that high WHR was associated with greater disturbance in reproductive hormones, particularly insulin, in PCOS as subsequently confirmed by others (Hollmann *et al.* 1997). Reproductive response to diet and infertility treatment is likely to be closely related to central fat as indicated by the data by Clark *et al.* (1995, 1998).

Effect of weight loss on menstruation and infertility

There were several reports in the 1950s that indicated that weight loss induces menstrual regulation in a proportion of women with obesity and anovulation. Later Bates and Whitworth (1982) were the first to show a reduction in plasma androgens with dieting and associated return of menstrual cycles. These endocrine and clinical observations have been confirmed by several studies including those by Pasquali *et al.* (1989a, b). Kiddy *et al.* (1989, 1990, 1992, Pettigrew and Hamilton-Fairley 1997) revisited dietary manipulation of subjects with obesity and PCOS showing that strict calorie restriction with a subsequent 5% or greater weight loss led to changes in insulin, IGF, SHBG, and menstruation. Menstrual regularity and hirsutism improved with some spontaneous pregnancies resulting. Since then there have been several studies confirming that weight loss improves clinical and biochemical parameters that are disordered due to weight problems (Pasquali *et al.* 1989a, Clark *et al.* 1995, 1998, Hollmann *et al.* 1997). All the above

studies, while showing the principle that dietary control leads to favorable reproductive outcomes, fail to address the issue of long-term compliance in a clinical situation. The exceptions are the work published by us in Adelaide that has shown how menstrual regularity and pregnancy can be restored by exercise and dietary advice without an emphasis on low calories (Clark *et al.* 1995, 1998) and that from Italy (Pasquali *et al.* 2000). More than 90% of obese, oligomenorrheic women showed a dramatic improvement in menstrual patterns with a high spontaneous conception rate and a lower miscarriage rate than before treatment. Even women with causes of infertility not related to anovulation (such as tubal blockage or a male partner with oligospermia) showed dramatic improvements in assisted reproduction pregnancies. Weight loss into the normal range is not required for a good clinical response indicating that weight loss per se was not the main reason for success. Mitchell and Rogers in 1953 had previously made the observation that "the onset of menses frequently precedes any marked loss of weight and occurs so soon after the start of the therapeutic regimen that the absolute degree of obesity cannot be the only factor involved." The Italian group have shown that weight loss inducing diets with or without metformin can induce substantial clinical and metabolic changes (Pasquali *et al.* 2000).

Several studies have reported that surgically induced weight loss (gastrojejunal anastomosis and gastric stapling) are successful in restoring menstruation and pregnancy but these operations may have significant morbidity and very poor neonatal outcome. However in one of these studies, the miscarriage rate was substantially reduced after the operation when compared to that before the operation (Bilenka *et al.* 1995).

Weight loss and exercise programs in lifestyle modification

We have described how overweight women can achieved sustained weight loss and alteration of reproductive function by encouraging women to join a group that meets weekly for 6 months. Having become frustrated by the lack of results when individual dietary advice was given by a dietician or medical practitioner, Clark pioneered the unique concept (at least in gynecology) of lifestyle modification within groups of overweight women seeking to achieve a pregnancy (Table 9.2). Women were encouraged to agree to join a group for 6 months during which they understood they would not be given any fertility treatment. At the first meeting, they were encouraged to bring their partners who were given the information about the group approach and subsequently meetings were held every week for about 2 hours. At these meetings, exercise and dietary advice were combined with other activities such as fashion information for the overweight, supermarket trips, and medical information on the pathophysiology of PCOS.

Table 9.2. The fertility fitness program

- Information about role of weight and body composition in reproductive disorders
- Agreement to seek lifestyle changes for at least 6 months
- Group meeting with partners to explain the course
- Weekly meetings for 2–2.5 h with women
- Gentle aerobic exercises for 1 h (walking, stepping, etc.)
- Lecture/seminar for 1 h (good eating – nutrition/alcohol/smoking/caffeine, psychological aspects, medical information, etc.)
- Put into practice for next 6 months
- If return of periods, pregnancy, etc., no further medical treatment
- If disorder persists after 6 months, offer appropriate medical treatment

The exercise component lasted approximately 1 hour with gentle exercises such as stepping and walking and is conducted by a keep-fit instructor who appreciates the problem of the overweight person. The exercise session is followed by a group session in which a lecture, seminar, or discussion concentrates on subjects of interest to the women. Initially, a dietician gives advice on healthy eating patterns without seeking to get women to follow a low-calorie diet. No attempt is made to induce massive caloric reduction and slow weight loss is the preferred option. Initially, many of the women expected diet sheets and advice similar to those obtained from commercial weight loss companies but in time they realize that sustained weight loss can only be achieved by a life-long alteration in eating patterns and not by crash diets.

Weight and fat redistribution is the first obvious sign before weight loss in this program. Menstrual regularity can be induced without any weight loss provided the dietary retraining and exercise is taken seriously. Even 2–5% weight change can be effective in restoring ovulation. In our first paper (Clark *et al.* 1995) we studied anovulatory women with PCOS; 12 of 13 ovulated by the end of 6 months and the majority became pregnant within a year, most spontaneously. In a subsequent report (Clark *et al.* 1998), we extended this success rate to all women with obesity who had a range of infertility conditions and showed the efficacy of this approach. In most of the women, weight loss has been sustained and it is likely that long-term health benefits will result. Our philosophy is that alteration of lifestyle, particularly weight, will lead to short-, medium-, and long-term benefits (Table 9.3).

Menstruation can be restored, fertility promoted naturally or by assisted reproduction with better results, the risks of diabetes mellitus, cardiovascular disease, and hyperlipidemia ameliorated, and the musculoskeletal and metabolic side effects reduced. Retraining of diet and exercise patterns can have life-long benefits and alter health outcomes significantly.

Table 9.3. Lifestyle modification suggested for treatment of PCOS in overweight women

- Moderate exercise (≥30 min/day).
- Dietary modification (fat ≤30% daily intake, ↓ saturated and *trans* fat and glycemic load, ↑ fibre and polyunsaturated fat)
- For weight loss, establishing an energy deficit of 500–1000 kcal/day
- Reduction of psychosocial stressors
- Cessation of smoking
- Moderate alcohol consumption
- Moderate caffeine consumption
- Group interaction/intervention to provide support and assist implementing changes

What diet for PCOS?

A low fat, moderate protein and high carbohydrate intake diet (30:15:55) with a restricted caloric input is the standard recommended diet in most countries. Concomitant exercise is essential for weight maintenance and contributes to reducing stress and improves the sense of well-being. Weight loss is maintained more effectively and compliance is increased when an ad libitum low fat high carbohydrate dietary pattern is followed over longer periods of time, compared to fixed energy diets (Toubro and Astrup 1997). There has also been increased community interest in a dietary protocol advocating a moderate increase in protein (to approximately 30% of total energy intake) and concomitant reduction in dietary carbohydrates (Skov *et al.* 1999). Furthermore, altering the type of carbohydrate to produce a lower glycemic response (low glycemic index, (GI)) is also proposed to improve satiety and metabolic parameters (Ludwig 2000) (Fig. 9.1).

High protein diets range from the medically acceptable 30% protein, 40% carbohydrate, 30% fat to the Atkins-type diet which is much higher in protein (50%) and is high in fat (Table 9.4). High protein diets are more likely to reduce ad libitum intake, increase subjective satiety, and decrease hunger compared to high carbohydrate diets (Skov *et al.* 1999, Johnston *et al.* 2004). Weight loss may be more substantial in the short term but is no better in other diets in the longer term. The evidence for improved insulin sensitivity with high protein diets is debatable and metabolic improvements are not better in PCOS when caloric intake is matched for low protein diets (Moran *et al.* 2003). Indeed there is some concern that metabolic changes and cardiovascular risk may increase with high protein diets, particularly with large amounts of red meat. Overall it appears as if dietary composition is not a key component of diets for PCOS provided caloric intake is reduced substantially. Ultimately, weight loss will result from a decrease in energy intake or increase in energy expenditure and this should be the key approach.

Table 9.4. Compensatory changes in the macronutrient composition of various diets

Diet description	Fat (% kJ)	Carbohydrate (% kJ)	Protein (% kJ)	Alcohol (% kJ)
Average	34	49	14	3
Low fat, high carbohydrate, low protein	30	55	15	–
Very low fat, very high carbohydrate	15	70	15	–
Moderate protein, moderate carbohydrate	30	40	30	–
Moderate protein, very low carbohydrate	55	15	30	–

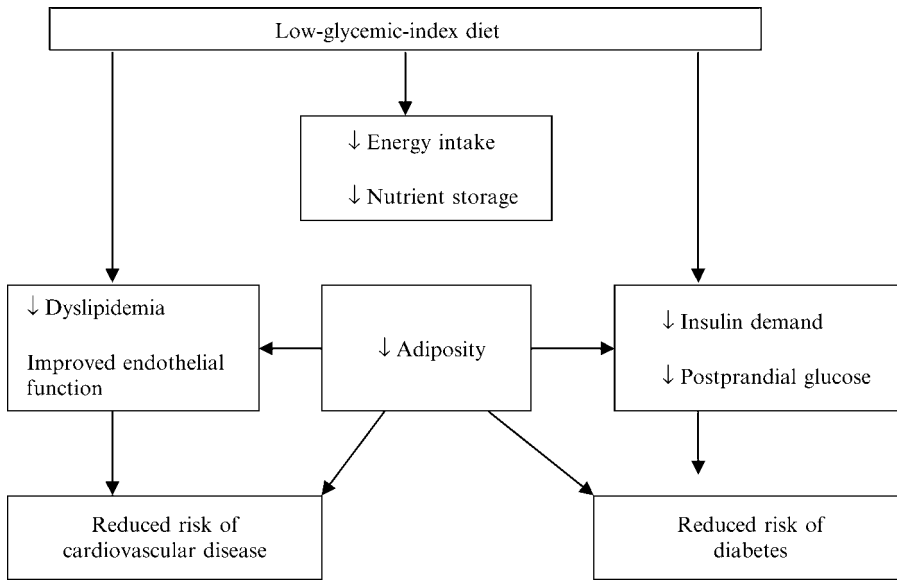

Fig. 9.1 Pathways by which a low glycemic index (GI) might be of benefit.

The potentially detrimental effects of a high carbohydrate diet might also be minimized through modifying the source of the dietary carbohydrate, achieved practically through changing the GI of the carbohydrate. The GI is a classification index of carbohydrate foods based on postprandial glucose response and is defined as the incremental area under the blood glucose curve produced by a standard amount of carbohydrates in a food relative to the incremental area produced by the same amount of carbohydrate from a standard source (Wolever *et al.* 1991). Claims have been made that low GI foods reduce postprandial insulin demand and thereby reduce hyperinsulinemia (Jarvi *et al.* 1999). There are no studies in the role of GI and diets for women with PCOS.

Dietary intervention and insulin sensitizing agents

Metformin alone or with clomiphene citrate is effective in increasing ovulation rates and pregnancy. Some studies have also suggested that metformin helps with weight loss (Pasquali *et al.* 2000). In a randomized controlled trial of diet with or without metformin, insulin sensitizing drugs were better than placebo in women with PCOS with respect to loss of weight, reduction in visceral fat, waist circumference, and testosterone. There was no differential effect on glucose or insulin levels. Other studies have not confirmed these observations when comparing metformin and placebo use (Acbay and Gundogdu 1996). Other glitazones are of no value in weight loss although they are effective in restoring ovulation.

Reasons for weight-related menstrual problems

Infertile, anovulatory obese women have higher plasma androgens, insulin, and LH concentrations and lower SHBG levels when compared to normal weight women or obese subjects with regular periods. It is possible that the increased estrogen production from peripheral tissues leads to a disorder of the hypothalamic–pituitary–ovarian axis. Insulin resistance is common in anovulatory women and together with reduced hepatic clearance of insulin and increased sensitivity of the beta cells to secretory stimuli is thought to be the major cause of hyperinsulinemia. Insulin in turn can induce androgen secretion from an ovary that is polycystic or genetically prone to excess androgen production. Current hypotheses suggest that hyperinsulinemia is a result of genetic or environmentally induced insulin resistance from peripheral tissues and this leads to increased androgen production from ovaries that are not resistant to the action of insulin. Reduction of hyperinsulinemia should lead to reduction of hyperandrogenemia and restoration of reproduction function. This hypothesis is clearly supported by the experimental observations by a number of investigators.

We have followed women participating in a weight loss program and have shown that return of ovulation coincides with a reduction in insulin resistance and a fall in central adiposity. In a group of anovulatory subjects who returned to ovulation with exercise and dietary restraint, waist circumference, central fat, LH, and insulin fell more than in those who remained anovulatory throughout (Buchholz *et al.* unpublished data). In a less extensive previous study we had shown that fasting insulin was significantly reduced by weight loss in anovulatory women who became ovulatory (Clark *et al.* 1995). While there is convincing evidence that insulin sensitivity can be restored in overweight women with PCOS who lose weight (Holte *et al.* 1994, 1995, Holte 1996), first phase insulin release remains significantly abnormal indicating an underlying problem in pancreatic

secretion in these subjects. Other investigators have disputed this observation. The return to ovulation associated with a reduction in insulin reinforces studies with insulin sensitizing agents such as troglitazone where improved insulin sensitivity without weight loss promotes ovulation and fertility (Dunaif *et al.* 1996, Ehrmann *et al.* 1997).

Luteinizing hormone pulse frequency and amplitude does not appear to alter during weight loss in obese subjects (Guzick *et al.* 1994) although absolute values of LH do decrease significantly in responders to diet as judged by ovulation.

Other factors involved may include androgens, hypothalmic endorphins, and leptin, all of which are increased in anovulatory overweight women. While leptin is increased in obese PCOS subjects, there is no increase over obesity not associated with PCOS and return of ovulation is not associated with a reduction in leptin concentrations prior to return of periods.

Depression is frequent in women with PCOS and infertility as shown by assessments performed in Adelaide women. Participation in the program was associated with an improvement in well-being and psychological parameters that may indicate restoration of reproductive potential is closely tied in with psychological changes (Galletly *et al.* 1996a, b). These may have an effect through the endorphin system and other neurotransmitters in the hypothalamic–pituitary axis.

Smoking

Many women with PCOS choose to smoke in response to stress or the desire to prevent further weight gain. Our experience shows that 40% of women in our unit who have PCOS are also smokers. There is convincing data that, apart from the well-known health hazards of smoking with respect to the cardiovascular and respiratory system, there are effects on reduction of fertility potential.

Studies show that the time to conception is increased by 30% for smokers and there is a two- to threefold increased risk of failing to conceive by 1 year of attempting pregnancy. Augood *et al.* (1998) have recently published a systematic review and meta-analysis of the effects of smoking on fertility and concluded that the risk of infertility in smokers was versus non-smokers was 1.60 (95% confidence interval 1.34–1.91). Women who subsequently went through a cycle of assisted reproduction were found to have an OR of 0.66 (0.49–0.88) for pregnancies per number of attempts in smokers versus non-smokers. This does not appear to be attributable to smoking in the male partner where the time to conception was not increased in male smokers when their partner's smoking status is taken into consideration.

Maternal smoking does not appear to affect the risk of spontaneous abortion but may alter the rate of abnormal placentation, abruptio placentae, and perinatal

Table 9.5. National Institutes of Health clinical guidelines for long-term treatment of overweight and obesity

- Sensible diet and changed eating habits for long term
- Effective physical activity program sustainable long term
- Behavior modification, reduction of stress, increased well-being
- Combination of dietary and behavioral therapy and increased physical activity
- Social support by physician, family, spouse, and peers
- Smoking cessation and reduction in alcohol consumption
- Avoidance of "crash diets" and short-term weight loss
- Minor roles for drugs involved in weight loss
- Avoidance of aggressive surgical approaches for majority
- Adaptation of weight loss programs to meet individual needs
- Long-term observation, monitoring, and encouragement of patients who have successfully lost weight

death after ante partum bleeding (Werler 1997). Growth of the fetus is definitely altered by smoking with birthweight reduced by an average of 200 g. There is a dose–response effect where birthweight decreases as numbers of cigarettes smoked increases. Perinatal mortality rates are increased by about 30% due to excesses in low birthweight, prematurity, and abnormal placentation.

All women with PCOS who are trying to become pregnant should be strongly advised to reduce or eliminate their smoking habit prior to therapeutic attempts at inducing ovulation with drugs. While this may require considerable effort, including the use of nicotine patches and hypnotherapy, the end results are well justified in terms of improved pregnancy rates, perinatal mortality, and health outcomes.

Stress reduction

Several studies have shown that women with PCOS are more likely to have a poor quality of life assessment, have eating disorders, and poor self-image (Jahanfer *et al.* 1995, Coffey and Mason 2003). Intervention by counseling and reassurance leads to improvement in these parameters and should be part of any program.

Conclusions

While the attending doctor may be tempted or pressurized to use fertility drugs for subjects with PCOS, lifestyle changes are critically important in these women, not only for successful management but also for long-term health. Overall recommendations are shown in Table 9.5.

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10

Ovulation induction for women with polycystic ovary syndrome

Roy Homburg

Introduction

Polycystic ovary syndrome (PCOS) is associated with approximately 75% of the women who suffer from infertility due to anovulation (Adams *et al.* 1986, Hull 1987) and is frequently diagnosed for the first time in the infertility clinic. The majority of women with anovulation or oligo-ovulation due to PCOS often have clinical and/or biochemical evidence of hyperandrogenism. Almost all these women will have a typical ultrasonic appearance of the ovaries (Adams *et al.* 1985).

Making the diagnosis of PCOS is important as this will dictate the treatment plan and the prognosis, and will serve in the avoidance of possible complications of treatment. Before embarking on ovulation induction therapy, although not theoretically essential for initial therapeutic decisions, for screening I usually take a blood sample for luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone, and fasting glucose and insulin concentrations. The ratio of fasting glucose to insulin levels gives a good indication of insulin sensitivity (Legro *et al.* 1998) and as hyperinsulinemia is present in about 80% of obese women and 30–40% of women of normal weight with PCOS (Dunaif *et al.* 1989) and is strongly associated with anovulation then it is certainly useful to know for possible therapeutic intervention. The LH value may be expected to be high in 40% of women with PCOS and is thought to be detrimental to successful ovulation induction and to the incidence of miscarriage (Balen *et al.* 1995).

In order to exclude other possible causes of oligo- or anovulation, total serum testosterone concentrations, together with a history of rapid progress of hyperandrogenic symptoms, are useful for the screening of androgen producing tumors; 17-hydroxy progesterone when highly elevated is pathognomonic for 21-hydroxylase deficiency; sex hormone binding globulin (SHBG) levels can be used in the

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calculation of the free androgen index; an oral or intravenous glucose tolerance test or even insulin clamp will give more accurate information on insulin metabolism; Cushing's syndrome, if suspected, can be diagnosed using the standard static and dynamic tests. Needless to say, a semen analysis of the partner is mandatory before starting treatment and screening for possible mechanical factors and endometriosis should be performed when indicated.

There are several modes of ovulation induction for patients who have PCOS. As hyperinsulinemia, lack of sufficient endogenous FSH activity, and high LH concentrations may all be instrumental in the pathology of anovulation in PCOS, the treatment modes described basically depend on either a reduction of insulin concentrations, FSH stimulation of the ovaries, or a reduction of LH concentrations or a combination of these.

Management of hyperinsulinism for ovulation induction

Insulin is of prime importance in the etiology of anovulation associated with PCOS. The rate of insulin resistance in women with PCOS is 50–80% and a large majority of these women are obese (Legro *et al.* 1998, Carmina and Lobo 1999). Abdominal obesity in women with PCOS exacerbates insulin resistance and its associated clinical sequelae. Indeed, central obesity and body mass index (BMI) are major determinants of insulin resistance, hyperinsulinemia, and hyperandrogenemia. As obesity is reaching epidemic proportions in some countries, we tend to see more expression of the stigmata of PCOS, including anovulation and infertility. Insulin stimulates LH and ovarian androgen secretion and decreases SHBG concentrations, therefore increasing the circulation of more free, biologically active testosterone [8]. Several other aspects of ovulation induction therapy are negatively influenced by obesity and hyperinsulinism in women with PCOS. More gonadotropins are required to achieve ovulation in insulin resistant women (Homburg 1998, Dale *et al.* 1998). Obese women being treated with low dose therapy have inferior pregnancy and miscarriage rates (Hamilton-Fairley *et al.* 1992). Both obese (White *et al.* 1996) and insulin resistant (Dale *et al.* 1998) women with PCOS, even on low dose FSH stimulation, have a much greater tendency to a multifollicular response and thus a relatively high cycle cancellation rate in order to avoid hyperstimulation. Fortunately, the deleterious effects of hyperinsulinemia in these patients are reversible. This may be achieved by weight loss in the obese and with insulin lowering medications.

Weight loss

Just as obesity expresses and exaggerates the signs and symptoms of insulin resistance, so loss of weight can reverse this process by improving ovarian

function and the associated hormonal abnormalities (Pasquali *et al.* 1989, Kiddy *et al.* 1992, Clark *et al.* 1995) and may alone induce ovulation and pregnancy. Loss of weight induces a reduction of insulin and androgen concentrations and an increase in SHBG concentrations. For obese women with PCOS, a loss of just 5–10% of body weight is enough to restore reproductive function in 55–100% within 6 months of weight reduction (Pasquali *et al.* 1989, Kiddy *et al.* 1992, Clark *et al.* 1995). Weight loss has the undoubted advantages of being effective and cheap with no side effects and should be the first line of treatment in obese women with anovulatory infertility associated with PCOS.

Metformin

The strong association between hyperinsulinemia and anovulation would suggest that a reduction of insulin concentrations could be of great importance (Velazquez *et al.* 1997, Fleming *et al.* 2002). For those who fail to achieve this by losing weight or who are of normal weight but hyperinsulinemic, an insulin sensitizing agent such as metformin may be indicated. Metformin is an oral biguanide, well established for the treatment of hyperglycemia, which does not cause hypoglycemia in normoglycemic patients. The sum total of its actions is often a decrease in insulin and androgen levels and, consequently, a resulting improvement of the clinical sequelae of hyperandrogenism.

Metformin alone

There are now a large number of studies published on the effect of metformin in a dose of 1500–2500 mg/day in women with PCOS. The majority of these studies have demonstrated an improvement in insulin concentrations, insulin sensitivity, and serum androgen concentrations accompanied by decreased LH and increased SHBG concentrations (Nestler *et al.* 2002). The restoration of regular menstrual cycles by metformin has been reported in the large majority of early, published series in which reinstatement of ovulation occurred in 78–96% of patients (Velazquez *et al.* 1997, Nestler *et al.* 1998, 2002, Moghetti *et al.* 2000, Ibañez *et al.* 2001, Fleming *et al.* 2002). Fleming *et al.* (2002), in a randomized controlled trial (RCT), demonstrated a significant but modest increase in the frequency of ovulation with metformin (850 mg, twice a day) compared to placebo in a group of 92 oligomenorrheic women with PCOS.

Metformin + clomiphene

In an RCT performed on clomiphene resistant infertile patients with PCOS, compared with placebo, metformin markedly improved ovulation and pregnancy rates with clomiphene citrate (CC) treatment (Vandermolen *et al.* 2001). In a further study, 46 anovulatory obese women with PCOS who did not ovulate on

metformin or placebo for 35 days were given 50 mg of CC daily for 5 days while continuing metformin or placebo. Of those on metformin, 19 of 21 ovulated compared with 2 of 25 on placebo (Nestler *et al.* 1998). In an interesting RCT, CC resistant women with PCOS received either metformin for 6 months and then CC, or human menopausal gonadotropin (hMG) alone for ovulation induction (George *et al.* 2003). In this small study, as metformin + CC was equally effective as hMG, less expensive, and more convenient, it was suggested as an intermediary step for CC resistant patients, worth trying before resorting to hMG.

Metformin + low-dose follicle stimulating hormone

When women with clomiphene resistant PCOS were administered FSH with or without pretreatment with metformin for 1 month in an RCT, those receiving metformin developed significantly less large follicles, produced less estradiol, and had fewer cycles canceled due to excessive follicular development. The reduction of insulin concentrations induced by metformin seemed to favor a more orderly follicular growth in response to exogenous gonadotropins for ovulation induction (De Leo *et al.* 1999). A similar RCT examined the effect of giving pretreatment with metformin or placebo for 6 weeks before a single cycle of low-dose FSH for patients with CC resistant PCOS but normal glucose tolerance (Yarali *et al.* 2002). Although insulin sensitivity remained unchanged, free testosterone concentrations decreased and 6 of the 16 patients in the metformin group ovulated before receiving FSH. Ovulation and pregnancy rates were insignificantly better in the metformin group who received FSH. This small study seems to indicate a direct effect of metformin on the ovary.

Metformin in in vitro fertilization

In an observational study in clomiphene-resistant patients undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI), those receiving metformin had a decreased total number of follicles but no difference in the mean number of oocytes retrieved. There were more mature oocytes, embryos cleaved, increased fertilization, and clinical pregnancy rates (70% vs. 30%) in the metformin group (Stadtmauer *et al.* 2001). However, in a prospective RCT on women with PCOS undergoing IVF (Kjotrod *et al.* 2003), overall metformin had no effect on the results of IVF, dose of FSH required, estradiol levels on the day of human chorionic gonadotropin (hCG), oocytes retrieved, or fertilization rate. The only positive effect of metformin was seen in the lean (but not obese) subgroup where it improved implantation and pregnancy rates. A recently completed single-center RCT in Leeds, UK, revealed a very similar doubling of pregnancy rate in patients treated from the beginning of a long-agonist regimen with metformin.

Metformin during pregnancy

The evidence so far is encouraging concerning the efficiency and safety of metformin as a single agent or in combination with CC or gonadotropins for induction of ovulation in women with hyperinsulinemic PCOS (Homburg 2002). Not only does metformin seem to be safe when continued throughout pregnancy but preliminary data suggest that this strategy can significantly decrease the high miscarriage rate usually associated with PCOS (Glueck *et al.* 2002, Jakubowicz *et al.* 2002). The apparent lack of teratogenicity and beneficial effect of metformin on miscarriage rates awaits confirmation by future, appropriately controlled, randomized, prospective studies.

It seems to be difficult to predict which individuals will respond well to metformin (Fleming *et al.* 2002). Furthermore, metformin has been shown to have a direct action on ovarian cells in vitro decreasing androgen production (Mansfield *et al.* 2003). These facts and the difficulties of accurately measuring insulin sensitivity in all PCOS patients have encouraged "blanket" treatment with metformin to all anovulatory PCOS patients in some centers. The wisdom of this strategy awaits ratification or, as noted by Harbourne *et al.* (2003) in a critical review of the evidence, clinical practice is ahead of the evidence. Another systematic review has come to a similar conclusion and calls for more well-designed RCTs using pregnancy or live birth rate as a primary end-point (Costello and Eden 2003).

The somewhat confusing early data regarding the use of metformin for ovulation induction was summed up in a Cochrane review (Lord *et al.* 2003). Metformin was found to be an effective treatment for anovulation in women with PCOS. As a first-line, single agent it was preferable to placebo with an odds ratio (OR) of 3.88 (confidence interval (CI) 2.25–6.69) in achieving ovulation and preferable to clomiphene alone when combined with clomiphene (OR 4.41, CI 2.37–8.22). Ovulation rates were higher when metformin was combined with clomiphene rather than used on its own (76% vs. 46%). An analysis of pregnancy rates suggested a significant treatment effect for metformin and clomiphene (OR 4.40, CI 1.96–9.85). This initial enthusiasm for using a combination of metformin and clomiphene may soon be tempered by the results of a recently completed Dutch collaborative study showing no increased efficiency over clomiphene alone.

Other compounds with the property of lowering insulin concentrations, the glitazones rosiglitazone and pioglitazone, and D-chiro-inositol, are under investigation. It is too premature to judge their effect on ovulation induction but early indications suggest a positive effect by rosiglitazone alone and more so when combined with clomiphene, on ovulation induction (Ghazeeri *et al.* 2003).

Stimulation of the ovaries with follicle stimulating hormone

Clomiphene citrate

Clomiphene citrate is a long-established first line treatment for women with PCOS who have absent or irregular ovulation. Paradoxically, its antiestrogen action in blocking estradiol receptors in the hypothalamus induces a change in gonadotropin releasing hormone (GnRH) pulse frequency, release of FSH from the anterior pituitary, and consequent follicular development and estradiol production. It is given in a dose of 50–250 mg/day for 5 days beginning on day 2–5 of spontaneous or induced bleeding starting with the minimum dose which may be raised in increments of 50 mg/day each cycle until an ovulatory cycle is achieved. I have found no advantage in using a daily dose of more than 150 mg which seems to increase significantly neither the ovulation rate nor follicular recruitment. The 20–25% who remain resistant to CC (i.e., remain anovulatory) are thus identified in three cycles. Patients who do not respond to clomiphene are likely to be more obese, insulin resistant, and hyperandrogenic than those who do respond (Imani *et al.* 1998). A course of six ovulatory cycles is usually sufficient to know whether pregnancy will be achieved using CC before moving on to more complex treatment, as approximately 75% of the pregnancies achieved with clomiphene occur within the first three cycles of treatment (Gysler *et al.* 1982). A treatment scheme for the administration of clomiphene is presented in Fig. 10.1.

Although ovulation is restored in approximately 80%, pregnancy is achieved in only about 35–40% of patients who are given clomiphene (MacGregor *et al.* 1968, Gysler *et al.* 1982, Imani *et al.* 2002). There are several possible explanations for this "gap." Clomiphene induces a discharge of LH as well as FSH so those with high basal LH levels are less likely to respond and conceive with clomiphene treatment (Homburg *et al.* 1988). However, the most probable factor involved in this large discrepancy between ovulation and pregnancy rates in patients treated with clomiphene is its antiestrogenic effect at the level of the endometrium and cervical mucus. While the depression of the cervical mucus, occurring in about 15% of patients, may be overcome by performing intrauterine insemination (IUI), suppression of endometrial proliferation, unrelated to dose or duration of treatment but apparently idiosyncratic, indicates a poor prognosis for conception in my experience when endometrial thickness remains <8 mm. Ultrasound evaluation of follicular growth and endometrial thickness and estimation of same-day serum estradiol concentrations on days 12–14 of the cycle are justified by the identification of those who are not responding or have depressed endometrial thickness and is helpful in the timing of natural intercourse or IUI. Although this monitoring implies added expense, this is neutralized by the prevention of protracted periods of possibly inappropriate therapy and delay in the inception of more efficient treatment.

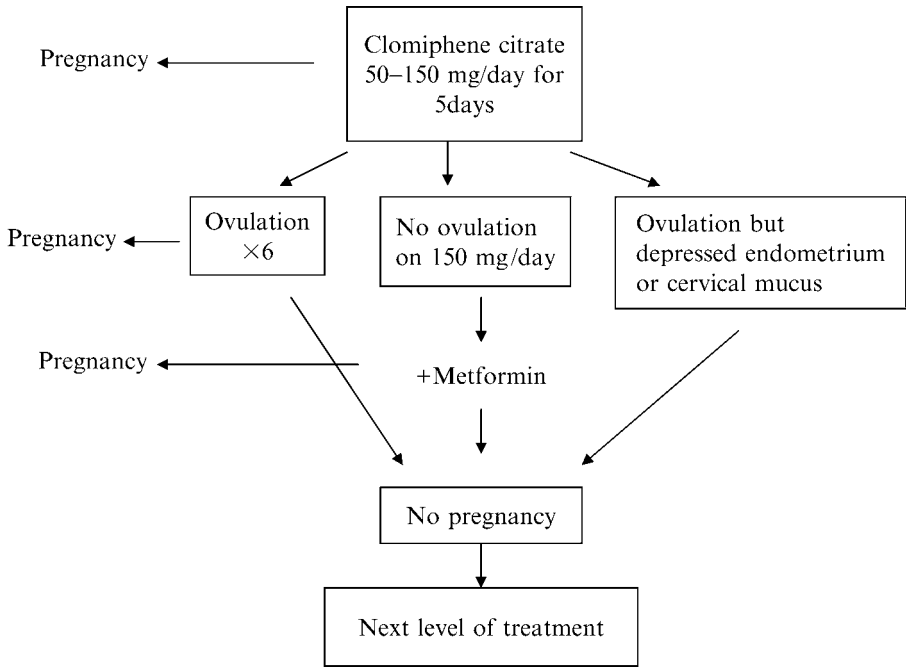

Fig. 10.1 Treatment scheme for clomiphene citrate.

Co-treatment with several proposed adjuvants has been advocated in an attempt to produce improved results from clomiphene treatment. The addition of an ovulation triggering dose of hCG, 5000–10 000 IU is only theoretically warranted when the reason for a non-ovulatory response is that the LH surge is delayed or absent despite the presence of a well developed follicle. Although the routine addition of hCG at midcycle seems to add little to the improvement of conception rates (Agrawal and Buyalos 1995). I have found it very useful, given when an ultrasonically demonstrated leading follicle attains a diameter of 18–24 mm, for the timing of intercourse or IUI. Dexamethasone, 0.5 mg/day at bedtime, as an adjunct to clomiphene therapy, suppresses the adrenal androgen secretion and may induce responsiveness to clomiphene in previous non-responders, mostly hyperandrogenic women with PCOS with elevated concentrations of dehydroepiandrosterone sulfate (DHEAS) (Diamant and Evron 1981, Daly *et al.* 1984). Although this method meets with some success, medium- to long-term glucocorticoid steroid therapy often induces side effects including increased appetite and weight gain which is counterproductive for women with PCOS.

The combined treatment of clomiphene with metformin has been dealt with in the section on metformin.

Aromatase inhibitors

Compared to clomiphene, aromatase inhibitors directly inhibit estrogen production and so activate the negative feedback mechanism to release FSH but avoid an antiestrogen effect on estrogen receptors. Theoretically, therefore, aromatase inhibitors should prove more efficient than clomiphene for attaining a pregnancy and, due to the fact that the negative feedback mechanism remains in working order, should also produce fewer multiovulations and fewer multiple pregnancies. In early trials, letrozole, an antiaromatase, has been examined to assess its efficacy for ovulation induction. It has been shown to be effective in inducing ovulation and pregnancy in women with anovulatory PCOS and inadequate clomiphene response (Mitwally and Casper 2001a) and improving ovarian response to FSH in poor responders (Mitwally and Casper 2001b). Although some encouragement may be taken from the solidity of the working hypothesis and the success of the preliminary results, hard evidence on efficacy and safety are awaited from larger trials.

Gonadotropin therapy

Clomiphene failure is regarded as a lack of response to clomiphene in a daily dose of 150 mg or six ovulatory cycles that have not resulted in a pregnancy. Gonadotropin (FSH) therapy is usually the next step following failure with clomiphene.

Polycystic ovaries contain twice as many small antral follicles as the normal ovary (Van der Meer *et al.* 1988). Their sensitivity to exogenous FSH makes anovulatory women with PCOS who receive gonadotropins particularly prone to multiple follicular development. It is multiple follicular development that is behind the main complications of gonadotropin therapy, ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. So, although polycystic ovaries may have a similar threshold of FSH stimulation to initiate a response, doses over and above the threshold will almost inevitably induce the development of multiple follicles by provoking the initial development of a large cohort, stimulating additional follicles, and even rescuing those follicles destined for atresia (Insler 1988). Experience using conventional step-up treatment with gonadotropins has demonstrated this problem well. Although cumulative conception rates have been acceptable for women with PCOS, this form of treatment, employing incremental dose rises of 75 IU every 5–7 days, characteristically induced multiple follicular development, resulting in a high frequency of multiple pregnancies and OHSS. In collected data (Hamilton-Fairley and Franks 1990) a mean multiple pregnancy rate of 34% and severe OHSS of 4.6% was reported. In a further study, a cumulative conception rate of 82% after six cycles using this protocol was tempered by an unacceptable rate of multiple pregnancies and OHSS (Homburg *et al.* 1995).

Prevention of ovarian hyperstimulation syndrome and multiple pregnancies

The aim of ovulation induction by direct FSH stimulation for women with PCOS is to achieve the development of a single dominant follicle rather than the development of many large follicles and thereby avoid the complications of OHSS and multiple pregnancies. This demands the attainment and maintenance of follicular development with exogenous FSH without exceeding the threshold requirement of the ovary. It was with this principle in mind that the chronic low dose regimen of FSH was designed. The classic chronic low-dose regimen employs a low starting dose for 14 days and then uses small incremental dose rises when necessary, at intervals of not less than 7 days, until follicular development is initiated (Seibel *et al.* 1984, Polson *et al.* 1987). The dose that initiates follicular development is continued until the criteria for giving hCG are attained (Fig. 10.2).

A comparative prospective study of the conventional regimen with chronic low-dose administration of FSH for anovulation associated with PCOS (Homburg *et al.* 1995) involved 50 participants treated with FSH, half of them using a conventional stepwise protocol (incremental dose rises of 75 IU every 5–7 days when necessary) and half with a regimen of chronic low dose as described above. Both methods of treatment had an initial dose of 75 IU FSH. Compared with the conventional dose protocol, the chronic low-dose regimen yielded slightly improved pregnancy rates (40% vs. 24%) while completely avoiding OHSS and multiple pregnancies, which were prevalent (11% OHSS and 33% multiple pregnancies) with conventional therapy. The key to success was the monofollicular ovulation rate of 74% in low-dose cycles compared with 27% on conventional therapy. The total number of follicles >16 mm and estradiol concentrations were half those observed on conventional therapy. A large French multicenter study

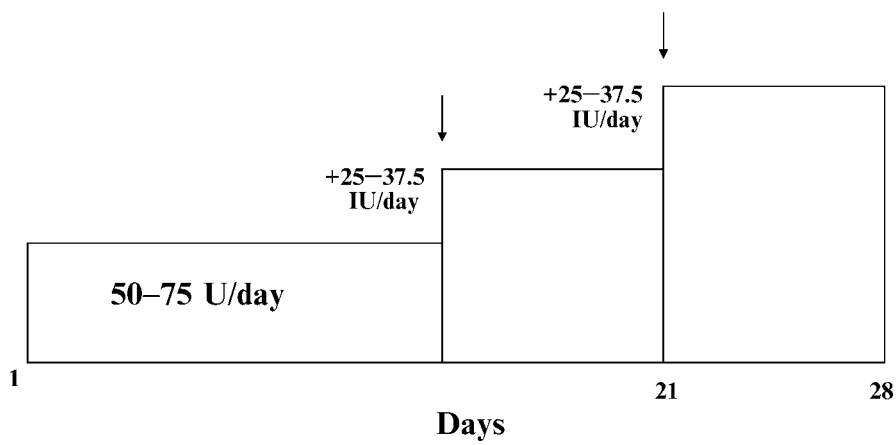

Fig. 10.2 Recommended scheme for first cycle of low-dose step-up FSH administration.

(Hedon *et al.* 1998) compared conventional and chronic low-dose regimens in 103 anovulatory euestrogenic women. The comparison of low with conventional dose revealed pregnancy rates of 33.3% versus 20% with a multiple (twin) pregnancy rate of 14% and 22%, respectively. The total number of follicles >10 mm and estradiol concentrations on the day of hCG in the low-dose group were half those seen on conventional therapy. Additionally, the low-dose regimen tended to produce a higher rate of mono- or bifollicular development in this study.

A summation of reported results (Homburg and Howles 1999) using a chronic low-dose protocol identical to that described above shows a remarkably consistent rate of mono-ovulatory cycles of around 70% and an acceptable pregnancy rate of 40% per patient and 20% per cycle. The real justification for the adoption of the chronic low-dose protocol may be seen in the almost complete elimination of OHSS and a multiple pregnancy rate of <6%.

In order to mimic more closely the events of the normal ovulatory cycle (decreasing FSH concentrations throughout the follicular phase), the Rotterdam group examined a step-down dose regimen with a starting dose of 150 IU and decreased the dose by 37.5 IU when a follicle of 10 mm was seen and by the same amount every 3 days if follicular growth continued (Schoot *et al.* 1995, Van Dessel *et al.* 1995). Compared with the classic low-dose step-up regimen a monofollicular growth rate of 88% of cycles was obtained for step-down as opposed to 56% with the step-up protocol (Van Santbrink and Fauser 1997). In the step-down group, duration of treatment and gonadotropin requirement were significantly reduced. However, a recent, randomized, French multicenter study comparing the step-up versus the step-down protocol demonstrated superiority of the step-up regimen as regards the rates of monofollicular development (68% vs. 32% of cycles), overstimulation (4.7% vs. 36%), and ovulation (70.3 % vs. 51.3%) [57].

Using the step-up protocol, how do the initial dose, the duration of its administration, and the incremental dose rise influence results? From the largest published series of chronic low-dose FSH therapy (White *et al.* 1996) it was possible to compare the results of a starting dose of 75 IU with that of 52.5 IU for an initial 14-day period with an incremental dose rise of 37.5 IU or 22.5 IU respectively. There were no significant differences between the two groups but pregnancy rate per patient, uniovulatory cycle rate, and miscarriage rate were all in favor of the smaller starting dose. The majority of patients reach the criteria for hCG administration within 14 days using 75 IU/day of urinary FSH (White *et al.* 1996) or 50 IU/day of recombinant FSH. However, a 14-day initial period with no dose change has been found taxing by some and prompted an attempt to cut down the initial period of 14 days without change of dose to 7 days. A comparison of 14-day versus 7-day starters (Homburg and Howles 1999) in 50 patients showed no significant differences other than a slightly higher rate of

multiple pregnancies in the 7-day group. As we regarded a multiple pregnancy as a "failure" of treatment, the 14-day initial period without any change of dose may be the safest. As for the size of the incremental dose rise, a recently completed multicenter study employing a step-up protocol starting with doses of 50 IU/day of recombinant FSH for a minimum of 7 days, compared two randomized groups using incremental dose rises of 25 IU or 50 IU when needed. The use of the smaller incremental dose rises was significantly more beneficial in terms of monofollicular development, ovulation rates, and cancellation rates.

To summarize, low-dose, step-up gonadotropin therapy should be preferred to the now outdated conventional therapy for patients with PCOS. Small starting doses in the first cycle for a 14-day initial period without a dose change and then a small incremental dose rise if required seem on present evidence to give the best results.

Reduction of luteinizing hormone concentrations

Gonadotropin releasing hormone agonists

Premature luteinization is an annoying and not infrequent occurrence during ovarian stimulation with gonadotropins. The ability of GnRH agonists to suppress LH concentrations before and during ovarian stimulation has earned them an undisputed place in IVF treatment protocols. Their possible application during ovulation induction should be particularly relevant in the presence of the chronic, tonic, high serum concentrations of LH observed in a high proportion of women with PCOS. Theoretically, by suppressing LH concentrations, GnRH agonists should not only eliminate premature luteinization but improve the relatively low pregnancy rates and the high miscarriage rates witnessed in this group of patients (Homburg 1998). In a large study (Christin-Maitre and Hugues 2003) 239 women with PCOS received hMG with or without GnRH agonist for ovulation induction or superovulation for IVF/embryo transfer (ET). Of pregnancies achieved with GnRH agonist, 17.6% miscarried compared with 39% of those achieved with gonadotropins alone. Cumulative live birth rates after four cycles for GnRH agonist were 64% compared with 26% for gonadotropins only.

Why, then, has the GnRH agonist not become standard treatment for ovulation induction in PCOS, despite the fact that our experience (Homburg *et al.* 1993b) and that of others has shown an increased pregnancy rate and lower miscarriage rate in women receiving combination treatment of agonist and gonadotropins when tonic LH concentrations are high? Co-treatment with GnRH agonist and low-dose gonadotropin therapy is more cumbersome, longer, requires more gonadotropins to achieve ovulation, has a greater prevalence of multiple follicle development, and consequently more OHSS and multiple pregnancies.

Combining GnRH agonist with gonadotropin stimulation will exacerbate the problem of multiple follicular development and therefore increase rates of cycle cancellation, OHSS, and multiple pregnancy (Homburg *et al.* 1990, Van der Meer *et al.* 1996). The loss of the endogenous feedback mechanism when using GnRH agonist and greater stimulation of follicles by the larger amounts of gonadotropins needed are probably responsible for the fact that GnRH agonists are not the solution to the problem of multiple follicular development.

In order to overcome the two main complications of ovulation induction for PCOS, multifollicular development and the possible deleterious effects of high LH levels, low conception rates, and high miscarriage rates, a combination of chronic low-dose FSH stimulation with GnRH agonist therapy should theoretically yield the best results. Scheele *et al.* (1993) studied women with PCOS undergoing ovulation induction with chronic low-dose FSH therapy, with and without adjuvant GnRH agonist therapy. A very low rate of monofollicular ovulation was achieved (14%) in the agonist cycles compared with 44% of those treated with low-dose FSH alone. Treatment with GnRH agonist abolished neither the inter- nor intraindividual variability of the FSH dose required to induce ongoing follicular growth but also seemed to induce an even further increase in the sensitivity of the polycystic ovary follicles to gonadotropin stimulation once the threshold FSH dose had been reached.

In summary, the combination of a GnRH agonist with low-dose gonadotropins should probably be reserved for women with high serum concentrations of LH who have repeated premature luteinization, stubbornly do not conceive on gonadotropin therapy alone, or who have conceived and had early miscarriages on more than one occasion.

Gonadotropin releasing hormone antagonists

Having defined the disadvantages of GnRH agonists in the treatment of anovulatory PCOS, it is to be hoped that GnRH antagonists can make a contribution. They have several theoretical advantages over the agonists as they act by the mechanism of competitive binding and this allows a modulation of the degree of hormonal suppression by adjustment of the dose. Further, antagonists suppress gonadotropin release within a few hours and have no flare-up effect, and gonadal function resumes without a lag effect following their discontinuation. If we apply these advantages to an ovulation induction protocol for PCOS, one can visualize that, used in combination with low-dose FSH administration, the antagonist could be given in single or repeated doses when a leading follicle of 13–14 mm is produced. This would theoretically prevent premature luteinization, protect the oocyte from deleterious effects of high LH concentrations, and still allow the follicle to grow unhindered to ovulatory size. Compared to agonist treated cycles

this would confer the advantages of a much shorter cycle of treatment, promise more conceptions and fewer miscarriages, reduce the amount of gonadotropin required, and increase the incidence of monofollicular ovulation with a consequent reduction in the prevalence of OHSS and multiple pregnancies.

To date, one trial employing a GnRH antagonist with recombinant FSH, specifically for women with PCOS, has been published (Elkind-Hirsch *et al.* 2003). Following pre-treatment with oral contraceptives, a GnRH antagonist was started in 20 patients on day 2 of the cycle. When LH concentrations were found to be suppressed, concurrent antagonist and recombinant FSH therapy was started and continued until the day of hCG. Luteinizing hormone was effectively suppressed by one dose of antagonist and all patients ovulated. Overall clinical pregnancy rates were 44% and ongoing pregnancy rates 28%. This was a preliminary trial but large RCTs are needed to confirm these results.

Laparoscopic ovarian drilling

The original treatment for PCOS, proposed by Stein and Leventhal (1935), was bilateral wedge resection of the ovaries. This met with remarkable success but was abandoned due to the high probability of inducing pelvic adhesions. Thanks to modern technology but using the same principles, laparoscopic ovarian drilling (LOD) by diathermy or laser now presents a further treatment option for women with anovulatory infertility associated with PCOS. This laparoscopic version of ovarian wedge resection employs a unipolar coagulating current or puncture of the ovarian surface with a laser, in four to ten places to a depth of 4–10 mm on each ovary.

An analysis of the first 35 reports, mostly uncontrolled series, showed that 82% of 947 patients ovulated following the operation and 63% conceived either spontaneously or after treatment with medications to which they had previously been resistant (Donesky and Adashi 1996). A Cochrane database analysis of six randomized controlled trials mostly comparing LOD with gonadotropin therapy showed similar cumulative ongoing pregnancy rates 6–12 months after LOD and after 3–6 cycles of gonadotropin therapy (Farquhar *et al.* 2001). A large, recently completed, multicenter study in The Netherlands showed parity in the results of LOD and low-dose FSH therapy (Bayram *et al.* 2004). However, those who did not respond in a short time after LOD were given clomiphene or FSH. The Cochrane analysis (Farquhar *et al.* 2001) highlighted the main advantage of LOD – a very high prevalence of monofollicular ovulation and therefore a significant reduction in multiple pregnancy rates compared with gonadotropin therapy.

Further possible advantages of LOD are a reported reduction in miscarriage rates (Abdel Gadir *et al.* 1990), the fact that it is an often successful "one-off"

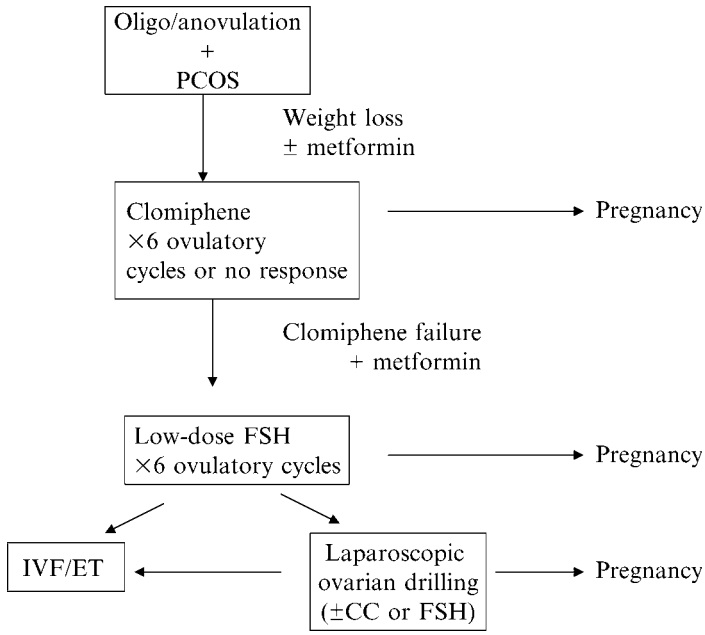

Fig. 10.3 Suggested stepwise treatment for infertility associated with PCOS.

procedure which may avoid the use of expensive medical therapy, and the exclusion of ovarian hyperstimulation syndrome. If ovulation is not forthcoming within 2–3 months following LOD, then ovulation induction can often be more successfully employed than preceding the operation. However, in a large number of cases spontaneous ovulation has been induced even for several years following LOD in a similar fashion to ovarian wedge resection, the "predecessor" of LOD (Lunde *et al.* 2001). A study of long-term follow-up after LOD showed that 54/110 women (49%) conceived spontaneously within a year and a further 42 women (38%) within 1 to 9 years following the operation (Amer *et al.* 2002). For those who respond to LOD but relapse into anovulation, a repeat procedure has been shown to be effective (Amer *et al.* 2003).

Those who are slim and have high LH concentrations seem to have the most favorable prognosis (Gjoanness 1994) and, although the mechanism involved in the restoration of ovulation is quite unknown, the principal endocrine change of a dramatic decrease in LH concentrations about 2 days after the operation seems to be an integral ingredient.

The place of LOD in the hierarchy and order of possible therapeutic regimes has not yet been fully determined and often depends on the expertise and experience of the treating clinic. A proposed treatment scheme is illustrated in Fig. 10.3.

In vitro fertilization and embryo transfer

Failure to conceive using the above therapeutic possibilities for the infertile PCOS patient still leaves them with a very viable "last resort" in IVF/ET, which provide excellent results. Compared with women undergoing IVF for tubal infertility, women with PCOS have a smaller percentage of recovered oocytes that are fertilized but the larger number of oocytes recovered balances this out, resulting in similar pregnancy rates (Homburg *et al.* 1993a). In vitro maturation of oocytes from women with PCOS may become a possible option (Child *et al.* 2002). However, it is proving technically difficult at present and concerns over the well-being of pregnancies achieved from in vitro maturation have not yet been fully answered.

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11

Laparoscopic surgical treatment of infertility related to PCOS revisited

Jean Cohen

Introduction

Polycystic ovarian syndrome (PCOS) – characterized by chronic anovulation, and/or androgen excess, hypersecretion of luteinizing hormone (LH), obesity, and infertility – is a relatively common condition in women during reproductive years. The consistent morphologic feature is a peripheral ring of small follicles in association with increased ovarian stroma. It remains an incompletely understood entity with varying degrees of severity and partial symptomatologies.

Wedge resection of ovaries has been proposed by Stein in 1935 (Stein and Leventhal 1935). For long it has been the only treatment of polycystic ovaries (PCO). When treatments by antiestrogens (clomiphene citrate) became available (Greenblatt 1961) and when the good results of these treatments were known, the surgical technique which had the inconvenience of periovarian adhesion formation disappeared. Subsequently numerous publications have indicated the interest of laparoscopic techniques (biopsy, cauterization, multi-electrocoagulation, laser, etc.) in cases of non-response to the medical treatments. The first attemps were tried in France thanks to Raoul Palmer's ovarian biopsy forceps (Palmer and Cohen 1965, Cohen *et al.* 1972a, b).

Techniques

Electrocautery

Biopsy

The first publication concerning pregnancies obtained after laparoscopic ovarian biopsies with cauterization with Palmer forceps date back to 1972. At this time, Cohen and colleagues (Cohen *et al.* 1972a) reported 21 pregnancies after 51

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successive ovarian biopsies. They came to the conclusion that this procedure has a therapeutic effect on some ovarian infertilities (Cohen *et al.* 1972b).

In 1989, B. M. Cohen reported 778 ovarian biopsies performed between 1971 and 1987 with a pregnancy rate (PR) of 31.8% distributed as 36.6% in less than 3 months, 32.2% between 3 and 8 months, and 31% over this period (Cohen 1989).

As early as 1972 numerous French authors confirmed spontaneous pregnancies after laparoscopic ovarian biopsy. Among those publications, Sykes (63% PR for 70 cases) (Sykes and Ginsburg 1972), Mintz (44% PR for 157 cases) (Mintz and De Brux 1971), Tescher (23% PR for 85 cases) (Tescher *et al.* 1972), Chassagnard (1974) (23% PR for 92 cases), Devaut (1977) (33% PR for 32 cases), Scarpa (55% PR for 29 cases) (Scarpa and Malaponte 1978), Fouqet (1978) (50% PR for 100 biopsies), Diquelou and colleagues (1988) practiced 68 ovarian biopsies and obtained 13 pregnancies in 3 months' time (19%) and 24 pregnancies in 12 months' time (35%). The rate of success was 34% in PCO and 40% in unexplained infertilities. As early as 1972, J. Cohen and colleagues indicated there was no relationship between pathological examination of the biopsied ovary and occurrence of pregnancy.

In the 778 cases of J. Cohen (Cohen and Audebert, 1989) the authors observed three complications due to the ovarian biopsy:

- two cases of ovarian hemorrhage linked to trauma of ovarian blood vessels, which were impossible to coagulate. One case was treated by immediate laparatomy and the second one by delayed laparotomy for hematocele.
- one bowel perforation due to an electric spark, leading to a perforation on the ninth day post laparoscopy. It was treated by a simple suture.

In all other cases where ovarian bleeding happened during the biopsy, hemostasis could be obtained either by cauterization or by pressure on the two sides of the biopsy with the forceps.

In some cases, the authors reviewed the biopsied ovary a few years later (on the occasion of Cesarean sections, ectopic pregnancy, or laparoscopy). The aspect usually observed is that of a simple depression on the surface of the ovary. In no cases have adhesions been observed on the biopsied capsule.

Multipunctures

Gjoanness (1984) proposed the procedure of laparoscopic multi-electrocauterization in PCOS. The ovulation rate was 92% and the pregnancy rate 69%. In a publication of 1989 (Gjoanness 1989) with a follow-up of 10 years, the same author reported the outcome of pregnancy of 89 women pregnant after electrocauterization. The miscarriage rate was 15%, which is less than after clomiphene or wedge resection.

Gjoanness (1994) reported the results concerning 252 women with PCOS treated with ovarian electrocauterization during the years 1979–1991: ovulation was obtained in 92% of the total series, and pregnancy in 84%. The response was influenced by body weight, with an ovulation range of 96–97% for the slim and moderately obese women decreasing to 70% for the very obese ones.

When ovulation was established, the pregnancy rate per se was independent of body weight, being 92% for slim and 95% for overweight women. In the responders (who ovulated following ovarian electrocautery), the annual rate of cessation of ovulation was only 3–4%. Even after a period of contraceptive use following ovarian electrocautery, ovulation was resumed and pregnancy obtained within a few months. Therefore the author proposes electrocautery as the primary treatment in women with PCOS undergoing laparoscopy for any reason, infertility being a present, future or hypothetical problem only.

Greenblatt and Casper (1987) proposed cauterization with small scissors. Eight to ten punctures were made on each ovary with a current of 4 A, until the cortex was penetrated. Six cases of PCO have been studied compared to six controls with regular cycles. Three to four days after laparoscopy a decrease of androtestosterone, testosterone, estradiol (E_2), and LH has been observed in the sole PCO group. An increase of follicle stimulating hormone (FSH) is also observed. These modifications are independent of anesthesia or laparoscopy as they do not appear in the control group. Four of six PCO women became pregnant during the same month.

Sumioko and Utsunomiya (1998) proposed multiple punch resection cautery of ovaries with monopolar forceps done on six to ten surface follicles. The authors estimated that this technique reduces 1/10 of the ovarian volume. Seven cases were observed. Four days after laparoscopy a decrease of LH, a diminution of LH pulse amplitude, and a decrease of androgens were observed. Follicle stimulating hormone and prolactin were not modified. The modifications observed persisted at the sixth week post laparoscopy. Four of the six women became pregnant between 12 and 44 weeks after their operation.

Pellicer and Remohi (1992) have treated 76 patients with PCOS. Of these, 58 had electrocautery and 6 had laser vaporization. All patients failed to respond to clomiphene or HMG treatment. Thirty-three patients had anovulatory cycles. The pregnancy rate after laparoscopic treatment was 52.6% and the ovulation rate 67.1%.

Armar and Lachelin (1993) made a study of 50 PCO women treated over a period of 3 years and 3 months. Diathermy was applied to each ovary for 4 s at a time in four separate places. Forty-three women (86%) ovulated in an average time of 23 days, and 33 women became pregnant (50 pregnancies with 8 spontaneous abortions). The abortion rate of 14% is very low; of the 22 women who had no pelvic abnormality other than PCO, 19 (86%) had one or more successful pregnancies.

Campo *et al.* (1993) treated 23 PCO women who failed to become pregnant with many kinds of ovulation induction. The technique was either multiple laparoscopic biopsies or a longitudinal incision on the surface of the ovary. Fifty-six percent of patients ovulated and 13 pregnancies occurred with a miscarriage rate of 8%.

Kovacs and colleagues (1991) treated 10 PCOS patients with ovarian electrocautery on 10 different points on each ovary. Seven women ovulated. The author observed a significant and persistent fall in serum testosterone levels and a transient fall with subsequent rise in inhibin levels.

Gadir and colleagues (1990) considers the laparoscopic electrocautery of ovaries as "the" treatment of PCOS. Eighty-eight patients who failed to respond to clomiphene were divided into three groups: (A) electrocautery, (B) HMG, and (C) pure FSH. After treatment the ovulation rates (as seen by hormone levels and ultrasound) were: A = 71.4%, B = 70.6%, C = 66.7%. The pregnancy rates per cycle were 9.5%, 12.6%, and 8.8% respectively. The miscarriage rates were 21.4%, 53.3%, and 40%. The birth rates were 37.9%, 23.3%, and 20.7%. The authors concluded that ovarian electrocautery is the best for treatment of PCOS.

Balen and Jacobs (1994) did a prospective study on 10 patients with resistant PCOS, comparing unilateral or bilateral laparoscopic diathermy. Diathermy was applied three or four times for 4 s. The results were evaluated within 6 weeks. Unilateral ovarian diathermy resulted in ovulation from both ovaries. Fifty percent of the patients responded to diathermy and those who responded had a significantly greater fall in serum LH concentrations than those who failed to respond.

Farhi and colleagues (1995) reported a retrospective study of 22 women to evaluate the effect of ovarian electrocautery on the ovarian response to gonadotropic stimulation and pregnancy rate in clomiphene citrate resistant women with PCOS and high basal serum LH levels. Markedly reduced basal serum LH concentrations and normal menstrual cyclicity in 41% of patients were recorded after laparoscopic ovarian electrocautery. Comparison of gonadotropin-stimulated cycles before and after electrocautery revealed significantly higher rates of ovulation and pregnancy after electrocautery as well as significant reduction in the number of ampoules, daily effective dose, and duration of the induction phase with HMG and in the daily effective dose with FSH. The results indicate an increased ovarian sensitivity to gonadotropins after laparoscopic ovarian electrocautery. The authors suggest a preference for laparoscopic ovarian electrocautery over medical treatment in all or selected group of clomiphene citrate resistant PCOS patients.

Costa *et al.* (2004) reviewed files of 100 infertile couples, where the woman was treated by laparoscopic ovarian drilling (LOD). There was a significant increase in

the rate of ovulatory cycles after LOD of 66% ($p < 0.0001$). The pregnancy rate in spontaneous cycles was 61.7% and the cumulative pregnancy rate adding ovulation induction was 81%.

Almeida and Risk (1998) reported a case of LOD by microlaparoscopy under local anesthesia.

Laparoscopic laser

Laparoscopic laser drilling has been introduced and used in the treatment of PCOS during the last 15 years. In the minds of those promoting it, the laser technique provides controllable power density, desirable depth of penetration, and predictable thermodamage of surrounding tissues. It may also diminish the risk of adhesions.

Few series have been published. All types of laser have been used: e.g., CO_2 laser, argon, and YAG. With the YAG laser Huber *et al.* (1988) performed three to five drills on each ovary (5–10 mm long and 4 mm deep). He obtained five spontaneous ovulations in eight patients treated. For the three others, clomiphene induced ovulation, contrary to the results obtained before laparoscopy.

Daniell and Miller (1989) treated 85 PCO women with different laser models. They were bad responders to clomiphene. During laparoscopy ovarian vaporization was performed by argon, CO_2, or potassium titanyl phosphate (KTP). A two-puncture technique was used to drain all the visible small subcapsular follicles of each ovary and to drill randomly placed craters in the ovarian stroma. Ovulation occurred spontaneously in 71%. Post-operatively, 56% conceived within 6 months of laparoscopy. The KTP laser at 20 W is used to vaporize multiple sites over each ovary. Small wells up to 2 cm deep are developed. The technique is very easy even in patients who are obese. The effect is transient. Kurtz and Daniell (1993) had no complication in over 120 cases.

Ostrzenski (1992) used translaparoscopic CO_2 laser ovarian wedge resection. The free ovarian surface was vaporized to a width of 1 cm. There was a 92% pregnancy rate and an 8% postsurgical adhesion rate, among 12 cases that were incorporated in the study.

Heylen and colleagues (1994) treated 44 anovulatory patients with laparoscopic argon laser. Spontaneous ovulation occurred in 80% of the women. Spontaneous conception occurred in 55% of the patients.

Donesky and Adashi (1995) reviewed 29 relevant studies identified in the English language. Pregnancies after laparoscopic ovulation induction procedures have been reported in an average 55% of treated subjects (range 20% to 65%).

Although laser techniques provide greater control over the type of damage induced in the ovary, this does not appear to translate into a clinical advantage. The impact of the different techniques on reducing adhesion formation remains theoretical.

Other techniques

Zullo *et al.* (2000) proposed bilateral ovarian drilling by minilaparoscopy without general anesthesia. Sixty-two infertile patients were operated. The pregnancy rate (65.6%) was comparable to the group with general anesthesia (60%). The intraoperative pain score was good and patients were discharged quickly.

Watrelot and Dreyfus (1999) carried out LOD through transvaginal hydrolaparoscopy.

Randomized controlled trials

Rimington *et al.* (1997) did a randomized controlled trial (RCT) on 50 women who required in vitro fertilization (IVF) for reasons other than anovulation.

They had all previously undergone ovarian stimulation with gonadotropin therapy which had failed to result in pregnancy or had been abandoned due to high risk of developing ovarian hyperstimulation syndrome (OHSS). Twenty-five women were treated by long-term pituitary desensitization followed by gonadotropin therapy, oocyte retrieval, and embryo transfer (group 1). Twenty-five women underwent laparoscopic ovarian electrocautery after pituitary desensitization followed by gonadotropin therapy, oocyte retrieval, and embryo transfer (group 2). A significantly higher number of women in group 1 had to have the treatment cycle abandoned due to impending or actual OHSS, determined by endocrine and clinical findings. In addition, the development of moderate or severe OHSS in completed cycles was higher in group 1. The pregnancy rate and miscarriage rate in the two treatment groups were similar. The authors propose that laparoscopic ovarian electrocautery is a potentially useful treatment for women who have previously had an IVF treatment cycle cancelled due to risk of OHSS or who have suffered OHSS in a previous treatment cycle.

Tozer *et al.* (2001) also considers that LOD does not appear to have a deleterious effect on controlled ovarian stimulation, and the outcome of IVF–embryo transfer may be beneficial in decreasing the risk of severe OHSS and improving the ongoing clinical pregnancy rate.

Farquhar *et al.* (2002) compared in an RCT the effectiveness of LOD diathermy ($n = 29$) with gonadotropin ovulation induction ($n = 21$) for women with clomiphene citrate resistant PCOS. Cumulative pregnancy rates were 28% at 6 months for LOD and 33% for three cycles of ovulation induction with gonadotropins. The authors concluded that LOD is a safe and effective alternative to ovulation induction with gonadotropins.

In a Cochrane meta-analysis (Farquhar and Lilford (2001) four randomized studies were reported. It was not possible to compare results of LOD with those of gonadotropin stimulation. However, in ovarian stimulation 9% to 40% of cycles were cancelled because of risk of hyperstimulation.

Table 11.1. Results of percoelioscopic treatments in PCOS

Authors	Year	Technique	Number of cases	Spontaneous ovulation (%)	Pregnancies (%)
Cohen *et al.*	1972	Biopsy	51		41
Gjoanness	1984	Cauterization	62	92.0	84
Greenblatt and Casper	1987	Cauterization	6	71.0	56
Huber *et al.*	1988	Laser	8	41.7	
Cohen	1989	Cauterization	778		31.8
Daniell and Miller	1989	Laser	85	83.8	66.7
Gadir *et al.*	1990	Cauterization	29	26.5	43.8
Tasaka *et al.*	1990	Cauterization	11	91	36
Utsonomiya *et al.*	1990	Biopsy	16	93.8	50.0
Gurgan *et al.*	1991	Cauterization	40	71	57
Kovacs *et al.*	1991	Cauterization	10	70	20
Gurgan *et al.*	1992	Laser	40	70	50
Ostrzenski	1992	Laser	12	92	92
Pellicer and Remohi	1992	Cauterization	131	67.1	52.6
Armar and Lachelin	1993	Cauterization	50	86	66
Campo *et al.*	1993	Resection coelio	23	56	56
Gjoanness	1994	Cauterization	252	92	84

Results

The results are presented in Table 11.1. They are homogeneous whatever the technique: more than 50% spontaneous ovulation and a mean percentage of 50% of pregnancies are obtained.

Lower spontaneous abortion rates with laparoscopic series compared to medical treatment appear in several studies. Cohen and Leal de Meirelles (1983) reported early pregnancy loss of 22 of 179 patients (13%), Gjoanness (1989) reported early pregnancy loss in only 13 of 89 patients (14.6%) who conceived after laparoscopic electrocautery. Gadir *et al.* (1990) reported early pregnancy loss in 3 of 14 (21.4%) patients randomized to undergo laparoscopic cautery. In contrast, 8 of 14 (55.4%) in the HMG group and 4 of 10 (40%) in the pure FSH group of the same study aborted.

Naether and colleagues (1994) evaluated 206 patients up to 72 months after laparoscopic surgery. Of these, 145 patients achieved a total of 211 conceptions giving a pregnancy rate of 70%. They showed that the effects are not temporary. There were 18% miscarriages and three ectopic implantations.

Complications

The risk of the technique is that of post-operative adhesions. In a review of 18 studies, El Helw and colleagues (1996) reported adhesion formation range between 0% and 100%. The wide variation can be explained by patient selection bias. Gjoanness (1984) has found no adhesion during Cesarean sections of pregnant patients. Dabirashrafi and colleagues (1991) published a complete study. Seventeen women were electrocauterized: eight second-look laparoscopies were performed, with no adhesions. In a second group of 21 patients who all had a second-look laparoscopy, four minimal adhesions and one moderate (according to the American Fertility Society classification) were observed.

Naether and Fischer (1993) evaluated the incidence and extent of previous adhesion formations subsequent to laparoscopic electrocoagulation from a total of 199 PCOS patients. Fifty cases of laparoscopy and 12 Cesarean sections served as second-look investigation. A subgroup of 30 patients had abdominal lavage and artificial ascitis after surgery: they underwent "early" second-look 2 to 14 days after laparoscopy. Adhesion formation was detected in 19.3% of these cases. The incidence was reduced to 16.5% with the use of abdominal lavage. The adhesions found were due to bleeding of the ovarian capsule. Adhesiolysis was easily possible during "early" second look.

Greenblatt and Casper (1993) observed periovarian adhesions of varying severity in eight women after laparoscopic cautery. Intercede adhesion barrier (Ethicon) showed no protective effect. Despite this finding, seven of eight women spontaneously conceived without any further therapy.

Gurgan and colleagues (1994) reported a review of 12 publications concerning adhesion formation as a complication of laparoscopic treatment of PCOS. Adhesion formation rates as assessed by second-look laparoscopy ranged from 0% to 100%. The mean adhesion score of the group treated with CO_2 laser was significantly higher than that of the electrocautery group (Table 11.2).

The rate of adhesions seem to be very different from one author or one technique to the other. The adhesions may be due to some bleeding on the ovarian surface or to premature contact between the ovary and the bowel after cauterization. In my practice the risk is very low, and less than the risk observed after laparotomy. All authors consider that adhesions do not exclude the possibility of pregnancy.

Ruiz Velasco (1996) considers that gonadal atrophy and/or premature ovarian failure caused by an excess of ovarian destruction is more common than is believed (cases are mostly unpublished).

El-Saeed (2000) evaluated the incidence and the degree of adhesion formation after LOD for anovulatory patients with clomiphene resistant PCOS. In a study of

Table 11.2. Periadnexal adhesion formation rates as assessed by second-look laparoscopy following surgical treatment of PCOS

Authors	Number of cases	Technique	Adhesion formation rate (%)
Portuondo *et al.* (1984)	24	Ovarian biopsy	0
Grochmal (1988)	30	ND: YAG laser	3
Lyles *et al.* (1989)	6	Cautery/ND: YAG laser	100
Daniell and Miller (1989)	8	CO_2 KTP laser	0
Keckstein (1989)	7	CO_2 laser	43
	4	ND: YAG laser	0
Dabirashafi *et al.* (1991)	8	Cautery	0
Gurgan *et al.* (1991)	7	Cautery	86
	10	ND: YAG laser	80
Gurgan *et al.* (1992)	20	ND: YAG laser	68
Armar and Lachelin (1993)	50	Cautery	24
Naether and Fischer (1993)	26	Cautery	35
Naether *et al.* (1994)	62	Cautery	19

46 patients, adhesions were detected in 67.5% following LOD on second-look laparoscopy.

The most effective ways to reduce the occurrence of post-operative adhesions are:

- a meticulous hemostasis if bleeding occurs;
- waiting a few minutes after cauterization before allowing the ovary to contact the peritoneum;
- irrigation with an isotonic solution.

Mode of action of laparoscopic procedures

The first post-operative endocrine alterations were described by Greenblatt and Casper (1987) and Sumioki and colleagues (1988). They observed a significant decrease of LH and androgen levels during the days immediately after surgery. The decrease is persistent after 6 weeks. This is enough to explain how the endocrinological disorders of PCOS are corrected and how pregnancies occur. The two authors agree that the trauma of the ovary is enough to induce a decrease in the production of local androgens, followed by a fall in estradiol, and a decrease of the positive feedback on LH.

Sumioki and Utsunomiya (1998) proposed a mechanism of monofolliculogenesis by ovarian drilling in PCOS:

- a vicious circle of high androgen and high LH pulsation;
- pituitary hyper-response and positive feedback from high androgen;
- high inhibition in atretic PCO follicle;
- high androgen and cytochrome P450 c 17 enzyme activity.

Greenblatt and Casper (1987) gives an important role to inhibin. Sakata and colleagues (1990) studied nine anovulatory patients having PCOS, submitted to a laparosocpic cauterization of ovaries. The levels of bioactive LH, immunoreactive LH, FSH, androstenedione, and testosterone were studied before and after cauterization and in five controls. Eight women ovulated spontaneously and three became pregnant. The authors noticed a decrease of androgens as well as immunoreactive LH, and they were the first to point out a decrease of bioactive LH.

Pellicer and Remohi (1992) studied 13 anovulatory patients after cauterization, and confirmed the decrease of the levels of LH, testosterone, and androstenedione ($p < 0.05$). Meanwhile the level of insulin and insulin-like growth factor 1 (IGF-1) remained unchanged.

Campo and colleagues (1993) confirmed the rapid decrease of the levels of androstenedione and plasma testosterone in all the cases after cauterization of the ovaries. However, the variations of these levels are not linked to clinical success. For this author there is no variation in the levels of LH. On the contrary, the mean values of FSH and its pulsatility increased significantly in the patients who became pregnant.

Amer *et al.* (2002) undertook a study of 116 anovulatory women with PCOS patients who underwent LOD between 1991 and 1999 and 34 anovulatory PCOS patients who had not undergone LOD.

The beneficial endocrinological (LH, LH/FSH, testosterone, free androgens) and morphocal (mean ovarian volume) effects of LOD appeared to be sustained for up to 9 years in most patients.

Duleba *et al.* (2003) evaluated the effects of laparoscopic ovarian wedge resection on hormonal and metabolic parameters of PCOS and compared profiles of women who achieved pregnancy with those who did not. Twenty-two women (67%) achieved clinical pregnancy within the mean of 4.9 months after surgery. Baseline parameters of women who became pregnant differed from those who did not: those who became pregnant were less obese, and had lower levels of total cholesterol, low-density lipoprotein, and triglycerides, higher levels of sex hormone binding globulin (SHBG), lower levels of fasting insulin, lower insulin area under the curve, and higher insulin sensitivity index. Subjects not pregnant by 12 weeks after surgery underwent repeat endocrine and metabolic evaluations. In these women, wedge resection was followed by declines in testosterone, LH, and insulin sensitivity index. Wedge resection had no significant effect on SHBG, dehydroepiandrosterone sulfate (DHEAS), or lipid profile.

It seems that all the authors agree on the fact that the ovarian traumas converge on: (a) a significant and immediate decrease of androgens; and (b) a secondary increase of FSH which could be related to a decrease of intraovarian inhibin. But the most difficult and unclear aspect of the problem is how to explain the fact that a physical trauma induces endocrine modifications. As all the authors do not perform the same technique (multiperforation, single or multiple biopsy, laser) one may think that it is not the volume of the injured tissues which plays a part. The only common point is the ovarian burn.

However, Mio and colleagues (1991) have shown in 18 patients with PCOS that only with transvaginal ultrasound-guided follicular aspiration could they obtain 87–100% ovulation rate per patient and 50% pregnancy. Most of the persistent follicles were punctured and their contents aspirated during the mid luteal phase. The same ovarian stimulation regimen as used in the previous cycles were administered in the cycles after the aspiration. A significant decrease of basal LH was observed. This method, simpler and less invasive, may be revolutionary, if further experiments confirm its efficacy.

Szilagyi and colleagues (1993) questioned whether restitution of menstrual cyclicity and ovulation were associated with changes in the opioidergic and dopaminergic activity, known to be aberrant in these women. Opioidergic and dopaminergic tone was assessed in patients with PCOS before and after laser vaporization ($n = 4$) or classical ovarian wedge resection ($n = 4$). Blood samples for the determination of LH, FSH, and prolactin were obtained frequently following opioidergic and/or dopaminergic antagonism effected by nalaxone (4 mg IU) or metoclopramide (10 mg IU). In response to either surgical approach, circulating LH levels decreased ($p < 0.01$) while FSH concentrations remained unaltered. Further, LH and FSH concentrations did not change following challenges with nalaxone or metoclopramide. This applied to conditions before and after surgery. Prolactin release in response to metoclopramide was markedly ($p < 0.01$) higher following ovarian surgery. Thus, both ovarian laser and classical wedge resection can restore normal menstrual cyclicity in PCOS patients, although they failed to alter opioidergic and dopaminergic activity. This suggests that ovarian surgery is effective in influencing gonadal control, but that the central opioidergic and dopaminergic control of gonadotropin secretion remains unaffected.

Graf and colleagues (1994) studied two patients after ovarian wedge resection. They found that testosterone decreased immediately and LH amplitudes were reduced in a PCOS patient.

Tulandi *et al.* (2000) evaluated the effects of LOD on serum vascular endothelial growth factor (VEGF) and on insulin response to the oral tolerance test in 27 women with PCOS. Levels of VEGF in women with PCOS are higher than

in normal women and ovarian drilling did not change these levels. The procedure did not change insulin responses.

In contrast, Amin *et al.* (2003) compared 25 women with PCOS and 20 women with regular menstrual cycles, after laparoscopic ovarian drilling. Higher serum levels of VEGF and IGF-1 may explain the increased vascularity that was demonstrated by Doppler blood flow in PCOS. Treatment by LOD reduced serum VEGF, IGF-1, testosterone, and LH, and reduced ovarian blood flow velocities.

One may consider again the two hypotheses that had already been formulated in 1983 (Cohen and Leal de Meirrelles 1983):

- either the burning of the ovary provokes a secondary hyperthermia inducing an increase of the concentration of gonadotropins by surface unity;
- or electrocoagulation stimulates the ovarian nerves which transmit the excitation to the superior centers.

Zaidi and colleagues (1995) studied the stromal blood flow in three groups of patients on day 2 or 3 of ovarian stimulation: group 1, 63 women with regular cycles; group 2, 13 women with PCO on ultrasound scan; group 3, 12 women with anovulatory cycles and PCO. A subjective assessment of the intensity and quantity of colored areas in the ovarian stroma appeared to be greater in the two last groups compared with group 1.

Mean (SEM) ovarian stromal peak systolic blood flow velocity (V_{max}) was 16.88 (1.79) and 16.89 (2.36) cm/s in groups 2 and 3 respectively. These velocities were significantly greater than the mean (SEM) ovarian stromal V_{max} of group 1: 8.74 (0.68) cm/s ($p < 0.001$). Mean (SEM) ovarian stromal time averaged maximum velocity (TAMX) was 10.55 (0.91) and 10.89 (1.80) cm/s in groups 2 and 3 respectively, both significantly greater than mean ovarian stromal TAMX of group 1 ($p < 0.001$). There was no significant difference in pulsatility index (PI) between the three groups. There thus appears to be significantly greater ovarian stromal blood flow velocity in women with polycystic ovaries as detected by color and pulsed Doppler ultrasound. But at present there is no study on possible changes of velocity after laparoscopic ovarian treatment of PCOS. It would permit the verification of our first hypothesis.

Shawki and colleagues (1998) studied the effect of ovarian drilling on ovarian blood flow in 35 patients. Assessment of PI of the ovarian artery before and after drilling showed a decrease of PI in 62% of cases by 30%. Spontaneous ovulation occurred in 58% of cases.

Brian Cohen (1989) made the hypothesis that drainage of androgens and inhibin from surface follicles could reverse the excessive collagenization of overlaying ovarian cortex and facilitate a softening of the ovarian tunica. Neighboring follicles that are not undergoing astresia may then mature and gain access to the ovarian surface, facilitating normal ovulation.

It remains a mystery how the laparoscopic techniques bring about the resumption of a disturbed endocrinological function. What is sure, anyway, is that laparoscopic techniques suppress less ovarian tissue than the wedge resection but act the same way.

Is re-electrocautery efficient when the first LOD did not induce pregnancy or ovulation? Kamel *et al.* (2004) studied 55 patients 6 months after the first procedure, when 30 were exposed to another LOD. The other 25 were treated with FSH for three cycles. In the first group 53.3% ovulated and 72% in the second. The cumulative pregnancy rate was 6.6% versus 16%. The authors concluded that redrilling is followed by a low pregnancy rate and possible hazards of adhesion formation.

The problem is different when the first LOD is followed by a pregnancy. In my own experience. I know at least 15 patients who asked for a second LOD after 2–3 years of secondary infertility and became pregnant after redrilling.

An economic evaluation of LOD versus gonadotropin therapy for women with clomiphene resistant PCOS has been published by Farquhar *et al.* (2004). They showed a significant reduction in both direct and indirect costs.

Conclusions

Considerable data have been collected on the impact of laparoscopic treatment of PCOS on the resumption of ovulation and the rate of pregnancy in infertile patients with a success rate of greater than 50%. A significant difficulty encountered in the evaluation of the studies is their lack of uniformity. There was a great variation in the diagnosis criteria used to define PCOS. None of the studies includes a treatment-independent control group. Some of the patients became pregnant with a medical treatment after laparoscopy (the same treatment having been ineffective before).

Nevertheless, so many reports of clinical experience permit to stress the advantages of laparoscopic surgical method:

- elimination of the risk of OHSS and multiple gestations;
- multiple ovulatory cycles from a single treatment;
- high pregnancy rate;
- usefulness of laparoscopy for the diagnostic of unexplained infertility;
- lower rate of spontaneous abortion;
- elimination of intensive monitoring and high cost treatment with gonadotropin therapy.

In any case, laparoscopic techniques have the following advantages over surgical wedge resection:

- cost savings;
- fewer post-operative adhesions.

They have the advantages over gonadotropin therapy of:

- serial repetitive ovulatory events resulting from a single treatment;
- no increased risk of OHSS or multiple pregnancies;
- lower incidence of spontaneous abortion;
- appreciation of the ovarian reserve by the count of the number of early follicles.

The disadvantages are:

- need for anesthesia;
- non-permanent ovulatory effect;
- possible post-operative adhesions.

The possible adverse effects (post-operative adhesions, bowel lesions) indicate that the technique must be performed by a well-trained gynecologist.

This procedure must not be considered as the first-line treatment. Clomiphene citrate remains the first line of therapy for the anovulatory patient with PCOS. For the resistant patients, laparoscopic techniques have many advantages over gonadotropin therapy, and must be offered. Clinical outcomes may be similar but the LOD differs in easiness, risks, and costs. On the other hand, when a gynecologist diagnoses a PCOS (by ultrasound imaging or hormonal results) and performs a laparoscopy for infertility, a cauterization of ovaries may be done at the same time in order to avoid a secondary surgical laparoscopy.

Recently the use of metformin has been proposed to improve reproductive and metabolic abnormalities in women with PCOS. There is no randomized study comparing metformin with LOD. However, Pirwany and Tulandi (2003) made a literature research and concluded that ovulation and pregnancy rates appear to be similar for both techniques. Both treatments decrease the incidence of ovarian hyperstimulation and the cancellation rates of IVF cycles.

This treatment option deserves more study by means of randomized controlled trials.

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12

In vitro fertilization and the patient with polycystic ovaries or polycystic ovary syndrome

Adam H. Balen

Introduction

In vitro fertilization (IVF) is not the first-line treatment for polycystic ovary syndrome (PCOS), but many patients with the syndrome may be referred for IVF, either because there is another reason for their infertility or because they fail to conceive despite ovulating (whether spontaneously or with assistance) – that is their infertility remains unexplained. Furthermore, approximately 30% of women have polycystic ovaries as detected by ultrasound scan. Many will have little in the way of symptoms and may present for assisted conception treatment because of other reasons (for example tubal factor or male factor). When stimulated these women with asymptomatic polycystic ovaries have a tendency to respond sensitively and are at increased risk of developing the ovarian hyperstimulation syndrome (OHSS). An understanding of the management of such patients is therefore important to specialists involved in IVF.

The association of enlarged, sclerocystic ovaries with amenorrhea, infertility, and hirsutism, as described by Stein and Leventhal (1935), is now described as the polycystic ovary syndrome (PCOS). In recent years it has become apparent that polycystic ovaries may be present in women who are not hirsute and who have a regular menstrual cycle. Thus, a clinical spectrum exists between the typical Stein–Leventhal picture (PCOS) and the symptomless women with polycystic ovaries. Even the clinical picture of patients with PCOS exhibits considerable heterogeneity (Balen *et al.* 1995). This heterogeneous disorder may present, at one end of the spectrum, with the single finding of polycystic ovarian morphology as detected by pelvic ultrasound. At the other end of the spectrum symptoms such

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as obesity, hyperandrogenism, menstrual cycle disturbance, and infertility may occur either singly or in combination. Biochemical disturbances (elevated serum concentrations of luteinizing hormone (LH), testosterone, insulin, and prolactin) are common. PCOS is a familial condition and a number of candidate genes have been implicated (Franks *et al.* 1997). Polycystic ovary syndrome appears to have its origins during adolescence, although it may present at any time and its expression is particularly associated with an increase in weight.

The definition of the syndrome has been much debated. There are many extraovarian aspects to the pathophysiology of PCOS yet ovarian dysfunction is central. At a recent joint ESHRE/ASRM consensus meeting a refined definition of the PCOS was agreed: namely the presence of two out of the following three criteria: (1) oligo- and/or anovulation; (2) hyperandrogenism (clinical and/or biochemical); (3) polycystic ovaries, with the exclusion of other etiologies (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

Diagnosis

Polycystic ovaries

The diagnosis of polycystic ovaries is best made not on the clinical presentation but rather on the ovarian morphology. With the advent of high-resolution ultrasound, identification of polycystic ovaries has been simplified, and ovarian biopsy, which is invasive and possibly damaging to future fertility because it can cause adhesions, is now unnecessary. Polycystic ovaries should be distinguished from multicystic ovaries, which occur normally during puberty and are associated with recovering weight loss-related amenorrhea. These ovaries do not contain increased stroma and the size of the cysts is usually larger than in polycystic ovaries (Adams *et al.* 1985).

For many years ovaries were described as polycystic if there were 10 or more cysts, 2–8 mm in diameter, arranged around a dense stroma or scattered throughout an increased amount of stroma (Adams *et al.* 1985). At a recent joint ASRM/ESHRE consensus meeting a refined definition of the PCOS was agreed, encompassing a description of the morphology of the polycystic ovary. According to the available literature, the criteria fulfilling sufficient specificity and sensitivity to define the the polycystic ovary should have at least one of the following: either 12 or more follicles measuring 2–9 mm in diameter or increased ovarian volume (>10 cm^3). If there is a follicle greater than 10 mm in diameter, the scan should be repeated at a time of ovarian quiescence in order to calculate volume and area. The presence of a single polycystic ovary is sufficient to provide the diagnosis. The distribution of the follicles and the description of the stroma are not required in the diagnosis. Increased stromal echogenicity and/or stromal volume are

specific to PCOS, but it has been shown that the measurement of the ovarian volume (or area) is a good surrogate for the quantification of the stroma in clinical practice (Balen *et al.* 2003).

The polycystic ovary is usually detected by ultrasound, the images correlating well with histopathological studies (Saxton *et al.* 1990, Takahashi *et al.* 1993). The original ultrasound definition was provided by transabdominal ultrasonography, which is still used in current publications.

Transabdominal ultrasound has been largely superseded by transvaginal scanning because of greater resolution and in many cases patient preference – as the need for a full bladder is avoided which saves time and may be more comfortable. Whilst this may be the case in the context of infertility clinics, where women are used to having repeated scans, it was found that 20% of women who were undergoing routine screening declined a transvaginal scan after having first had a transabdominal scan (Fulghesu *et al.* 2001).

The transvaginal approach gives a more accurate view of the internal structure of the ovaries, avoiding apparently homogeneous ovaries as described with transabdominal scans, particularly in obese patients. With the transvaginal route, high-frequency probes (>6 MHz) having a better spatial resolution but less examination depth can be used because the ovaries are close to the vagina and/or the uterus and because the presence of fatty tissue is usually less disruptive (except when very abundant).

Recent studies with computerized three-dimensional reconstructions of ultrasound images of the polycystic ovary have shown that the major factor responsible for the increase in ovarian volume is an increase in the stroma, with little contribution from the cysts themselves (Kyei-Mensah *et al.* 1998). Zaidi and co-workers (1995), using color and pulsed Doppler ultrasound, have shown that the stroma of the polycystic ovary has a very high rate of blood flow, consistent with histological studies showing increased stromal vascularity (Goldzieher and Green 1962) and the recent finding of large amounts of vascular endothelial growth factor (VEGF) in the theca cells of the polycystic ovary (Kamat *et al.* 1995).

High-resolution ultrasound scanning has made it possible for an accurate estimate to be made of the prevalence of polycystic ovaries in the general population. Several studies have estimated the prevalence of polycystic ovaries in "normal" adult women and have found rates of approximately 20–33% (Polson *et al.* 1988, Clayton *et al.* 1992, Farquhar *et al.* 1994, Michelmore *et al.* 1999). It is important to differentiate between polycystic ovaries and PCOS. The former term describes the morphological appearance of the ovary whereas the latter term is only appropriate when polycystic ovaries are found in association with a menstrual disturbance, most commonly oligomenorrhea, the complications of hyperandrogenization (seborrhea, acne, and hirsutism), and obesity.

Features of polycystic ovary syndrome

Polycystic ovary syndrome is often associated with endocrinological abnormalities and in particular with alterations in the serum concentrations of LH, prolactin, estrogens, and androgens (in particular, testosterone and androstenedione). In about 40% of cases, plasma concentrations of LH are raised. In a proportion of patients with PCOS, moderate hyperprolactinemia (usually 600–2000 mU/l) is present. Hyperprolactinemia may be caused by the stimulation of pituitary lactotrophs by acyclical estrogen production (Franks *et al.* 1985) rather than by a primary pituitary defect. Estrogen levels may be altered in PCOS patients who are anovulatory. Estradiol levels are similar to those found in normal women during the early follicular phase of the cycle. Estrone levels are raised, mostly because of extraovarian conversion of androstenedione (Baird *et al.* 1977), which largely takes place in adipose tissue. Finally, the polycystic ovary tends to produce an excess of androgens. As with the clinical picture, these endocrine changes are variable and patients with PCOS may have serum hormone concentrations in the normal range.

Hypersecretion of LH is particularly associated with menstrual disturbances and infertility. Indeed, it is this endocrine feature that appears to result in reduced conception rates and increased rates of miscarriage in both natural and assisted conception (Balen *et al.* 1993). The finding of a persistently elevated early to mid-follicular phase LH concentration in a woman who is trying to conceive suggests the need to suppress LH levels by either pituitary desensitization, with a gonadotropin releasing hormone agonist (GnRH-a) or laparoscopic ovarian diathermy. There are, however, no large, prospective randomized trials that demonstrate a therapeutic benefit from a reduction in serum LH concentrations during ovulation induction protocols.

The patient's body mass index (BMI) correlates with an increased rate of hirsutism, cycle disturbance, and infertility (Kiddy *et al.* 1992, Balen *et al.* 1995). Obese women with PCOS hypersecrete insulin, which stimulates ovarian secretion of androgens, and is associated with hirsutism, menstrual disturbance, and infertility. It is seldom necessary to measure the serum insulin concentration, as this will not overtly affect the management of the patient, but the prevalence of diabetes in obese women with PCOS is 11% (Conway 1991), so a measurement of impaired glucose tolerance is important in these women. Obese women ($BMI > 30$ kg/m^2) should therefore be encouraged to lose weight. Weight loss improves the symptoms of PCOS and improves the patient's endocrine profile (Kiddy *et al.* 1992, Clark *et al.* 1995).

There have been many reviews over the years which have attempted to piece together the complexities of the syndrome (Franks 1989, Insler and Lunenfeld 1991, Homburg 1996), but that is beyond the scope of this chapter. Because

a consensual definition has not, until recently, been accepted, when we turn to the literature on the treatment of the condition it proves impossible to compare studies from different centers that use differing starting points.

Prevalence

The prevalence of polycystic ovaries in women with ovulatory disorders has been well documented. With the use of high-resolution ultrasound, it is now apparent that as high a proportion as 87% of patients with oligomenorrhea and 26% with amenorrhea have polycystic ovaries (Adams *et al.* 1986). Polson and colleagues (1988) found a prevalence of 22% in a volunteer "normal" population and a number of other studies have found a similar prevalence (see above). A study of 224 normal female volunteers between the ages of 18 and 25 years identified polycystic ovaries using ultrasound in 33% of participants (Michelmore *et al.* 1999). Fifty percent of the participants were using some form of hormonal contraception, but the prevalence of polycystic ovaries in users and non-users of hormonal contraception was identical. Polycystic ovaries in the non-users of hormonal contraception were associated with irregular menstrual cycles and significantly higher serum testosterone concentrations when compared with women with normal ovaries; however, only a small proportion of women with polycystic ovaries (15%) had "elevated" serum testosterone concentrations outside the normal range. Interestingly there were no significant differences in acne, hirsutism, BMI, or body fat percentage between women with polycystic and normal ovaries and hyperinsulinism and reduced insulin sensitivity were not associated with polycystic ovaries in this group.

The prevalence in patients referred for in vitro fertilization (IVF) is not so well known. Several studies have suggested that many patients have polycystic ovaries, for example it was shown that 33% of women attending for IVF had polycystic ovaries (MacDougall *et al.* 1993). Polycystic ovaries with or without clinical symptoms are therefore a common finding in patients referred for IVF. It must be stressed that the first-line treatment for PCOS is not IVF. Occasionally, the IVF specialist will be presented with a patient with PCOS, referred for IVF, who either has never had induction of ovulation or has been inadequately stimulated. Provided there is no other cause for their infertility, for example tubal damage, then induction of ovulation is first-line therapy. Options include clomiphene citrate, aromatase inhibitors, gonadotropin therapy or laparoscopic ovarian diathermy (see Chapter 11). In vitro fertilization may be necessary in patients with PCOS who have failed to conceive despite at least six ovulatory cycles (i.e., those who have coexisting "unexplained" infertility).

The response of the polycystic ovary to stimulation for IVF

The response of the polycystic ovary to stimulation in the context of ovulation induction aimed at the development of unifollicular ovulation is well documented and differs significantly from that of normal ovaries. The response tends to be slow, with a significant risk of ovarian hyperstimulation and/or cyst formation (Dor *et al.* 1990, MacDougall *et al.* 1993, Balen *et al.* 1994). Conventional IVF currently depends on inducing multifollicular recruitment. It is thus to be expected that the response of the polycystic ovary within the context of an IVF program should also differ from the normal, but this has previously been assumed rather than documented. Jacobs and co-workers (1987) described an increase in follicle production in patients with polycystic ovaries, and others have referred to the "explosive" nature of the ovarian response (Smitz *et al.* 1991).

There are several possible explanations for this "explosive" response. There are many partially developed follicles present in the polycystic ovary and these are readily stimulated to give rise to the typical multifollicular response. Thecal hyperplasia (in some cases with raised levels of LH and/or insulin) provides large amounts of androstenedione and testosterone, which act as substrates for estrogen production. Granulosa cell aromatase, although deficient in the "resting" polycystic ovary, is readily stimulated by follicle stimulating hormone (FSH). Therefore, normal quantities of FSH act on large amounts of substrate (testosterone and androstenedione) to produce large amounts of intraovarian estrogen. Ovarian follicles, of which there are too many in polycystic ovaries, are increasingly sensitive to FSH (receptors for which are stimulated by high local concentrations of estrogen) and as a result there is multiple follicular development associated with very high levels of circulating estrogen. In some cases, this may result in OHSS, to which patients with polycystic ovaries are particularly prone.

There are two additional factors to be considered. The first is that many women with PCOS, particularly those who are obese, have compensatory hypersecretion of insulin in response to the insulin resistance specifically related to PCOS and that caused by obesity. Since the ovary is spared the insulin resistance, it is stimulated by insulin, acting, as it were, as a co-gonadotropin. Insulin augments theca cell production of androgens in response to stimulation by LH and granulosa cell production of estrogen in response to stimulation by FSH (Adashi *et al.* 1985).

The second factor to be considered relates to the already mentioned widespread expression of VEGF in polycystic ovaries. Vascular endothelial growth factor is an endothelial cell mitogen that stimulates vascular permeability, hence its involvement in the pathophysiology of OHSS; it is normally confined in the ovary to the blood vessels and is responsible there for invasion of the relatively

avascular graafian follicle by blood vessels after ovulation. The increase of LH at midcycle leads to expression of VEGF, which has recently been shown to be an obligatory intermediate in the formation of the corpus luteum (Ferrara *et al.* 1998). In polycystic ovaries, however, Kamat and colleagues (1995) have shown widespread expression of VEGF in theca cells in the increased stroma. More recent studies (Agrawal *et al.* 1998) have shown that, compared with women with normal ovaries, women with polycystic ovaries or PCOS have increased serum VEGF, both before and during LH releasing hormone (LHRH) analog therapy and gonadotropin treatment.

The above data serve to remind us of the close relationship of polycystic ovaries with OHSS and also provide a possible explanation for the multifollicular response of the polycystic ovary to gonadotropin stimulation. One of the mechanisms that underpins the unifollicular response of the normal ovary is diversion of blood flow within the ovaries, first from the non-dominant to the dominant ovary and, second, from cohort follicles to the dominant follicle. This results in diversion of FSH away from the cohort follicles and permits them to undergo atresia. The widespread distribution of VEGF in polycystic ovaries may prevent this diversion of blood flow, leaving a substantial number of small and intermediate sized follicles in "suspended animation" and ready to respond to gonadotropin stimulation. The distribution of VEGF in the polycystic ovary therefore helps to explain one of the fundamental features of the polycystic ovary, namely the loss of the intraovarian autoregulatory mechanism that permits unifollicular ovulation to occur.

A study of the outcome of IVF in 76 patients diagnosed as having polycystic ovaries on pre-treatment ultrasound scan was compared with 76 control patients who had normal ovaries. The subjects were matched for age, cause of infertility, and stimulation regimen (MacDougall *et al.* 1993). Despite receiving significantly less human menopausal gonadotropin (hMG), patients with ultrasound- diagnosed polycystic ovaries had significantly higher serum estradiol concentrations on the day of human chorionic gonadotropin (hCG) administration, developed more follicles, and produced more oocytes. Fertilization rates, however, were reduced in patients with polycystic ovaries. There was no significant difference in cleavage rates. The pregnancy rate per embryo transfer was 25.4% in the polycystic ovary group and 23.0% in the group with normal ovaries. There were three high-order multiple pregnancies in the polycystic ovary group but none in the group with normal ovaries. Of the patients with polycystic ovaries, 10.5% developed moderate/severe OHSS compared with none in the controls ($p = 0.006$). Patients with and without polycystic ovaries undergoing IVF had similar pregnancy and live birth rates, as each had similar numbers of good-quality embryos for transfer. The study indicated the importance of the diagnosis of polycystic

ovarian morphology prior to "controlled" ovarian stimulation, because it is less likely to be controlled in women with polycystic ovaries and these patients are more likely to develop OHSS and multiple pregnancy. Similar observations in women with polycystic ovaries undergoing IVF have been reported by others (Homburg *et al.* 1993a).

There are a small number of patients with polycystic ovaries who are poor responders, rather than over-responders. Such patients are often very resistant to gonadotropin stimulation and may benefit from the addition of growth hormone (Owen *et al.* 1991).

Preconception counseling

Women with polycystic ovaries encounter specific problems during assisted conception treatment cycles. By our diagnostic criteria, many women are unaware that their ovaries are polycystic and may have presented with another cause of subfertility. When polycystic ovaries have been diagnosed by ultrasound, it is helpful to discuss this finding with the patient, as a preliminary knowledge of the behavior of the polycysic ovary in response to superovulation regimens acts as a foundation for both an explanation of the drugs chosen and advice about potential problems – specifically OHSS and multiple pregnancy.

Some women will already have been diagnosed as having either polycystic ovaries or PCOS and may be aware of the sequelae. The latter group will usually have had endocrinological and metabolic problems, and for them, preconception counseling should involve more than an outline of the consequences of treatment. There are additional problems that may occur during pregnancy and there may be a chance to reduce their risk by appropriate measures, such as weight loss, even before embarking upon assisted conception regimens.

There are thus two aspects to the counseling and subsequent management of women with polycystic ovaries: first, the general behavior of the polycystic ovary itself; and second, the additional features of PCOS. Ovaries that are morphologically polycystic contain multiple antral follicles and are extremely sensitive to stimulation. It is the woman with PCOS who will benefit most from preconception counseling. She has not only an endocrine disorder but also a metabolic one. She may therefore have hyperandrogenism and insulin resistance – it is the consequences of the latter that have a particular bearing on pregnancy. Hyperinsulinemia may lead to obesity, which in turn is associated with hypertension, pre-eclampsia, and gestational diabetes. Although hypertension and pre-eclampsia have been directly associated with PCOS (Diamant *et al.* 1982), it appears that it is the resultant obesity that is the prime factor (Gjoanness 1989). Dietary restriction and reduction of weight gain during pregnancy do not reduce the

incidence of pre-eclampsia, yet if an ideal weight can be attained before conception, pre-eclampsia may be avoided. The role of insulin lowering drugs in the context of IVF are still being evaluated.

It is established that women with polycystic ovaries exhibit insulin resistance, particularly if they are obese, and it has been postulated that hyperinsulinemia may have an etiological role in PCOS, possibly through effects on ovarian insulin-like growth factor 1 (IGF-1) receptors. Even if exposure to high insulin or IGF-1 levels is not etiological, it is thought to result in increased ovarian androgen secretion. Fasting insulin levels are raised in two-thirds of obese and one-third of lean women with PCOS; those women with hyperinsulinemia are more likely to present with menstrual disturbances and hyperandrogenism than those with normal insulin levels. The most effective management is advice on diet and weight loss. One should also be aware that type 2 diabetes mellitus may be precipitated by some treatments, such as synthetic sex steroids (Fox and Wardle 1990). Screening women with PCOS for glucose intolerance should now be standard pratice, with a 75g oral glucose tolerance test being performed in all women with a BMI of greater than 30 kg/m^2. Gestational diabetes is also more prevalent amongst women with PCOS, our study quoting a prevalence of 8.1%, as compared with a population prevalence of 0.25%. Of the 89 pregnancies studied, the rate was greatest (19%) in the obese women; none of those with a normal weight became diabetic in pregnancy. Although dietary restriction is employed in the management of obesity, active steps towards weight loss during pregnancy itself are not advised.

Obesity in pregnancy also leads to an increased incidence of urinary tract infections, fetal malpresentations and dystocia, postpartum hemorrhage, and thromboembolism. The perinatal mortality rate in the infants of obese women (>110th percentile, Metropolitan Life Insurance tables) is also double that of the normal population. Current research suggests that in later life women with PCOS may be at risk from hypertension, type 2 diabetes mellitus, and cardiovascular disease, because insulin resistance is also associated with a reduction of the cardioprotective high-density lipoprotein 2 (HDL_2). Women with endometrial hyperplasia and carcinoma are traditionally obese, with hypertension and diabetes, and they are likely to have polycystic ovaries. Therefore, preconception counseling is important not only to advise short-term weight loss, in order to reduce maternal and neonatal morbidity, but also to prevent later morbidity by encouraging obese women to become slim, and non-obese women to stay slim.

There has been disagreement in the past about the association of PCOS with congenital abnormalities, especially as treatment regimens may have an influence (Ahlgren *et al.* 1976). The miscarriage rate is increased in women with polycystic ovaries (Sagle *et al.* 1988). This is thought to be secondary to an abnormal

endocrine environment, specifically hypersecretion of LH, affecting either oocyte maturation or endometrial receptivity (Regan *et al.* 1990). There is, however, no evidence of an increased incidence of congenital abnormalities either in women with PCOS or in women undergoing ovulation induction and IVF. That PCOS does not cause congenital anomalies is also supported by the high prevalence of the syndrome in our clinic, whose statistics are included in the reports of the Medical Research Council (1990) and Beral and colleagues (1990). Couples can thus be given appropriate reassurance at the time of their first consultation.

Implications of PCOS in relation to fertility

Anovulation is the main cause of infertility in women with PCOS. Many regimens have been evolved to induce ovulation and the interested reader is referred to Balen *et al.* (2003). There is a body of evidence indicating that hypersecretion of LH is inimical both to fertility and to pregnancy outcome (Balen *et al.* 1993b). Luteinizing hormone has several functions in the control of the developing follicle. In the early follicular phase, low levels of LH induce a change in function of the theca interstitial cells from progesterone to androgen production (Erickson *et al.* 1985). Follicle stimulating hormone then promotes the conversion of androgen to estradiol by the granulosa cells. Not only does LH initiate theca cell androgen production, but it is also involved in the reversal to progesterone secretion at the time of the preovulatory surge. Luteinizing hormone is also involved in the suppression of oocyte maturation inhibitor (OMI). The action of OMI is to maintain the meiotic arrest of the oocyte at the diplotene stage of prophase I. The precise nature of OMI is uncertain, but it is known that cyclic adenosine monophosphate (cAMP) activates OMI or is itself OMI. By reducing cAMP in the oocyte, LH enables the reactivation of meiosis and hence the attainment of oocyte maturity prior to ovulation. Inappropriate release of LH may profoundly effect this process such that the released egg is either unable to be fertilized (Homburg *et al.* 1988), or, if fertilized, miscarries (Regan *et al.* 1990).

It was first demonstrated in 1985 that oocytes obtained from women undergoing IVF who had a serum LH value greater than one standard deviation above the mean on the day of hCG administration had a significantly reduced rate of fertilization and cleavage (Stanger and Yovich 1985). It was shown not only that ovulation and fertilization are affected by high tonic LH levels, but also that miscarriage is more likely (Homburg *et al.* 1988). There has been some disagreement over the significance of an elevated LH, with one group suggesting no deleterious effect in IVF cycles (Thomas *et al.* 1989). In this study it was considered that only cycles that result in a pregnancy should be used to provide the normal range of LH concentrations and that by taking LH levels above the 75th percentile,

no adverse effect on fertilization or cleavage was detected. An effect on miscarriage was not addressed.

To assess the risk of miscarriage after IVF with respect to age, cause of infertility, ovarian morphology, and treatment regimen, a retrospective analysis was performed of 1060 pregnancies conceived between July 1984 and July 1990 as a result of 7623 IVF cycles (Balen *et al.* 1993a). This was at a time before the routine use of GnRH agonists and so it was possible to assess the different modalities of stimulation without the absolute suppression of serum gonadotropin levels. Ovarian stimulation had been achieved either by the administration of clomiphene citrate (100 mg/day from day 2 to 6 day of the menstrual cycle) and hMG (Pergonal, Serono Ltd., UK) and/or FSH (Metrodin, Serono Ltd., UK); or by the combined use of the GnRH-a buserelin (Suprefact, Hoechst, UK) and hMG or FSH. Three regimens of administration of buserelin (500 g/day given subcutaneously) and hMG/FSH were used. In the "long" protocol, buserelin was administered from day 1 of the menstrual cycle and treatment with hMG or FSH commenced after pituitary desensitization had been achieved at least 14 days later. In the "short" protocol, buserelin was administered from day 2 and hMG or FSH from day 3 of the menstrual cycle and both continued until the day of hCG administration. In the "ultrashort" protocol, buserelin was administered on days 1–3 and hMG or FSH were given from day 2 of the cycle until the day of hCG administration.

Of the 1060 pregnancies, 724 (68.3%) were ongoing, 282 (26.6%) ended in a spontaneous miscarriage, and 54 (5.1%) were ectopic. There were four heterotopic pregnancies (0.004%). The mean age of women who had ongoing pregnancies (32.18 SD 3.86 years) was significantly lower ($p < 0.008$) than that of the women who miscarried (33.17 SD 4.09 years). There was a highly significant difference ($p = 0.001$, 95% confidence interval (CI) 5.42–18.28%) between the miscarriage rates in patients who received the long buserelin regimen (19.1%) compared with those who received clomiphene citrate, but no difference between those who received short (28.0%) or ultrashort (24.7%) buserelin regimens and clomiphene citrate. There was no significant difference in the miscarriage rate in patients who received hMG (21%) or FSH (16.5%) and a long buserelin regimen. The rate of miscarriage in patients who received clomiphene citrate was 47.2% in those with polycystic ovaries and 20.3% in those with normal ovaries ($p < 0.00005$, 95% CI 15.59–38.33%). In patients who received the long buserelin regimen, there was no significant difference in miscarriage rates between those with polycystic ovaries (20.3%) and those with normal ovaries (25.5%). There was also no difference in the miscarriage rates in women with normal ovaries who received clomiphene citrate (20.3%) or the long buserelin regimen (25.5%). There was, however, a highly significant difference ($p = 0.0003$,

95% CI 13.82–40.09%) in miscarriage rates in women with polycystic ovaries who received clomiphene (47.2%) and those who received the long buserelin regimen (20.3%). Miscarriage rates were not affected by treatment with hMG versus FSH in patients with normal (24.6% vs. 28%) or polycystic ovaries (18% vs. 25%) who were treated with the long buserelin regimen and similarly between hMG versus FSH in patients with normal (19.3% vs. 23.5%) or polycystic ovaries (47.6% vs. 46.2%) who were treated with a clomiphene citrate regimen (Balen *et al.* 1993a).

The high rate of miscarriage in those who received clomiphene citrate may be related to the deleterious effects of elevated serum LH levels. Clomiphene citrate causes an exaggerated early follicular phase release of both gonadotropins, and the resultant elevated level of LH may reduce the chance of conception and increase the risk of miscarriage (Shoham *et al.* 1990). The protective effect of GnRH agonists, such as buserelin, is presumably mediated by the functional hypogonadotropic hypogonadism and suppressed LH levels that they induce. This notion is consistent with the observation that it was the long protocol of treatment with buserelin, but not the short or ultrashort protocols, that was associated with the reduction in miscarriage rates. It is well documented that the use of the short or ultrashort protocols of GnRH-a is associated with a rise of LH concentrations to pre-ovulatory surge levels (Tan *et al.* 1992) so that the developing ovarian follicle may be exposed to inappropriately high LH levels, especially in patients with polycystic ovaries, in whom return to baseline levels of LH takes longer than average. In this respect, the use of the short or ultrashort protocols of GnRH-a exposes the patient to the same adverse effects as when clomiphene citrate is used.

Homburg and colleagues (1993b) also studied the outcome of 97 pregnancies in women with PCOS. The patients were treated by either ovulation induction or IVF with either hMG alone or hMG after pituitary desensitization with the GnRH-a Decapeptyl. The miscarriage rate in the agonist-treated patients (17.6%) was significantly lower than the miscarriage rate in the women treated with hMG alone (39.1%; $p = 0.03$). The study demonstrates that pituitary desensitization is the important factor in reducing miscarriage rates in women with polycystic ovaries, rather than clomiphene citrate being the adverse factor, as clomiphene was not given to the patients in that study.

The use of a GnRH agonists to achieve pituitary desensitization has become almost routine in IVF clinics because of the flexibility in programming oocyte recovery (Tan *et al.* 1992) and superior outcomes (Fleming *et al.* 1985, Frydman *et al.* 1988). The new generation of GnRH antagonists is finding a role in routine clinical practice and may have additional potential benefits for patients with polycystic ovaries (see below).

Superovulation strategies for women with polycystic ovaries and/or PCOS

When ovarian stimulation is required for IVF, a different approach to therapy is required, because the objective is to achieve multifollicular development, resulting in the collection of several appropriately mature eggs, but without causing OHSS. The latter is a particular problem in women with PCOS as they usually exhibit greater sensitivity than women with normal ovaries to exogenous stimulation (Salat-Baroux and Antoine 1990).

The initial experience in ovulation induction for IVF was with a combination of clomiphene citrate with either or both hMG and FSH, or sometimes with a high-dose gonadotropin alone (Fleming and Coutts 1990). Irrespective of ovarian morphology, these treatment regimens do not suppress pituitary responsiveness to the secretory products of the developing follicle. Premature luteinization and a premature LH surge may both occur, with deleterious effect on the developing oocytes (Gemzell *et al.* 1978) or ovulation prior to oocyte recovery. These problems are more often encountered in women with PCOS (Fleming and Coutts 1988).

It is interesting to note that, contrary to earlier beliefs, ovarian stimulation resulting in the collection of large numbers of oocytes (more than 10) results in a poor outcome, the optimum number being between seven and nine (Sharma *et al.* 1988). This is of particular relevance to women with polycystic ovaries in whom there are often a high number of oocytes, yet poor rates of fertilization and implantation – the overall effect being to achieve an equivalent pregnancy rate to a control group (Dor *et al.* 1990) but a higher miscarriage rate.

There are few studies that have specifically compared different treatment regimens for women with and without polycystic ovaries, and those that have vary in their definition and diagnosis of the syndrome (Salat-Baroux *et al.* 1988a, Tanbo *et al.* 1990). The two particular aims of therapy in this group of women are the correction of the abnormal hormone milieu, by suppressing elevated LH and androgens, and the avoidance of ovarian hyperstimulation. Pituitary desensitization avoids the initial surge of gonadotropins with the resultant ovarian steroid release that occurs in the short GnRH protocol. Although the long protocol theoretically provides controlled stimulation, the polycystic ovary is still more likely than the normal ovary to become hyperstimulated (Salat-Baroux and Antoine 1990). With both long and short protocols, significantly more eggs are collected from women with polycystic than normal ovaries (Jacobs *et al.* 1987) and, interestingly, the total dose of exogenous gonadotropins is the same for either regimen. It has also been proposed that a longer period of desensitization (30 instead of 15 days) is of benefit by reducing androgen levels (Salat-Baroux *et al.* 1988b); in the latter study, the longer duration of treatment did not improve pregnancy rates but did apparently decrease the incidence of hyperstimulation.

The other debate in ovulation induction for women with PCOS is whether the use of FSH alone has any benefit over hMG: is the hypersecretion of LH responsible for the exaggerated response to stimulation of the polycystic ovary? Does minimizing circulating LH levels by giving FSH alone improve outcome? Preparations of purified urinary FSH contain some LH activity, usually less than 1%, and preliminary work has suggested that ovulation induction can be achieved without exogenous LH (Jones *et al.* 1984). In patients with hypogonadotropic hypogonadism, however, follicular maturation is often incomplete and inconsistent (Couzinet *et al.* 1988, Shoham *et al.* 1991), as LH, by its action on the thecal cells, is required for full ovarian steroidogenesis. Thus the presence of some LH facilitates normal follicular development. Most studies have found no benefit over hMG from the use of FSH alone in ovulation induction for either in vivo (Messinis *et al.* 1986, Salat-Baroux *et al.* 1988a) or in vitro fertilization (Bentick *et al.* 1988, Larsen 1990). The most probable reason is that there are only 75 units of LH activity in each ampoule and, when hMG is given in standard doses to patients who are receiving treatment with buserelin, the serum LH levels barely rise to above 5 IU/l. In patients with PCOS the serum LH concentration is usually 2–4 times that level – that is, the serum level represents a higher "secretion rate" than that mimicked by injections of hMG.

So far as in vitro fertilization is concerned a meta-analysis of randomized controlled comparisons of urinary derived FSH and hMG (Agrawal *et al.* 2000) showed that, in studies in which the long protocol of GnRH desensitization was used, there was no difference in outcome between ovarian stimulation with urinary derived FSH or with hMG preparations.

In recent years, recombinant follicle stimulating hormone (rFSH) has increasingly been used in ovulation induction and IVF treatments. Although there is pituitary suppression by GnRH agonists during the IVF treatment, a low endogenous LH level is sufficient to permit an adequate steroidogenesis in the mature follicles. Recombinant FSH is synthesized by transfecting Chinese hamster ovary cell lines with both FSH subunit genes. It has a higher bioactivity than urinary FSH (Out *et al.* 1995) and results in a higher number of oocytes retrieved in IVF treatment, and a shorter duration of treatment in clomiphene resistant anovulatory patients (Coelingh Bennink *et al.* 1998). It has been reported that the initial dose of rFSH can be reduced to 100 IU, achieving a similar response as the usual starting dose of 150–225 IU (Devroey *et al.* 1998). Marci *et al.* (2001) demonstrated that a low-dose stimulation protocol with rFSH can lead to high pregnancy rates in IVF patients with polycystic ovaries who are at risk of a high ovarian response to gonadotropins. This protocol may potentially reduce the risk of OHSS. Teissier *et al.* 1999 demonstrated that women with PCOS undergoing IVF cycles using hMG had higher testosterone and estradiol levels compared

with those using rFSH, due to higher serum LH levels. A recent meta-analysis of 18 randomized trials on the effectiveness and the outcomes of IVF cycles with a long protocol using GnRH agonist, between ovarian stimulation with rFSH and urinary FSH, was published in the Cochrane Database of Systematic Reviews (European and Middle East Orgalutran Study Group 2001). This review concluded that rFSH is more effective, and has greater batch-to-batch consistency and higher bioactivity than the urinary FSH. The total amount of gonadotropins required in IVF treatment was significantly lower with rFSH than with urinary FSH. Additionally, the clinical pregnancy rates per cycle started were also higher with rFSH, although the magnitude of the observed difference was small, 3.7%. No significant differences were detected in the rates of miscarriage, multiple pregnancy and OHSS. It seems then rFSH may have some additional benefit over hMG in IVF treatment.

We recommend the long protocol of pituitary desensitization for women identified as having polycystic ovaries and a dose of 75–100 units of FSH or hMG, which is intentionally lower than our usual starting dose of 150 units. The dose may, of course, be modified if the patient has exhibited either an exuberant or a poor response in a previous cycle. Follicular development is then monitored principally by daily ultrasonography from day 8 of stimulation, with additional measurements of serum estradiol being helpful in some cases.

The recent introduction of schedules of gonadotropin stimulation that incorporate treatment with GnRH antagonists holds promise for patients with PCO and PCOS, although the results of specific trials in this condition are not yet available. Gonadotropin releasing hormone antagonists do not activate the GnRH receptors and produce a rapid suppression of gonadotropin secretion within hours. The new IVF protocol using GnRH antagonists can offer a shorter and simpler treatment in comparison with the long protocol using GnRH agonists (Olivennes *et al.* 1996, European Orgalutran Study Group *et al.* 2000, North American Ganirelix Study Group 2001). A systematic review in the Cochrane Database showed that there is a trend of reduction of ovarian hyperstimulation syndrome in the GnRH antagonist treatment groups with the combined odds ratio of 0.47 (95% CI 0.18–1.25) (Al-Inany and Aboulghar 2002).

Another advantage of using GnRH antagonists is that the native GnRH or GnRH agonist can displace the antagonist from the GnRH receptors at the pituitary level. Therefore, in a GnRH antagonist IVF cycle, GnRH agonist can be administered to induce an LH surge and to trigger the final oocyte maturation and ovulation. Itskovitz-Eldor *et al.* (2000) demonstrated a rapid rise of LH concentrations after administration of GnRH agonist, and a peak in LH levels at 4 h after the injection. The pattern of the induced LH surge was similar to those observed in the natural cycle. Fauser *et al.* (2002) showed that the outcomes of

IVF treatment in terms of the number of oocytes retrieved, the proportion of metaphase II oocytes, the fertilization rates, the number of good-quality embryos, and the implantation rates were comparable to using hCG triggering ovulation. Triggering of ovulation with GnRH agonist is potentially more physiological and can reduce the risk of OHSS compared with using hCG, due to a shorter half-life of LH (60 min vs. 32–34 h). Exogenous hCG has been known to associate with OHSS, and in fact hCG activity can still be detected 8 days after adminstration. Further studies are needed to evaluate this potential advantage, especially among higher responders, women with PCO and PCOS.

A recent multicenter double-blind study revealed that new recombinant human LH can be as effective as hCG in inducing the final follicular maturation in IVF treatment (European Recombinant LH Study Group 2001) with a lower incidence of OHSS. This clinical effect can be beneficial for women with polycystic ovaries undergoing either GnRH antagonist or agonist IVF cycles.

Obesity and insulin resistance

Patients with PCOS undergoing IVF appear to have an increased risk of miscarriage (Kodama *et al.* 1995). Patients with android obesity, which is a common feature of PCOS, and a high BMI (>25 kg/m^2) were found to have low pregnancy rates after IVF (Wass and Rossner 1997, Fedorcsak *et al.* 2001). These observations were consistent with the early studies on pregnancy outcomes after ovulation induction with gonadotropins in obese PCOS women (Dale *et al.* 1998). Fedorcsak *et al.* (2001) concluded that obesity (BMI >25 kg/m^2), independent of insulin resistance, is associated with gonadotropin resistance. Fewer oocytes were also retrieved from obese women. The number of oocytes collected and the quality of transferred embryos were positively correlated. In other words, embryo quality declined along with the number of oocytes recovered. Therefore, obese patients should be advised to lose weight before IVF treatment.

Insulin resistance and compensatory hyperinsulinemia contribute to the pathogenesis of PCOS. A number of studies have investigated the effects of using the insulin lowering agents, mainly metformin, on women with PCOS. The initial studies were small, and non-randomized. A recent meta-analysis of appropriately conducted randomized studies has confirmed a beneficial effect of metformin in improving rates of ovulation when compared with placebo and also improving both rates of ovulation and pregnancy when used with clomiphene citrate compared with clomiphene citrate alone (Lord *et al.* 2003). The data indicate that serum concentrations of insulin and androgens improve although, contrary to popular belief, body weight does not fall.

There are no guidelines for the use of metformin in women with anovulatory PCOS. It is the author's practice to consider metformin therapy for overweight PCOS women wishing to conceive, initially alone for 3 months in order to acclimatize to side effects and then combined with clomiphene citrate, although it is preferable to prescribe the latter once the patient's BMI has fallen to 30–32 kg/m^2. Metformin therapy may be commenced after appropriate screening and advice about diet, lifestyle, and exercise for anovulatory women with PCOS who wish to conceive. The usual dose is either 850 mg twice daily or 500 mg tds. Baseline investigations should include an oral glucose tolerance test, FBC, U and E and LFTs. Side effects are predominantly gastrointestinal (anorexia, nausea, flatulence, and diarrhea) and may be reduced by taking metformin just before food and gradually increasing the dose from 850 mg at night to 850 mg twice daily after 1 week. Metformin therapy is not thought to cause lactic acidosis in non-diabetic women with PCOS and normal renal and liver function. Metformin should be discontinued for 3 days after iodine-containing contrast medium has been given. Metformin is usually discontinued in pregnancy although there is no evidence of teratogenicity and preliminary reports from retrospective studies of a reduced rate of gestational diabetes.

There is also some evidence suggesting that metformin can improve response to clomiphene and gonadotropin ovulation induction therapy (Nestler *et al.* 1998, Vandermolen *et al.* 2001, Kocak *et al.* 2002). Hyperinsulinemia is often associated with hyperandrogenism. Teissier *et al.* (2000) suggested that the follicular endocrine microenvironment is related to oocyte quality in women undergoing IVF. The study showed that testosterone levels in the follicular fluid were significantly elevated in PCOS follicles compared with the normal patients. They also demonstrated that significantly higher levels of follicular testosterone concentrations in those follicles with meiotically incompetent oocytes compared with follicles with meiotically competent oocytes. It was concluded that the excess follicular androgen concentration could affect oocyte maturation and quality. Hence, high androgen levels may contribute to a lower fertilization rate among the oocytes retrieved from women with PCOS compared to those without. Therefore, co-treatment with metformin in IVF treatment may also improve the response to exogenous gonadotropins. A recent publication by Stadtmauer *et al.* (2001) demonstrated that the use of metformin in patients with PCOS undergoing IVF treatment improved the number of mature oocytes retrieved, and the overall fertilization and pregnancy rates. However, caution is required to interpret the retrospective observational data. We are currently performing a randomized controlled trial to evaluate the extent of potential benefit of using metformin in women with PCO and PCOS undergoing IVF treatment.

In vitro maturation of oocytes

In recent years, in vitro maturation (IVM) has attracted a lot of interest as a new assisted reproductive technique (Trounson *et al.* 1994, Child *et al.* 2001). The immature oocytes are retrieved from antral follicles of unstimulated (or minimally stimulated) ovaries via the transvaginal approach. The oocytes are subsequently matured in vitro in a special formulated culture medium for 24–48 h. The mature oocytes are fertilized, usually by intracytoplasmic sperm injection (ICSI), and the selected embryos are transferred to the uterus 2–3 days later.

Although IVM is labor-intensive compared with conventional IVF treatment, there are a number of clinical advantages by the avoidance of exogenous gonadotropins, most importantly by avoiding the risk of OHSS. Since patients with PCOS have more antral follicles and a higher risk of developing OHSS compared with those without, IVM may be a promising alternative to conventional IVF.

Some studies reported that the maturation rate of immature oocytes recovered from patients with PCOS were lower than those from women with normal regular menstrual cycles (Child *et al.* 2002). However, Chian *et al.* (2000) demonstrated that priming with hCG before the retrieval of immature oocytes from unstimulated women with PCOS improved the maturation rates. A prospective observational study of 180 cycles carried out by Child *et al.* (2001) demonstrated that significantly more immature oocytes were retrieved from PCO (10 ± 5.1) and PCOS (11.3 ± 9.0) groups than from women with normal ovaries (5.1 ± 3.7) ($p < 0.05$). The overall oocyte maturation and fertilization rates were similar among the three groups. The subsequent pregnancy and live birth rates per transfer were significantly higher in the PCO and PCOS groups. This could be partially explained by the fact that there was a greater choice in the embryos selected for transfer in these two groups. However, women with PCO and PCOS were significantly younger and had more embryos transferred than women with normal ovaries.

Furthermore, Child *et al.* (2002) also reported a case-control study comparing 170 IVM and 107 IVF cycles for women with PCOS. In vitro maturation yields significantly fewer mature oocytes than IVF cycles (7.8 vs. 12, $p < 0.01$) and fewer embryos per retrieval (6.1 vs. 9.3, $p < 0.01$). The pregnancy rates per retrieval were similar between the groups. However, the implantation rates in the IVM group was significantly lower than IVF group (9.5% vs. 17.1%, $p < 0.01$) with the fact that patients in IVM cycles received more embryos than in the IVF cycles (3.2 ± 0.7 vs. 2.7 ± 0.8, $p < 0.01$). The lower implantation rates may be due to a reduced oocyte potential or a reduced endometrial receptivity. Interestingly, they also reported that the incidence of OHSS in the IVF group was 11.2%. Continuous improvements in the culture medium and synchrony between endometrial and embryonic development will hopefully result in better IVM success rates in

the future. It is also important that the infants born after IVM treatment should have a long-term follow-up to ensure the safety of this new technology.

Luteal support and the ovarian hyperstimulation syndrome

The OHSS is a well-recognized complication of ovulation induction. In its severe form, it is characterized by ascites, ovarian enlargement with cyst formation, pleural effusion, and electrolyte disturbances (Schenker and Weinstein 1978). Oliguria and vascular complications may ensue and there have been fatalities. The syndrome is believed to be relatively rare in patients undergoing ovarian stimulation for IVF, despite the multiple follicular development and high estrogen levels that commonly occur, and it has been suggested that the protective mechanism is mediated through aspiration of ovarian follicular fluid at the time of oocyte collection (Friedman *et al.* 1984). However, OHSS does occur in IVF (Golan *et al.* 1989, MacDougall *et al.* 1992) and, when severe, is the only life-threatening condition associated with ovarian stimulation in IVF.

It has been apparent for some time that patients with polycystic ovaries undergoing straightforward ovulation induction are particularly at risk of developing OHSS. Recently this has been confirmed in IVF as well. In a total population of 1302 patients, 15 patients were described who underwent ovarian stimulation for IVF and developed OHSS of sufficient severity to merit hospital admission (prevalence of 1.2% with 0.6% having severe OHSS) (MacDougall *et al.* 1992). Of these patients, 53% had ultrasonically diagnosed polycystic ovaries and 87% were undergoing their first attempt at IVF. All had received luteal support in the form of hCG. Although the pregnancy rate in this group was very high (93.3%), the multiple pregnancy rate was 57% with a miscarriage rate of 14.3% (MacDougall *et al.* 1992). It is therefore recommended that all patients undergoing IVF have a pelvic ultrasound scan performed either prior to or early in the treatment cycle. If polycystic ovaries are identified, the dose of gonadotropins should be minimized.

It has been observed that OHSS is rare in patients with hypopituitary hypogonadism. The use of GnRH agonists has been recommended in patients with polycystic ovaries, in the hope that, by converting the patient to a hypogonadal state, OHSS might be prevented. Unfortunately, this does not seem to be the case and, in fact, a number of reports suggest that OHSS is actually more common when LHRH agonists are used, especially if patients have polycystic ovaries (Charbonnel *et al.* 1987).

Recent studies have suggested that the increased propensity of polycystic ovaries to become overstimulated is due to increased expression of VEGF in the stroma of the polycystic ovary, which itself has increased blood flow, as assessed

by color Doppler. Careful monitoring of estrogen levels and numbers of follicles by ultrasound scanning during stimulation for IVF can also help to identify those at risk. In patients thought to be at risk of OHSS (age less than 30 years, and/or polycystic ovaries, and/or estrogen levels greater than 8000 pmol/l, and/or more than 20 follicles at oocyte collection), luteal support in the form of hCG should be withheld. It is now standard practice to use progesterone pessaries instead of hCG as luteal support in all patients. Transfer of a maximum of two embryos in this group reduces the multiple pregnancy rate with its attendant obstetric and neonatal problems. Alternatively, embryos may be frozen for transfer at a later date, and, in the case of patients on GnRH analogs, the analog is continued until the onset of menstruation (by giving a long-acting depot) and hormone replacement therapy for the frozen embryo replacement cycle is commenced at that stage.

Conclusion

Women with PCOS may have presented with symptoms of endocrine or metabolic disturbance prior to seeking assisted conception or they may be diagnosed at their first attendance at the infertility clinic, a proportion of them having polycystic ovaries on ultrasound scan, but no symptoms. Women with polycystic ovaries require careful management to achieve follicular maturation in an environment free from elevated LH levels in order to enhance fertilization and pregnancy outcome. The sensitivity of the polycystic ovary to exogenous stimulation and the risk of OHSS have been emphasized. The association between insulin resistance and the pathogenesis of PCOS has resulted in interest in the potential for insulin lowering drugs, such as metformin, which appear to benefit anovulatory women with PCOS and may also enhance ovarian response to stimulation. More research is required in this area. In addition to offering assisted conception, we feel that the infertility clinic is an ideal place to offer preconceptional counseling and advice as to how to minimize the metabolic sequelae of PCOS that may occur later in life.

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13

Role of hyperinsulinemic insulin resistance in polycystic ovary syndrome

Maria J. Iuorno

Introduction

Hyperinsulinemic insulin resistance is now recognized as a prominent feature of polycystic ovary syndrome (PCOS). Only recently has its role in the pathophysiology of PCOS been recognized. Both obese and lean women with PCOS appear to have some degree of insulin resistance and understanding the role of insulin resistance in PCOS has led to the successful use of insulin sensitizing drugs in the treatment of this disorder.

Insulin resistance is defined as the reduced ability of insulin to stimulate glucose uptake. Insulin actually has a number of subcellular actions not related to glucose uptake Figure 13.1 below outlines two major pathways of insulin subcellular signaling. Clinically, insulin stimulation of the GLUT-4 transporter increases glucose uptake intracellularly (Sivitz *et al.* 1989, Douen *et al.* 1990, Klip and Paquet 1990, Koranyi *et al.* 1990). However, a number of additional subcellular insulin signaling pathways also exist, such as the mitogen activated protein (MAP) kinase activation pathway that primarily regulate mitogenesis. In vitro data in a number of different cellular models of insulin resistance and diabetes have demonstrated that while glucose uptake is relatively downregulated in insulin resistance, MAP kinase pathways and alternate pathways may be upregulated leading to increased de novo lipogenesis in type 2 diabetes mellitus (Nikoulina *et al.* 2001, Bouzakri *et al.* 2003, Carlson *et al.* 2003, Koistinen *et al.* 2003). Therefore, generally when defining insulin resistance, it becomes necessary to specify that decreased insulin mediated intracellular uptake of glucose is the "resistant" pathway of insulin action.

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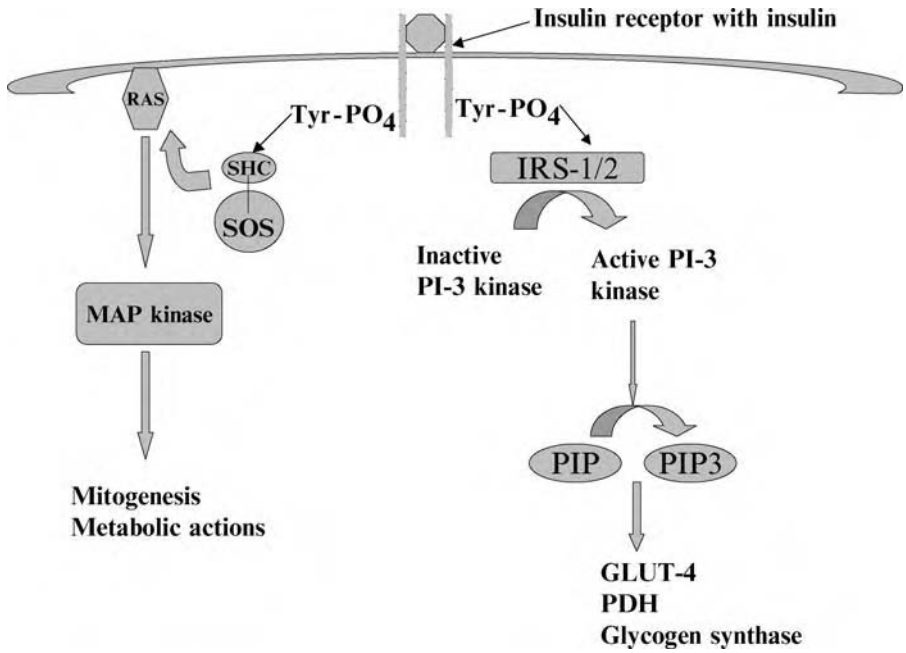

Fig. 13.1 Schematic diagram of two classical insulin signaling pathways. PIP/PIP3, phosphotidylinositol phosphate-3; GLUT-4, GLUT-4 transporter; IRS, insulin receptor substrate; PDH, pyruvate dehyrdrogenase; Tyr-PO_4, tyrosine phosphate; MAP, mitogen activated protein.

Epidemiology of insulin resistance in PCOS

Several studies have documented that the prevalence of obesity or overweightness in PCOS is at least 30–50% (Diamanti-Kandarakis *et al.* 1999, Azziz *et al.* 2004). In addition, among certain societies, obesity is a more common phenotype (Azziz *et al.* 2004) than among women in Europe (Asunción *et al.* 2000) and East Asia (Carmina *et al.* 1992, Wijeyaratne *et al.* 2002). Although obesity is associated with PCOS, it is clear that not all women with PCOS are obese. Dunaif *et al.* (1989) demonstrated that women who are lean (with body mass index (BMI) <25 kg/m^2) are in fact more insulin resistant than their normal counterparts when matched for BMI. In this study, euglycemic insulin clamps (a highly precise and sensitive method for measuring insulin resistance) were performed in lean normal women and lean women with PCOS. The normal women demonstrated relatively greater insulin sensitivity than those with PCOS, despite being matched for BMI. This evidence supports the concept that insulin resistance may be pathognomonic for this disorder.

The insulin resistance phenotype is most likely inherited genetically. Legro *et al.* (2002) studied 336 women with PCOS and 304 sisters of these probands and

compared them to 47 control women with regard to insulin resistance. Even after adjustment for BMI and age, sisters of women with PCOS had significantly more insulin resistance than control women independently of PCOS itself as reflected by higher fasting insulin levels and higher glucose to insulin ratios. A number of recent studies have also found that brothers of women with PCOS have increased insulin resistance as measured by fasting insulin levels and by a method known as homeostatic measurement assessment of insulin resistance (HOMA IR) (Yildiz *et al.* 2003, Kaushal *et al.* 2004). Thus, the genetic pathways of insulin resistance are manifested independently of the PCOS phenotype, which lends credence to the hypothesis that insulin resistance is a prominent feature of PCOS. However, it is not likely the only genetic mutation needed to produce the entire syndrome, as not all women with insulin resistance have PCOS.

The insulin resistance syndrome, defined as the metabolic syndrome, is prevalent among women with PCOS. The metabolic syndrome is defined by the National Cholesterol Education Program (2001) Third Consensus Report (NCEP ATP III) as increased waist circumference greater than 35 inches in women, serum triglycerides greater than 150 mg/dl, high-density lipoprotein (HDL) cholesterol less than 50 mg/dl in women, blood pressure greater than 130/85, or fasting glucose greater than 110 mg/dl. Having two or more of these characteristics defines the metabolic syndrome in any one individual. Among 161 women with PCOS in a retrospective analysis, 43% of these women satisfied the NCEP ATP III criteria for the metabolic syndrome (Apridonidze *et al.* 2005). This prevalence is twofold higher than that identified among age matched, eumenorrheic women of the National Health and Nutrition Examination Survey (NHANES) cohort. Since the metabolic syndrome is considered a major cardiovascular risk factor and a major risk factor for type 2 diabetes mellitus, women with PCOS are considered at increased risk for these comorbidities.

Type 2 diabetes mellitus is extremely prevalent among women with PCOS. Insulin resistance is a major contributing factor towards the development of type 2 diabetes mellitus among women with PCOS. There are additional other risk factors that lead to diabetes in women with PCOS, including obesity, pancreatic beta cell dysfunction, dyslipidemia, and family history of type 2 diabetes in first-degree relatives.

Recent data by Ehrmann *et al.* (1999) and Legro *et al.* (1999) suggest that the prevalence rate of glucose intolerance is as high as 40% in women with PCOS by World Health Organization criteria. Similarly, Dunaif *et al.* (1987) found that 20% of women had impaired glucose tolerance or type 2 diabetes mellitus using the less stringent national Diabetes Data Group Criteria. Our group has found that among premenopausal women with type 2 diabetes mellitus, 25% also carried the diagnosis of PCOS (Peppard *et al.* 2001). These cohort studies have

shown that the rate of undiagnosed diabetes is as high as 10% and usually affects these women as early as the third decade of life.

Based upon this evidence, it might be prudent to regularly screen women with PCOS for type 2 diabetes mellitus with a 2-h oral glucose tolerance test in order to identify the disease process early and avoid complications from longstanding untreated disease.

In addition to type 2 diabetes, women with PCOS may have increased risk of developing coronary atherosclerosis, not only by virtue of displaying the metabolic syndrome and carrying additional risk factors such as obesity or hypertension but by merely having the syndrome itself. A recent study (Vryonidou *et al.* 2005) compared young women with PCOS (ages 17–35 years) to age matched and BMI matched normal eumenorrheic women with respect to carotid intimal medial thickness (IMT), a known marker of early atherosclerosis. Women with PCOS had increased IMT as compared to normal women and the study also found that PCOS was an independent risk factor for increased carotid IMT. Earlier studies involving slightly older women with PCOS have had similar findings (Talbott *et al.* 2000). Women with PCOS also display increased endothelial markers of inflammation such as plasminogen activator inhibitor-1 (Atiomo *et al.* 1998, Macut *et al.* 2001, Ibañez *et al.* 2002, Sills *et al.* 2003, Diamanti-Kandarakis *et al.* 2004) and highly sensitive C-reactive protein (Sampson *et al.* 1996, Kelly *et al.* 2001, Bahceci *et al.* 2004, Boulman *et al.* 2004, Talbott *et al.* 2004). Although these surrogate markers suggest increased risk for coronary artery disease in women with PCOS, a prospective epidemiologic study demonstrating increased risk for mortality from PCOS due to coronary artery disease has yet to be published.

Cellular mechanisms of insulin resistance in PCOS

Although insulin resistance is a clear and specific characteristic associated with PCOS, the exact cellular mechanisms of insulin resistance in PCOS is not known. Insulin receptor numbers and affinities of receptors for insulin have been shown to be normal in women with PCOS (Conway *et al.* 1994, Tarkun *et al.* 2005). Therefore, abnormalities in subcellular signaling of insulin are a more probable mechanism for insulin resistance. Several investigators have found that adipocytes from women with PCOS have downregulated insulin stimulated glucose transport early in the insulin signaling pathway (Rosenbaum *et al.* 1993, Ciaraldi *et al.* 1997, Dunaif *et al.* 2001). These cells also have decreased insulin stimulated lipolysis (Rosenbaum *et al.* 1993). Dunaif *et al.* (1995) have also found that fibroblasts isolated from women with PCOS displayed decreased serine autophosphorylation of the insulin receptor. These data suggest that there is a defect in the serine kinase regulating this activity in women with PCOS. Other studies support

the concept that increased free fatty acids in women with PCOS at least contributes to the insulin resistance of muscle, liver, and adipose tissue.

More recently, there is evidence that women with PCOS have abnormalities in processing a non-classical mediator of insulin action called D-chiro-inositolphosphoglycan (DCI-IPG); this is an inositol phosphoglycan known to be present in the muscle and adipose tissue of primates and humans (Asplin *et al.* 1993). Insulin stimulates the intracellular transport of this mediator via G-protein activation (Sleight *et al.* 2002) and once intracellular, DCI-IPG can directly increase pyruvate dehydrogenase activity and increase glucose utilization (Ortmeyer *et al.* 1993, 1995). Type 2 diabetic men have been shown to deficient in DCI-IPG as compared to normal men (Asplin *et al.* 1993). In one landmark study, Nestler *et al.* found that women with PCOS treated with D-chiro-inositol (DCI), a synthetic mediator of DCI-IPG, can reduce insulin resistance and androgen production and increase ovulatory rates in women with PCOS (Nestler *et al.* 1998b). In another study, these same authors found that metformin increases insulin stimulated DCI-IPG activity in women with PCOS (Baillargeon *et al.* 2004). These data support the concept that more than one pathway may lead to insulin resistance in PCOS, including a non-classical mechanism of insulin action.

The question still remains unanswered regarding the true cellular link between insulin resistance and increased androgen production in PCOS. Since not all women with insulin resistance have PCOS or hyperandrogenism, this implies that an ovarian abnormality must also be present in order for hyperinsulinemia to increase ovarian androgen production. Nestler *et al.* (1989, 1990, 1991) found that administering diazoxide to normal and PCOS women to directly inhibit production of insulin from the pancreas without affecting insulin sensitivity significantly reduces testosterone production from the theca cell of PCOS women without affecting testosterone secretion from the ovaries of normal women. This seems to imply that there is a specific ovarian defect in PCOS women that incurs an abnormal or heightened response to insulin with increased testosterone secretion from the theca cell.

Insulin may directly increase theca cell production of testosterone by affecting subcellular substrates of androgen production. For instance, metformin given to women with PCOS decreases activity of the rate-limiting enzyme in testosterone synthesis, cytochrome P450 17 α (gene *CYP17*). In line with this hypothesis, Nelson-Degrave *et al.* (2005) demonstrated decreased insulin-stimulated phosphorylation of mitogen activated protein kinase kinase (MEK) 1/2 and extracellular regulated kinase (ERK) 1/2 in the MAP kinase pathway from theca cells of women in PCOS as compared to normal women, resulting in increased expression of CYP17. Other in vitro studies have also confirmed upregulation of CYP17 activity in theca cells of women with PCOS, although a mutation in CYP17 itself has not been identified (Daneshmand *et al.* 2002).

In addition to insulin resistance per se, there are a number of epiphenomena that occur in response to improving insulin in women with PCOS that seem to suggest that insulin's action on the ovary may not be the only mechanism by which insulin acts to induce hyperandrogenism in these women. For example, insulin-like growth factor 1 (IGF-1) can also stimulate ovarian androgen production (Homburg *et al.* 1992, Mason *et al.* 1993, Nahum *et al.* 1995) and it has been suggested that insulin sensitizing drugs reduce ovarian androgen production by decreasing free IGF-1 levels (De *et al.* 2000, Kowalska *et al.* 2001, Stadtmauer *et al.* 2001, Ibañez *et al.* 2003, Pawelczyk *et al.* 2004). Insulin itself has also been shown to decrease sex hormone binding globulin (SHBG) production from the liver and can in turn increase free testosterone concentrations in women with PCOS (Buyalos *et al.* 1993, Fendri *et al.* 1994, Ebeling *et al.* 1995, Pasquali *et al.* 1997, Ciampelli *et al.* 1999). Indeed insulin sensitizing therapy has been shown to generously increase SHBG and decrease free testosterone in women with PCOS (Crave *et al.* 1995, Nestler *et al.* 1996, 1997, 1998a, Pasquali *et al.* 1997, Diamanti-Karandakis *et al.* 1998, Chou *et al.* 2003, Glueck *et al.* 2003).

Summary

Although the mechanisms of insulin resistance in PCOS have not been specifically elucidated, it is clear that insulin resistance plays a significant role in the pathogenesis of this disease, both from the metabolic and gynecologic point of view. Unfortunately, there are really no reliable outpatient tests of insulin resistance to quantify this in any one individual as random insulin levels and fasting glucose to insulin ratios have notoriously poor correlation to the gold standard test, the euglycemic clamp. Since it seems clear that insulin resistance is present in almost all women with PCOS, it is of questionable value to measure insulin resistance per se in PCOS. Given the risk for the metabolic syndrome and diabetes in these women, it behooves the practitioner to screen patients for dyslipidemia and abnormal glucose tolerance even in young, lean patients with this disorder in order to prevent atherosclerosis and type 2 diabetes. This emphasizes the goal of making the clinical manifestations of insulin resistance in this population one of the major endpoints of therapy beyond normalization of menses and fertility.

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14

Novel treatments for polycystic ovary syndrome, including in vitro maturation

William M. Buckett and Seang Lin Tan

Introduction

Polycystic ovary syndrome (PCOS) was first described by Stein and Leventhal (1935), although the overweight, hirsuit, amenorrheic phenotype they originally described, we now realize, probably reflects the severe end of the spectrum of the syndrome. Diagnosis is made primarily by ultrasound and endocrine changes, although there remains some controversy about the significance of ultrasound-only diagnosed PCOS when the clinical phenotype and endocrine pattern are normal (Polson *et al.* 1988, Abdel Gadir *et al.* 1992). The ultrasound features of PCOS are enlarged ovaries, thickened ovarian stroma, multiple peripheral cysts (2 –10 mm diameter), and an increased intraovarian stromal blood flow (Adams *et al.* 1986, Zaidi *et al.* 1995, Buckett *et al.* 1999). The endocrine pattern is characterized by raised serum luteinizing hormone (LH) levels in the early follicular phase or raised ratio of LH to follicle stimulating hormone (FSH) and raised levels of testosterone and androstenedione, often associated with reduced levels of sex hormone binding globulin (SHBG) (Hull 1987, DeVane *et al.* 1975). However, not all these features are present in every case of PCOS and it is clear that the syndrome, primarily anovulation associated with hyperandrogenemia, is an end-point of multiple causes and this is reflected in the unknown etiology of the syndrome.

This heterogeneity has spawned many avenues for treatment of PCOS such as weight loss and lifestyle management, ovulation induction with varieties of agents, the treatment of hyperinsulinemia, the treatment of hyperandrogenemia, and the surgical treatment of PCOS. All these are described elsewhere in this volume.

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Novel treatments for PCOS

Surgical treatment

Treatments for PCOS have broadly followed the understandings of PCOS. Therefore, after the modern description of the syndrome in the 1930s, the mainstay of treatment was laparotomy (to establish the diagnosis) and ovarian wedge resection (Stein and Cohen 1939, Leventhal and Scommegna 1963). Although the exact mechanism of the therapeutic effect of ovarian wedge resection remains unclear, ovarian stromal hypersecretion of some factors is reduced by this destructive surgery and its modern equivalent – laparoscopic ovarian drilling (be it by diathermy/cautery, laser, or harmonic scalpel) continues to be used with varying degrees of therapeutic effect (Greenblatt and Casper 1987, Daniell and Miller 1989, Takeuchi *et al.* 2002).

More recently, novel developments using the same destructive principle include attempts at transvaginal ultrasound-guided ovarian diathermy (Ferraretti *et al.* 2001) and transvaginal ultrasound-guided hydrocoagulation by injecting heated saline into the ovarian stroma (Ramzy *et al.* 2001).

These developments would seem to offer the benefits of surgical treatment of PCOS in an outpatient/office setting. Following these initial reports, their efficacy and risk profile need to be assessed in larger studies.

Ovulation induction

The development of clomiphene citrate in the late 1950s and its use to induce ovulation in the 1960s marked the beginning of ovulation induction in PCOS (Whitelaw 1963). This was followed by the development of urinary and then recombinant gonadotropins and many protocols with gonadotropins, clomiphene citrate, or other antiestrogens have been used for ovulation induction in women with PCOS.

More recently, the aromatase inhibitors such as letrozole (Mitwally and Casper 2001, Healey *et al.* 2003) and more recently anastrozole (Al-Omari *et al.* 2004) have been used to induce ovulation in women with PCOS. The unifollicular ovarian response – and therefore low multiple pregnancy rates (Mitwally *et al.* 2005) – as well as the purported improved endometrial response of these may indicate a role for these early in the treatment of anovulation due to PCOS.

Insulin sensitizing agents

The association of hyperinsulinemia with PCOS and the synergistic role of insulin in the development of the phenotype of PCOS (Shoupe *et al.* 1983, Pasquali *et al.* 1987) led to the use of metformin in the treatment of PCOS (Nestler *et al.* 1998). Treatment reduces the hyperinsulinemia and hyperandrogenemia associated with

PCOS as well as inducing "spontaneous" ovulation. Other insulin sensitizing agents, such as rosiglitazone, have also been developed and used with some success in the treatment of PCOS (Baillargeon *et al.* 2004).

In vitro fertilization

If all other infertility treatments fail, then in vitro fertilization (IVF) is an effective treatment for women with PCOS and anovulation as well as any additional causes of infertility (Homburg 2003).

Nevertheless, women with polycystic ovaries (PCO) produce more follicles and eggs than normovulatory patients stimulated by a similar protocol (Dor *et al.* 1990, MacDougall *et al.* 1993, Buyalos and Lee 1996). Although pregnancy rates are similar to those of normovulatory patients, there is reduced fertilization and cleavage rates due to the poorer quality of the retrieved oocytes (Franks, 1997). In addition, the ovarian hyperstimulation syndrome (OHSS) that can affect these patients is a potentially life-threatening condition of iatrogenic origin (MacDougall *et al.* 1992, Avecillas *et al.* 2004).

In vitro maturation (IVM) of immature oocytes from women with PCOS without any stimulation is an alternative to conventional IVF which avoids the risks of ovarian stimulation. The remainder of this chapter will discuss this new technique for women with PCO/PCOS.

In vitro maturation

Control and initiation of oocyte maturation in vivo

Human oocytes are arrested in prophase I of meiosis during fetal life. These immature oocytes can be found in the non-growing follicles. As each cohort is recruited and follicular dominance is established, the oocyte acquires the ability to reinitiate meiosis (Pincus and Enzmann 1935) (Fig. 14.1).

Following resumption of meiosis, the nuclear membrane dissolves and the chromosomes progress from metaphase I to telophase I. The dissolution of the nuclear membrane is known as germinal vesicle breakdown (GVBD). Following the completion of the first meiotic division and the extrusion of the first polar body, the second meiotic division starts and is arrested at metaphase II (MII) until ovulation and fertilization. Immature oocytes (i.e., those that have not reached MII) are unable to undergo fertilization and subsequent embryo cleavage. Oocyte maturation, therefore, can be defined as the reinitiation and completion of the first meiotic division from the germinal vesicle (GV) stage to the MII stage, with the accompanying cytoplasmic maturation.

Nuclear maturation begins with GVBD. This is initiated in vivo either by the atretic degeneration of the follicle or by the pre-ovulatory gonadotropin surge

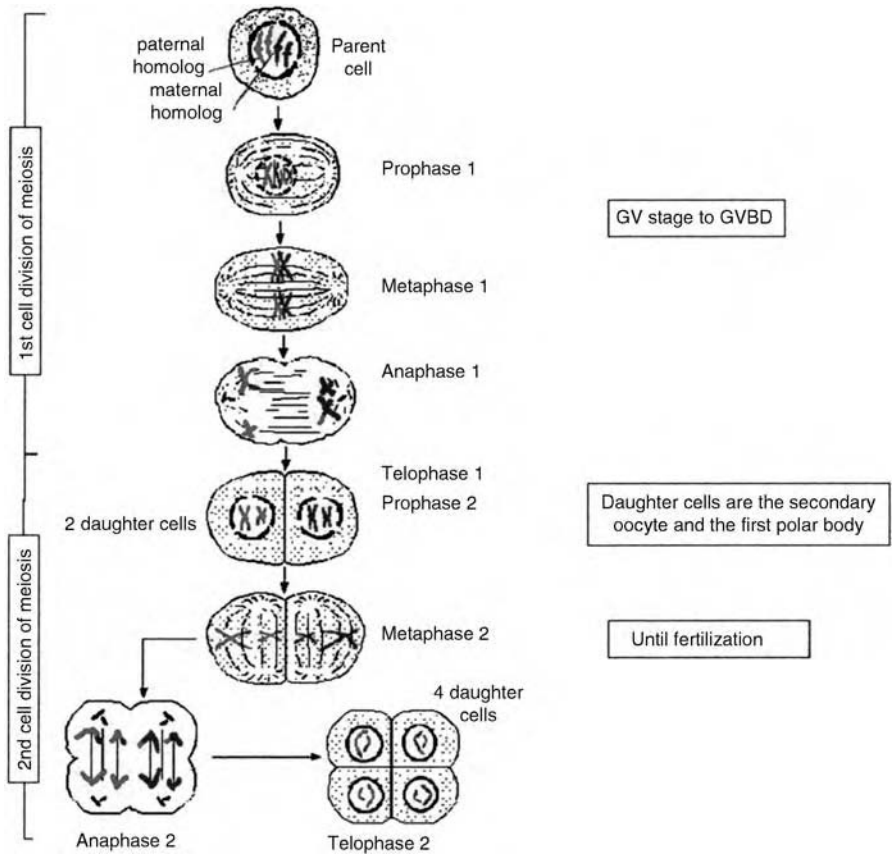

Fig. 14.1 Schematic representation of meiosis and the relevant changes pertaining to oocyte maturation. GV, germinal vesicle; GVBD, germinal vesicle breakdown.

(FSH and primarily LH). The LH surge provides the classic hormonal endocrine trigger for ovulation and luteinization of the follicle, as well as leading to the resumption of oocyte meiosis (Channing *et al.* 1978, Seibel *et al.* 1982). The establishment of pregnancies following IVF of oocytes aspirated from the ovary required identification of the LH surge prior to collection before yielding mature oocytes capable of fertilization (Edwards *et al.* 1980) and the use of human chorionic gonadotropin (hCG) has subsequently been used pharmacologically as a homolog for the LH surge to produce mature oocytes for many forms of assisted reproduction treatment (Steptoe and Edwards 1970). However, there are no LH receptors on the oocyte, therefore nuclear maturation must be mediated either through the granulosa cells and follicular fluid, or via the cumulus cells which are intimately connected with the oocyte through numerous gap junctions.

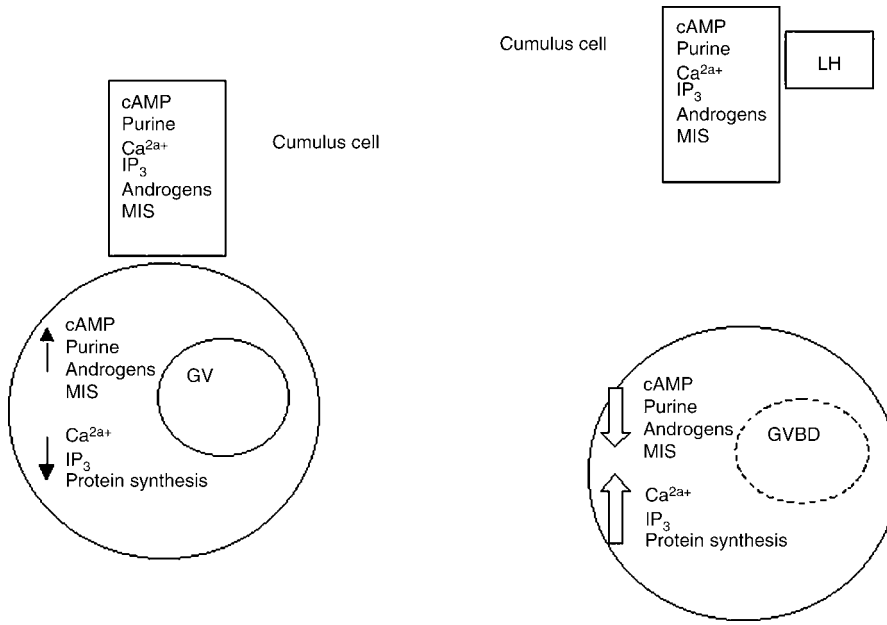

Fig. 14.2 Schematic representation of the hypothetical model for the participation of various factors involved in the initiation of germinal vesicle breakdown (GVBD). Luteinizing hormone (LH) binding to the cumulus cells leads to a loss of the cumulus–oocyte communication and a change in the cytoplasmic oocyte factors. MIS, mullerian inhibiting substance; IP_3, inositol 1,4,5-triphosphate; cAMP, cyclic adenosine monophosphate.

Many factors and regulatory molecules have been shown to either inhibit or promote nuclear and cytoplasmic oocyte maturation (Chian *et al.* 2004a), either directly or via the cumulus cells (Fig. 14.2).

Maturation of oocytes in vitro

Oocytes from unstimulated ovaries

Oocytes from unstimulated ovaries have been obtained from women undergoing tubal surgery, Cesarean section, and oophorectomy as part of an organized oocyte donation program (Cha *et al.* 1991) or as an adjunct to natural cycle IVF (i.e., with unstimulated or minimally stimulated ovaries) (Paulson *et al.* 1994). However, maturation rates, fertilization rates, even with intracytoplasmic sperm injection (ICSI), and ultimately pregnancy rates initially were disappointingly low (Cha and Chian 1999), although improvements in retrieval technique and maturation culture have led to significantly improved clinical pregnancy rates (Chian *et al.* 2004b).

Factors that have been reported to affect the maturational competence of aspirated immature oocytes include the timing of retrieval and the size of the

ovarian follicles. Early evidence suggested that immature oocytes collected in the luteal phase had significantly higher maturation rates (Cha *et al.* 1992). However, it has been found that there is a decreased maturation rate in oocytes from small follicles (3–4 mm) when compared with those from larger follicles (9–15 mm) (Tsuji *et al.* 1985) and similarly there is decreased chance of obtaining oocytes as the follicle becomes mature/dominant in the absence of a spontaneous gonadotropin surge or administered hCG (Templeton *et al.* 1986).

More recent evidence suggests that aspiration in the mid-follicular phase in women with regular menstrual cycles of the antral follicles (8–10 mm) prior to the emergence of the dominant follicle offer the best oocytes for maturation and ultimately pregnancy (Mikkelsen *et al.* 1999).

Initial in vitro culture of immature human oocytes was with standard TCM-199 or Ham's F-10 media supplemented with 10% fetal calf serum and this resulted in maturation rates (to MII) of around 30% (Shea *et al.* 1975). Maturation rates improved to around 60% when this was substituted with human follicular fluid or peritoneal fluid (Cha *et al.* 1991) and currently maturation rates of around 80% are achieved with IVM oocyte medium with human serum albumin (HSA) or IVM oocyte medium without HSA which needs supplemented protein source (usually inactivated maternal serum) as well as FSH and LH (either urinary or recombinant preparations) (Chian *et al.* 1999a, Smith *et al.* 2000). Some authors also routinely use antibiotic supplements in the culture media.

Oocytes from women with PCO or PCOS

Immature oocytes from the increased number of antral follicles found in women with PCO or PCOS retain their maturational and developmental competence (Trounson *et al.* 1994) and pregnancies have been reported (Barnes *et al.* 1995, Chian *et al.* 1999a). Although early studies suggested that the developmental capacity of immature oocytes seemed higher in regularly cycling women compared to women with irregular and anovulatory cycles (Barnes *et al.* 1996), later studies showed comparable maturation and fertilization rates (Suikkari *et al.* 2000, Cavilla *et al.* 2001) and the success of many IVM programs in treating women with PCO and PCOS demonstrates that indeed the reverse may be true (Chian *et al.* 2004a).

Clinical application of in vitro maturation

Development of in vitro maturation as a clinical treatment for infertility

The first successful IVM birth was in Korea. In this case immature oocytes were collected at Cesarean section for oocyte donation (Cha *et al.* 1991). Following this, the first pregnancy in a woman with anovulatory infertility using her own immature oocytes was reported in Australia (Trounson *et al.* 1994) and a further

pregnancy in a woman with PCOS was reported by the same group, using IVM combined with ICSI, assisted hatching, and blastocyst culture (Barnes *et al.* 1995).

As discussed earlier, women with PCO or PCOS undergoing IVF have an increased risk of OHSS from gonadotropins compared to women with normal ovaries (MacDougall *et al.* 1992); these women were an obvious group who might benefit from IVM and thereby avoid ovarian stimulation.

Initial studies indicated that although immature oocytes recovered from unstimulated patients with PCO or PCOS can be matured, fertilized, and developed in vitro, the implantation rate of these cleaved embryos was disappointingly low (Trounson *et al.* 1998, Cha *et al.* 2000). However, recent data indicate that IVM using hCG priming before immature oocyte retrieval improves clinical pregnancy and implantation rates (Chian *et al.* 2004b). Further studies and current data show that pregnancy rates of 30–40% are achieved in women with the higher numbers of antral follicles associated with PCO or PCOS (Tan *et al.* 2002).

Pre-retrieval priming with human chorionic goandotropin

Although germinal vesicle stage oocytes have been retrieved from the standard stimulated IVF cycles 36 h after hCG administration and successful pregnancies have been established using such in vitro matured oocytes (Veeck *et al.* 1983, Nagy *et al.* 1996, Edirisinghe *et al.* 1997, Check *et al.* 2001) the pregnancy rates are low.

However, in women with PCOS undergoing IVM without ovarian stimulation, it has been demonstrated that the time course of oocyte maturation in vitro is hastened and the rate of oocyte maturation is increased by priming with 10 000 IU hCG 36 h before retrieval of immature oocytes from women with PCO or PCOS (Chian *et al.* 2000).

Initial reports also suggested that pregnancy rates may be improved by priming with hCG prior to immature oocyte retrieval (Chian *et al.* 1999b) and this has been confirmed by subsequent reports (Nagele *et al.* 2002, Son *et al.* 2002, Buckett *et al.* 2003; Lin *et al.* 2003).

Based on more than 1000 IVM cycles in a multicenter study with hCG priming before immature oocyte retrieval from women with PCO or PCOS, the pregnancy and implantation rates reached 30–35% and 10–15% respectively (Chian *et al.* 2004b). Interestingly, recent findings show that in women with PCO or PCOS, the time course and maturation rates are different when germinal vesicle stage oocytes were divided into different groups based on the morphology of cumulus cells after hCG priming (Yang *et al.* 2001). Therefore, it seems that hCG priming both promotes some oocytes to undergo maturation to the metaphase I stage, when derived from the relatively larger follicles (>10 mm in diameter), and also enhances some germinal vesicle stage oocytes from small follicles to acquire maturational and developmental competence in vivo.

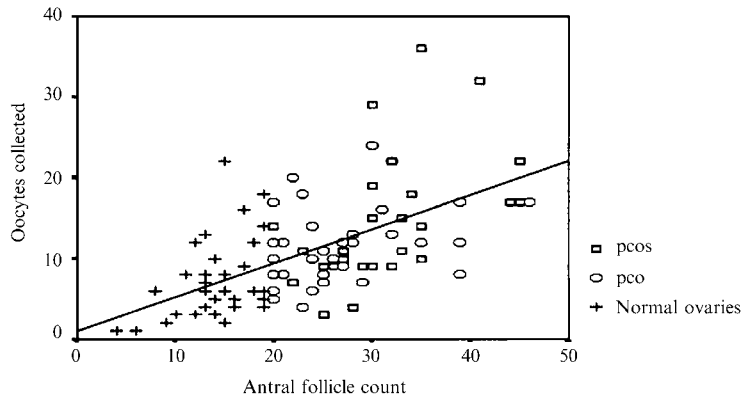

Fig. 14.3 Number of immature oocytes collected and early follicular phase antral follicle count. After Tan *et al.* (2002).

Priming with follicle stimulating hormone

As an alternative approach, a truncated course of ovarian stimulation with FSH before immature oocyte retrieval has been applied, suggesting that FSH pre-treatment promotes efficient recovery of immature oocytes and maturation in vitro (Wynn *et al.* 1998). It has also been reported that priming with recombinant FSH during the follicular phase before harvesting of immature oocytes from patients with PCOS improves the maturational potential of the oocytes leading to increased implantation and pregnancy rates (21% and 29% respectively) (Mikkelsen and Lindenberg 2001).

However, since the main benefit of IVM in women with PCOS would be the avoidance of any ovarian stimulation, it would be preferable to avoid FSH completely.

Indications for in vitro maturation

As detailed above, both IVM protocols currently used report the highest pregnancy rates in women with PCO or PCOS. Therefore women with PCOS, ultrasound-only PCO, or a combined antral follicle count of greater than 20 who need IVF are the best candidates for IVM. The antral follicle count is the best clinical predictor of the number of immature oocytes that may be retrieved and the best predictor of clinical pregnancy (Tan *et al.* 2002) (Fig. 14.3). These women are also at increased risk of OHSS and therefore would benefit most from avoiding ovarian stimulation, as well as having the highest success rates with IVM.

Other indications for IVM in women with PCOS include women undergoing ovarian stimulation for conventional IVF, who develop an over-response and would otherwise be canceled because of the risk of developing OHSS. Initially, one live

birth was reported from immature oocytes that were collected from a patient at risk of developing OHSS (Jaroudi *et al.* 1997). More recently, eight (out of 17 cycles) clinical pregnancies were achieved from patients with potential risk of developing OHSS followed by immature oocyte retrieval and IVM (Lim *et al.* 2002).

Procedure of in vitro maturation

Cycle initiation

In women with oligo- or amenorrhea the treatment cycle is initiated by the administration of oral, intravaginal, or intramuscular progesterone and the timing of the start of treatment can be planned. Withdrawal bleeding usually occurs within 3 days after the last dose. On day 2–4 following the onset of menstrual bleeding, the women undergo a baseline ultrasound scan to ensure that there are no ovarian cysts.

Transvaginal ultrasound scans should be repeated on day 7–9 to plan the immature oocyte retrieval and confirm the absence of a dominant follicle. In women with anovulatory cycles the retrieval can be performed on day 10–14 of the cycle. Prior to immature oocyte retrieval, women are primed with 10 000 IU hCG subcutaneously 36 h before oocyte retrieval.

Immature oocyte retrieval

Transvaginal ultrasound-guided oocyte collection is performed using a specially designed 19G single-lumen aspiration needle (K-OPS-7035-RWH-B-ET, Cook, Australia) with an aspiration pressure of 7.5 kPa. Aspiration of all small follicles is performed under intravenous sedation with paracervical block or under spinal anesthesia. A multiple puncture technique is used. Oocytes are collected in 10-ml culture tubes containing 2 ml warm 0.9% saline with 2 IU/ml heparin. The collection needle should be flushed regularly to prevent blockage with debris or blood clot. All aspirates are examined under the microscope for cumulus–oocyte complexes (COCs). Prior to discarding the fluid, it should be poured through a filter (Cell Stainer 352350, Falcon, USA) then rinsed with IVM washing medium and re-examined under the microscope for further COCs.

In vitro oocyte maturation

Following oocyte collection, the oocytes are evaluated for the presence or absence of a GV in the cytoplasm of the oocyte (Fig. 14.4), and the immature oocytes are then transferred into the maturation medium for culture. If no GV is seen in an immature oocyte, the oocyte is defined as germinal vesicle breakdown (GVBD) (Fig. 14.5). The mature (MII) oocytes are determined by the presence of a first polar body (Fig. 14.6). All oocyte handling procedures are conducted on warm stages and plates at 37 °C. Cumulus–oocyte complexes are rinsed in IVM washing

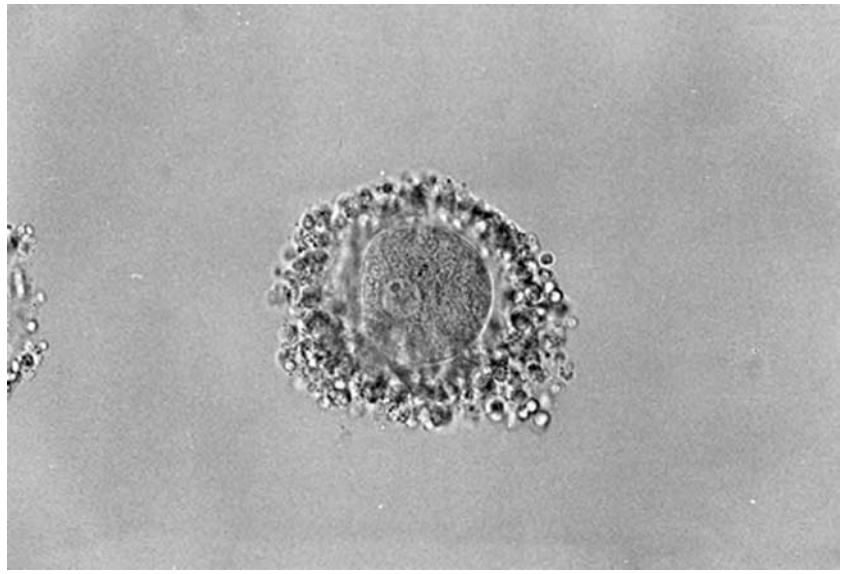

Fig. 14.4 A germinal vesicle (GV) stage immature oocyte. The nucleolar germinal vesicle can be clearly seen in the immature oocyte and the cumulus is compacted.

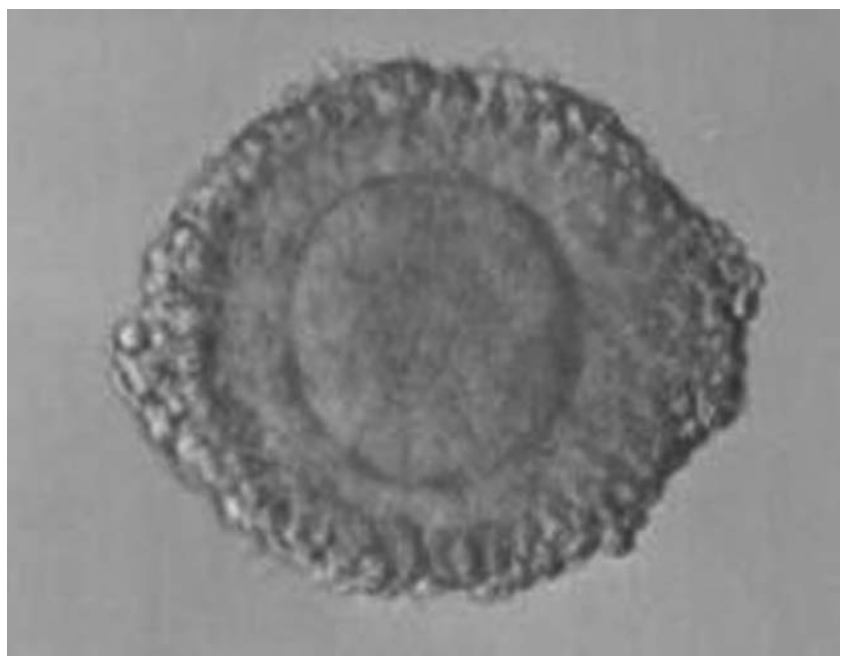

Fig. 14.5 A germinal vesicle breakdown (GVBD) stage immature oocyte. The germinal vesicle is no longer visible, although the cumulus is still compacted and there is no polar body.

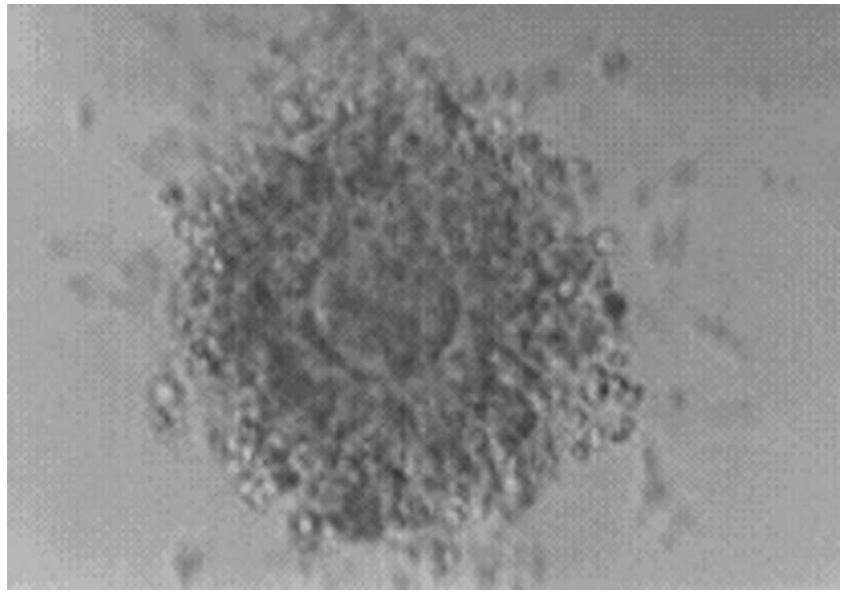

Fig. 14.6 A mature metaphase II (MII) oocyte. The cumulus is expanded and there is extrusion of the first polar body.

medium. Immature oocytes are then incubated in an organ tissue culture dish (60 × 15 mm; Falcon) containing 1 ml of IVM oocyte medium supplemented with 75 mIU/ml FSH and 75 mIU/ml LH at 37 °C in an atmosphere of 5% CO_2 and 95% air with high humidity. Following culture, the maturity of the oocytes is determined under the microscope at 12-h intervals for up to 48 h following the retrieval.

Fertilization

Oocytes that are mature at the time of checking are then denuded of cumulus cells using finely drawn glass pipettes following 1min exposure to 0.1% hyaluronidase solution ready for ICSI. Spermatozoa for ICSI is prepared by mini-Percoll separation (45% and 90% gradients) at 560 *g* for 20 min. Following Percoll separation, the sperm pellet is washed twice (200 *g*) with 2 ml of Medi-Cult IVF medium. A single spermatozoon is then injected into each metaphase II oocyte. Following ICSI, each oocyte is transferred into 20 fl droplet of Medi-Cult IVF medium in a tissue culture dish (35 × 10 mm; Falcon) under mineral oil. Fertilization is assessed 18 h after ICSI for the appearance of two distinct pronuclei and two polar bodies.

Embryo transfer

Following the fertilization, embryos with two pronuclei are transferred into 1.0 ml of Medi-Cult IVF medium in the organ tissue culture dish (60 × 15 mm;

Falcon) for further culture. Because implantation rates are lower with IVM, compared with ovarian stimulation and conventional IVF, we recommend transferring one or two more embryos at IVM than would be transferred for conventional IVF in the same age group. In our experience, this leads to the same rate of multiple pregnancy for IVM cycles as for IVF cycles. Since oocytes are not necessarily mature and inseminated at the same time following maturation in culture, the developmental stages of embryos may be variable both within and between patients. Before transfer, all embryos for each patient are pooled and selected for transfer. Embryo transfer technique is the same as that employed for conventional IVF.

Endometrial preparation and luteal support

For the preparation of the endometrium, women are given estradiol valerate at a dosage depending on the endometrial thickness on the day of oocyte retrieval in divided doses, starting on the day of oocyte retrieval. If the endometrial thickness on the day of oocyte retrieval is <4 mm, a 10–12-mg dose is administered daily; if it is between 4 and 6mm a dose of 8 mg is given, and if it is >6 mm, a 6-mg dose is given.

Luteal support is started on the day that MII maturation is achieved and ICSI is performed. Previously this was provided by 200mg intravaginal progesterone three times daily for 16 days, although 50 mg intramuscular progesterone is currently given.

On the day of embryo transfer, the endometrial thickness is measured again by transvaginal ultrasound scan. If the endometrial thickness is <8 mm, the couple are offered embryo cryopreservation and transfer in a subsequent cycle.

Disadvantages of in vitro maturation

Although the clinical application of IVM in women with PCOS is an exciting development in the field of assisted reproductive technologies, there have been several concerns regarding potential disadvantages associated with IVM.

Increased cost

In vitro maturation represents a significant reduction in the cost of IVF to the couple seeking treatment by virtue of avoiding the costs of ovarian stimulation; these can be significant, particularly when the gonadotropin releasing hormone (GnRH) analog long protocols are used either with or without recombinant gonadotropins typically ranging from CAD$2000 to CAD$2500 per cycle started.

However, there are increased laboratory costs. These include the direct and indirect costs of oocyte maturation, ICSI, and often assisted hatching. As the

immature oocytes need to be checked every 12 h, this also increases laboratory personnel costs.

Finally, there is a need for training, both for clinicians to learn the immature oocyte retrieval, which may be technically more demanding than conventional IVF because the follicles are smaller and the ovaries are often more mobile, as well as for embryologists to learn the maturation techniques.

Lower success rates

At present, many North American centers report clinical pregnancy rates in excess of 40% per oocyte retrieval for conventional IVF (Society for Assisted Reproductive Technology 2004). Although, some IVM programs reports success rates approaching this (Chian *et al.* 2004b), many do not. The only study that has tried to compare IVM with conventional IVF was a case-matched controlled study of women with PCO or PCOS. This showed clinical pregnancy rates of 38% with conventional IVF and 26% with IVM (Child *et al.* 2002), although obviously there were no cases of OHSS in the IVM group.

All centers that perform conventional IVF as well as IVM report higher clinical pregnancy and implantation rates with conventional IVF, although IVM has a much higher clinical pregnancy rate per cycle compared with natural cycle IVF (Nargund *et al.* 2001). The reduced costs, monitoring, and risks associated with IVM still make this treatment, in appropriate patients, an attractive alternative to conventional IVF with ovarian stimulation and its attendant risks.

Chromosomal abnormalities

Some authors have concern regarding the risk of karyotypic abnormalities in in vitro matured oocytes, particularly as there may be higher rates of karyotypic abnormalities amongst immature oocytes (Hardy *et al.* 2000). More recent evidence, however, suggests that there is no increase in the karyotypic abnormality rates in successfully matured oocytes, although these data are still preliminary (Picton *et al.* 2002).

Lack of outcome data

The successful establishment of effective IVM programs for the treatment of infertility is still relatively new. Therefore the number of babies born worldwide is still relatively small. The two studies so far have shown no increase in the obstetric, perinatal, and neonatal outcomes in women who conceived following IVM (Mikkelsen *et al.* 2003, Buckett *et al.* 2004).

Nevertheless, caution and appropriate counseling is needed regarding the unknown long-term effects of IVM on any children conceived as a result of treatment and continued follow-up is advised.

Conclusions

In vitro maturation has been shown to be an effective treatment for infertility in women with PCO or PCOS who have not conceived following ovulation induction or who need IVF for other indications. It avoids the costs, monitoring, and risks associated with ovarian stimulation, particularly OHSS. This makes it an attractive treatment option not only for many women undergoing treatment themselves, but also for women wishing to donate oocytes.

Although IVM is a relatively new treatment, early evidence from in vitro studies and from pregnancy outcome studies suggest that there is unlikely to be significant risk to children conceived with this treatment.

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15

The pediatric origins of polycystic ovary syndrome

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Introduction

The polycystic ovary syndrome (PCOS) is a common disorder characterized by chronic anovulation and hyperandrogenism, and frequently associated with insulin resistance and type 2 diabetes mellitus. Signs and symptoms of PCOS usually develop during, or soon after, puberty (Ehrmann *et al.* 1995, Apter 1998), and several features of the PCOS can be recognized in girls with premature pubarche and adolescents with hyperandrogenism (Ibañez *et al.* 1993).

In the last decade, the concept of PCOS has evolved from being mostly a reproductive disorder to a metabolic disorder. Nowadays, PCOS is described as a heterogeneous disorder presenting with a constellation of different manifestations throughout the life of women (Ibañez *et al.* 1998c). The familial clustering of women with PCOS suggests that heredity is involved in the pathogenesis of the syndrome, but the underlying mechanisms are unknown.

Polycystic ovary syndrome is the first component of the metabolic syndrome to be detected in many women (Dunaif 1997). The early identification of the syndrome will have important implications in the prevention and treatment of affected women, especially since progression of the full-blown syndrome might be ameliorated and prevent from the lifelong sequelae of PCOS. This chapter reviews the origin of PCOS during childhood and adolescence.

Physiology of puberty in the female

Puberty is the transition period from the sexually immature state to becoming capable of reproducing, which is marked by the appearance of secondary sexual characteristics and acceleration in growth. Two physiological processes, gonadarche and adrenarche, drive the transition to puberty.

Polycystic Ovary Syndrome, 2nd edn, ed. Gabor T. Kovacs and Robert Norman. Published by Cambridge University Press.

Several features of puberty resemble those of PCOS, including menstrual irregularities, hyperandrogenism, and insulin resistance (Nobels and Dewailly 1992). Many of the endocrine changes that occur during puberty are also observed, yet exaggerated, in PCOS. First, the secretion of luteinizing hormone (LH) in response to gonadotropin releasing hormone (GnRH) is increased. Second, secretion of androgens increases in the adrenal glands during adrenarche, and later in the ovaries due to an increase of the activity of 17α-hydroxylase and 17,20-lyase. Third, puberty is associated with a relative insulin resistance and increased insulin levels, together with the activation of the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis and a decrease in sex hormone binding globulin (SHBG) and IGF-binding protein-1 (IGFBP-1) (Amiel *et al.* 1986, Bloch *et al.* 1987, Hindmarsh *et al.* 1988, Holly *et al.* 1989, Argente *et al.* 1993).

Physiology of the gonadal axis from birth to normal puberty

Gonadarche consists of the growth and maturation of the gonads and is associated with increased secretion of sex steroids. Gonadarche results from the resurgence of activity in the hypothalamic–pituitary axis, which has been relatively quiescent since early childhood. Pubertal changes in girls involve thelarche and maturation of breast, and menarche and the establishment of regular menstrual cycles. The age of onset and the tempo of the progression of puberty vary considerably within adolescents.

During fetal and perinatal life, the hypothalamic secretion of GnRH is insensitive to the negative feedback of sex hormones, and the pituitary–gonadal axis functions at a pubertal level, with elevated levels of LH, follicle stimulating hormone (FSH), and estrogens. During childhood, the hypothalamic–pituitary–gonadal system is sensitive to the negative feedback of sex steroids and is downregulated, maintaining a minimal GnRH secretion. Both LH and FSH levels are low at this time, with FSH levels being relatively higher than LH levels. In late prepuberty, the gonadostat begins to relinquish its inhibition and episodic secretion of GnRH and an intermittent secretion pattern of gonadotropins occurs. The onset of puberty in girls is characterized by an increase in LH mean concentrations and pulse amplitude or frequency (Penny *et al.* 1977, Apter *et al.* 1993), which is followed by a fall after the onset of ovulatory cycles in adolescent girls (Venturoli *et al.* 1992).

Adrenarche

Adrenarche is the onset of increased adrenal androgen production that precedes the pubertal rise of gonadotropins and gonadarche. Androgens are responsible for changes such as the development of sexual hair, acne, and apocrine glands.

The adrenal androgens, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), are mainly produced by the zona reticularis. The zona reticularis is

thought to be morphologically equivalent to the fetal zone of the adrenal cortex that disappears after the first few months of life, and its development correlates with a low expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) (Hornsby 1997). During adrenarche, the activity of 17-hydroxylase, 17,20-lyase, sulfokinase, and sulfatase are increased, and the levels of 17-OH pregnenolone, DHEA, and DHEAS increase strikingly. No changes in pituitary adrenocorticotropin (ACTH) secretion or in other adrenal stimulatory factors have been identified to be responsible for the enzyme shifts observed during adrenarche.

Insulin resistance during puberty

Pubertal development is associated with an approximate 25–30% reduction in insulin sensitivity and a compensatory rise in circulating insulin levels (Caprio *et al.* 1989, Amiel *et al.* 1991). The peak fasting insulin levels are reached at mid-puberty, regardless of chronological age, and is followed by a recovery to pre-pubertal levels again when Tanner stage V is reached. The insulin resistance is selective for glucose metabolism and occurs both in lean and obese children, being restricted to peripheral tissues. The levels of GH increase in parallel to the fall in insulin sensitivity during puberty, possibly contributing to insulin resistance (Amiel *et al.* 1986). Sex hormones are not thought to be responsible for the changes in insulin action and secretion during puberty because sex steroids remain high after puberty, whereas insulin sensitivity is restored to prepubertal levels.

The IGF-1 axis in children and during puberty

Insulin-like growth factor-1 mediates the growth-stimulating effects of GH and circulates in serum bound to IGF-binding proteins (IGFBPs). Serum IGF-1 levels increase slowly during childhood, exhibiting a sharp increase during puberty and a progressive decline during adulthood (Argente *et al.* 1993). Serum IGFBP-3 levels show a pattern similar to that of IGF-1. In contrast, serum IGFBP-1 levels decrease during childhood, attain a nadir during puberty, and slightly increase towards adulthood, being inversely related to the secretory pattern of insulin (Holly *et al.* 1988). The hyperinsulinemia of puberty may promote growth through direct anabolic effects, but also through suppression of liver IGFBP-1 production, thereby increasing the bioavailability of unbound IGF-1 to target tissues.

Hyperandrogenism in children and adolescents

Premature pubarche

Premature pubarche precedes the development of PCOS in some patients and, to date, premature pubarche can be considered as the earliest PCOS-like phenotype

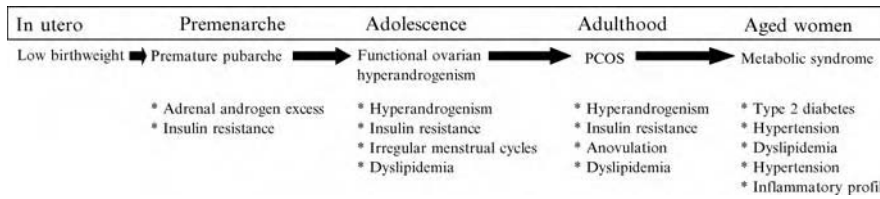

Fig. 15.1 Manifestations of hyperandrogenism and the polycystic ovary syndrome at different ages: premature pubarche and insulin resistance as forerunners of PCOS and the metabolic syndrome.

that may be recognized (Ibañez *et al.* 1993, 1997b, Vuguin *et al.* 1999, Kent and Legro 2002, Silfen *et al.* 2002).

Premature pubarche is defined as the appearance of pubic hair, axillary hair, and/or body odor prior to age 8 years in girls and age 9 years in boys. Premature pubarche often results from premature adrenarche – a premature increase in adrenal androgen production (Ibañez *et al.* 2000a). Typically, the adrenal androgen concentrations and, especially, the levels of DHEAS are increased for chronological age, but fall within the reference range for the stage of pubic hair development. Because the enzymes 17-hydroxylase and 17,20-lyase are encoded by a single gene (*CYP17*), a posttranslational abnormality in this gene was proposed to play a role in the pathogenesis of premature pubarche (Zhang *et al.* 1995).

Premature pubarche is not usually associated with other signs of sexual development (Ibañez *et al.* 1992). Some children present with a transient growth spurt and an advanced skeletal maturation, which have no impact on the onset of puberty or on the final height attained. Menarche occurs earlier than usual for normal girls of the same ethnic background, but within the normal range for the general population (Ibañez *et al.* 1992, Miller *et al.* 1996). Of note, overweight and weight gain may trigger adrenarche regardless of basal adrenal androgen secretion and chronological age (Pang 1984, Rosenbaum and Leibel 1989, Remer and Marz 1999).

Several studies suggest that premature pubarche may be considered as a forerunner of adult disease (Ibañez *et al.* 2000a) (Fig. 15.1). Peripubertal and postpubertal girls diagnosed with premature pubarche during childhood present an increased incidence of hirsutism (Yen 1986). After gonadarche, as many as 45% of girls with a history of premature pubarche develop functional hyperandrogenism (Ibañez *et al.* 1993). Some endocrine–metabolic abnormalities typical of PCOS, such as hyperinsulinemia, insulin resistance, and dyslipidemia are already apparent in girls with premature pubarche (Apter *et al.* 1995, Miller *et al.* 1996, Ibañez *et al.* 1997a), and this subset of girls with metabolic alterations are apparently those at risk to develop PCOS later in life.

Hyperandrogenism and polycystic ovary syndrome

Polycystic ovary syndrome is a lifelong process resembling in adults the physiological oligo-ovulation of adolescence including increased LH and androgen levels, irregular menstrual cycles, a similar ovarian morphology, and insulin resistance with increased insulin levels (Nobels and Dewailly 1992).

Polycystic ovary syndrome is one of the most common endocrinopathies in premenopausal women (Carmina and Lobo 1999), and is characterized by hyperandrogenism and chronic oligo-ovulation. The symptoms, signs, and biochemical features of PCOS are heterogeneous, and for a particular individual may change over time. Common clinical features include hirsutism, acne, and menstrual irregularity, and PCOS is often associated with hyperinsulinemia, insulin resistance, and dyslipidemia (Zawadski and Dunaif 1992). Acanthosis nigricans may appear as a sign of hyperinsulinemia and insulin resistance, especially in obese girls and women (Oppenheimer *et al.* 1995). With aging, PCOS increases the risk for diabetes and cardiovascular disease (Ehrmann *et al.* 1999, Legro 2003).

Although no particular criteria for the diagnosis of PCOS have been universally accepted, those most widely used for the past decade were derived from a conference sponsored in 1990 by the National Institutes of Health–National Institute of Child Health and Human Development (NIH–NICHD) (Zawadski and Dunaif 1992). These criteria included: (1) menstrual dysfunction, (2) clinical hyperandrogenism (hirsutism, acne, androgenic alopecia), and/or biochemical evidence of hyperandrogenism, and (3) exclusion of other disorders, such as non-classic adrenal hyperplasia, hyperprolactinemia, thyroid or Cushing's disease, or androgen secreting tumors. Insulin resistance was not considered a major criterion. However, in many Commonwealth countries and others from northern Europe, the definition of PCOS requires the presence of polycystic ovaries on ultrasonography (Homburg 2002).

The estimated prevalence of PCOS in women of reproductive age is 5–10% (Franks 1995). Using NIH–NICHD criteria, reports of the prevalence of PCOS in a reproductive-aged population are consistently in the 6% to 7% range in Northern American and Mediterranean populations (Knochenhauer *et al.* 1998, Asunción *et al.* 2000, Diamanti-Kandarakis *et al.* 1999). The actual prevalence of PCOS in adolescents is unknown, but previous studies estimate that PCOS has the same overall incidence in adolescent and adult women (Goldzieher 1973).

The identification of the onset of PCOS in prepubertal girls is hampered by the fact that ovulatory function is not established in these girls, and therefore is not a valid criterion for the diagnosis of hyperandrogenism. Women with PCOS may present with irregular menses from menarche, suggesting oligo-ovulation, and hirsutism appears soon after puberty (Dunaif 1997). Cycle irregularity and anovulation are common during the first years after menarche, but ovulatory

cycles tend to occur within 3–5 years (Metcalf *et al.* 1983). Some hyperandrogenic adolescents with oligomenorrhea will later have regular ovulatory cycles (Venturoli *et al.* 1994), but menstrual irregularities during the postmenarcheal years may be an early clinical sign of PCOS in many girls (Avvad *et al.* 2001). Furthermore, the persistence of menstrual irregularity 2 years after menarche is associated with a 50% risk for future ovulatory dysfunction (Southam and Richart 1996).

Adolescent girls with a history of premature pubarche are at increased risk to develop anovulation several years after menarche (Ibañez *et al.* 1999b). The prevalence of polycystic ovaries in adolescents has been correlated with the irregularity of the menstrual cycle pattern (Van Hooff *et al.* 2001). Girls with regular menstrual cycles show a prevalence of polycystic ovaries of 9%, rising to 28% when the cycles are irregular, and to 45% in the presence of oligomenorrhea. The persistence of anovulatory cycles in adolescents has been associated with elevated levels of LH and androgens (Venturoli *et al.* 1992).

Ehrmann *et al.* (1995) suggested that functional ovarian hyperandrogenism, defined by an exaggerated ovarian and LH response to the GnRH analog nafarelin, is one of the major mechanisms underlying PCOS (Barnes *et al.* 1989, Ehrmann *et al.* 1992). However, this response probably reflects an increased tonic LH stimulation, and should be considered to be a diagnostic test, not a pathogenic hypothesis. As many as 50% of women with functional ovarian hyperandrogenism have also evidence of functional adrenal hyperandrogenism determined by an ACTH stimulation test (Ehrmann *et al.* 1992). An abnormal regulation of CYP17 in the ovary and adrenal glands seems to be responsible for the excessive production of androgens (Ehrmann *et al.* 1995). Adolescent girls with a history of premature pubarche also present an exaggerated ovarian response to GnRH analogs throughout puberty (Ibañez *et al.* 1997b).

Insulin resistance is prevalent in women with PCOS (Ehrmann *et al.* 1995), and although not present universally, insulin resistance appears to play a central role in the pathogenesis of PCOS in many patients (Dunaif 1997). Hyperinsulinism and insulin resistance have been reported in obese and lean adult women with PCOS and hyperandrogenic adolescents (Chang *et al.* 1983, Dunaif *et al.* 1987, Ibañez *et al.* 1995). When insulin resistance occurs, the beta cell of the pancreas secrete greater amounts of insulin to prevent hyperglycemia, leading to compensatory hyperinsulinemia until beta cell compensation fails and type 2 diabetes ensues.

Insulin stimulates ovarian and adrenal steroidogenic enzymes. In vitro studies demonstrate that insulin stimulates LH release by the pituitary gland (Adashi *et al.* 1981), and increases ovarian LH receptors (Adashi *et al.* 1985), facilitating

androgen synthesis at the ovary. Insulin and insulin-like growth factors, together with other peptides, increase the androgenic response of theca cells and adrenal cells to LH and ACTH respectively, increasing CYP17 activity and steroidogenesis (Penhoat *et al.* 1989). Insulin also decreases hepatic production of SHBG (Nestler *et al.* 1991) and of IGFBP-1 (Nobels and Dewailly 1992), thus increasing the availability of testosterone and IGF-1 to target tissues.

Women with PCOS have an increased risk for developing glucose intolerance and type 2 diabetes. The prevalence of impaired glucose tolerance in PCOS patients is 31–35%, and that of type 2 diabetes is 7.5–10% (Ehrmann *et al.* 1999). Obesity exacerbates the progression to type 2 diabetes by exacerbating insulin resistance (Dunaif *et al.* 1987). The conversion rate from impaired glucose tolerance to type 2 diabetes in women with PCOS is increased five- to tenfold compared to non-hyperandrogenic subjects, and 30–50% of obese women with PCOS will develop impaired glucose tolerance or type 2 diabetes by the age of 30 years (Ehrmann *et al.* 1999). Insulin resistance or hyperinsulinemia are often present in the early stages of PCOS (Lewy *et al.* 2001), and adolescent girls with PCOS are at increased risk for impaired glucose tolerance and type 2 diabetes (Palmert *et al.* 2002). The impaired insulin action and secretion may be influenced by genetic and/or environmental factors.

Pediatric origins of polycystic ovary syndrome

The Barker hypothesis

Although a complete description and full discussion of this hypothesis will be provided in the next chapter of the book, we will summarize some aspects which are essential for the understanding of the pediatric aspects of female hyperandrogenism.

The Barker hypothesis proposes that intrauterine malnutrition predisposes to insulin resistance, imprinting future metabolic derangements in affected fetuses (Hales and Barker 1992, Barker *et al.* 1993). Intrauterine growth retardation alters the development of adipose tissue during fetal life (Lapillonne *et al.* 1997). In conceptual agreement, low birthweight increases the risk of type 2 diabetes, dyslipidemia, and hypertension in adults (Barker and Clark 1997, Phillips and Barker 1997). Low birthweight and rapid childhood weight gain also predict abnormalities of glucose tolerance (Crowther *et al.* 1998).

Ibañez *et al.* (1998c) suggested a prenatal origin of hyperandrogenism because they observed that intrauterine growth retardation was frequently associated with the development, later in life, of premature pubarche and hyperinsulinism in girls, and functional ovarian hyperandrogenism and disorders of glucose tolerance in adult women.

However, the hypothesis of a common in utero programming of hyperandrogenism and hyperinsulinism has not been confirmed by others (Jaquet *et al.* 1999, Laitinen *et al.* 2003). In fact, the original description of an influence of prenatal imprinting and the subsequent development of PCOS by Barker and associates (Cresswell *et al.* 1997) reported that obese women with polycystic ovaries and increased concentrations of plasma LH and testosterone were actually born with an above-average birthweight, whereas a second subgroup of lean women with polycystic ovaries and increased plasma LH but normal testosterone concentrations had normal birthweight but were born after term. Barker and colleagues (Cresswell *et al.* 1997) concluded that the two forms of PCOS had different origins in intrauterine life, with thin women presenting an altered hypothalamic control of LH release resulting from prolonged gestations.

Premature pubarche and insulin resistance as forerunner of PCOS

The diagnosis of PCOS in prepubertal girls, based on the NIH–NICHD criteria (Zawadski and Dunaif 1992), is not possible because menstrual cycles are not established. A PCOS-like phenotype is observed in some girls with premature pubarche who present with several features of PCOS before menarche (Ibañez *et al.* 1993, 1997b, Vuguin *et al.* 1999, Kent and Legro 2002, Silfen *et al.* 2002) and premature pubarche may be considered an early presentation of PCOS in these patients (Ibañez *et al.* 2000a). Girls with premature pubarche have insulin resistance, dyslipidemia, and elevated androgens (Oppenheimer *et al.* 1995, Ibañez *et al.* 1998b, DiMartino-Nardi 1999, Vuguin *et al.* 1999, Silfen *et al.* 2002).

Postpubertal outcome of girls diagnosed of premature pubarche during childhood shows an increased incidence of functional ovarian hyperandrogenism at adolescence (45% vs. 3–6% in normal population) (Ibañez *et al.* 1993), hyperinsulinism, and insulin resistance (Ibañez *et al.* 1997a).

Ibañez *et al.* (1997b) assessed the ovarian hyper-response of 17-hydroxyprogesterone to the GnRH analog leuprolide in adolescents who had had premature pubarche. Pubertal girls with premature pubarche had an exaggerated ovarian androgen synthesis compared with Tanner stage and bone age matched controls (Ibañez *et al.* 1997b). The baseline levels of DHEAS and Δ^4-androstenedione at diagnosis of premature pubarche correlated with 17-hydroxyprogesterone levels after GnRH analog stimulation during puberty, suggesting that functional ovarian hyperandrogenism is more common in girls with pronounced premature pubarche (Ibañez *et al.* 1993). Both peak and incremental increases in response to GnRH analog stimulation in serum 17-hydroxypregnenolone and DHEA levels throughout puberty, and of 17-hydroxyprogesterone and Δ^4-androstenedione levels in late puberty, were significantly higher in premature pubarche girls compared with non-hyperandrogenic girls (Ibañez *et al.* 1997b).

Moroever, the adrenal responses to ACTH stimulation in postpubertal girls with a history of premature pubarche also showed an increased secretion of DHEA, 17-hydroxypregnenolone, and Δ^4-androstenedione (Ibañez *et al.* 1994). This pattern of responses to GnRH analog and ACTH stimulation suggested an abnormal activity of ovarian and adrenal 17α-hydroxylase and 17,20-lyase functions, and supports a pubertal onset of ovarian and adrenal hyperandrogenism.

In addition to primary tendency towards exaggerated androgen secretion by the ovary and the adrenals, other genetic and environmental factors are probably needed for these adolescents to develop PCOS. The metabolic syndrome and the endocrine characteristics of PCOS appear early in adolescent girls with hyperandrogenism, and adolescents in whom hyperinsulinemia persists after puberty are at a higher risk to develop PCOS (Ibañez *et al.* 1998a).

There are very few data regarding the prevalence and mechanisms responsible for insulin resistance in adolescents with PCOS. In a study by Palmert and co-workers (Palmert *et al.* 2002), 27 adolescents with PCOS underwent an oral glucose tolerance test. Eight of them had impaired glucose tolerance, and another patient had type 2 diabetes, reaching an overall prevalence of disorders in glucose tolerance close to the 40% figure reported in adults with PCOS (Ehrmann *et al.* 1999, Legro *et al.* 1999). Compared with a control group, adolescents with PCOS have higher fasting and stimulated insulin responses to intravenous glucose tolerance test, but not higher plasma glucose levels (Apter *et al.* 1995, Mauras *et al.* 1998), suggesting adequate compensatory beta cell function.

Obese adolescent girls with PCOS present approximately a 50% reduction in peripheral tissue insulin sensitivity, hepatic insulin resistance, and compensatory hyperinsulinemia when compared with obese non-hyperandrogenic girls, and these abnormalities appear early in the course of the syndrome (Lewy *et al.* 2001). In another study by Arslanian *et al.* (2001) obese PCOS adolescents with or without impaired glucose tolerance were compared using the 2-h hyperglycemic clamp to assess first- and second-phase insulin secretions, and the 3-h hyperinsulinemic–euglycemic clamp to assess hepatic glucose production and insulin stimulated glucose disposal. In these obese adolescents with PCOS, glucose intolerance was associated with decreased first-phase insulin secretion, decreased glucose disposition index, and increased hepatic glucose production (Arslanian *et al.* 2001) (Fig. 15.2).

Initially the beta cell function was able to compensate for the insulin resistance by increasing insulin secretion, precluding the development of impaired glucose tolerance (Arslanian *et al.* 2001). The short duration of PCOS and insulin resistance in these adolescents with PCOS might explain this difference between adolescents and adults with PCOS, the latter frequently developing impaired glucose tolerance (Dunaif 1997, Ehrmann *et al.* 1999).

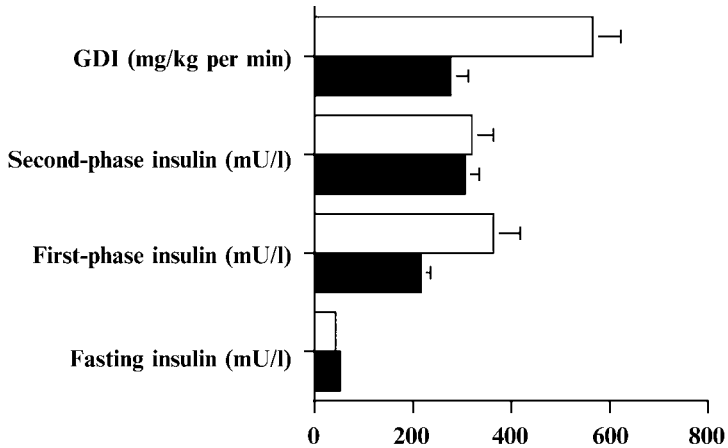

Fig. 15.2 Fasting insulin, first- and second-phase insulin secretion, and glucose disposition index (GDI) during a hyperinsulinemic–euglycemic clamp, in obese PCOS adolescents with or without glucose intolerance. White bars indicate girls with normal glucose tolerance, and black bars indicate girls with impaired glucose tolerance. Adapted from Arslanian *et al.* (2001).

However, in these girls beta cell exhaustion might also occur later in life leading to impaired glucose tolerance and type 2 diabetes. The finding of severe insulin resistance in adolescents with PCOS points to an increased risk for type 2 diabetes in these girls, strongly supporting the convenience of considering early interventions directed towards amelioration of insulin resistance (Arslanian *et al.* 2001, 2002).

Adolescent girls with hyperandrogenism present with low levels of IGFBP-1 and SHBG (Apter *et al.* 1995, Ibañez *et al.* 1995, 1997a, Silfen *et al.* 2003), and there is an inverse relationship between the levels of IGFBP-1 and SHBG with insulin. Although IGFBP-1 levels were lower in obese adolescent girls with PCOS as compared with obese non-hyperandrogenic girls, no differences in IGF-1 levels were detected (Lewy *et al.* 2001). Insulin may act by decreasing hepatic production of both IGFBP-1 and SHBG, which increases the bioavailability of IGF-1 and free testosterone to target tissues (Nestler *et al.* 1991, Nobels and Dewailly 1992).

Non-obese girls with premature pubarche present hyperinsulinemia after an oral glucose tolerance test during pubertal development compared with Tanner stage and bone age matched girls (Ibañez *et al.* 1997a) (Fig. 15.3). These patients also have lower levels of SHBG and IGFBP-1 (Ibañez *et al.* 1997a). Using the frequent sampling intravenous glucose tolerance test and the ACTH stimulation test, adrenal steroid levels appeared to be increased in prepubertal girls with premature pubarche and insulin resistance in two different studies (Oppenheimer *et al.* 1995, Vuguin *et al.* 1999). The ACTH stimulated steroid levels were inversely

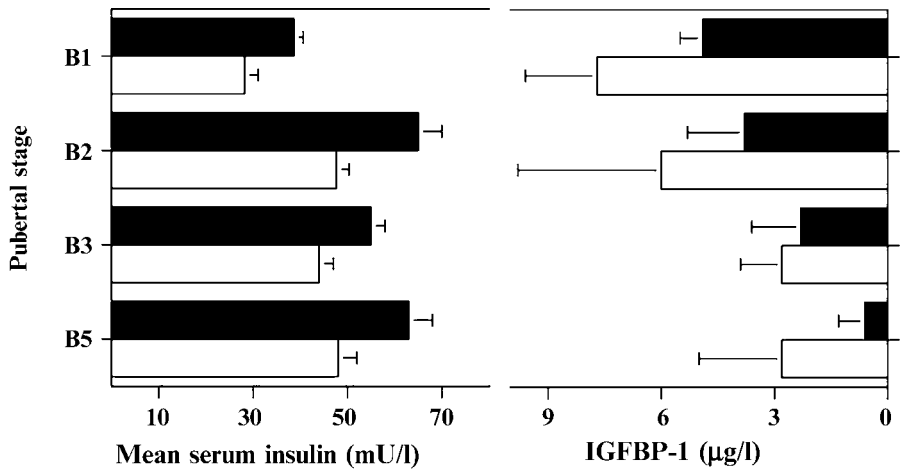

Fig. 15.3 Mean serum insulin during an oral glucose tolerance test and IGFBP-1 levels at different pubertal stages (B1 to B5) in girls with a history of premature pubarche. White bars indicate control girls and black bars indicate girls with a history of premature pubarche. Please note the inverse relationship between the changes in serum insulin and IGFBP-1 levels. Adapted from Ibañez *et al.* (1997a).

correlated with insulin sensitivity and IGFBP-1 and positively correlated with IGF-1 levels, whereas IGFBP-3 levels were within the normal range. Preliminary data suggest that girls with premature pubarche who remain obese are at higher risk of developing PCOS (DiMartino-Nardi 1999), especially when many of these girls had a strong family history of type 2 diabetes (DiMartino-Nardi 1999).

Of note, ethnicity plays a role not only on the development of PCOS, but also in premature pubarche (Dunaif *et al.* 1989, Sorbara *et al.* 1991). African American and Caribbean Hispanic girls with premature pubarche are frequently obese, presenting with marked hyperandrogenism and insulin resistance (Oppenheimer *et al.* 1995, Banerjee *et al.* 1998, DiMartino-Nardi 1999, Vuguin *et al.* 1999).

The studies referred above support the hypothesis that, in some girls with premature pubarche, insulin resistance may precede the clinical presentation of PCOS. Hyperinsulinemia, together with IGF-1 and other trophic hormones, would induce increased androgen synthesis by the adrenal cortex and ovarian theca cells (Adashi *et al.* 1985, L'Allemand *et al.* 1996). The decreased hepatic production of SHBG and IGFBP-1 by insulin might be an additional risk factor, increasing the bioavailability of unbound testosterone and IGF-1 to target tissues as stated earlier (Nestler *et al.* 1991, Nobels and Dewailly 1992).

Hyperandrogenic adolescents may present with other metabolic disorders, including dyslipidemia and abnormal blood pressure regulation. Circulating lipid concentrations in adolescents with PCOS have been reported to be normal

(Arslanian *et al.* 2001), or to present several abnormalities (elevated total cholesterol, low-density lipoprotein (LDL) and triglyceride levels, and decreased high density lipoprotein levels) that may evolve progressively in the presence of insulin resistance (Ibañez *et al.* 1998b, Silfen *et al.* 2003). Blood pressure is usually within the normal range in young women with PCOS (Legro 2003). However, adolescent girls with PCOS and impaired glucose tolerance have abnormalities in nocturnal blood pressure regulation, consisting of the absence of the nocturnal decline (non-dipper pattern) in blood pressure (Arslanian *et al.* 2001). This finding might be the earliest risk marker of cardiovascular disease in women with PCOS.

Role of the GH/IGF-1 axis and of gonadotropins in the development of hyperandrogenism and PCOS

The hypothalamic–pituitary abnormalities observed in adult women with PCOS have been studied also in adolescents with the syndrome (Apter *et al.* 1995, Silfen *et al.* 2003). The levels and pulsatile characteristics of GH and the circulating levels of total IGF-1 appear to be normal in hyperandrogenic adolescents (Apter *et al.* 1995, Ibañez *et al.* 1995), whereas the levels of IGFBP-3 may be either normal (Apter *et al.* 1995) or decreased (Ibañez *et al.* 1995), the latter enhancing IGF-1 bioavailability. Accordingly, serum concentrations of free IGF-1 are higher in non-obese adolescents with PCOS (Silfen *et al.* 2003). However, a recent study found amplified GH secretion in non-obese adolescents with PCOS (García-Rudaz *et al.* 2002).

Dysregulation of the insulin–IGF system may be one of the most important mechanisms involved in the pathogenesis of premature pubarche and its progression to PCOS (Silfen *et al.* 2002). Prepubertal girls with premature pubarche have increased total and free levels of IGF-1 and decreased levels of IGFBP-1 (Ibañez *et al.* 1997a, Silfen *et al.* 2002). Total and free levels of IGF-1 correlate with Δ^4-androstenedione levels in girls with premature pubarche, and this correlation together with insulin resistance suggests that in this subgroup of premature pubarche patients the IGF system plays a causative role in the development of hyperandrogenism (Silfen *et al.* 2002). Further studies are required to determine whether IGF-1 levels predict a subset at risk for the development of PCOS.

The pattern of increased LH secretion typical of puberty is also found in adolescents and adult women with PCOS, even in early pubertal – yet still premenarcheal – girls with features of the syndrome (Apter *et al.* 1994). Adolescent girls with PCOS demonstrate higher levels of LH, Δ^4-androstenedione, and DHEAS when compared with controls, and this abnormality is greater in the non-obese group of patients (Silfen *et al.* 2003), suggesting that the neuroendocrine system may be more affected in non-obese adolescents with PCOS and play an additional role, in addition to insulin resistance and exaggerated androgen

secretion, in this subgroup of patients. However, there are not enough data supporting a primary role for LH in the pathogenesis of premature pubarche, hyperandrogenism, and adolescent PCOS (Zumoff *et al.* 1983, Apter *et al.* 1994). Moreover, in adult women the increase in LH is not sensitive or specific for the diagnosis of PCOS, considering that only half of the patients with functional ovarian hyperandrogenism present the abnormality, and that increased levels of LH are also found in patients with congenital adrenal hyperplasia (Ehrmann *et al.* 1992).

Ovarian morphology

Ultrasonography is difficult to perform in children, and on many occasions the result relies on the skill of the operator. The transabdominal approach also limits the visualization of pelvic organs (i.e., in case of obesity).

Prepubertal ovaries are small, with some follicular development going on. The follicular number and size increase when approaching puberty. The presence of multifollicular ovaries with normal ovarian size should not be considered abnormal during puberty (Adams *et al.* 1985). Girls at age 6 years have a prevalence of polycystic ovaries in ultrasound of 6% (Bridges *et al.* 1995), a figure that increases with age until puberty, when it reaches a prevalence similar to the 20–25% reported in unselected adult women (Polson *et al.* 1988).

On the contrary, the prevalence of polycystic ovaries among girls with premature pubarche is 41% (Battaglia *et al.* 2002). Girls with premature pubarche and polycystic ovaries present with increased ovarian volumes (similar to those of girls at Tanner stage II–B2), a hyperechogenic stroma, and a higher number of subcortical small follicles, all these features resembling the ultrasound criteria for polycystic ovaries described for adult women by Adams *et al.* (1985). No data are available about a possible association of polycystic ovaries in girls with premature pubarche with insulin or IGF-1 levels.

Genetics

Hyperandrogenism, insulin resistance, and abnormalities of carbohydrate metabolism cluster among PCOS families, suggesting a genetic predisposition to these traits (Norman *et al.* 1996, Legro *et al.* 2002a, Yildiz *et al.* 2003). The prevalence of impaired glucose tolerance and type 2 diabetes is higher in first-degree relatives of girls with premature pubarche (Ibañez *et al.* 1999a). The hyperandrogenic sisters of PCOS patients are more insulin-resistant than the non-affected sisters (Norman *et al.* 1996, Legro *et al.* 1998, 2002a, Yildiz *et al.* 2003), and male members of families of PCOS are more frequently insulin resistant compared with those of controls (Legro *et al.* 2002b, Yildiz *et al.* 2003). However, genetic approaches to the pathogenesis of PCOS are hampered by the phenotypic

heterogeneity of the syndrome, the lack of uniform criteria for diagnosis, relative infertility of affected individuals, partial penetrance, and imprecise male phenotype (Escobar-Morreale *et al.* 2005).

The molecular basis of PCOS and the insulin resistance and metabolic abnormalities associated to the syndrome remain unknown (Urbanek and Spielman 2002, Escobar-Morreale *et al.* 2004, Roldán *et al.* 2004). Polycystic ovary syndrome is a complex disorder, and no single gene has been identified as the susceptibility gene. The syndrome may develop because of the interaction of several genes contributing simultaneously to the pathogenesis, with environmental risk factors such as prenatal nutrition, diet, or lifestyle. To date, very few data are available in pediatric populations.

Witchel and associates (Witchel *et al.* 1997, Escobar-Morreale *et al.* 1999, Witchel and Aston 2000) showed that the prevalence of heterozygosity for mutations in the *CYP21* gene is increased among children with premature pubarche, adolescent girls with hyperandrogenism, and hirsute hyperandrogenic women, compared with healthy control subjects. In another study of these authors, five candidate loci were studied (*CYP21*, *HSD3B2*, *IRS-1*, *ADRB3*, and *GRL*) (Witchel *et al.* 2001). Only 11 of 40 children presenting with premature pubarche (27.5%) and 9 of 29 hyperandrogenic girls (31%), compared with 9 of 15 healthy women (60%) showed no variants for any of the loci examined (Witchel *et al.* 2001). Twenty percent of premature pubarche children and adolescent girls with hyperandrogenism presented simultaneously two or more allelic variants (Witchel *et al.* 2001). In all of the premature pubarche children and in most of the hyperandrogenic girls carrying two or more variants, at least one involved *CYP21* (Witchel *et al.* 2001). These data support the hypothesis that multiple genetic variants are involved in the pathogenesis of premature pubarche and PCOS.

Patients with premature pubarche present a trend towards increased frequency of the R972 variant of the *IRS-1* gene (Witchel *et al.* 2001), and hyperandrogenic adolescent girls with a history of premature pubarche who do not carry the variant R972 of the *IRS-1* gene show lower SHBG concentrations than hyperandrogenic adolescent girls carrying the variant (Ibañez *et al.* 2002b). The R972 variant of the *IRS-1* gene is a loss-of-function mutation, which has been associated with impaired insulin secretion and PCOS in adult women (El Mkadem *et al.* 2001). More studies are required to confirm the importance of the R972 variant in the progression of girls with premature pubarche or hyperandrogenic adolescents to PCOS.

Hypothesis for the peripubertal onset of PCOS

In light of current concepts of the mechanisms responsible for hyperandrogenism and insulin resistance during the premenarcheal, pubertal, and adolescent stages,

different theories have been proposed (Nobels and Dewailly 1992, Zhang *et al.* 1995, Miller 1997). New approaches to the study of PCOS are also arising such as the impact of inflammatory cytokines or the importance of free fatty acids and fat metabolism in the genesis of obesity, insulin resistance, and glucose tolerance impairment.

The insulin/IGF-1 hypothesis

Nobels and Dewailly (1992) postulate that the hyperinsulinemia of puberty, together with IGF-1 and other trophic hormones, would induce increased androgen synthesis and a PCOS-like syndrome in predisposed adolescent girls (Adashi *et al.* 1985, L'Allemand *et al.* 1996). Girls in whom the insulin and IGF-1 levels of puberty do not decline would develop the syndrome.

The onset of pulsatile GH secretion during early puberty induces the production of IGF-1 by the liver and other tissues. GH also induces insulin resistance that affects peripheral glucose metabolism (Amiel *et al.* 1991). The resulting hyperinsulinemia decreases the hepatic production of IGFBP-1 and SHBG, thus increasing the bioavailability of free IGF-1 and free testosterone to target tissues (Nestler *et al.* 1991, Nobels and Dewailly 1992). Insulin and IGF-1 accomplish mitogenic effects on the theca cells of the ovaries and favor ovarian and adrenal steroidogenic synthesis (Ehrmann *et al.* 1995). The intraovarian androgen excess would induce an unfavorable environment for follicle maturation, leading to follicular atresia and anovulation. The arrested follicles would impair estrogen production and the elevated circulating estrone levels resulting from the aromatization of Δ^4-androstenedione in adipose tissue sensitize the pituitary to secrete excessive LH, which maintains the excessive stimulation of the ovary (Fig. 15.4).

The serine/threonine phosphorylation hypothesis

Potential mechanisms suggested for hyperandrogenism and insulin resistance include increased serine/threonine phosphorylation of ovarian and adrenal CYP17, and an increase in the serine phosphorylation of the insulin receptor, a potential unifying hypothesis to explain the presence of hyperandrogenism and insulin resistance in premature adrenarche and PCOS (Zhang *et al.* 1995, Miller 1997).

Zhang *et al.* (1995) reported that during adrenarche, an increase in serine/threonine phosphorylation of CYP17 increases the activity of the 17,20-lyase enzyme, resulting in a shift of the enzyme activity towards androgen production. Dunaif (1995) suggested that the insulin resistance observed in women with PCOS is secondary to having an excessive serine phosphorylation of the insulin receptor ß chain, inhibiting tyrosine phosphorylation. Thus, given that serine

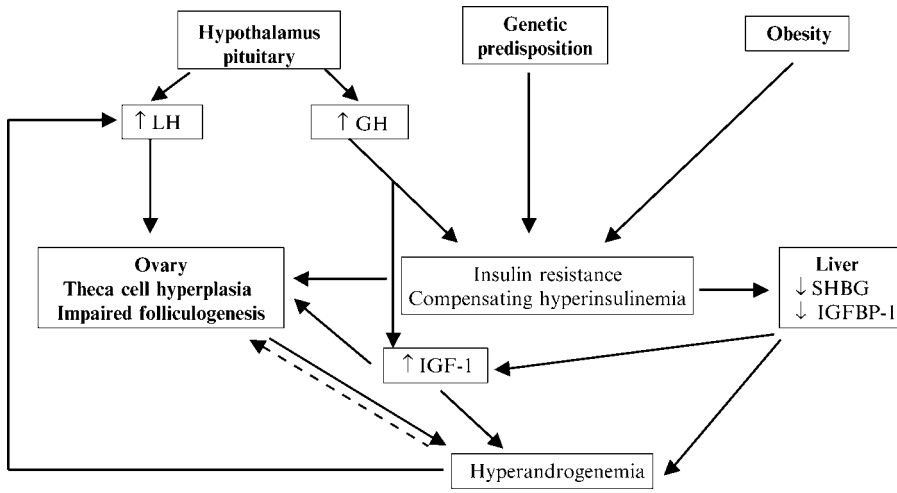

Fig. 15.4 Pathogenesis of PCOS: the insulin/IGF-1 hypothesis. Increased IGF-1 and insulin levels collaborate in inducing hyperandrogenism in predisposed women. Adapted from Nobels and Dewailly (1992).

phosphorylation might cause an increased activity of a key enzyme in androgen synthesis together with the induction of insulin resistance, several authors speculated that this process might provide a common molecular pathway for the hyperandrogenism and hyperinsulinism observed in premature pubarche and PCOS (Dunaif *et al.* 1995, Zhang *et al.* 1995, Auchus *et al.* 1998). However, this hypothesis could not be confirmed in analysis of 17,20-lyase activity in cultured fibroblasts obtained from women with PCOS (Martens *et al.* 2000), and the mechanism involved in the increased phosphorylation of serine and threonine residues has not been identified.

The adipose tissue and insulin resistance

The ectopic fat storage and lipotoxicity hypothesis concerns the role of adipokines and cytokines in the regulation of insulin sensitivity. Over the past few decades, profound lifestyle changes have occurred. Obesity is increasing its prevalence in the pediatric population, and this increase has been paralleled by an increase in the prevalence of the metabolic syndrome, type 2 diabetes, and cardiovascular disease (Goran *et al.* 2003, Miller *et al.* 2004). Childhood risk factors such as increased body mass index and LDL cholesterol levels predict the later development of adult cardiovascular disease (Davis *et al.* 2001, Raitakari *et al.* 2003), and even damage to the arterial wall and endothelial dysfunction, which are early risk markers of cardiovascular events, may develop during childhood (Tounian *et al.* 2001).

Polycystic ovary syndrome has been considered an early manifestation of the metabolic syndrome (Ibañez *et al.* 1998c), and obesity has been associated with a higher incidence of premature pubarche (Pang 1984, Rosenbaum and Leibel 1989). Both lean and obese adolescents and women with PCOS are insulin resistant when compared with non-hyperandrogenic controls (Chang *et al.* 1983, Dunaif *et al.* 1987, Palmert *et al.* 2002, Escobar-Morreale *et al.* 2003), although obesity markedly exacerbates insulin resistance (Dunaif *et al.* 1987).

The relationship between obesity, insulin resistance, type 2 diabetes, and cardiovascular risk in adults has been explained in part by the lipotoxicity hypothesis (Ravussin and Smith 2002, Schaffer 2003). Obese adolescent girls have impaired glucose disposal and fail to increase glucose oxidation and suppress lipid oxidation in response to insulin infusion (Caprio *et al.* 1995). Adolescents and adult women with PCOS may manifest a metabolic phenotype predisposed to increased plasma free fatty acids, followed by increased storage of fat especially in skeletal muscle and liver (Ravussin and Smith 2002). This aberrant fat storage (lipotoxicity) would alter insulin signaling, insulin metabolism, and pancreatic beta cell function, contributing to the development of insulin resistance (Schmitz-Peiffer 2000).

It is also known that the adipose tissue secretes several bioactive molecules, termed adipokines, which include proteins (leptin, adiponectin, resistin, etc.) and cytokines (tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), etc.), that play a central role in the relationship between adipose tissue and insulin resistance (Diamond and Eichler 2002, Fernandez-Real and Ricart 2003). Insulin resistance and serum markers of inflammation, including cytokines, are increasingly being considered as predictors of cardiovascular disease (Van Lente 2000, Fernandez-Real and Ricart 2003).

Leptin and adiponectin improve insulin sensitivity, whereas TNF-α and IL-6 are proinflammatory cytokines that favor insulin resistance by inhibiting insulin action (Diamond and Eichler 2002).

Girls with premature pubarche and prepubertal obesity show elevated leptin levels, which suggests a role for leptin in the development of premature pubarche (Cizza *et al.* 2001, Teixeira *et al.* 2004). The concentrations of the anti-inflammatory adiponectin are low, and those of IL-6 are increased, in girls with premature pubarche and in adolescents with PCOS and a previous history of premature pubarche (Ibañez and de Zegher 2004, Ibañez *et al.* 2004b). Increased plasma levels of plasminogen activator inhibitor-1 (PAI-1) have also been observed in early pubertal premature pubarche girls, and might be a marker indicating later risk to develop the hyperinsulinemic and hyperandrogenic features of PCOS (Ibañez *et al.* 2002a).

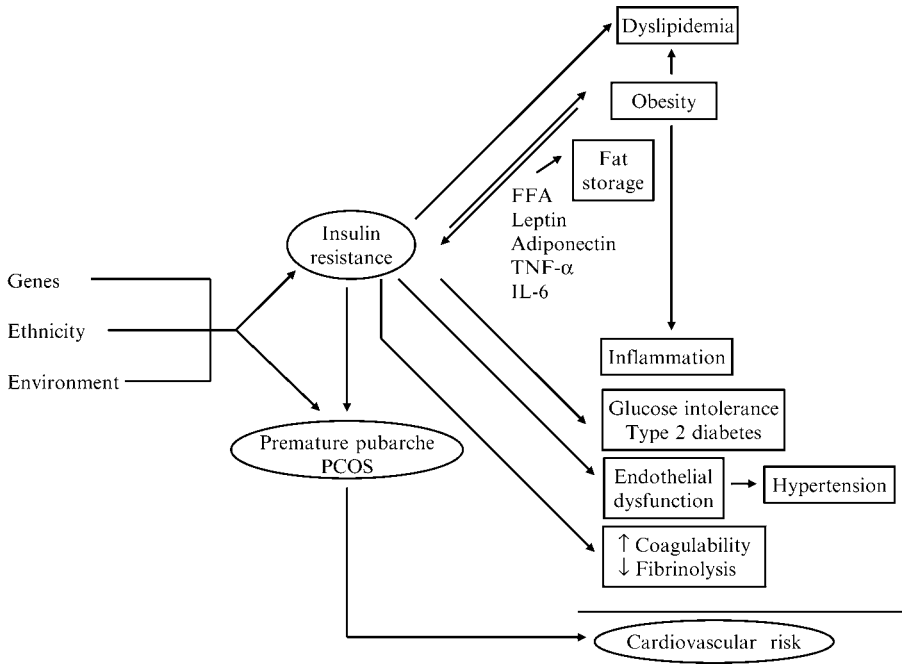

Fig. 15.5 Pathogenesis of PCOS: insulin resistance and the adipose tissue. Children with premature pubarche and adolescents with PCOS are insulin resistant and have an increased risk for cardiovascular events later in life. Obesity exacerbates insulin resistance and predisposes these patients to develop a metabolic phenotype characterized by increased plasma free fatty acids and fat deposits in aberrant sites, further contributing to insulin resistance. Adipokines and cytokines secreted in the adipose tissue also play a central role in the relationship between adipose tissue and insulin resistance. FFA, free fatty acids; TNF, tumor necrosis factor; IL, interleukin.

All together these data suggest that the subset of girls with premature pubarche and adolescents PCOS patients share with adult PCOS patients the increased risk and the mechanisms influencing the development of the metabolic syndrome and cardiovascular disease at older ages (Fig. 15.5).

Consequences for the management of hyperandrogenic girls

Polycystic ovary syndrome is a common disorder in women, which becomes clinically apparent soon after puberty (Apter 1998). Intrauterine growth retardation and/or premature pubarche have been proposed to predispose to PCOS in affected girls (Ibañez *et al.* 1998c, 1999c). In women with PCOS, menstrual cycle irregularities and signs of hyperandrogenism can be observed during adolescence

(Apter 1998). Polycystic ovary syndrome is also the first evidence for several features of the metabolic syndrome, and an early manifestation during life of an adverse genotypic and phenotypic metabolic profile in affected adolescents and adult women (Dunaif 1997). Hyperinsulinemia, insulin resistance, and an altered regulation of the insulin–IGF system may be detected in girls with premature pubarche, in adolescents with functional ovarian hyperandrogenism and a previous history of premature pubarche, and in young women with PCOS (Apter *et al.* 1995, Oppenheimer *et al.* 1995, Ibañez *et al.* 1996, Vuguin *et al.* 1999, Arslanian *et al.* 2001, Lewy *et al.* 2001, Palmert *et al.* 2002, Silfen *et al.* 2003).

Since insulin resistance is the primary abnormality early in the course of type 2 diabetes, the finding of insulin resistance in adolescents with PCOS would predict that this group could be at an increased risk for impaired glucose tolerance and type 2 diabetes (Lewy *et al.* 2001). The early identification of PCOS could have an important impact on the prevention and treatment of associated disorders, and the progression of the full-blown syndrome might be ameliorated with proper intervention.

The American Diabetes Association (2000) recommends that clinicians screen adolescents with PCOS for abnormal glucose tolerance with an oral glucose tolerance test. Although no screening test is universally accepted, recent studies propose that indexes using fasting insulin and glucose levels serve as surrogate measures of insulin sensitivity and pancreatic beta cell function in young subjects (Conwell *et al.* 2004, Gungor *et al.* 2004).

Early intervention should be considered in adolescents with PCOS and insulin resistance with still compensating beta cell function, before severe abnormalities in glucose metabolism occur. Healthy lifestyle modifications and weight reduction improve insulin sensitivity and obesity, decrease androgen levels and hirsutism, and restore regular menses in adolescents with PCOS (Pasquali *et al.* 1989, Ibañez *et al.* 2000b). Metformin, an insulin sensitizer approved by the Food and Drug Administration to treat type 2 diabetes, has demonstrated its efficacy in the treatment of adolescents with PCOS and impaired glucose metabolism (Glueck *et al.* 2001, Ibañez *et al.* 2000b, Arslanian *et al.* 2002). Metformin therapy improves hyperinsulinemia, hirsutism, hyperandrogenism, and dyslipidemia in hyperandrogenic adolescents, inducing regular menses and ovulation within 4 months (Ibañez *et al.* 2000b, 2001). Metformin also prevents progression from premature pubarche to PCOS in the high-risk group of formerly low birthweight girls followed by Ibañez *et al.* (2004a, b). Factors predicting response to metformin include elevated baseline insulin levels and menstrual irregularity as well as elevated baseline androgen levels (Moghetti *et al.* 2000).

More traditionally, the treatment for PCOS has been symptom-based, and these approaches are still valid. Oral contraceptive pills are helpful in suppressing

the ovarian hyperandrogenism and in restoring regular menstrual cycles, but have minimal effect on insulin sensitivity. Other benefits of oral contraceptive pills include decreasing terminal hair growth, ameliorating acne, and reducing the risk for endometrial hyperplasia and endometrial carcinoma, a substantial concern in patients with chronic oligomenorrhea. Antiandrogen drugs such as spironolactone, flutamide, or finasteride have been used in combination with oral contraceptive pills, and the use of flutamide in conjunction with metformin in non-obese adolescents with hyperandrogenism and hyperinsulinism has been demonstrated to improve insulin sensitivity, lipids profile, and androgen levels more markedly than both drugs administered separately (Ibañez *et al.* 2002c).

Conclusion

Women with PCOS have an increased risk of developing the metabolic syndrome, type 2 diabetes, and even cardiovascular events (Dunaif 1997, Legro 2003). Hence, early diagnosis and interventions are required in girls and adolescents with hyperandrogenic features. The progression to adult disease probably depends on the interaction of environmental factors with a certain genetic background resulting in androgen excess and insulin resistance. Therefore, future efforts should be dedicated to elucidate the mechanisms involved in the pathogenesis of PCOS, and to detect the factors implicated in the progression from the pediatric manifestations of hyperandrogenism to the adult full-blown PCOS.

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16

Fetal programming of polycystic ovary syndrome

David H. Abbott, Cristin M. Bruns, Deborah K. Barnett, and Daniel A. Dumesic

Introduction

Readily available, highly calorific foods (Briefel and Johnson 2004), together with an increasingly sedentary lifestyle (Winkleby and Cubbin 2004), are causing progressive detriments in human health (Mokdad *et al.* 1999, Katzmarzyk and Ardern 2004, Rigby *et al.* 2004). In the USA alone, overweight or obesity afflicts approximately one in three adult women and contributes to a rapidly increasing incidence of type 2 diabetes (NHANES 2006). The current epidemiological evidence suggests that such an escalating prevalence of obesity and diabetes will continue for the foreseeable future (Zimmet 1999, Zimmet *et al.* 2003). Such a prediction is of particular concern for women's reproductive health because obesity and diabetes contribute markedly to anovulatory infertility (Norman and Clark 1998, Norman *et al.* 2004), the most frequent cause of infertility in women (Abbott *et al.* 2004).

Metabolic dysfunction has considerable consequences for polycystic ovary syndrome (PCOS), a highly prevalent metabolic and infertility disorder of reproductive-aged women that is exacerbated by obesity (Ehrmann *et al.* 1995, Franks 1995, Dunaif 1997, Escobar-Morreale *et al.* 2004, Carmina *et al.* 2005). The syndrome has a highly heterogeneous presentation that can include androgen excess, amenorrhea, insulin resistance, and obesity, among other general health disorders (Abbott *et al.* 2002a, Dumesic *et al.* 2005). The most recent PCOS consensus diagnosis (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004), however, requires the presence of only two out of three specific androgenic and reproductive criteria: (1) hyperandrogenism, as determined biochemically from circulating total or unbound testosterone levels or clinical signs of hyperandrogenism, (2) intermittent or absent menstrual cycles,

Polycystic Ovary Syndrome, 2nd edn, ed. Gabor T. Kovacs and Robert Norman. Published by Cambridge University Press.

and (3) polycystic ovaries, as visualized by ultrasound. The diagnosis of PCOS is one of exclusion, and can only be reached when phenotypically similar, but mechanistically different disorders, such as classical and non-classical 21-hydroxylase deficiency, Cushing syndrome, and androgen secreting tumors, have been excluded (Zawadzki and Dunaif 1992).

PCOS, however, has no consistent phenotype (Legro 2003, Adams *et al.* 2004, Escobar-Morreale *et al.* 2005, Dumesic *et al.* 2005), lacks a genetically defined basis (Legro 1998, Escobar-Morreale *et al.* 2004), and has no known etiology (Legro 1998, Abbott *et al.* 2002a). Since PCOS is the leading endocrinopathy of reproductive-aged women (Mishell *et al.* 1991) and might increase in prevalence from the increasing frequency of obesity, there is an urgent clinical need to determine the elusive etiology of the syndrome and to develop therapeutic approaches to prevent its onset in adolescence (Homburg 2005, Ibañez and de Zegher 2005) before adult pathology occurs.

A variety of hypotheses have been proposed suggesting either a genetic or environmental origin for PCOS. There is strong evidence for the former, since PCOS is readily transmitted across generations, through both male and female family members, as if regulated by an autosomal dominant gene with inconsistent penetrance (Legro 1998, Strauss 2003). Most candidate genes, however, have failed to maintain repeated and reliable association with the PCOS phenotype (Urbanek *et al.* 2000, Gaasenbeek *et al.* 2004, Powell *et al.* 2005), except for a gene locus on chromosome 19 (i.e., *D19S884*), centromeric to the insulin receptor gene (Urbanek *et al.* 1999, Tucci *et al.* 2001, Villuendas *et al.* 2003).

Environmental mechanisms, however, may also provide an etiology for the PCOS syndrome as insulin resistance and compensatory hyperinsulinemia stimulate ovarian androgen excess, and may lead to PCOS (Dunaif, 1997, Hopkinson *et al.* 1998). Since insulin resistance and its clinical sequelae are associated with low birthweight (Hales *et al.* 1991, Barker *et al.* 1993, Phipps *et al.* 1993), and are common features of PCOS (Ehrmann *et al.* 1995, Franks *et al.* 1996, Holte, 1996, 1998, Dunaif 1997), PCOS may be programmed in utero, possibly as a consequence of poor intrauterine growth. Such fetal programming may be determined by environmental factors, such as poor maternal nutrition (Barker 1998), but also may include genetic components since genomic variants related to the expression of insulin resistance are associated with PCOS (San Millan *et al.* 2004). Evidence for multiple regulatory factors in utero is provided by girls with low birthweight and precocious puberty (Ibañez *et al.* 1996, 1998), high birthweight (Cresswell *et al.* 1997), or prolonged gestation (Cresswell *et al.* 1997), all of whom frequently express PCOS symptoms in adolescence or adulthood.

In order to better understand a fetal origin for PCOS, and the potential implications of therapeutic strategies targeting in utero development, this chapter

examines the evidence for fetal programming, emphasizing our findings from prenatally androgenized female rhesus monkeys. While there is currently no identified fetal origin for PCOS in humans, the weight of evidence from non-human primate (Abbott *et al.* 1997, 1998, 2002a), ovine (West *et al.* 2001, Birch *et al.* 2003), and rodent (J. E. Levine, pers. comm.) models for PCOS overwhelmingly suggests a fetal origin for the PCOS syndrome, as determined by in utero exposure to androgen excess (Abbott *et al.* 2002a, 2005). Since intra-uterine growth retardation is associated with the development of PCOS in Spanish women (Ibañez *et al.* 1996, 1998, Ibañez and de Zegher 2005) and of PCOS-like traits in ovine and rodent models (sheep: Manikkam *et al.* 2004; rats: Slob *et al.* 1983), we also examine the relevance of the Barker hypothesis for the development of a PCOS phenotype.

Fetal programming, the Barker hypothesis, and PCOS

Epidemiological studies of human populations have repeatedly demonstrated an association between low birthweight and the subsequent adult development of cardiovascular disease, hypertension, insulin resistance, and type 2 diabetes mellitus (Barker 1994, 1995, 1998). Fetal programming has been proposed as the causal mechanism functionally linking impaired fetal growth with adult disease. The fetal programming (fetal origins or Barker) hypothesis (Barker 1994) posits that a stimulus or insult occurring at a critical gestational age for target tissue growth and development permanently alters tissue structure and function, as well as organ size. Whether such deviations from normal fetal development are genetically (heritable) or environmentally determined or both remains debatable (Drake and Walker 2004). In this regard, fetal undernutrition (Barker and Osmond 1986, Bertram and Hanson 2001, Rhind *et al.* 2001) or fetal exposure to glucocorticoid excess (Edwards *et al.* 1993, Seckl and Meaney 2004) cause low birthweight and cardiovascular disease in several mammalian species from rodents to humans, and such environmentally induced insults are capable of being perpetuated to subsequent generations (Drake and Walker 2004, Drake *et al.* 2005), thereby mimicking genetically determined traits. On the other hand, a variety of genetic loci have been identified as the causal mechanism responsible for both small size at birth and onset of type 2 diabetes, as well as cardiovascular disease in adulthood (Dunger *et al.* 1998, Hattersley *et al.* 1998).

The hypothesis that episodic fetal undernutrition selects for genes important in energy conservation over many generations, i.e., the "thrifty genotype" hypothesis, was first described by Neel (1962). Although these genes would be advantageous in times of food scarcity, they would lead to obesity and diabetes when food is abundant. The "thrifty phenotype" or Barker hypothesis, in contrast,

posits that intrauterine malnutrition leads to small size at birth, and predisposes to diabetes and its precursors in later life (Hales and Barker 2001). Barker interprets fetal responses to undernutrition as an organized and programmed process whereby fetal brain development is spared to the detriment of other organ systems (Barker 1994). Barker's hypothesis has raised important mechanistic questions as to whether fetal programming is (1) an adaptive response for postnatal survival in a food-scarce environment, (2) the consequence of normal fetal development constrained by undernutrition, or (3) bona fide fetal pathology (Ellison 2005). The answers to such questions are crucial (Brakefield *et al.* 2005) since therapeutic interventions may well exert differential effects on improving each of these processes.

The relevance of the Barker hypothesis to PCOS, in terms of fetal response to undernutrition, is unclear. Certainly, poor intrauterine growth and low birthweight are associated with the development of PCOS in northern Spanish women (Ibañez *et al.* 1996, 1998, Ibañez and de Zegher 2005), in whom precocious puberty also exists (Hokken-Koelega 2002). Such associations between low birthweight and PCOS outcome, however, are not found in larger studies of Finnish (Laitinen *et al.* 2003) and Dutch (Sadrzadeh *et al.* 2003) women. Such heterogeneity in association between fetal outcome and PCOS symptomatology is not surprising given the heterogeneity of the PCOS phenotype, perhaps with the Spanish women representing a distinct subset of PCOS patients. Results from animal models of PCOS are equally mixed regarding the association of adult traits with low birthweight. Clear evidence of intrauterine growth retardation and low birthweight exists in prenatally androgenized female rats (Slob *et al.* 1983, J. E. Levine, pers. comm.) and ewes (Mannikam *et al.* 2004). Interestingly, male rats and rams, exposed to the same fetal manipulation as prenatally androgenized females, also exhibit fetal growth retardation and low birthweight.

In contrast, prenatally androgenized female rhesus monkeys, although extremely close mimics of adult PCOS symptomatology (Abbott *et al.* 2002a, 2005), do not exhibit low newborn (within 5 days of birth) weight (Fig. 16.1). When compared with untreated females born during the same time period (1978–84) at the National Primate Research Center, University of Wisconsin–Madison (WPRC), female rhesus monkeys exposed to fetal androgen excess all clearly lie well within the mid-ranges of untreated female newborn weights. Female rhesus monkeys were exposed to fetal androgen excess, equivalent to fetal male serum testosterone levels (Resko *et al.* 1987), when their mothers received consecutive daily subcutaneous injections of 10 mg testosterone propionate during early gestation (for 15–35 days starting on gestation days 40–44; early treated; gestation = 165 days) or late gestation (for 15–25 days starting on gestation days 100–115; late treated) (Goy and Robinson 1982, Goy and Kemnitz 1983). Notably, four prenatally

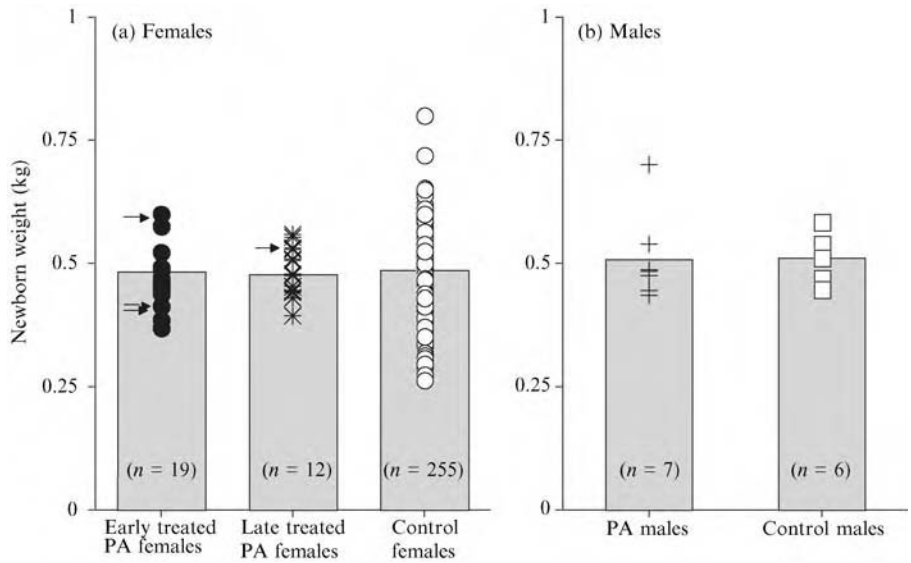

Fig. 16.1 Mean (bars) and individual newborn (postnatal days 1–5) body weights of (a) early and late treated prenatally androgenized (PA) and control female rhesus monkeys and (b) prenatally androgenized and control male rhesus monkeys. The arrows indicate three early treated prenatally androgenized females and one late treated prenatally androgenized female that were identified as diabetic (see text) in adulthood. The male data are modified from Bruns *et al.* (2004). Individual values: solid circles, early treated prenatally androgenized females; asterisks, later treated prenatally androgenized females; open circles, control females; plus signs, prenatally androgenized males; open squares, control males.

androgenized, but no untreated females of similar age, subsequently developed adult type 2 diabetes (up to 14–25 years of age; fasting serum glucose >140 mg/dl) Young *et al.* 1999, Hotta *et al.* 2001), and none of these diabetic females exhibited low newborn weight (indicated by arrows in Fig. 16.1). Studies of prenatally androgenized female rhesus monkeys conducted separately from those at WPRC confirm normal fetal growth and newborn weight (Herman *et al.* 2000, Abbott *et al.* 2005). The heterogeneity in association of low birth or newborn weight with PCOS traits in different animal models may help determine the functional basis for the complex relationship between low birthweight and PCOS in women.

Male monkeys exposed to the same prenatal androgen treatment as prenatally androgenized females also do not exhibit low birthweight (Bruns *et al.* 2004) (Fig. 16.1). Fetal males exposed to the same prenatal androgen excess as fetal females appear to suffer from similar fetal programming, as discussed in more depth below. Since, in the non-human primate model, prenatally androgenized

males have fetal serum testosterone levels similar to control or untreated males (Resko *et al.* 1987), the biologically effective component of prenatal androgen treatment may not be mediated through increased fetal blood levels of testosterone. Instead, it may involve an estrogenic or androgenic metabolite of testosterone, or an effect of maternal androgen excess on placental or maternal physiology that alters, or impairs, fetal development (Bruns *et al.* 2004). Similar issues have arisen in studies of prenatally androgenized rams (Mannikam *et al.* 2004). Regardless of final mechanism, it is interesting to note that the same fetal origins result in similar glucoregulatory dysfunction in both male and female prenatally androgenized rhesus monkeys (Bruns *et al.* 2004). Such parallel outcomes for both sexes in the monkey studies may provide insight into possible fetal origins for glucoregulatory dysfunction in fathers and brothers of women with PCOS (Fox 1999, Colilla *et al.* 2001, Sir-Petermann *et al.* 2002a, Yildiz *et al.* 2003).

Birthweight is particularly associated with insulin resistance and obesity in adulthood when low weight at birth is followed by postnatal weight gain, or catch-up growth (Ong *et al.* 2000, Ong and Dunger 2002, Barker 2004). Such a scenario is found in the prenatally androgenized ewes (Mannikam *et al.* 2004), but not in the prenatally androgenized female rhesus monkeys (Goy *et al.* 1977, Wilen *et al.* 1977, Goy and Robinson 1982) (Fig. 16.2). Early treated prenatally androgenized female monkeys, however, do exhibit a transient increase in body weight compared to normal females between approximately 3–4 years of age (Wilen *et al.* 1977) (Fig. 16.2), during late adolescence and early adulthood, at an age when normal males start to exhibit greater body weight than normal females Kemnitz *et al.* 1988 (Fig. 16.2). Whether this transient excess body weight in early treated prenatally androgenized females reflects an increase in body fat, and whether it reflects fetal programming or an aspect of masculinization, remain to be determined. Certainly, age at menarche is delayed in early treated prenatally androgenized females (Fig. 16.2) and is closer than normal to the age at onset of male puberty in rhesus monkeys (Goy *et al.* 1977, 1988).

Predictions from the Barker hypothesis for the non-human primate model for PCOS related to growth and metabolic abnormalities

Predictions from the Barker hypothesis for the presence of abnormal growth and metabolic traits in prenatally androgenized female and male monkeys are illustrated in Table 16.1. As previously discussed, low birthweight and catch-up growth are not found in this animal model for PCOS, and low birthweight is not found in large population studies of PCOS (Laitinen *et al.* 2003, Sadrzadeh *et al.* 2003). The remaining four predictors for abdominal obesity and glucoregulatory

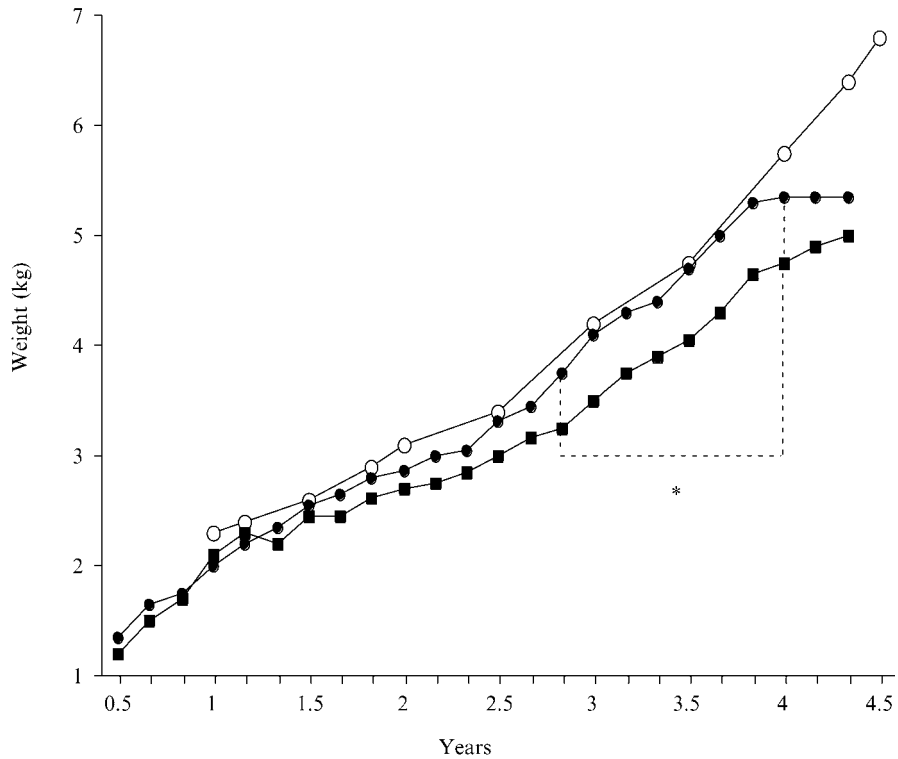

Fig. 16.2 Mean body weights of young, ovary intact, early treated prenatally androgenized ($n = 9$, closed circles) and control ($n = 18$, closed squares) female rhesus monkeys and gonadally intact control male rhesus monkeys ($n = 7$, open circles). *$p < 0.05$, prenatally androgenized versus control female body weights at ages 2.8–4.0 years. Female data are modified from Wilen *et al.* (1977) and the male data are modified from Kemnitz *et al.* (1988).

impairments, however, are all found in early treated prenatally androgenized females, and 1–2 of the glucoregulatory predictions are met in late treated females and prenatally androgenized males (Table 16.1 and detailed below). As noted previously by Barker (1994), intrauterine catch-up growth may obscure earlier fetal growth restriction and may thus lead to fetal programming outcomes without any indication of low birthweight. Thus, while low birthweight is not induced in the non-human primate model for PCOS, we need to await results from current ultrasound studies of fetal development in prenatally androgenized female rhesus monkeys to finally determine whether any intrauterine growth restriction and catch-up growth occur during gestation.

The remaining sections of this chapter examine PCOS traits exhibited by prenatally androgenized female and male rhesus monkeys and interpret their relevance to PCOS in women and to the Barker hypothesis when appropriate.

Table 16.1. Prediction of abnormal traits expected in prenatally androgenized female and male rhesus monkeys from the Barker hypothesis; details of the traits found in the monkeys are discussed in the text

Abnormal traits	Barker hypothesis prediction	Early treated PA female observation	Late treated PA female observation	PA male observation
Low birthweight	+	−	−	−
Catch-up growth	+	−	?	?
Visceral obesity	+	+[a]	?	?
Insulin resistance	+	+[b]	−[c]	+[d]
Insulin insufficiency	+	+[c]	−[c]	+[d]
Glucose intolerance	+	+[b]	+[b]	−[d]

Notes:

+, trait present; −, trait absent; ?, trait yet to be assessed.

[a] Eisner *et al.* (2003).

[b] Abbott *et al.* (2003).

[c] Eisner *et al.* (2000).

[d] Bruns *et al.* (2004).

Polycystic ovary syndrome outcomes in female rhesus monkeys following fetal androgen excess programming

Reproductive traits in PCOS: anovulation, hyperandrogenism, and polycystic ovaries

Prenatally androgenized female rhesus monkeys ovulate, on average, at 50–60% the frequency of normal females (Table 16.2), regardless of whether they are exposed to androgen excess during early or late gestation. Between 33% and 40% of prenatally androgenized females are anovulatory (Table 16.2), in contrast to 3–4% of age and size matched controls. Prenatally androgenized females exhibit approximately double the normal incidence of polyfollicular ovaries (Table 16.2), with polyfollicular ovaries in prenatally androgenized females being ~60% greater in volume than ovaries with similar morphology in normal females (Abbott *et al.* 2002b); 33–50% of anovulatory prenatally androgenized females have such polyfollicular ovaries (Table 16.2). Thus, prenatally androgenized females demonstrate intermittent or absent menstrual cycles and large, polyfollicular ovaries, regardless of the timing of prenatal androgen excess exposure.

Assigning clinical signs of hyperandrogenism, such as hirsutism, is impractical in rhesus monkeys, especially since hair-pulling (Reinhardt *et al.* 1986) distorts any systematic scoring. Circulating levels of testosterone, however, can be reliably determined in the early follicular phase (Dumesic *et al.* 1997) or in an equivalent 30-day anovulatory period (Abbott *et al.* 1998), and are elevated in early treated prenatally androgenized females alone (Fig. 16.3). While serum estradiol levels are

Table 16.2. PCOS-like abnormalities in ovarian function and morphology in prenatally androgenized female rhesus monkeys

	Prenatally androgenized females		
PCOS trait[a]	Early treated	Late treated	Normal females
Number of ovulatory cycles across 6 months[b]	3.83 ± 0.79* (6)	3.2 ± 1.24# (5)	6.33 ± 0.42 (6)
Percent anovulatory females	40.0 (4/10)	33.3 (3/9)	3.6 (1/28)
Percent polyfollicular ovaries	35.7 (5/14)	28.6 (2/7)	14.8 (4/27)
Percent anovulatory females with polyfollicular ovaries	50.0 (2/4)	33.3 (1/3)	0.0 (0/1)

Notes:

[a] Animal numbers vary between traits due to animal availability for procedures and assessments during and between experiments.

[b] Abbott *et al.* (2005), mean ± SEM (*n*); *, $p < 0.04$, # $p < 0.02$ versus normal females.

normal in both groups of prenatally androgenized females, the testosterone : estradiol ratio is elevated in early treated prenatally androgenized females (Fig. 16.3). Nevertheless, when injected with recombinant human chorionic gonadotropin (rhCG) during the early follicular phase or an anovulatory period, both early (Eisner *et al.* 2002) and late (Abbott *et al.* 2005) treated prenatally androgenized females display excessive serum testosterone responses, indicative of ovarian hyperandrogenism.

Whether such ovarian hyperandrogenism is intrinsic to a reprogrammed, prenatally androgenized ovary or is secondary to abnormal ovarian stimulation has not yet been determined. In studies of PCOS ovarian function, there is evidence for both an intrinsic ovarian abnormality (Gilling-Smith *et al.* 1997, Jonard and Dewailly 2004) and abnormal luteinizing hormone (LH) and insulin stimulation of the ovary (Willis *et al.* 1998, Veldhuis *et al.* 2002). In studies of prenatally androgenized ovarian function, evidence for an intrinsic ovarian abnormality awaits long-term treatment of such female monkeys with gonadotropin releasing hormone (GnRH) analogs to downregulate LH secretion. Evidence for abnormal ovarian stimulation, however, is currently available. Early, but not late, treated prenatally androgenized females exhibit LH hypersecretion during the early follicular phase or equivalent timing during a 30-day anovulatory period (Fig. 16.3) and show reduced sensitivity to estradiol-mediated LH negative feedback (Steiner *et al.* 1976, Abbott *et al.* 2005). Late treated prenatally androgenized females, nevertheless, display reduced sensitivity to progesterone-mediated LH negative feedback in a similar fashion to early treated females (Levine *et al.* 2005).

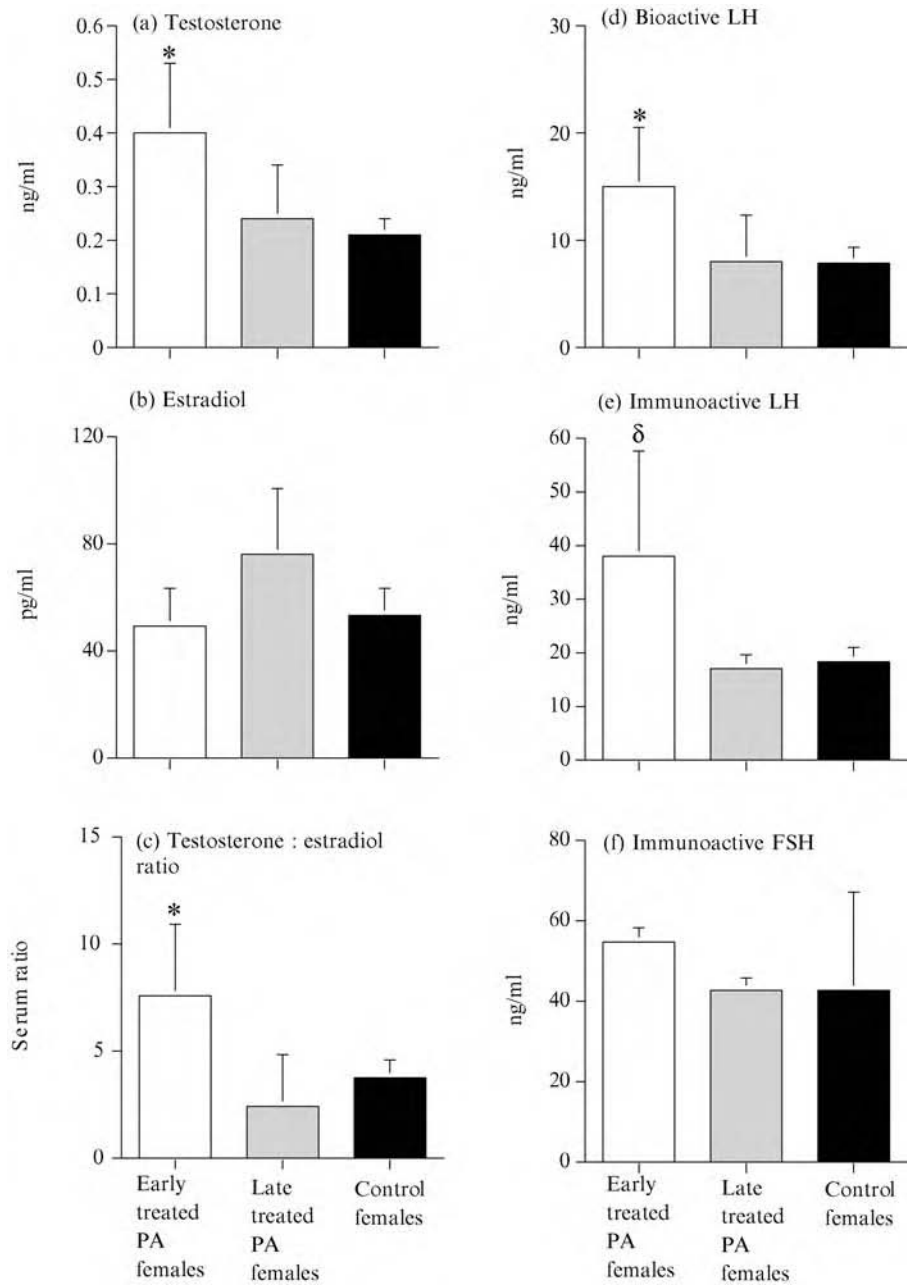

Fig. 16.3 Mean (±SEM) serum values in early (open bars) and late (gray bars) treated prenatally androgenized and control (black bars) adult female rhesus monkeys reflecting (a) testosterone, (b) estradiol, (c) testosterone : estradiol ratio, (d) bioactive luteinizing hormone (LH), (e) immunoactive LH, and (f) immunoactive follicle stimulating hormone (FSH) during either the early follicular phase of the menstrual cycle or equivalent time during a 30-day anovulatory period (Abbott *et al.* 1998). *$p < 0.05$ versus controls, δ $p < 0.08$ versus controls.

Thus, like the PCOS ovary (Rosenfield *et al.* 1994, Franks 1995, Dunaif 1997), the prenatally androgenized ovary is exposed to abnormal LH stimulation. In addition, compensatory hyperinsulinemia from insulin resistance results in abnormal ovarian stimulation in prenatally androgenized female monkeys (Eisner *et al.* 2000, Dumesic *et al.* 2002) similar to that demonstrated in PCOS women (Dunaif *et al.* 1996, Nestler and Jakubowicz 1996, Willis *et al.* 1998, Veldhuis *et al.* 2002). As in PCOS women (Dunaif *et al.* 1996, Nestler and Jakubowicz 1996, Baillargeon *et al.* 2004, Brettenthaler *et al.* 2004) anovulation in prenatally androgenized monkeys is alleviated by insulin sensitizer treatment (Abbott *et al.* 2004), implicating insulin in the mechanism of abnormal ovarian function.

Reproductive traits in PCOS: impaired fertility

Reduced oocyte quality presents an additional reproductive abnormality in both prenatally androgenized female rhesus monkeys (Dumesic *et al.* 2002) and PCOS women (Ludwig *et al.* 1999, Urman *et al.* 2004, Hwang *et al.* 2005). Prenatally androgenized female rhesus monkeys exhibit abnormal follicle and oocyte responses to controlled ovarian hyperstimulation for in vitro fertilization (IVF) (Dumesic *et al.* 2002, 2003, 2005), while reduced oocyte quality in PCOS patients undergoing IVF contributes to increased rates of implantation failure and pregnancy loss (Homburg *et al.* 1988, Sagle *et al.* 1988, Dor *et al.* 1990, Tarlatzis *et al.* 1995). While early treated female monkeys exhibit the most obvious oocyte developmental impairment in vitro after controlled ovarian hyperstimulation for IVF, all prenatally androgenized females demonstrate abnormal intrafollicular steroidogenesis with some degree of reduced blastocyst formation (Dumesic *et al.* 2002, 2003) (Fig. 16.4) suggesting an adverse effect of prenatal androgenization on oocyte development. None of these intrafollicular steroid and embryonic abnormalities accompanying controlled ovarian hyperstimulation for IVF can be predicted from circulating hormone levels, or from number and maturity of oocytes collected.

Abnormalities in intrafollicular steroidogenesis occur when prenatally androgenized females are stimulated with either recombinant human follicle stimulating hormone (rhFSH) alone (Dumesic *et al.* 2003) or with rhFSH stimulation followed by rhCG administration before oocyte retrieval (Dumesic *et al.* 2002). In late treated prenatally androgenized females, low follicular fluid estradiol and androstenedione levels accompany rhFSH therapy alone (Dumesic *et al.* 2003); consistent with the ability of estrogen to enhance oocyte development in primates (Tesarik and Mendoza 1995, Zheng *et al.* 2003), subtle embryonic developmental impairment occurs by the blastocyst stage following combined rhFSH/rhCG treatment in these females (Dumesic *et al.* 2002). In early treated prenatally androgenized females, low follicular fluid estradiol and androstenedione levels occur after both stimulation protocols, and are accompanied by an elevated

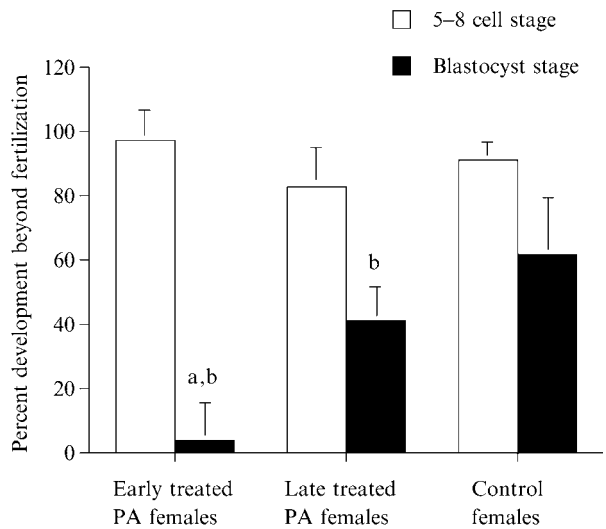

Fig. 16.4 Mean (upper 95% confidence limit) percentage of zygotes developing to the 5–8 cell (open bars) and blastocyst (solid bars) stages in five early treated, five late treated, and five control adult female rhesus monkeys following ovarian hyperstimulation for in vitro fertilization. Primate embryonic genome activation occurs after the 5–8 cell stage (Edwards 1997). (a) $p < 0.05$ versus control and late treated prenatally androgenized females at the same stage; (b) $p < 0.05$ versus 5–8 cell stage. Data are modified from Dumesic *et al.* (2002).

intrafollicular progesterone : estradiol ratio following combined rhFSH/rhCG therapy. Early treated females exhibit a pronounced impairment in embryonic development after the fetal genome activation stage (5–8 cells), with most embryos failing to form blastocoeles, suggesting as in humans that both the absolute amount of estradiol and the progesterone to estradiol ratio in the follicle affects oocyte development (Kreiner *et al.* 1987, Rom *et al.* 1987). Furthermore, all prenatally androgenized females receiving combined rhFSH/rhCG therapy for IVF exhibit abnormal steroidogenesis in follicles containing mature oocytes that fertilize and develop to blastocysts, raising concerns regarding the potential adverse effects of any prenatal androgen exposure on oocyte development beyond the blastocyst stage.

Equally important, abnormal follicle differentiation and oocyte maturation in early treated, but not late treated, prenatally androgenized females undergoing rhFSH therapy followed by rhCG administration is associated with LH hypersecretion and the inability to normally suppress serum insulin levels between day one of rhFSH treatment and the day of oocyte retrieval (Dumesic *et al.* 2002). The combination of these metabolic and reproductive abnormalities in early treated prenatally androgenized females has important implications on folliculogenesis because insulin enhances FSH-induced upregulation of LH receptors in granulosa

cells, thereby increasing their ability to produce progesterone in response to LH (Willis and Franks 1995, Willis *et al.* 1996, Eppig *et al.* 1998). Therefore, timing of prenatal androgen excess also might influence susceptibility of the oocyte to androgen programming in utero by altering follicle differentiation through metabolic and/or neuroendocrine dysfunction.

Metabolic traits in PCOS

Insulin resistance and impaired pancreatic beta cell function are central to the development of type 2 diabetes (DeFronzo 1992). Increased adiposity in PCOS women compounds the risk of glucose abnormalities such that ~50% of PCOS women have impaired glucose tolerance or type 2 diabetes by the fourth decade of life (Legro *et al.* 1999). Fat accumulation in the abdominal compartment is particularly deleterious to metabolic function (Wagenknecht *et al.* 2003). Prenatally androgenized female monkeys harbor similar metabolic problems as PCOS women (Eisner *et al.* 2000). Although early treated prenatally androgenized females exhibit insulin resistance and impaired insulin secretion, females exposed in late gestation have preserved insulin secretory capacity and show decrements in insulin sensitivity only with increasing body mass index (BMI) (Eisner *et al.* 2000, Abbott *et al.* 2005). Consistent with a more severe metabolic phenotype, early treated prenatally androgenized females accumulate more visceral fat than controls, as measured by computed tomography combined with dual x-ray absorptiometry, even when corrected for BMI and total body fatness (Eisner *et al.* 2003). Furthermore, early treated prenatally androgenized females exhibit a greater circulating concentration of free fatty acids than controls during a frequently sampled intravenous glucose tolerance test (Abbott *et al.* 2002c).

Amelioration of impaired insulin action has beneficial glucoregulatory effects in both women with PCOS and prenatally androgenized female monkeys. Metformin and a variety of thiazolidinediones (trogliatzone, pioglitazone, and rosiglitazone) improve insulin action and glucose regulation in women with PCOS (Dunaif *et al.* 1996, Nestler and Jakubowicz 1996, Baillargeon *et al.* 2004, Brettenthaler *et al.* 2004). Initial studies suggest that pioglitazone therapy also might improve insulin action and glucose regulation in both early and late treated prenatally androgenized monkeys (Abbott *et al.* 2004). Overall, these findings raise the possibilities that (1) insulin secretion and insulin action are perturbed in prenatally androgenized female rhesus monkeys and in PCOS women, and (2) such insulin impairments in both species can develop from preferential visceral fat accumulation and can be ameliorated by insulin sensitizing agents. Such parallels in metabolic dysfunction between prenatally androgenized female monkeys and women with PCOS provide evidence in support of fetal androgen excess programming of metabolic function in both cases.

Males

Close male relatives of women with PCOS demonstrate metabolic abnormalities, including an increased prevalence of impaired glucose tolerance and diabetes, hyperinsulinemia (Fox 1999, Colilla *et al.* 2001, Sir-Petermann *et al.* 2002a, Yildiz *et al.* 2003), and endothelial dysfunction (Kaushal *et al.* 2004), suggesting that the metabolic deficits of PCOS are not female-specific. Prenatal androgen excess may therefore reprogram metabolic function regardless of sex. Consistent with this, male rhesus monkeys exposed to testosterone excess in utero develop metabolic abnormalities similar to those seen in prenatally androgenized females (Bruns *et al.* 2004). Adult male monkeys, exposed to testosterone excess during various stages of gestation, demonstrate insulin resistance and impaired pancreatic beta cell function compared to age and size matched controls. Unlike prenatally androgenized females, exposed males do not exhibit elevations in basal androgens, though provocative testing has not been explored. Recent evidence, however, supports that some brothers of PCOS women exhibit adrenal hyperandrogenism (Legro *et al.* 2002) and exaggerated 17α-hydroxylase activity after leuprolide administration (Sir-Petermann *et al.* 2002b). Therefore, prenatal hyperandrogenism may be central to the pathogenesis of the metabolic deficits in PCOS women and their close male relatives, as has recently been shown in prenatally androgenized male and female monkeys (Bruns *et al.* 2004).

Fetal programming hypothesis for PCOS

Fetal androgen excess may, therefore, provide the elusive etiology for PCOS in women, and the metabolic defects found in their close male kin. Prenatally androgenized female rhesus monkeys manifest many of the adult traits found in PCOS women, regardless of the timing of gestational exposure. Similar fetal exposure in male monkeys results in metabolic defects found in male close relatives of PCOS women. Variation in the gestational timing of androgen exposure may also contribute to variation in adult phenotype. Females exposed to androgen excess during early gestation exhibit a greater preponderance of reproductive and metabolic PCOS traits when compared to females exposed during late gestation (Tables 16.1 and 16.3). This more pronounced expression of PCOS in early treated females may reflect their exposure to androgen excess when many fetal organ systems are in the midst of differentiation (Abbott *et al.* 2005). Late treated females, in contrast, are exposed to androgen excess when most organ systems have completed differentiation, but are still undergoing functional maturation. It is, therefore, not surprising that these two different gestational exposures to androgen excess yield different programming outcomes, with early gestational exposure changing structure and multiple aspects of

Table 16.3. Summary of reproductive and metabolic PCOS traits demonstrated by early and late treated prenatally androgenized female rhesus monkeys, and metabolic traits of close male relatives of PCOS women exhibited by prenatally androgenized male monkeys

PCOS trait	Prenatally androgenized females		Prenatally androgenized males
	Early treated	Late treated	
Reproductive			
Ovarian (or male extraovarian) hyperandrogenism	Yes	Yes	No hyperandrogenism
Anovulation (or male infertility)	Yes	Yes	Fertility unknown
Enlarged polyfollicular ovaries	Yes	Yes	N/A
LH hypersecretion	Yes	No	Unknown
Reduced steroid negative feedback on LH	Yes	Yes	Unknown
Impaired embryonic development	Yes	Yes	N/A
Metabolic			
Insulin resistance	Yes	No	Yes
Impaired insulin response to glucose	Yes	No	Yes
Hyperglycemia	Yes	Yes	No
Increased type 2 diabetes	Yes	No	No
Increased abdominal fat	Yes	Unknown	Unknown
Hyperlipidemia	Yes	Unknown	Unknown

function, and late gestational exposure limited to modifying maturation (Abbott *et al.* 2005) (Fig. 16.5, Table 16.3). The lack of frank impairments in LH and insulin secretion, and oocyte developmental competence in late treated female monkeys (Table 16.3), may provide the best examples of such differential effects of androgen excess exposure. Such heterogeneity of PCOS phenotype in female rhesus monkeys may help to explain the perplexing heterogeneity in expression of PCOS phenotype in women.

Our overall hypothesis for fetal programming of PCOS traits in early treated prenatally androgenized female rhesus monkeys is illustrated in Fig. 16.5. Androgen excess programming appears to inflict two distinct "hits" on female reproductive and metabolic physiology. Whether much of the reproductive programming is secondary to altered hypothalamic GnRH regulation of pituitary LH secretion, or involves additional abnormalities that are primarily ovarian, remains to be determined. A parallel debate still occurs regarding the basis of PCOS reproductive abnormalities (ovarian component, e.g., Gilling-Smith *et al.* 1997, McCartney *et al.* 2004; hypothalamic component, e.g., McCartney *et al.* 2002, Chhabra *et al.* 2005). An analogous situation may also exist with regards to metabolic

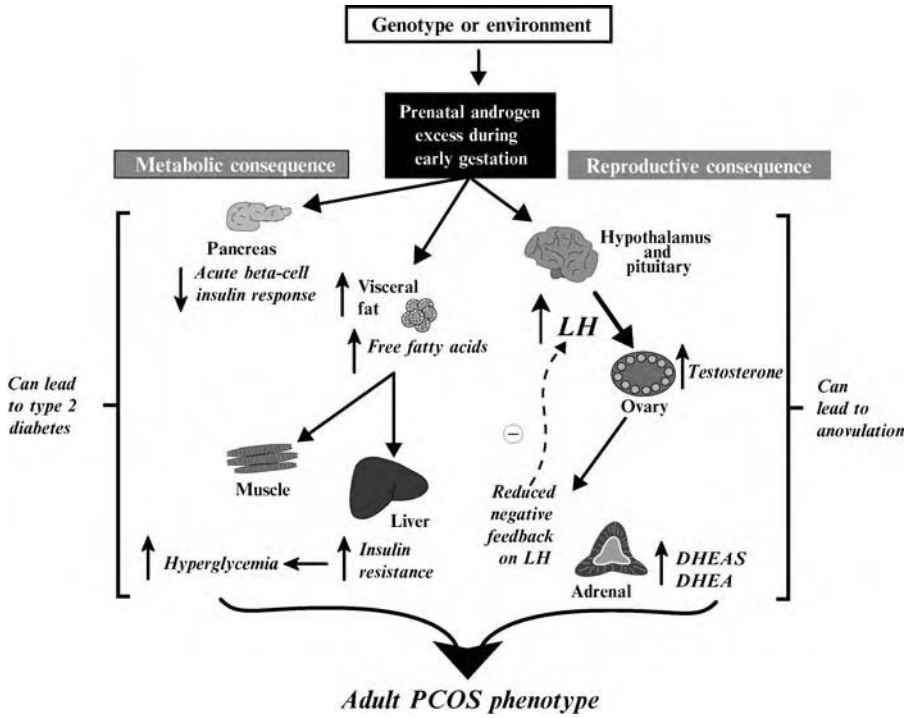

Fig. 16.5 Diagrammatic representation of our hypothesis for early gestation fetal androgen excess programming of adult PCOS traits. Genetic or environmental mechanisms induce fetal hyperandrogenism (see text) that result in permanent changes in both reproductive and metabolic function. Reproductive consequences include: (1) altered hypothalamic–pituitary function leading to LH hypersecretion, (2) ovarian hyperandrogenism that may or may not be the result of LH hypersecretion, (3) reduced steroid hormone negative feedback regulation of LH, which may be a component of the initial permanent alteration in hypothalamic–pituitary function, (4) adrenal hyperandrogenism, and (5) increased anovulation. Metabolic consequences include: (1) increased abdominal adiposity that may be responsible for increased circulating total free fatty acid levels, (2) impaired pancreatic insulin secretory response to glucose, (3) impaired insulin action and compensatory hyperinsulinemia, (4) hyperglycemia, and (5) increased incidence of type 2 diabetes. Insulin resistance and compensatory hyperinsulinemia may be functionally implicated in the anovulatory mechanism. DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate.

programming: is it secondary to altered abdominal adiposity, or does it involve additional abnormalities that are either primarily pancreatic or insulin receptor mediated? Whichever is the case, the basis for fetal androgen excess in PCOS women may still be determined by genetic or environmental mechanisms, or by a combination of both (Abbott *et al.* 2002a) (Fig. 16.5). Our recent findings in prenatally androgenized male rhesus monkeys (Bruns *et al.* 2004) (Table 16.3)

raise the intriguing possibility that metabolic abnormalities prevalent in both PCOS women and their male close relatives may have a common fetal origin.

Our fetal androgen excess hypothesis for PCOS agrees with the increased prevalence of PCOS in women with fetal androgen excess disorders, including classical congenital adrenal hyperplasia from 21-hydroxylase deficiency and congenital adrenal virilizing tumors (Barnes *et al.* 1994, Phocas *et al.* 1995, Merke and Cutler 2001, Stikkelbroeck *et al.* 2003). Such commonality in phenotypic abnormalities provides further support for our contention that altered fetal environment may affect differentiation in the female fetus, permanently programming physiology and modifying genetic susceptibility to pathology after birth. Our hypothesis also agrees with the increased prevalence of insulin resistance in men with a possible fetal androgen disorder from 21-hydroxylase deficiency (Hautanen *et al.* 1997), and may apply to male close relatives of women with PCOS. Certainly, fetal female androgen excess may occur with reasonable frequency in humans, since umbilical cord sampling of 10 human female fetuses during early gestation showed that 40% exhibited high serum testosterone levels well into the normal fetal male range (Beck-Peccoz *et al.* 1991).

The prenatally androgenized female and male rhesus monkeys, as models for PCOS women and their male close relatives, respectively, implicate hyperandrogenism (or its fetal consequence) during critical exposure times of primate fetal development in the pathogenesis of the adult PCOS phenotype in women and metabolic derangements in their male kin. The hypothesis that genetically or environmentally determined hyperandrogenism (Fig. 16.5), beginning in intrauterine life, programs human fetal development for PCOS, and its male equivalent, in adulthood, opens new directions in the treatment of PCOS through increased understanding of hormonal environmental disruption during intrauterine life and how it programs target tissue differentiation in the developing fetuses of both sexes.

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17

Adrenocortical dysfunction in polycystic ovary syndrome

Bulent O. Yildiz and Ricardo Azziz

Introduction

Androgen excess is the most common endocrine disorder of reproductive-aged women, with the majority of patients having a functional abnormality, namely polycystic ovary syndrome (PCOS) (Azziz *et al.* 2004a). We and others have reported the estimated prevalence of this syndrome to be approximately 6–8% (Knochenhauer *et al.* 1998, Diamanti-Kandarakis *et al.* 1999, Asunción *et al.* 2000, Azziz *et al.* 2004b), using the 1990 National Institute of Child Health and Human Development (NICHD) conference diagnostic criteria for PCOS (Zawadzki and Dunaif 1992). This conference concluded that the diagnostic criteria of PCOS should include: (1) clinical and/or biochemical signs of hyperandrogenism, (2) oligo-ovulation, and (3) exclusion of other known disorders such as Cushing's syndrome, hyperprolactinemia, and non-classic congenital adrenal hyperplasia (NCAH) (Zawadzki and Dunaif 1992). A recent expert meeting sponsored by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) expanded this definition, noting that PCOS should be diagnosed when at least two of the following three criteria are present: (1) oligo- and/or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, or (3) polycystic ovaries on ultrasonography, after the exclusion of related disorders (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004a, b).

The adrenal androgens (AAs) are primarily secreted by the zonae reticulares of the adrenal cortex, and include dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), Δ^5-androstene-3β, 17β-diol (androstenediol), and 11β-hydroxyandrostenedione (11-OHA_4). While androstenedione (A_4) can also be considered an AA, it is significantly less specific since in adult women approximately 50% of

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this steroid is secreted by the ovary. The adrenal cortex also accounts for about 25% of the circulating testosterone (T) levels. Following, we review the prevalence of AA excess, validity of DHEAS as a measure of AA excess, underlying steroidogenic patterns, effects of extra-adrenal factors on adrenal steroidogenesis, and heritability of AA excess of PCOS.

Epidemiology of adrenal androgen excess in PCOS

While the majority of patients with PCOS demonstrate an ovarian source for their high circulating androgen levels, many also display AA excess suggesting adrenocortical hyperfunction in PCOS (Gallagher *et al.* 1958). For example, serum levels of DHEAS and 11-OHA$_4$ are elevated in 20–50% of patients with PCOS (Wild *et al.* 1983, Hoffman *et al.* 1984, Steinberger *et al.* 1984, Carmina *et al.* 1986, Moran *et al.* 1999a, Kumar *et al.* 2005). However, it is also clear that AAs begin to decline after the age of 30 years, both in normal women and those with PCOS (Azziz and Koulianos 1991, Kumar *et al.* 2005) (Fig. 17.1). In a retrospective study of 145 hyperandrogenic patients with hirsutism and/or oligo-ovulation, we noted that patients with high DHEAS levels were younger, thinner, and more hirsute than hyperandrogenic women with lower DHEAS levels (Moran *et al.* 1999a). These findings suggested that the diagnosis of AA excess probably requires the use of age-adjusted normative values. Furthermore, the impact of race on the prevalence of AA excess in PCOS is unclear. In one report, the prevalence of AA excess among PCOS patients was found to be similar among Italian, US Hispanic American, and Japanese women (Carmina *et al.* 1992).

To determine what the prevalence of AA excess was in PCOS, taking into account race and the age-related changes in AAs, we undertook a study of 213 (27 Black and 186 White) women with PCOS and 182 (88 Black and 94 White) age-matched healthy eumenorrheic non-hirsute women (controls) (Kumar *et al.* 2003). As expected, mean total T, free T, A$_4$, DHEAS and fasting insulin levels, and body mass index (BMI), were higher in women with PCOS than control women. DHEAS levels were significantly lower in Black than White controls, whereas fasting insulin and BMI were higher in Black controls. DHEAS levels decreased similarly with age in control and PCOS women of either race (Fig. 17.1). In control women of both races and Black PCOS women, DHEAS levels did not correlate with BMI, waist-to-hip ratio (WHR), or fasting insulin; alternatively among White women with PCOS, DHEAS levels negatively correlated with BMI and fasting insulin. For each race and age group the upper 95% normative values for log DHEAS was calculated, and the number of PCOS subjects with log DHEAS values above this level were assessed. The prevalence of supranormal DHEAS levels was 33% and 20% among Black and White women with PCOS, respectively, not a

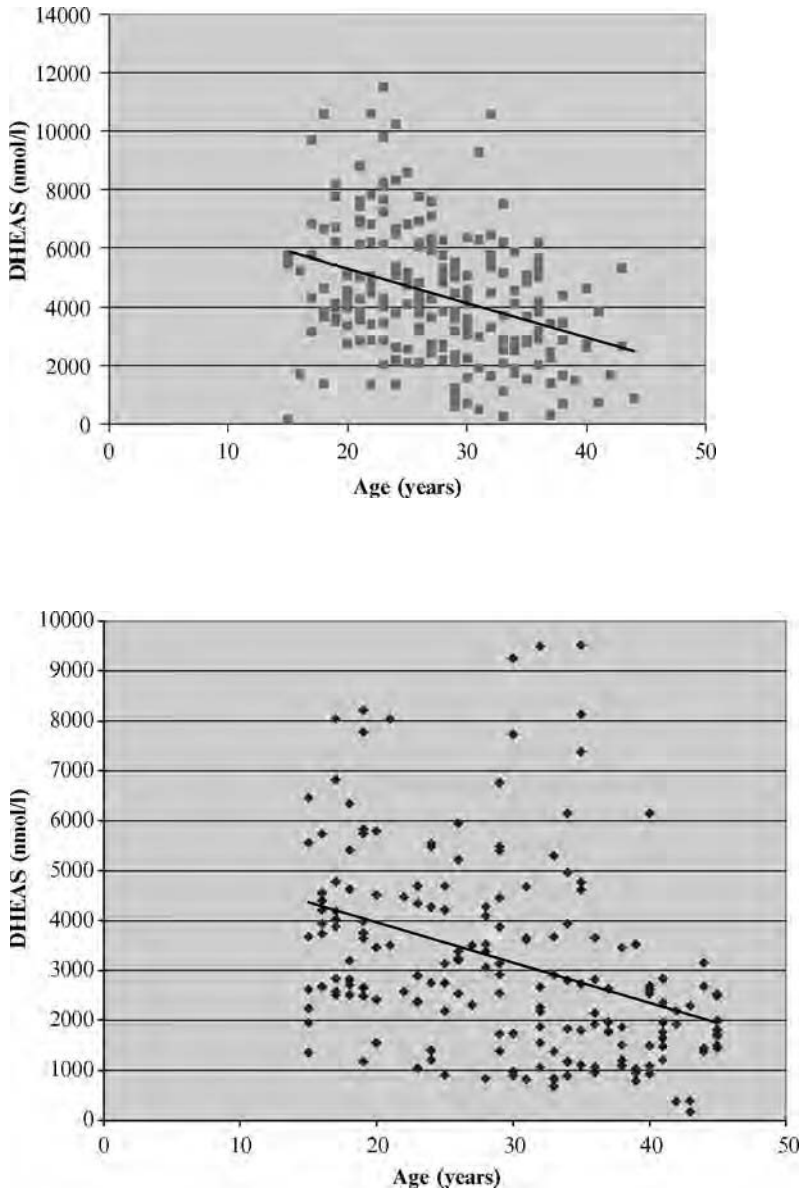

Fig. 17.1 Scatter diagrams and linear regression trend line for dehydroepiandrosterone sulfate (DHEAS) levels according to age in (a) PCOS ($r = -0.34$, $p < 0.0001$) and (b) healthy controls ($r = -0.38$, $p < 0.0001$). Note that DHEAS levels in PCOS and controls decrease with age at similar rates. Reprinted with permission from Kumar *et al.* (2005).

significant difference. Cluster analysis did not reveal any specific subpopulations of DHEAS levels among PCOS patients.

These data indicate that the age-associated decline in DHEAS levels is observable and similar in both control and PCOS women, regardless of race. Furthermore, there may be significant differences in mean DHEAS levels between White and Black control women. Whether these differences are also present among women of other racial/ethnic groups (e.g., Hispanics and Asians) remains to be determined. Since AA secretion appears to have strong genetic component (see below), it is possible that race/ethnicity will play a role in determining the prevalence of AA excess in PCOS. Overall, the prevalence of DHEAS excess is approximately 20% among White and 30% among Black PCOS patients, when using age and race adjusted normative values. The present study also indicates that while BMI and fasting insulin had little impact on circulating DHEAS levels in healthy women, among White PCOS patients these parameters were negatively associated with circulating DHEAS levels. Finally, these data need to be considered in light of the fact that DHEAS levels appear to be only loosely associated with the biosynthesis and secretion of AAs, and are subject to changes in DHEA sulfotransferase (DHEA-ST) activity, which may not affect adrenocortical biosynthesis in a similar fashion (see next section).

Validity of dehydroepiandrosterone sulfate as a measure of adrenocortical dysfunction in PCOS

Clinically, the measurement of circulating levels of the metabolite DHEAS has been traditionally used as the marker for AA excess (Korth-Schutz *et al.* 1976, Lobo *et al.* 1981, Feher *et al.* 1985), since this hormone is: (1) 97–99% of adrenocortical origin (Abraham and Chakmakjian 1973, Abraham 1974, Vermeulen 1983), (2) the second most abundant steroid, after cortisol (F), (3) relatively stable throughout the day and the menstrual cycle (Nieschlag *et al.* 1973, Abraham 1974, Mikulecky *et al.* 1995, Rosenfield *et al.* 1975), due to its relatively long-half life (Baulieu *et al.* 1965, Wang *et al.* 1967, Gant *et al.* 1971, Longcope 1996, Legrain *et al.* 2000), and (4) is easily measured. We should note, however, that DHEAS levels might not always reflect the status of adrenocortical steroidogenesis.

Several studies, including ours, have used DHEAS as a marker of AA secretion in PCOS. However, DHEAS as a marker of AA secretion, particularly in PCOS, should be used with caution, as DHEAS levels may not always reflect alterations in adrenocortical steroidogenesis. First, the relationship of DHEAS to adrenocortical biosynthesis, as measured by the response to adrenocorticotropic hormone (ACTH) stimulation, is generally weak. DHEAS levels are only weakly positively associated with the adrenocortical secretion of 11-deoxycortisol in response to

acute ACTH stimulation (Azziz *et al.* 1991a). DHEAS levels have correlated with the 17-hydroxypregnenolone (17-HPREG) and DHEA response to ACTH stimulation in one study (Azziz *et al.* 1993) but not in another (Moran *et al.* 1999b). Levels of DHEAS were also related to the overall adrenocortical responsivity (degree of response to ACTH stimulation) for A_4, but only weakly with the responsivity of DHEA and F (Azziz *et al.* 1998a).

Second, the response of DHEAS to extra-adrenal factors may differ from that of AA secretion. For example, when oophorectomized women are treated with exogenous T, the DHEAS to DHEA ratio increases without accompanying change in the adrenocortical secretion of DHEA or A_4 in response to ACTH stimulation (Azziz *et al.* 1991b). In addition, PCOS women treated with gonadotropin releasing hormone analog (GnRH-a) suppression experienced a decrease in basal DHEAS levels without accompanying changes in basal DHEA levels, or the steroid response to ACTH stimulation (Azziz *et al.* 1998a). In vitro studies also support the dichotomy of DHEAS and DHEA production. For example, the production of DHEAS, in contrast to that of DHEA, was highly affected by the type of tissue preparation (adrenocortical slices, minces, or cell suspensions) (Hines and Azziz 1999). Insulin within physiologic levels appeared to increase DHEAS production, while alternatively decreasing the secretion of DHEA (Hines *et al.* 2001).

Third, DHEAS levels are not always supranormal in patients with inherited adrenal dysfunction, such as 21-hydroxylase (21-OH) deficient NCAH. For example, we studied 13 patients with untreated 21-OH deficient NCAH and observed that 92.3% and 100% of NCAH patients had A_4 and DHEA basal levels, and 100% had ACTH-stimulated levels of A_4 and DHEA above normal. Alternatively, only 53.8% of patients with NCAH had DHEAS levels above normal (Huerta *et al.* 1999).

Fourth, classifying PCOS patients according to whether they have DHEAS levels above the upper normal limit or not does not clearly distinguish patient groups that have distinct adrenal behaviors. For example, few differences in steroidogenesis, estimated from the steroid response to ACTH stimulation, were observed between PCOS women with and without DHEAS excess (Moran *et al.* 2004). In addition, a decrease in circulating DHEAS levels was observed in PCOS patients treated with a GnRH-a, regardless of whether they had absolute DHEAS excess or not (Azziz *et al.* 1998a), although the decrease was greater in patients with elevated DHEAS levels than in their counterparts (23% vs. 11%, $p < 0.0001$). Likewise, the administration of troglitazone resulted in a decrease in DHEAS levels in PCOS patients regardless of initial circulating DHEAS level (Azziz *et al.* 2003).

Overall, these data suggest that DHEAS levels are loosely associated with the adrenocortical secretion of other adrenal products, basally and in response to

ACTH. However, alterations in DHEAS production may also occur independent of changes in adrenocortical steroidogenesis, possibly due to selective effects on adrenal or hepatic DHEA-ST. These data also caution against the artificial classification of PCOS patients into those with and without AA excess based solely on the circulating DHEAS levels, as adrenocortical function appears to represent a continuum and is generally enhanced in PCOS. Consequently, studies of adrenocortical function in PCOS should consider evaluating alterations in adrenocortical steroidogenesis and metabolism (e.g., DHEA-ST) separately.

In vivo characterization of adrenocortical steroidogenesis in androgen excess and PCOS

Adrenal androgen excess in PCOS may represent dysregulation of adrenocortical biosynthesis, principally in response to ACTH stimulation, or abnormalities in the metabolism of adrenal products, including DHEA and cortisol. We have already reviewed potential abnormalities of DHEA sulfation, and how this may impact on our assessment of AA excess in PCOS. Below we review potential abnormalities in steroidogenesis or metabolism of adrenal products.

Abnormalities of adrenocortical biosynthesis in PCOS

Adrenocorticotropic hormone stimulates the adrenocortical release of androgens and glucocorticoids in vivo (Azziz *et al.* 1990) and in vitro (Hines and Azziz 1999). To obtain an estimation of the relative adrenocortical enzymatic activities in vivo, acute adrenal stimulation by intravenous or intramuscular administration of ACTH-(1–24) is currently the preferred method. Because there is an excess of ACTH receptors within the adrenal cortex, maximal adrenocortical secretion can be obtained with the acute administration of ACTH-(1–24) doses as low as 0.01 mg (Graybeal and Fang 1985). Hence, commercially available doses of 0.25 mg provide maximum adrenal stimulation, regardless of body weight (Azziz *et al.* 1990).

The interpretation of the acute ACTH stimulation test results requires various assumptions: (1) steroidogenesis follows a two-dimensional cascade, (2) the enzyme in question is the only limiting factor, (3) ACTH acts primarily at the cholesterol cleavage site, (4) there is unlimited hormonal precursor, (5) the clearance of measured steroids does not vary over test time, (6) the test is reproducible, and (7) the serum concentrations of steroids reflect intra-adrenal gradients (Azziz 1997). Obviously, many of these assumptions are not absolute, and appropriate interpretation of the ACTH stimulation test results requires recognizing these limitations. However, overall estimating adrenocortical enzymatic activity by

measuring the circulating steroid levels during the acute administration of ACTH is a useful tool in the study of adrenal function in women with androgen excess.

Women with PCOS demonstrate a generalized hypersecretion of adrenocortical products, basally and in response to ACTH, including pregnenolone (PREG), 17-HPREG, DHEA, A_4, 11-deoxycortisol (S), and possibly F (Azziz *et al.* 1991a, 1993, 1998b, Moran *et al.* 1999b, 2004). Circulating DHEAS levels are positively, albeit weakly, associated with the degree of hypersecretion. The adrenocortical dysfunction observed does not appear to be the result of genetic defects affecting 21-OH (P450c21, encoded by *CYP21*) (Azziz *et al.* 1991c, Azziz and Owerbach 1995), 11-OH (P450c11, encoded by *CYP11B1*) (Joehrer *et al.* 1997), or 3β-hydroxysteroid dehydrogenase (3β-HSD) (encoded by *HSD3B2*) (Lutfallah *et al.* 2002, Carbunaru *et al.* 2004). Previous impressions that AA excess in PCOS was due to subtle enzymatic deficiencies might be the result of investigators studying only a limited number of steroids during the ACTH stimulation, without the benefit of confirmatory molecular genetic studies. In fact, the single steroidogenic difference observed between PCOS women and healthy controls appears to be a greater estimated $\Delta^5$17-OH activity (Azziz and Owerbach 1995), primarily observed in PCOS patients with high DHEAS levels (Moran *et al.* 2004). A common polymorphism of *CYP17*, the gene encoding P450c17, did not appear to have an important modulatory role on circulating DHEAS levels (Kahsar-Miller *et al.* 2004), although more extensive analysis of this gene for other potential variations remains to be performed.

It is possible that AA excess in PCOS is due to a change in the responsiveness or sensitivity of the adrenal cortex to ACTH stimulation. We studied the hypothalamic–pituitary–adrenal (HPA) axis sensitivity and responsivity to stimulation in 12 PCOS women with and 12 without DHEAS excess, and in 12 controls. Each subject underwent an acute 90-min ovine corticotropin releasing hormone (oCRH) stimulation test (1 μg/kg) and an 8-h incremental intravenous stimulation with ACTH-(1–24), with doses ranging from 20 to 2880 ng/1.5 m^2 per hour, and a final bolus of 0.25 mg (Azziz *et al.* 1998b). During the acute oCRH stimulation test no significant differences in the net maximal response (i.e., change from baseline to peak level) for ACTH, DHEA, A_4, or F were observed; although the area under the curve (AUC) for the DHEA response was higher among PCOS women with DHEAS excess compared to those patients without DHEAS excess or healthy controls (Fig. 17.2). We did not observe a difference in the sensitivity (i.e., threshold or minimal stimulatory dose) to ACTH between the groups for any of the steroids measured (Fig. 17.3). The mean responsivity (slope of response to incremental ACTH stimulation) of A_4, and the net maximal and overall (i.e., AUC) response of DHEA, was greater in PCOS patients with DHEAS excess.

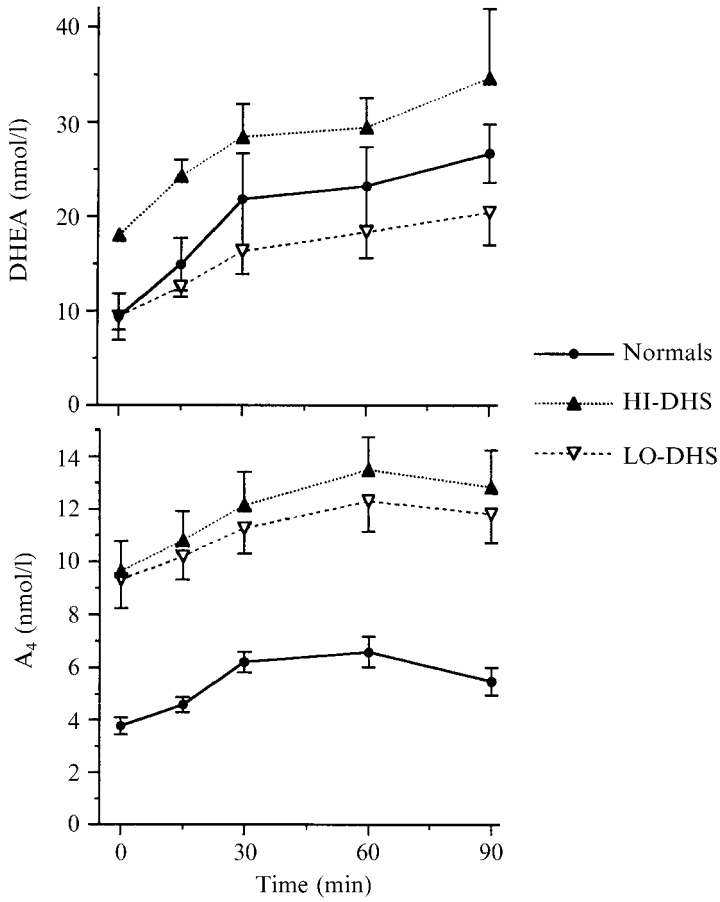

Fig. 17.2 The mean (±SD) androstenedione (A_4) and dehydroepiandrosterone (DHEA) response curves during acute oCRH (1μg/kg) stimulation in PCOS women with excess DHEAS (HI-DHS) ($n = 12$) and PCOS women without excess DHEAS (LO-DHS) ($n = 12$) and in age and weight matched normal controls ($n = 11$). Reprinted with permission from Azziz *et al.* (1998b).

Abnormalities in the metabolism of cortisol in PCOS

An interesting proposition is that AA excess and the enhanced adrenocortical function observed in PCOS is the result of increased peripheral metabolism of cortisol. The principal pathways for metabolism of cortisol include irreversible hepatic inactivation by 5α-reductase (5α-RA) type 1 and 5β-reductase (5β-RA), and reversible interconversion with cortisone catalyzed by 11β-HSD. Stewart and colleagues studied 11 patients with PCOS, without evidence of 21-OH or 3β-HSD deficiency (Stewart *et al.* 1990). Urinary total cortisol metabolites were higher in patients than controls, with a high ratio of 5α to 5β cortisol metabolites in the urine. These data suggested that the PCOS women studied had abnormal

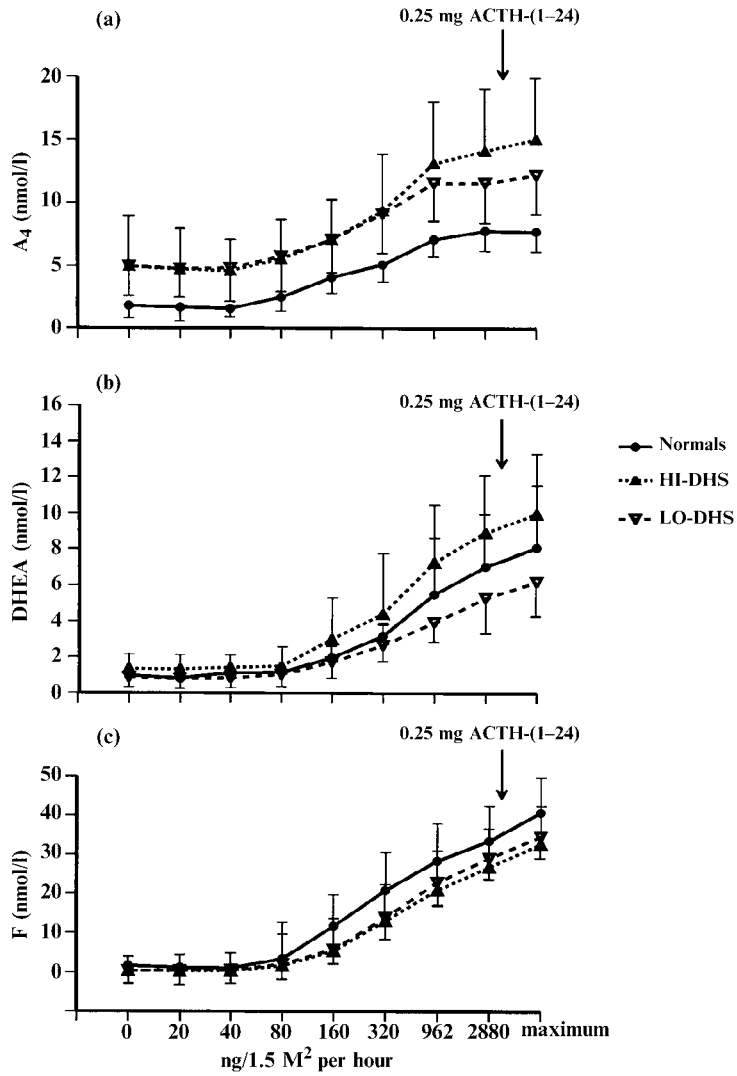

Fig. 17.3 The mean (±SD) androstenedione (A_4), dehydroepiandrosterone (DHEA), and cortisol (F) response curves during incremental ACTH stimulation in PCOS patients with high (DHEAS >3000 ng/ml (HI-DHS) $n = 12$) and low (DHEAS <2750 ng/ml (LO-DHS) $n = 12$) levels of DHEAS, and in age and weight matched normal controls ($n = 11$). (a) Mean A_4 measurements for normal subjects significantly lower ($p < 0.05$) than for HI-DHS or LO-DHS subjects, and mean A_4 values for HI-DHS and LO-DHS subjects not significantly different. (b) Mean DHEA measurements for HI-DHS subjects significantly higher ($p < 0.05$) than for LO-DHS patients, and mean DHEA values for normal subjects not significantly different from HI-DHS or LO-DHS subjects. (c) Mean F measurements for normal subjects significantly higher ($p < 0.05$) than for LO-DHS or HI-DHS women, and mean F values for HI-DHS and LO-DHS subjects not significantly different. Reprinted with permission from Azziz *et al.* (1998b).

cortisol metabolism, potentially indicating enhanced 5α-RA and/or impaired 5β-RA activity. These investigators did not find an abnormality in the interconversion of cortisol to cortisone in their patients. Similar findings were reported by other investigators (Chin *et al.* 2000, Tsilchorozidou *et al.* 2003).

The interconversion of cortisol and hormonally inactive cortisone is catalyzed by at least two isoforms of 11β-HSD. The type 2 11β-HSD (11β-HSD2), a nicotinamide-adenine dinucleotide (NAD)-dependent, unidirectional dehydrogenase found predominantly in the placenta and kidneys, converts cortisol to cortisone; the type 1 enzyme (11β-HSD1), which has both nicotinamide-adenine dinucleotide phosphate (NADP)-dependent dehydrogenase and 11-oxo-reductase activity, and is found predominantly in the liver and gonads, exerts 11-reductase activity converting cortisone back to cortisol (Agarwal *et al.* 1989, 1994). Consequently, dysregulation of the activity 11β-HSD1 could result in decreased cortisol production (i.e., reactivation), and consequently increased adrenocortical function, in PCOS.

Rodin and colleagues measured the 24-h urinary excretion of steroid hormone metabolites by high-resolution capillary gas chromatography in 65 women with PCOS and 45 normal women matched for BMI (Rodin *et al.* 1994). In contrast to the findings of other investigators (Stewart *et al.* 1990, Chin *et al.* 2000), after adjustment for BMI the urinary excretion of cortisol metabolites was found to be 1.5 and 1.3 times higher in PCOS than in controls. Women with PCOS also had significantly higher ratios of 11-oxo (oxygenated) metabolites to 11-hydroxy metabolites of cortisol and of 11-oxo to 11-hydroxy metabolites of corticosterone. These investigators suggested that their results could be explained by either enhanced 11β-dehydrogenase activity or impaired 11β-reductase activity causing increased oxidation of cortisol to cortisone, which could not be accounted for by obesity. Finally, the observed abnormality in cortisol metabolism could not be explained by the presence of endogenous inhibitors of 11β-HSD1 activity (Walker *et al.* 2000).

Whether these abnormalities represent a primary or a secondary abnormality is unclear. For example, the expression of 11β-HSD1 is highly regulated, including by sex steroids and growth hormone (Low *et al.* 1994) and insulin (Hammami and Siiteri 1991). Stewart suggested that the findings of 11β-HSD1 dysregulation might reflect the increased android obesity of patients with PCOS (Stewart and Edwards 1994). These investigators reported that 11β-HSD1 activity is reduced in patients who have android or central obesity, but not in those with gynoid obesity, regardless of gender (Stewart *et al.* 1999). However, Tsilchorozidou and colleagues (2003) observed both enhanced 5α-RA and reduced 11β-HSD1 activities among 18 lean PCOS women compared with 19 lean controls. Nonetheless, sex steroids may be playing a role in the observed abnormalities of cortisol metabolism. For example, the urinary ratio of 5α-tetrahydrocortisol to total tetrahydrocortisol

in PCOS, reflecting 5α-RA activity, decreased following 3 months of ovarian suppression with a long-acting GnRH-a (Szilagyi *et al.* 2000). Tsilchorozidou and colleagues (2003) observed that insulin seemed to enhance the 5α-reduction of steroids in PCOS, but was not associated with the reduced 11β-HSD1 activities observed in their lean PCOS women. Consequently, it is possible that the observed alterations in cortisol metabolism in PCOS may reflect abnormalities of adiposity, insulin action, or sex steroids in the disorder.

Overall, these data indicate that PCOS women have a generalized adrenocortical hypersecretion of adrenocortical products, basally and in response to ACTH stimulation, similar to the hypersecretion in response to human chorionic gonadotropin (hCG) or GnRH analog observed in the ovary of these patients. The increased responsivity of AAs to ACTH does not appear to be due to altered pituitary responsivity to CRH, or increased sensitivity of the adrenal to ACTH stimulation. Whether it is secondary to increased zonae reticulares mass or to differences in P450c17α activity remains to be determined. However, since the most significant steroidogenic difference between PCOS women with and without DHEAS excess is a greater estimated $\Delta^5$17-OH activity in the former, the possibility that P450c17 function, whether due to inherited or regulatory factors, is abnormal in PCOS and is principally responsible for the AA excess observed is likely. It is also possible that AA excess and the enhanced adrenocortical function observed in PCOS is the result of increased peripheral metabolism of cortisol, either through enhanced 5α-RA or impaired 11β-HSD1 activities. Finally, in general adrenocortical and AA hypersecretion in PCOS is not the result of inherited mutations of *CYP21*, *CYP11B1*, or *HSD3B2*.

Effects of extra-adrenal factors on adrenal steroidogenesis

A number of extra-adrenal factors may play a role in the AA excess of PCOS, including ovarian products, and factors related to insulin action and/or obesity.

Interaction of the ovary and adrenal in PCOS

Adrenocortical dysfunction in PCOS patients may represent an acquired defect secondary to abnormal ovarian secretion. Various investigators, including ourselves, have used ovarian suppression using long-acting GnRH analogs, to elucidate the role of gonadal steroids on adrenocortical function in PCOS. In general, most investigators have observed a 20–25% decrease in mean DHEAS levels following long-acting GnRH-a suppression in PCOS women with elevated levels of this AA, although the elevated AA levels rarely normalize (Gonzalez *et al.* 1991, Carmina *et al.* 1995, Fruzzetti *et al.* 1995, Azziz *et al.* 1998a). Overall, only hyperandrogenic patients with elevated levels of DHEAS demonstrated a significant decrease

in basal DHEA levels (Azziz *et al.* 1998a). There also appears to be limited effect of GnRH-a suppression on the adrenal response to acute ACTH stimulation (Azziz *et al.* 1998a). Overall, these data suggest that ovarian factors may be increasing AA secretion in PCOS, possibly including androgens and estrogens.

Studies in hypoestrogenic women have suggested that exogenous estrogen administration can alter the adrenocortical response to ACTH stimulation, although there is little consensus as to the extent, significance, or type of alteration (Gonzalez and Speroff 1990). Ditkoff *et al.* (1995) observed that transdermal estradiol (E_2) replacement for 1 week in long-acting GnRH-a treated PCOS patients with AA excess was sufficient to restore the hyper-responsiveness of androgens to oCRH stimulation. Alternatively, we determined the steroid responses to a continuous incremental ACTH-(1–24) infusion (20–1280 ng/1.5 m^2 per hour), followed by an ACTH-(1–24) bolus of 0.25 mg, before and after 3 months of transdermal E_2 therapy (0.05 mg/day) in 14 postmenopausal women. Estradiol administration had no effect on basal, postdexamethasone, or maximally stimulated serum levels of F, DHEA, A_4, or 17-hydroxyprogesterone (17-HP). Furthermore, E_2 did not affect adrenal sensitivity or responsiveness to ACTH-(1–24) stimulation. Finally, the steroid ratios reflecting 3β-HSD (i.e., the A_4/DHEA ratio) and $\Delta^4$17,20-lyase (i.e., the A_4/17-OHP ratio) activities also were unaffected by E_2 therapy. Consequently, these data suggest that it is unlikely that extra-adrenal estrogens have a significant impact on adrenocortical function, and are unlikely to be responsible for mediating the ovarian effects on DHEAS levels.

Studies in female-to-male (F–M) transsexuals have been conflicting regarding the effect of exogenous testosterone. One set of investigators noted no change in four subjects after 6–12 months of testosterone enanthate treatment (Futterweit *et al.* 1992), and another noted an increase in the response of F, A_4, and DHEA to ACTH stimulation in 20 F–M transsexuals (Polderman *et al.* 1994). We prospectively studied the effect of 3 weeks of exogenous T administered in healthy oophorectomized women (Azziz *et al.* 1991b). A significant change in adrenocortical steroidogenesis was not observed, with the exception of an apparent increase in the metabolism of DHEA to DHEAS suggesting that T increases DHEA-ST activity.

These data suggest that ovarian sex steroids contribute modestly to circulating DHEAS levels (10–20%), possibly by enhancing hepatic or adrenal DHEA-ST activity, with limited change in the adrenocortical response to stimulation. Consistent with this hypothesis, in female but not male rats, gonadectomy decreases the amount of DHEA-ST messenger RNA (mRNA) in liver (Labrie *et al.* 1994). It is unclear what mediates this effect. Overall, the ovary appears to have a limited impact on the adrenal in PCOS, with the exception of a possible effect on DHEA-ST.

Effect of abnormalities of the glucose–insulin axis

Approximately 50–70% of PCOS patients have insulin resistance and hyperinsulinemia (Marin *et al.* 2003), and many have evidence of glucose intolerance (Legro *et al.* 1999). Hyperinsulinemia in PCOS stimulates androgen secretion by ovarian theca cells and increases the hormonally active free androgen fraction by reducing the hepatic production of sex hormone binding globulin (SHBG) (Dunaif 1997). Consequently, it is possible that factors relating to, or arising from, the metabolic abnormalities observed in PCOS may also result in the adrenocortical abnormalities observed in these patients.

Insulin resistance in young women generally results in the development of compensatory hyperinsulinemia, which results in the stimulation of androgen secretion by the ovary (Barbieri *et al.* 1986, Nestler *et al.* 1987), and perhaps by the adrenal. However, insulin levels appear to be negatively correlated with, or to actually acutely suppress, circulating DHEAS levels in normal women (Nestler *et al.* 1987) and men (Nestler *et al.* 1989, 1994), and diabetic subjects (Yamaguchi *et al.* 1998). Nonetheless, Nestler and colleagues subsequently indicated that insulin suppression by diazoxide or weight loss had different effects in men, where a decrease in circulating insulin resulted in an increase in DHEAS levels, and in women, where no change was observed (Beer *et al.* 1994, Jakubowicz *et al.* 1995).

Studying 213 consecutive untreated women with PCOS and 165 age and race matched healthy eumenorrheic non-hirsute controls, we were unable to detect a significant association between circulating DHEAS and fasting insulin levels (Kumar *et al.* 2003). However, these cross-sectional data do not preclude the possibility of a more subtle effect of insulin or other insulin resistance associated factors on other AAs or adrenocortical steroidogenesis. We compared seven hyperandrogenic severely hyperinsulinemic (i.e., peak insulin levels during a 2-h oral glucose tolerance test (OGTT) $>$ 500 μU/ml) women with eight hyperandrogenic normoinsulinemic (i.e., peak insulin levels during a 2-h OGTT $<$ 190 μU/ml) patients and nine healthy BMI matched controls (Azziz *et al.* 1995). Basal A_4, DHEA, and DHEAS circulating levels were higher in the severely hyperinsulinemic patients, although the difference in DHEA did not reach statistical significance. Alternatively, the mean peak post-stimulation A_4 level was higher in normoinsulinemic compared to the hyperinsulinemic hyperandrogenic patients (11.0 ± 2.0 vs. 8.1 ± 2.1 ng/ml, respectively; $p < 0.02$). There was no significant difference before and after ACTH stimulation for any of the other steroids measured. Lanzone and colleagues studied 11 normoinsulinemic and 21 hyperinsulinemic PCOS patients but did not observe differences in the baseline levels of DHEAS (DHEA was not studied) between the two groups (Lanzone *et al.* 1992). Although, the response of F, DHEAS, and T to acute ACTH stimulation did not differ between the groups, peak post-stimulation A_4 levels, in contrast to our

results, were higher in hyperinsulinemic compared to normoinsulinemic subjects. The difference in results between these studies may rest, at least in part, on the small number of subjects studied.

In order to determine whether more subtle abnormalities of insulin action are related to the adrenocortical dysfunction of PCOS Falcone and colleagues studied 19 women with PCOS and nine age and weight matched controls using the tolbutamide-modified frequently sampled intravenous glucose tolerance test (FSIVGTT) analyzed by the minimal model (Falcone *et al.* 1990). A significant decline in DHEA levels were observed in control subjects, and in PCOS women with normal insulin sensitivity, 3 h after glucose administration. Alternatively, no significant change in DHEA occurred in insulin resistant PCOS subjects. DHEAS levels were not measured. It was hypothesized that the failure of glucose-stimulated endogenous insulin secretion to significantly depress DHEA levels in insulin resistant women with PCOS may account in part for their androgen excess.

We studied nine reproductive-aged patients with PCOS and nine age, race, and BMI matched controls with an insulin-modified FSIVGTT and an acute 60-min ACTH-(1–24) stimulation test (Farah-Eways *et al.* 2004). The fasting insulin and fasting glucose levels were not correlated to any of the adrenal parameters studied, with the exception of a positive association between the basal 17-HP and the fasting glucose level ($r = 0.85$, $p < 0.004$). The insulin sensitivity index (S_I) and the acute insulin response to glucose (AIR_G) had a limited correlation with adrenocortical parameters in both groups. Alternatively, glucose effectiveness (S_G), a measure of the ability of glucose to control its own production/uptake (i.e., glucose-mediated glucose disposal), was positively associated in PCOS patients with the basal levels of F, DHEA, and DHEAS, the ACTH-stimulated peak levels of F, DHEA, and 17-HPREG, and the net increment following ACTH administration for F, DHEA, and 17-HPREG. No such relationship was observed in control women. These data suggest that adrenocortical biosynthesis, basally and in response to ACTH, may be more closely associated with glucose-mediated glucose disposal than with the degree of hyperinsulinemia or the sensitivity of insulin-mediated glucose disposal.

Additional evidence that insulin resistance associated factors play in regulating adrenocortical biosynthesis arises from studies examining the effect of insulin sensitizers. We studied 305 PCOS women randomized to receive placebo, or the insulin sensitizer troglitazone in doses of 150 mg/day, 300 mg/day, or 600 mg/day, for 20 weeks (Azziz *et al.* 2003). In these women the circulating DHEAS levels decreased 18–26% with troglitazone 600 mg/day, regardless of basal DHEAS level (Fig. 17.4). Guido and colleagues treated 11 obese PCOS women with another thiazolidinedione, pioglitazone, 45 mg/day for 6 months and observed that the peak post-ACTH stimulation levels of 17-HP and A_4 were lower after treatment,

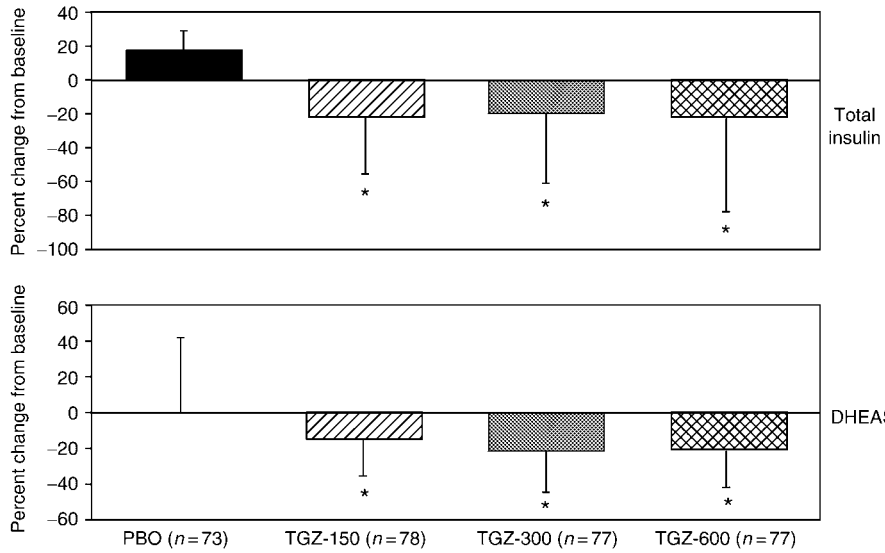

Fig. 17.4 Change in basal fasting total insulin and DHEAS levels with placebo (PBO) or troglitazone 150 mg/day (TGZ-150), 300 mg/day (TGZ-300), or 600 mg/day (TGZ-600). Mean ± SD noted; *, $p < 0.05$. Reprinted with permission from Azziz *et al.* (2003).

although there was no change in the basal or ACTH-stimulated DHEAS or F levels (Guido *et al.* 2004). These investigators did not assess DHEA. Furthermore, in this study the net increment in steroids following ACTH stimulation was not calculated, an important omission as changes in the basal levels of steroids arising from the suppression of ovarian androgen production in response to decreasing insulin levels can also result in decreased peak levels following ACTH stimulation. In fact, in this study a measurable decrease in basal A_4 levels with pioglitazone treatment was observed.

It is possible that the effect of thiazolidinediones observed on steroid levels may be the result of direct inhibition of steroid biosynthesis by these drugs (Arlt *et al.* 2001). Alternatively, metformin does not appear to have this effect. La Marca and colleagues studied 14 women with PCOS before and 30 days after treatment with metformin 1500 mg/day (la Marca *et al.* 1999). These investigators noted that metformin treatment did not result in a significant change in the basal F, A_4, and DHEAS levels, nor in the net increment in F. However, significant reductions occurred in the secretion of A_4, and 17-HP in response to ACTH stimulation. DHEA was not measured. In another study 15 adolescents with PCOS and impaired glucose tolerance received 3 months of metformin 1500 mg/day (Arslanian *et al.* 2002). In this study, no change in circulating DHEAS levels was observed. However, the net increases in A_4, 17-HP, and 17-HPREG following

acute ACTH stimulation was also lower after metformin treatment. Again, DHEA was not measured.

Finally, in a small preliminary study using human adrenal minces we observed that insulin in physiologic levels resulted in an increase in DHEAS, and a decrease in DHEA, with no change in F secretion (Hines *et al.* 2001). These data suggest that insulin may primarily increase the activity of DHEA-ST. Other investigators using primary cultures of human adrenocortical cells from donors of ages 19–77 years observed that insulin at physiological concentrations increased the mRNA levels for p450c17α and HSD3B2 (Kristiansen *et al.* 1997). Insulin had lesser effects on p450c21 and p450scc mRNA levels, and was without effect on CYP11A mRNA. Consistent with our findings, the effects of insulin were accompanied by decreases in the ratio of DHEA/F synthesized from PREG by the cultures. Taken together these in vitro data suggest that, contrary to what is observed in vivo, insulin may actually suppress the production of DHEA, possibly increasing its conversion to DHEAS.

Overall, there are conflicting data regarding the interaction between the insulin–glucose axis and adrenocortical dysfunction in PCOS. Insulin itself may have a modest stimulatory effect on DHEA-ST, but may actually result in steroidogenic changes reducing the adrenal production of DHEA. Preliminarily, insulin-mediated glucose disposal (i.e., insulin sensitivity) appears to play a limited role in the adrenocortical hyper-responsiveness of PCOS. Alternatively, our data suggests the novel concept that adrenocortical dysfunction in PCOS may be more closely linked to those mechanisms underlying glucose-mediated glucose disposal (i.e., glucose effectiveness).

Effect of obesity on adrenocortical steroidogenesis

Obesity may impact on adrenocortical function by decreasing insulin sensitivity and increasing circulating insulin levels, as discussed above. However, obesity may also alter adrenal function through the secretion of adipocytokines and other inflammatory products (Ehrhart-Bornstein *et al.* 1998), and by increasing the circulating levels of estrogens (see above), through increased aromatization by adipose tissue stromal cells (Ackerman *et al.* 1981, Forney *et al.* 1981, Bulun and Simpson 1994).

In order to determine the effect of obesity on adrenocortical biosynthesis in vivo, we studied 30 healthy, eumenorrheic, non-hirsute female volunteers weighing between 90% and 110% of their ideal body weight (IBW) and 27 weighing greater than 120% (obese) their IBW using acute ACTH stimulation (Azziz *et al.* 1991d). Obese volunteers demonstrated higher free T levels and a higher DHEAS : DHEA ratio. With ACTH stimulation the net increment in A_4 was almost twofold higher in obese volunteers ($p < 0.001$), although no other differences were observed in either basal or adrenal response measures. In agreement, Brody *et al.* (1987) noted among

29 postmenopausal women that degree of obesity was correlated to the net response of DHEA to ACTH stimulation. Komindr and colleagues (1986) studied 10 normal and 16 obese eumenorrheic non-hirsute women matched for age, and reported that the mean DHEA response slope was significantly raised, and the minimal ACTH threshold dose for A_4 was significantly lower (increased sensitivity), in obese women. Overall F responses were no different between obese and normal weight subjects.

These results contrast with those of Vicennati and colleagues (1998), who studied 12 women with abdominal and 13 with peripheral obesity, and seven healthy normal-weight women. Although basal F levels were similar, the response of F (as AUC) to ACTH stimulation was higher in women with abdominal obesity than in the other groups. Alternatively, there were no significant differences in basal and stimulated serum levels of DHEA, A_4, and 17-HP among the three groups, and no significant correlation was observed between basal and stimulated androgen levels and BMI. In another study, the A_4 response to ACTH stimulation was also not correlated with the BMI in healthy women (Rittmaster *et al.* 1993). A number of other investigators have observed a greater degree of cortisol secretion and metabolism in obese subjects with visceral adiposity (Vicennati *et al.* 1998, Stewart *et al.* 1999, Vicennati and Pasquali 2000).

These findings suggest that the adrenal in obese healthy women may hypersecrete F, and possibly A_4 and DHEA, in response to ACTH, although not all investigators agree. Whether this is due to the higher circulating levels of insulin (see above) in obese individuals, or whether this reflects the effects of adipocyte-generated adipocytokines or similar factors, remains unknown. Future studies need to more clearly document the effect of obesity on adrenocortical function, including the production and metabolism of glucocorticoids, the effect of body fat distribution, the effect of weight loss, the mechanisms underlying such relationships, and its role in the adrenocortical hyper-responsivity of PCOS.

Heritability of adrenal steroidogenesis in PCOS

There is growing evidence that inheritance plays a significant role in determining the circulating AA levels in normal individuals (Akamine *et al.* 1980, Rotter *et al.* 1985, Meikle *et al.* 1988, Rice *et al.* 1993). Circulating AA levels and their response to ACTH are highly individualized, compared to the secretion of glucocorticoids in normal women (Azziz *et al.* 2001). For example, both basal and ACTH-stimulated levels of DHEA demonstrated a high level of between-subject variability (60–70%) compared to a between-subject variability for F of 15–40% (Azziz *et al.* 2001). Various investigators have observed a significant degree of heritability for the AA metabolite DHEAS (Rotter *et al.* 1985), with the relative basal levels of DHEA and DHEAS varying little over time (Thomas *et al.* 1994, Nafziger *et al.*

1998). Consequently, it appears that heritability plays a significant role in determining the circulating levels of AAs. Whether this represents the inheritance of factors regulating adrenocortical biosynthesis, or of factors determining AA metabolism or clearance is unclear.

Adrenal androgen levels among the general population appear to be highly variable, and this variability may reflect variations in the inherited ability of the adrenal to secrete AAs in response to ACTH. To begin testing this hypothesis we studied the between-subject variability of the response of DHEA and F to acute ACTH stimulation in 56 healthy eumenorrheic non-hirsute healthy women (Azziz *et al.* 2001). After controlling for age, basal F, but not DHEA, was negatively correlated to its net increment or response ($r = -0.54$; $p < 0.001$). The coefficients of variation between subjects were 61.4% and 16.9%, for the peak DHEA and F levels 60 min after stimulation, respectively (Fig. 17.5). We concluded that among normal women, the ability to secrete AA (i.e., DHEA) varies widely compared to F, and that this between-subject variability in DHEA levels is, at least in part, due to a variable response of AAs to ACTH stimulation. These data suggest that there is a wide variation in the ability of the adrenal to secrete DHEA, basally and in response to ACTH, compared to that of F. Whether the population variance in the production of DHEA is due to the effect of inherited factors, or alternatively due to the ability of extra-adrenal factors to affect AA secretion, and whether the AA excess frequently observed in PCOS is due to the greater risk of those women with higher AA levels to develop the disorder, remains to be determined.

Another approach to determining whether AA secretion, basally or in response to ACTH, represents an inherited factor is to determine whether it is relatively constant in an individual over time. We have noted that the secretion of DHEA in response to ACTH was widely variable in the normal population (see above) (Azziz *et al.* 2001). To determine the stability of adrenocortical steroidogenesis, at least as measured by the acute response to ACTH stimulation, we initially studied 10 healthy volunteers, and noted that over an average of 4.0 ACTH stimulations, performed monthly, the variability for ACTH-stimulated values of A_4 and F was only ~10%, while that of DHEA was ~20% (Azziz *et al.* 1990). More recently, we prospectively studied 23 untreated PCOS patients (age 29.3 ± 8.1 yrs, BMI 33.5 ± 6.3 kg/m^2) and seven age and BMI matched control women on two occasions 3–5 years apart (Yildiz *et al.* 2004). A decrease in mean DHEAS values was observed in PCOS, possibly due to the effect of age on this androgen metabolite. However, a comparison of the initial and repeat studies for both PCOS patients and controls indicated that there were no significant differences in any of the responses of DHEA, A_4, or F over the period of study.

The heritability of AA secretion was also demonstrated by Legro and colleagues (2002) who studied 119 brothers of 87 unrelated women with PCOS

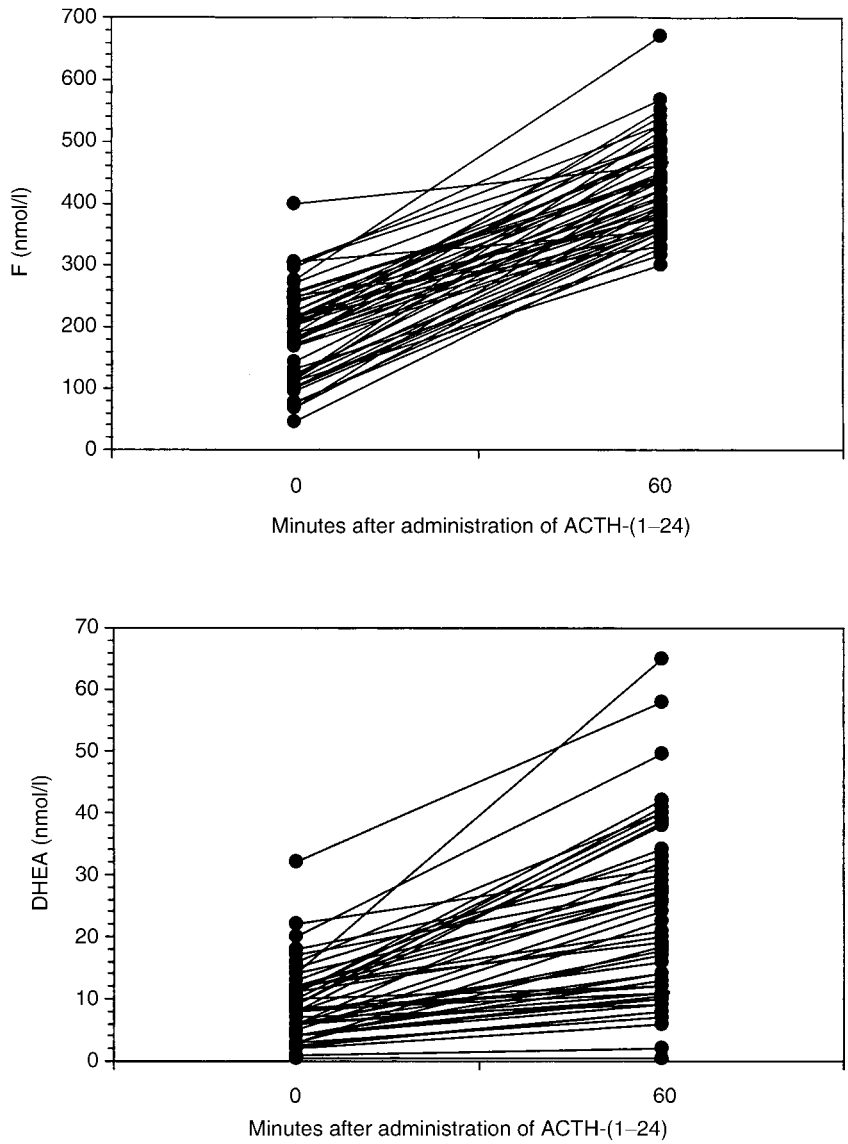

Fig. 17.5 Levels of cortisol (F) and dehydroepiandrosterone (DHEA) before (0 min) and after the administration of 1 mg ACTH-(1–24) intravenously (60 min). Note the wide intersubject variation in the 60-min DHEA levels compared with those of F. Reprinted with permission from Azziz *et al.* (2001).

and 68 weight and ethnicity comparable unrelated control men. Brothers of women with PCOS had significantly elevated DHEAS levels compared to control men, and there was a significant positive linear relationship in DHEAS levels between PCOS probands and their brothers. These data suggested a familial

clustering of elevated DHEAS levels in the families of PCOS women, suggesting that this may reflect an inherited abnormality in the disorder.

Finally, we should indicate that candidate gene analysis to date has not revealed any significant effect of a T to C substitution in the 5′ promoter region of CYP17 (52), the N363S allele representing a variant of the glucocorticoid receptor (GRL) (Kahsar-Miller *et al.* 2000), and a G972R variant of the *IRS-1* gene (Witchel *et al.* 2005). We also did not find an association between eight common mutations of CYP21 and AA excess in PCOS (112), in agreement with others (Dolzan *et al.* 1999).

Overall, these data indicate that a wide variation in the ability of the adrenal to secrete DHEA, basally and in response to ACTH, compared to that of F, exists in the normal population. Consequently, it is possible that those women with a greater ability to secrete AAs in response to ACTH are also at greater risk for developing PCOS, and hence AA excess is over-represented in this population of patients. Adrenal androgen secretion, basally and in response to ACTH stimulation, is relatively constant over time, potentially representing an inherited trait and a risk factor for PCOS. In agreement, girls with premature pubarche and exaggerated AA secretion appear to be at increased risk for developing PCOS in adulthood (Ibañez *et al.* 1993). Candidate gene analysis has been limited, and has yet to yield significant findings.

Summary

A wide variation in the ability of the adrenal to secrete DHEA, basally and in response to ACTH, is present in the normal population. Whether this populational variance in the production of DHEA is due to the effect of inherited factors, or alternatively due to the ability of extra-adrenal factors to affect AA secretion, remains to be determined. However, it is possible that the AA excess frequently observed in PCOS is due to the greater risk of those women with higher AA levels to develop the disorder. Overall, between 20% and 33% of patients with PCOS demonstrate AA excess, as reflected by the circulating DHEAS levels. We should note that age- and possibly race-specific normative values should be used to determine this prevalence. Furthermore, we should note that alterations in DHEAS levels do not necessarily reflect changes in adrenocortical steroidogenesis, possibly due to selective effects of extra-adrenal factors on adrenal or hepatic sulfotransferase. Consequently, studies of adrenocortical function in PCOS should evaluate alterations in adrenocortical steroidogenesis and DHEA-ST separately.

Patients with PCOS demonstrate a generalized increase in circulating DHEAS levels, and a generalized hypersecretion of adrenocortical products, basally and in response to ACTH stimulation, primarily due to hyper-responsivity of AAs to ACTH stimulation and to increased activity of $\Delta^5$17-OH. The mechanisms

underlying this upregulation remain unclear. To date, no specific genetic defects have been identified. The production of AAs in response to ACTH appears to be closely related to those factors regulating glucose-mediated glucose disposal (glucose effectiveness), and somewhat less to the effect of extra-adrenal androgens, insulin resistance, or hyperinsulinemia. In contrast, while DHEAS levels are also strongly associated with glucose effectiveness, the production of this metabolite appears to also be increased by extra-adrenal androgens and insulin, although this regulatory mechanism remains to be demonstrated conclusively. Finally, DHEAS levels and the response of AAs to ACTH stimulation are relatively constant over time and appear to be an inherited trait in PCOS.

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18

Polycystic ovary syndrome in Asian women

Ernest Hung Yu Ng, Carina Chi Wai Chan, and Pak Chung Ho

Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder in women of reproductive age as this syndrome may affect 5–10% of premenopausal women in Western countries (Franks 1995). Women with this syndrome may present with one, all, or any combination of menstrual irregularities, chronic anovulation, infertility, obesity, and hyperandrogenism. There is substantial heterogeneity of symptoms and signs among women with PCOS and different criteria have been used to confirm the diagnosis. Ultrasound assessment of ovarian morphology is considered to be essential and the gold standard for defining polycystic ovaries in Europe (Adams *et al.* 1986, Balen 1999). Characteristic ovarian morphology is not required in the American definition, which states that PCOS is the association of hyperandrogenism with chronic anovulation in women without specific underlying diseases of the adrenal or pituitary glands (Dunaif 1997).

Recently, a revised definition of PCOS was agreed and required the presence of two from the following three diagnostic criteria: (1) oligo- and/or anovulation; (2) clinical and/or biochemical features of hyperandrogenism; and (3) the presence of polycystic ovary (PCO) morphology (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). In recent years, transvaginal ultrasound has become the most commonly used diagnostic method for the identification of PCO. In order to make a diagnosis of PCO, >10 follicles of 2–10 mm in diameter and increased density of ovarian stroma (Adams *et al.* 1986) are required. These ultrasound criteria have been revised and either 12 or more follicles measuring 2–9 mm in diameter in at least one ovary, or increased ovarian volume (>10 cm^3) should be present on scanning (Balen *et al.* 2003).

Polycystic Ovary Syndrome, 2nd edn, ed. Gabor T. Kovacs and Robert Norman. Published by Cambridge University Press.

Ethnic background of women with PCOS may affect the clinical, hormonal, and metabolic characteristics of this condition. This chapter summarizes studies related to the clinical, hormonal, and metabolic characteristics and response to the treatment of PCOS in Asia, which may be different from those in Western countries.

Clinical, hormonal, and metabolic characteristics

Polycystic ovaries

In Western countries, PCO is a common finding in normal women (Polson *et al.* 1988) and is found in 26% of patients with amenorrhea, in 87% of those with oligomenorrhea, and in 92% of women with hirsutism (Adams *et al.* 1986). The prevalence of PCO in Indian subcontinent Asian women aged 18–40 years and recruited from the lists of local general practitioners was 52% (110/212). There were significant associations between PCO and menstrual irregularity, infertility, the Ferriman–Gallwey score for body hair distribution, the presence of acanthosis nigricans, and the fasting blood glucose concentration (Rodin *et al.* 1998).

We studied the effects of age on ovarian markers in normal Chinese women who had history of spontaneous conception, regular monthly cycles, and no significant gynecological disorders. Using the ultrasound criteria proposed by Adams *et al.* (1986), PCO (Fig. 18.1) was encountered in 5.6% (18/332) of fertile Chinese women (Ng *et al.* 2003, 2004). PCO seems to be more common in our patients presenting with infertility as it was found in 26 (12.2%) out of 213 infertile women scanned (Ng *et al.* 2005a), although PCO was demonstrated in one (0.8%) out of 128 infertile women recruited in our earlier study (Ng *et al.* 2000).

In women with previous gestational diabetes (GDM), a higher prevalence of PCO has been described. Using the revised ultrasound criteria (Balen *et al.* 2003), we compared the prevalence of PCO between 70 Chinese women with previous GDM and 70 age, parity, and delivery-year matched women who had a normal oral glucose tolerance test (OGTT) during their index pregnancy (Chan *et al.* 2006a). The GDM group had a higher though not statistically significant prevalence of PCO than the control group (34% versus 23% respectively). Within the GDM group, there was no difference in the metabolic profiles between those with and without PCO, although the body mass index (BMI) and Ferriman–Gallwey score tended to be higher in women with PCO. The hormonal profile was also comparable except that women with PCO had a lower serum follicle stimulating hormone (FSH) level.

Ovarian stromal blood flow

Subjective assessment of the intensity and quantity of colored areas during color or power Doppler analysis usually appears to be higher in PCO than normal ovaries. Increased ovarian stromal blood flow has been considered to be a new

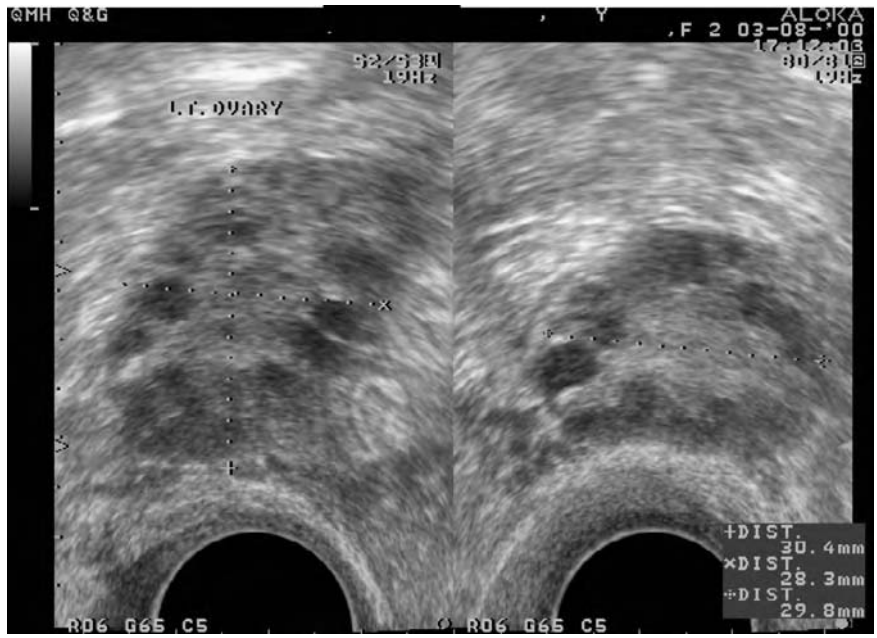

Fig. 18.1 Polycystic ovary on transvaginal scanning.

parameter to assist in the ultrasound diagnosis of PCO. Zaidi *et al.* (1995) first reported a significantly greater mean ovarian stromal peak systolic blood flow velocity and time-averaged maximum velocity blood flow velocity in PCOS women than infertile women with normal ovaries. Higher serum concentrations of vascular endothelial growth factor (VEGF) were found in PCOS women and were related to the increased ovarian stromal velocities, when compared with women with normal ovaries (Agrawal *et al.* 1998).

Ovarian stromal blood flow can be assessed by color Doppler and power Doppler ultrasound. Power Doppler imaging is more sensitive than color Doppler imaging at detecting low-velocity flow and hence improves the visualization of small vessels. In combination with three-dimensional (3D) ultrasound, power Doppler provides a unique tool with which to examine the ovarian stromal blood supply as a whole as opposed to analysis of small individual stromal vessels in two-dimensional planes (Fig. 18.2). The built-in VOCAL (virtual organ computer aided analysis) imaging program for the 3D power Doppler histogram analysis can be used to determine the ovarian volume and indices of vascularization and blood flow. Vascularization index (VI) measures the number of color voxels representing the blood vessels in the ovary and is expressed as a percentage (%) of the ovarian volume.

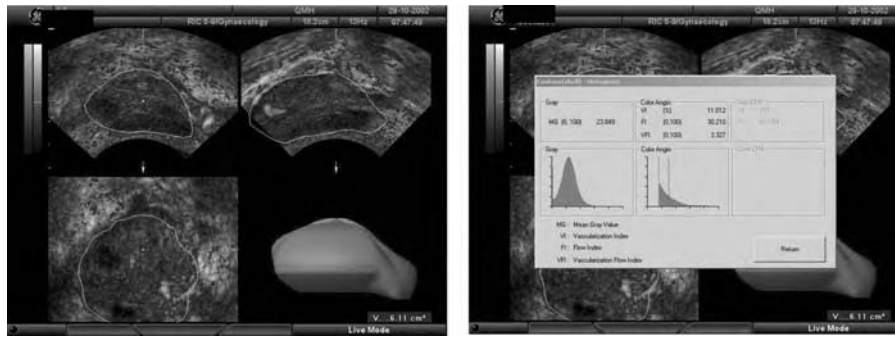

Fig. 18.2 Ovarian stromal blood flow of polycystic ovary measured by three-dimensional ultrasound with power Doppler.

Flow index (FI) is the mean color value in the color voxels and represents the average intensity of flow inside the ovary. Vascularization flow index (VFI) made by multiplying VI and FI is a combination of vascularity and flow (Pairleitner *et al.* 1999).

Using 3D ultrasound with power Doppler, Pan *et al.* (2002) demonstrated significantly higher ovarian stromal blood flow in PCOS women while Järvelä *et al.* (2002) found similar ovarian stromal blood flow between PCOS women and women with normal ovaries. Therefore, conflicting information exists in the literature with respect to ovarian stromal blood flow in PCOS women. Infertile women with normal ovaries were chosen as normal controls in the above studies on ovarian stromal blood flow in PCOS. It is possible that ovarian stromal blood flow is reduced in infertile women and therefore fertile women with normal ovaries may be a much better choice for comparison.

We conducted a prospective study to compare the ovarian stromal blood flow measured by 3D power Doppler ultrasound and serum VEGF concentration between fertile women with normal ovaries and infertile women with PCOS and to compare the ovarian stromal blood flow and hormonal parameters of PCOS women in relation to their BMI and antral follicle count (AFC) (Ng *et al.* 2005b). Women with PCOS were significantly younger and had significantly higher total AFC, total ovarian volume, and total ovarian VI/FI/VFI than those of normal fertile controls (Table 18.1). Serum luteinizing hormone (LH) concentration was significantly higher and serum FSH concentration was significantly lower in PCOS women than normal fertile controls. Body mass index and serum VEGF concentrations were similar between the two groups. After adjusting for the difference in the age of women between the two groups, significant differences were found in total AFC, total ovarian volume, and serum LH concentration only between normal fertile controls and PCOS women (Table 18.1).

Table 18.1. Comparison of age, total antral follicle count, total ovarian 3D power Doppler flow indices, and hormonal parameters between normal fertile controls and PCOS women (data given as median (range))

			P value[a]	
	Normal fertile controls ($n = 107$)	PCOS women ($n = 32$)	Mann–Whitney U test	Two-way ANOVA[b]
Age (years)	36.0 (25–40)	31.0 (22–38)	<0.001	–
Body mass index (kg/m^2)	21.95 (16.34–36.84)	23.00 (18.52–36.14)	0.133	0.539
Total antral follicle count	12.0 (2–20)	38.5 (18–75)	<0.001*	<0.001*
Total ovarian volume (cm^3)	10.47 (4.24–38.10)	21.04 (8.75–42.12)	<0.001*	<0.001*
Total ovarian VI (%)	2.73 (0.08–36.97)	3.79 (0.19–24.73)	0.023*	0.285
Total ovarian FI (0–200)	52.28 (19.18–95.64)	58.54 (45.09–74.22)	0.001*	0.068
Total ovarian VFI (0–200)	0.671 (0.01–17.62)	1.310 (0.04–19.13)	0.024*	0.347
Serum FSH (IU/l)	6.20 (1.0−13.7)	5.10 (1.0−9.7)	0.046*	0.131
Serum LH (IU/l)	3.30 (1.0−9.3)	6.10 (1.0−22.0)	<0.001*	<0.001*
Serum VEGF (pg/ml)	279.1 (52.3−1288.8)	228.8 (50.2−765.3)	0.216	0.07

Notes:

[a] *Statistically significantly different.

[b] After adjusting the difference in the ages of the women.

Ovarian 3D power Doppler flow indices were negatively correlated with BMI and were significantly higher in normal weight PCOS women than their overweight counterparts (Table 18.2). Ovarian 3D power Doppler flow indices were similar in PCOS women with total AFC <38 or ≥38, which is the median total AFC in PCOS women (Fig. 18.3).

Our results demonstrated that normal fertile controls and PCOS women had similar total ovarian 3D power Doppler flow indices and serum VEGF concentration after adjusting the difference in age of women. Normal weight PCOS patients had significantly higher total ovarian 3D power Doppler flow indices than their overweight counterparts. Patients with PCOS are more sensitive to the stimulation of gonadotropins and are at higher risk of ovarian hyperstimulation syndrome (OHSS) (Aboulghar and Mansour 2003). Increased ovarian stromal blood flow in PCOS patients, especially in normal weight PCOS women shown in our study, may lead to a greater delivery of gonadotropins to the granulosa cells of the developing follicles. Therefore, we should incorporate the assessment of the ovarian stromal blood flow in the management of PCOS women undergoing ovulation induction or ovarian stimulation in order to reduce the associated risk of OHSS.

Table 18.2. Comparison of age, total antral follicle count, total ovarian 3D power Doppler flow indices, and hormonal parameters between PCOS women with body mass index <25 kg/m^2 and ≥ 25 kg/m^2 (data given as median (range))

	BMI <25 kg/m^2 ($n=21$)	BMI ≥ 25 kg/m^2 ($n=11$)	P value[a]
Age (years)	30.0 (26–38)	32.0 (28–37)	0.112
Body mass index (kg/m^2)	21.00 (18.52–24.30)	29.2 (25.79–36.14)	<0.001*
Total antral follicle count	43.5 (22–75)	36.0 (18–72)	0.412
Total ovarian volume (cm^3)	21.52 (13.17–42.12)	17.54 (8.75–40.55)	0.340
Total ovarian VI (%)	5.67 (2.12–24.73)	1.82 (0.19–7.18)	0.001*
Total ovarian FI (0–200)	60.86 (52.90–74.22)	49.50 (45.09–65.42)	0.001*
Total ovarian VFI (0–200)	1.767 (0.59–19.13)	0.448 (0.04–2.43)	0.001*
Serum FSH (IU/l)	5.10 (1.0–9.0)	5.20 (3.3–8.1)	0.721
Serum LH (IU/l)	9.5 (1.0–22.0)	5.6 (2.0–8.3)	0.148
Serum VEGF (pg/ml)	226.9 (50.2–765.3)	293.8 (171.3–533.1)	0.432
Serum testosterone (nmol/l)	2.24 (0.19–6.65)	1.90 (0.67–5.48)	0.697
Serum androstenedione (nmol/l)	2.63 (1.62–5.28)	3.26 (2.06–5.80)	0.498
Serum DHEAS (μmol/l)	1.88 (0.84–2.80)	1.56 (0.96–2.12)	0.498
Serum SHBG (nmol/l)	227.08 (80.80–374.90)	102.10 (93.92–108.60)	0.1
Serum fasting leptin (μg/l)	11.40 (5.49–43.92)	32.03 (27.14–43.85)	0.047

Note:

[a] *Statistically significantly different.

Metabolic aspect

Women from Japan with PCOS were less obese and did not have hirsutism when compared with PCOS women from the United States and Italy (Carmina *et al.* 1992). Indian PCOS and non-obese reference subjects had higher insulin responses after an OGTT than Whites (Norman *et al.* 1995). South Asians with anovulatory PCOS have more severe symptoms, and have higher fasting insulin concentrations and lower insulin sensitivity than Caucasians (Wijeyaratne *et al.* 2002).

Medical induction of ovulation

Clomiphene citrate

Clomiphene citrate (CC) is usually used as the first-line drug to induce ovulation in women with PCOS. Successful ovulation is achieved in approximately 70–85 % of women and 40–50 % will conceive (The ESHRE Capri Workshop 1997). In 47 PCOS women receiving CC 50–200 mg daily for ovulation induction, Takahashi

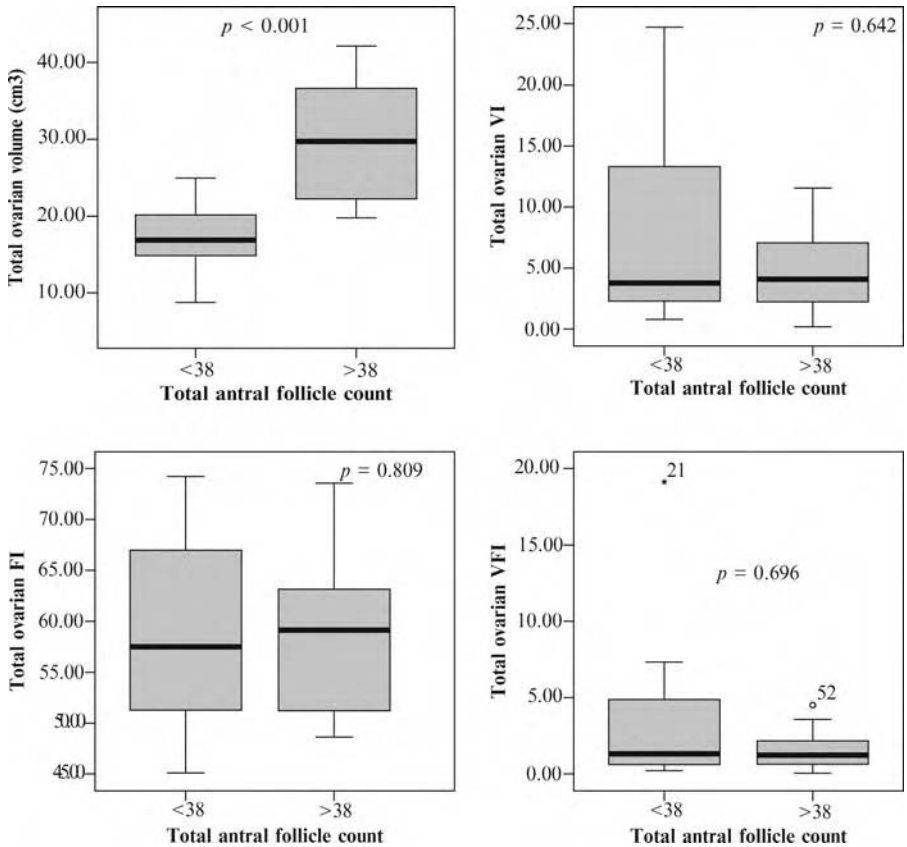

Fig. 18.3 Boxplot of total ovarian volume and total ovarian VI, FI, and VFI of PCOS women with AFC <38 and ≥38 antral follicle count.

et al. (1994) revealed that small multiple follicles (≥10) and enlarged ovarian volume (>6.2 ml) were the most prominent transvaginal ultrasound findings associated with non-responsiveness to CC. Only 47% of the CC responders and 79% of the CC non-responders had bilaterally enlarged ovaries (>6.2 ml) whereas 96% of the CC non-responders had a significantly increased number of follicles (≥10 follicles) in each ovary compared with the incidence (16%) in the CC responders. Furthermore, none of the CC non-responders had bilaterally normal ovaries, and 96% of patients with PCOS with bilaterally abnormal ovaries were CC non-responsive.

The response to human corticotropin releasing hormone (hCRH) in PCOS women may predict the response to CC (Kondoh *et al.* 1999). In women with PCOS, plasma adrenocorticotropic hormone (ACTH) and cortisol levels were significantly higher and the plasma ACTH level after the administration of hCRH

was higher than in controls. Based on the response to hCRH, patients with PCOS could be classified into three categories: those with a normal response to hCRH (group 1), those with an exaggerated response of ACTH to hCRH (group 2), and those with a high basal level of cortisol and a poor response to hCRH (group 3). In groups 2 and 3, levels of dehydroepiandrosterone sulfate (DHEAS) were significantly higher, suppression of androstenedione by dexamethasone was significantly greater, and ovulation rates with CC were significantly lower than in group 1. The results of this study also highlight the disturbance of the hypothalamic–pituitary–adrenal axis in some women with PCOS.

Gonadotropin

Gonadotropin treatment can be offered when these anovulatory women fail to respond to CC. The use of gonadotropin is more expensive and associated with a much higher risk of multiple pregnancy and developing OHSS. Different protocols of administering gonadotropins such as fixed-dose, low-dose step-up, and step-down regimens have been developed. Andoh *et al.* (1998) concluded that the low-dose step-up regimen of gonadotropin for patients with PCOS may be the safest protocol among these three regimens. Serum FSH levels on the day of human chorionic gonadotropin (hCG) administration were significantly higher in the fixed-dose regimen group than in the step-down and the low-dose step-up regimen groups, and the number of growing follicles ($\geq$11 mm) in the low-dose step-up regimen group was significantly smaller than in the fixed-dose regimen group.

Insulin sensitizing agents

Insulin resistance and compensatory hyperinsulinemia are prominent features of PCOS (Dunaif 1995) and occur when there is oligo/amenorrhea (Robinson *et al.* 1993). Increased insulin concentrations lead to hyperandrogenism because of increased production of ovarian androgen and decreased synthesis of sex hormone binding globulin (SHBG). As hyperinsulinemia plays a significant role of anovulation in women with PCOS, clinical improvement can be anticipated by reducing serum insulin concentrations. Metformin, an insulin sensitizing agent, has been extensively used to induce ovulation in women with PCOS. A recent meta-analysis (Lord *et al.* 2003) showed that metformin is effective in achieving ovulation in women with PCOS, with odds ratios of 3.88 (95% confidence interval 2.25 to 6.69) for metformin compared with placebo and 4.41 (2.37 to 8.22) for metformin and CC compared with CC alone.

However, our prospective, randomized, double-blind, and placebo-controlled study comparing the ovulation rate following metformin and placebo for 3 months did not show any improvement in women resistant to CC (Ng *et al.* 2001). Twenty infertile Chinese women aged <40 years, who had ultrasound features

Table 18.3. Comparison of hormonal and metabolic parameters before and after taking placebo and metformin for 3 months (data given as median (range))

	Placebo ($n=7$)		Metformin ($n=9$)	
	Before	After	Before	After
Body mass index (kg/m^2)	22.7 (17.9–28.9)	23.1 (18.8–29.1)	24.1[a] (19.6–34.2)	23.0[a] (18.9–32.4)
FSH (IU/l)	6.2 (1.7–9.6)	5.6 (3.4–8.9)	6.7 (3.7–7.8)	5.4 (3.0–8.7)
LH (IU/l)	11.3 (5.9–14.4)	13.0 (9.0–22.5)	10.0 (5.3–23.2)	9.7 (3.1–24.6)
Testosterone (nmol/l)	1.3[b] (0.5–2.2)	1.5[b] (1.0–2.6)	1.8[b] (1.0–3.7)	1.2[b] (0.6–2.3)
Androstenedione (nmol/l)	8.7 (6.4–13.2)	9.8 (7.3–12.6)	9.2 (6.6–12.6)	8.5 (4.6–11.3)
DHEAS (μmol/l)	4.9 (2.4–9.1)	3.8 (1.0–6.8)	5.9 (0.8–9.7)	7.0 (3.8–9.6)
SHBG (nmol/l)	36.6 (15.5–52.7)	32.9 (14.3–60.2)	28.7 (9.0–47.1)	26.6 (10.4–45.9)
Fasting insulin (mIU/l)	12.1 (2.8–17.0)	7.3 (5.2–21.9)	10.8 (4.7–19.7)	8.2 (3.9–9.0)
Fasting leptin (μg/l)	10.4 (2.3–16.2)	10.5 (3.1–17.9)	10.2[a] (7.8–24.3)	7.9[a] (3.8–13.5)
Fasting sugar (mmol/l)	5.3 (3.2–6.1)	4.9 (4.4–5.7)	5.2 (4.6–7.1)	5.1 (4.6–5.6)
2-h sugar after OGTT (mmol/l)	6.4 (5.1–15.1)	5.8 (3.5–7.7)	7.4 (3.5–15.1)	7.7 (5.6–11.2)
Fasting cholesterol (mmol/l)	4.7 (3.7–5.4)	4.9 (3.7–8.4)	5.1 (3.8–5.7)	4.4 (3.5–6.1)
Fasting triglycerides (mmol/l)	1.1 (0.5–1.6)	1.1 (0.4–2.2)	1.0 (0.4–2.6)	1.0 (0.4–1.5)
Fasting HDL (mmol/l)	1.2 (0.8–1.6)	1.2 (0.9–1.9)	1.1 (0.7–2.1)	1.6 (0.8–1.9)
Fasting LDL (mmol/l)	3.0 (2.0–3.5)	3.4 (1.9–6.2)	3.0 (2.2–3.8)	2.5 (1.8–4.3)

Notes:

[a] $p < 0.01$ by Wilcoxson ranked sum test.

[b] $p < 0.05$ by Wilcoxson ranked sum test.

of PCO and remained anovulatory on CC, were randomized to receive placebo or metformin 500 mg three times a day for 3 months. Hormonal and metabolic profiles were determined before the therapy and were repeated at 3 months in women not pregnant within this period. Clomiphene citrate was then added for one cycle to those not ovulatory on placebo or metformin alone. The median ovulation rate in the placebo group was 0% (range 0–50.0%) after placebo only and 6.9 % (range 0–50.0%) after placebo and CC whereas the corresponding rates in the metformin group were 0% (range 0–22.2%) and 0% (range 0–22.2%) respectively. There was no improvement in the ovulation rate despite significant reduction of BMI, serum testosterone, and fasting leptin concentrations in the metformin group (Table 18.3). Our results implied that metformin treatment may result in successful ovulation only in certain subgroups of these women.

Hyperadrogenism, characterized clinically by hirsutism or biochemically by elevated serum concentrations of androgens, was included as one of selection

criteria in many studies on the use of metformin in PCOS. Metformin treatment seemed to have no effect on ovarian function in women with normal testosterone concentrations whereas patients with elevated pre-treatment testosterone concentrations showed the most marked increase in ovulation rate (Pirwany *et al.* 1999). The majority of our patients did not have clinical or laboratory features of hyperandrogenism. This may also explain the absence of ovarian response to metformin in Chinese PCOS women in Hong Kong.

Other insulin sensitizing agents have also been tried with success. Hasegawa *et al.* (1999) administered troglitazone (400 mg/day) for 12 weeks to 13 women with PCOS and insulin resistance. Before troglitazone administration, the ovulation rate during CC was 34.9% per cycle (15/43) and increased significantly to 72.7% (8/11) during troglitazone coadministration. Further, an ovulation rate of 42.3% (11/26) was achieved with troglitazone alone. The mean (±SD) fasting insulin concentration was significantly reduced, from 18.3 ± 8.9 to 10.5 ± 7.1 μU/ml. The LH level was reduced from 9.7 ± 3.4 to 4.8 ± 3.9 mIU/ml and the testosterone level was reduced from 0.9 ± 0.5 to 0.5 ± 0.3 ng/ml in accordance. Atherosclerotic lipid levels also were normalized. However, this drug has been withdrawn from the market because of hepatotoxicity caused by an idiosyncratic reaction.

Herbal medicine

Obese women with PCO are more likely to have hirsutism, amenorrhea, and lower serum SHBG level. Obesity in PCOS is associated with anovulation, resistance to CC or FSH, lower pregnancy rate, and increased cardiovascular problems. Weight loss of >5% in these women with dietary treatment has been shown to improve reproductive function, increase SHBG, decrease free testosterone, and decrease hyperinsulinemia (Kiddy *et al.* 1992).

Green tea, a very commonly consumed beverage especially in Asia, has been found to exert beneficial effects in thermogenesis, glucose, and lipid metabolism as well as the hormonal system, which are all very relevant in the management of patients with PCOS. It has been shown that green tea can effectively reduce body weight in rats (Han *et al.* 1999, Kao *et al.* 2000) and humans (Chantre and Lairon 2002), mediated via catechins (Dulloo *et al.* 1999). Although there are numerous catechins in green tea, the most influential is probably epigallocatechin gallate (EGCG), which cannot be obtained in appreciable amounts from any other food source. Lung Chen, a Chinese green tea, contains the most abundant EGCG as compared to other Chinese teas and Japanese green tea (Yang and Koo 1997).

We randomized 34 obese Chinese PCOS women into green tea extract made into capsules versus placebo for 3 months (Chan *et al.* 2006b). When compared

before and after treatment, the body weight of the green tea group did not show any difference, whereas all the anthropometric measurements of the control group were significantly higher after 3 months. With the exception of a reduced free testosterone level in the green tea group, there were no differences in all the hormone levels in both groups. The biochemical profiles of the two groups were also similar except that there was a small but significant rise in the triglyceride level in the green tea group. Fewer patients in the green tea group remained amenorrheic but this was not significantly different from the control group. Therefore, green tea supplementation did not significantly reduce body weight in obese PCOS women, nor alter the glucose or lipid metabolisms.

The lack of a positive finding in our study may be related to the inadequate dose of green tea. A daily equivalent of 540 mg of EGCG was used in our study, which was much higher than those used in other studies. Most of the studies involving the use of green tea in cancer or cardiovascular disorders have shown a chronic consumption in an amount of 4 to 10 cups daily being protective (Imai *et al.* 1997). Furthermore, it is uncertain whether the response to EGCG is different in different ethic groups, especially for those groups with a strong habit of taking tea in their daily lives.

Other herbal medicines have been investigated to reduce testosterone concentration, induce ovulation, and achieve pregnancy (Wang *et al.* 1988, Takahashi and Kitao 1994, Sakai *et al.* 1999, Ushiroyama *et al.* 2001, Xiao 2003). More studies are definitely required in this area.

Surgical induction of ovulation

Laparoscopic ovarian drilling has now replaced open ovarian wedge resection as the surgical treatment for women with CC-resistant PCOS and appears to be as effective as gonadotropin treatment in these women (Farquhar *et al.* 2005). In general, laparoscopic ovarian drilling in patients with PCOS resulted in 55–93% spontaneous ovulation and 0–84% pregnancy rates. This is thought to work by damaging part of the hormone-producing ovarian tissue, thus reducing androgen and inhibin levels.

Insulin resistance commonly seen in PCOS women may also be affected. In a prospective, controlled study, Wu *et al.* (1996) demonstrated that insulin resistance in non-obese Chinese women with PCOS was reverted following wedge resection. During the OGTT, non-obese Chinese women with PCOS had a significantly higher mean serum area of the curve of glucose, insulin, C-peptide, insulin/glucose levels, and C-peptide/insulin values, when compared with these women following wedge resection and normal women with similar age and BMI.

Conclusion

There is substantial heterogeneity of symptoms and signs among women with PCOS. Ethnic background of women with PCOS may affect the clinical, hormonal, and metabolic characteristics of this condition. It is important to take into consideration the ethnic background of patients in studies related to PCOS.

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19

Obesity surgery and the polycystic ovary syndrome

John B. Dixon and Paul E. O'Brien

Introduction

Obesity is a serious disease, which is associated with an array of comorbid conditions that have a major impact on both physical and mental health, and on quality of life. Obesity surgery provides the only reliable method of achieving and sustaining significant weight for those with severe obesity (body mass index (BMI) >35 kg/m^2). The combination of an obesity epidemic, relatively ineffective conservative therapy, and advances in modern laparoscopic surgery has generated a demand for effective safe surgical methods to achieve significant weight loss. It is therefore not surprising that obesity surgery is one of the most rapidly developing and expanding areas of surgery today. Obesity, especially central obesity with insulin resistance, is commonly associated with features of the polycystic ovary syndrome (PCOS), namely disturbance of ovulatory function, infertility, and evidence of androgen excess (Hartz *et al.* 1979) and it is not surprising that weight loss has become an important goal in obese women with this condition.

Obesity surgery: mechanism of action

The traditional division of obesity surgery into malabsorptive and restrictive has been misleading as it has implied knowledge regarding the mechanism of action utilized to achieve and sustain weight loss. Currently two procedures make up the vast majority of bariatric surgical procedures throughout the world: these are the laparoscopic adjustable gastric banding (LAGB) (Fig. 19.1) and Roux-en-Y gastric bypass (RYGB) (Fig. 19.2), with neither producing malabsorption of

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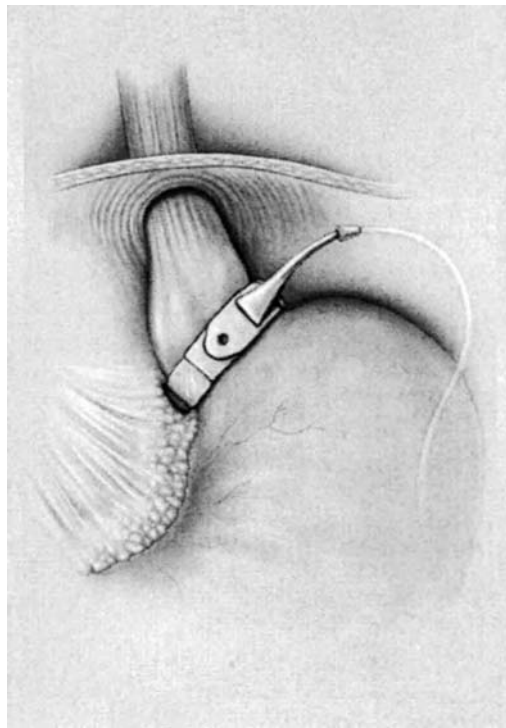

Fig. 19.1 Laparoscopic adjustable gastric banding.

macronutrients. A third procedure, biliopancreatic diversion (BPD) (Fig. 19.3), is used far less frequently and has a component of macronutrient malabsorption as part of its action. Increasingly the more invasive procedures, RYGB and BPD, are being performed laparoscopically.

The great challenge in managing obesity and its related diseases is achieving and sustaining significant weight loss. The prevention of starvation is fundamental to survival of all species and not surprisingly we have powerful central mechanisms to respond to any weight loss (Schwarz *et al.* 2003). Obesity surgery appears to be the only therapy that allows early and prolonged satiety following a small meal despite very significant weight loss. Following obesity surgery patients should be hungry but they are not. The adjustability of the LAGB procedure has allowed us to switch off the procedure and by opening the stoma of the band an increase in appetite is soon experienced (A. F. Dixon *et al.* 2005). The mechanism of action of bariatric surgery remains largely undiscovered despite a growing number of candidate gut hormones being found (Small and Bloom 2004). Interest in this area of research is expanding as minor alterations to the gut hold at least one key to sustainable treatment for obesity.

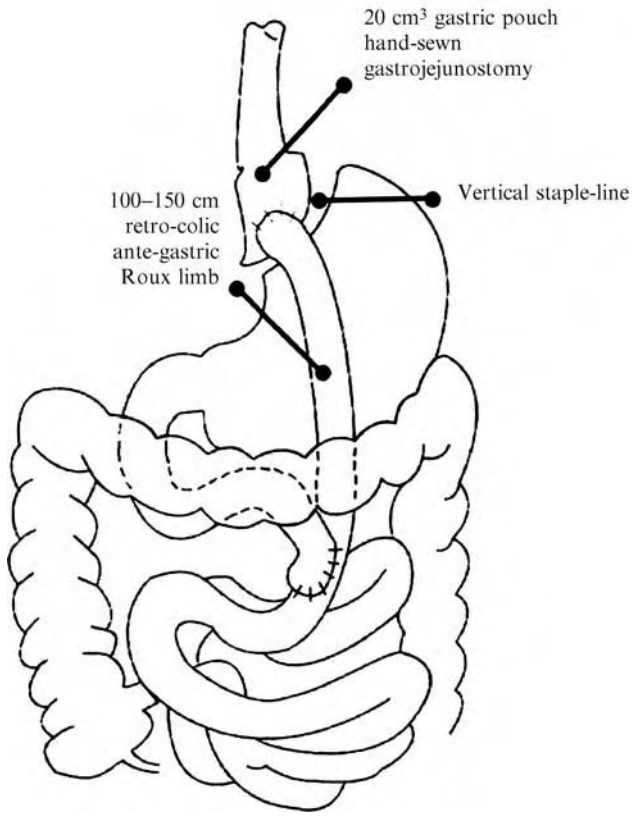

Fig. 19.2 Roux-en-Y gastric bypass.

Obesity surgery: weight loss

Weight loss following current obesity surgery procedures has been subject to several recent systematic reviews (Buchwald *et al.* 2004, Chapman *et al.* 2004, Maggard *et al.* 2005). All procedures have been able to achieve more than 50% loss of excess weight. That is over 50% of weight in excess of ideal weight as defined by medium build from the Metropolitan Life Tables (Metropolitan Life Foundation 1983). Fifty percent of excess weight loss represents a fall in BMI for an average surgical patient from 45 kg/m^2 to 33 kg/m^2. The most invasive malabsorptive procedures such as BPD provide greatest weight loss with a sustained loss of 65–70% of excess weight, but at significant nutritional risk. The Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP–S) systematic review showed greater weight loss by RYGB than LAGB during the first 2 years after operation, but no difference after that (Chapman *et al.* 2004). In a recent review (O'Brien *et al.* 2006a) we have extended

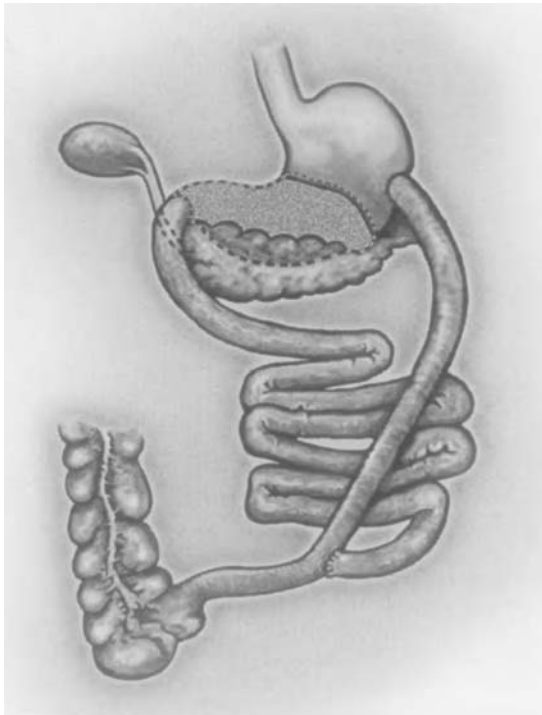

Fig. 19.3 Biliopancreatic diversion.

the data of the ASERNIP-S data by including all studies reported up to December 2005 in which more than 100 patients were included. RYGB and LAGB are effective procedures with greater initial weight loss for RYGB, but at 4, 5, and 6 years there is no difference. There are very limited weight loss data beyond 6 years for any procedures; however, all data to date indicate sustainability of significant weight loss. These data indicate that at 5 years surgery attains substantial weight loss compared with the truly marginal effect of all other weight loss therapies.

Weight loss: effect on obesity related metabolic factors

The success and durability of weight loss following bariatric surgery has provided a major opportunity to explore the effects of weight loss in a broad range of obesity related conditions. Weight loss is accompanied by improved insulin sensitivity and a reduction in markers of chronic inflammation, key factors thought to drive the metabolic syndrome (Dixon *et al.* 2003a). Substantial weight loss through obesity surgery provides unparallel changes in obesity related disease. More than two-thirds of those with type 2 diabetes will be off all treatment and have normal fasting blood glucose, hemoglobin A_{1c}, and serum insulin, and

remission rates are greater if the disease is treated early, that is before irreparable beta cell damage has occurred (Pories *et al.* 1995, Dixon and O'Brien 2002a, Schauer *et al.* 2003). There are also major improvements or complete resolution of hypertension and metabolic syndrome (O'Brien *et al.* 2006b). The sleep problems, including obstructive sleep apnea and increased daytime sleepiness, that are associated with obesity improve dramatically or resolve completely with weight loss (J. B. Dixon *et al.* 2005). The liver disease of the metabolic syndrome, non-alcoholic steatohepatitis, undergoes an amazing transformation with weight loss, with improvement reductions in liver fat or steatosis, inflammation, and fibrosis (Dixon *et al.* 2004, Kral *et al.* 2004). All of these above conditions are closely related to central or truncal adiposity and insulin resistance, and of course are frequently seen in obese women with PCOS. In addition, weight loss provides much better quality of life and body image, and fewer symptoms of depression (Dixon *et al.* 2001a, 2002, 2003b). Recent publications also indicate that obesity surgery increases life expectancy (Christou *et al.* 2004, Flum and Dellinger 2004).

Weight loss as a therapy for PCOS

Given the very strong association between central adiposity, insulin resistance, and the PCOS, it is understandable that weight loss may be seen as potentially the most effective management of overweight or obese women suffering this condition. Briefly, it is hypothesized that the pathogenesis of the sex hormone abnormalities in obese women with PCOS are secondary to insulin resistance and hyperinsulinemia, mediated by insulin-like growth factor-1 and insulin-like growth factor binding proteins (Poretsky *et al.* 1988, Dunaif 1997). The effect is an increase in ovarian androgen production and reduced hepatic production of sex hormone binding globulin (SHBG), which result in an increase in unbound testosterone (Farquhar 2000). Indeed, weight loss appears to be important in resolving this hormonal imbalance. Intentional diet-induced weight loss often leads to normal menstruation and improved fertility (Mitchell and Rogers 1953) in association with raised SHBG and lower free testosterone levels (Botwood *et al.* 1995). There are also reports of reduced hirsutism, acne, and acanthosis nigricans (Pasquali *et al.* 1989). Improved insulin sensitivity through lifestyle changes to diet and physical activity forms the cornerstone of initial management of women with PCOS who seek fertility (Huber-Buchholz *et al.* 1999).

Unfortunately a characteristic of those with insulin resistance is difficulty in losing weight (Khan *et al.* 2000, Dixon *et al.* 2001b, Norris *et al.* 2005a, b). We will not detail the broader range of studies regarding weight loss and its effect on PCOS, as this is the topic of another chapter, but will focus on weight loss following obesity surgery. It should be noted, however, that optimal obesity surgery

programs provide indefinite follow-up and have a strong emphasis regarding behavioral change to improve dietary intake, and to maximize opportunities for increasing physical and reducing sedentary activities.

Obesity surgery and the PCOS

There are a limited number of observational series and reports of ovarian functional improvement and improved fertility following weight loss surgery (Bilenka *et al.* 1995) in association with lower androgen levels (Deitel *et al.* 1987, Bastounis *et al.* 1998). Twenty-four women with a diagnosis of PCOS prior to RYGB were surveyed clinically following significant weight loss; all developed regular menstruation at a mean of 3.4 ± 2.1 months following surgery and 52% had resolution of hirsutism and most of the remainder reporting that the condition had improved (Eid *et al.* 2005).

In a study of 107 severely obese premenopausal women (BMI >35 kg/m^2) presenting for LAGB surgery we found a significant relationship between increasing obesity, especially central obesity, and lower SHBG concentrations. An increased neck circumference provided the best correlation with lower SHBG concentrations. After controlling for neck circumference no other anthropometric measures or biochemical measures influenced variance of SHBG. Testosterone concentrations were lower with increasing age and were not influenced by increasing BMI. This suggests that the raised free androgen index in severely obese women is largely driven by the lower SHGB levels. Thirty of these women (28%) had a clinical diagnosis of the PCOS and these women were more insulin resistant, had greater pancreatic beta cell insulin secretion, and had higher alanine aminotransferase levels, suggesting hepatic dysfunction, all biochemical features associated with the metabolic syndrome (Dixon and O'Brien 2002c).

Forty-two of these women had repeat measures taken at 12 months following LAGB surgery (Table 19.1). In association with weight loss there was a significant rise in SHBG and fall in serum testosterone leading to a substantial fall in the free androgen index. Fourteen of the 42 women followed for at least 1 year gave a pre-surgery history of menstrual irregularity. Twelve had developed regular menstruation and two previously infertile women conceived without specific fertility treatment and have now had uneventful pregnancies. Twelve of the 42 women had symptoms for a clinical diagnosis of PCOS pre-operatively. At 1 year ten had regular menstruation and eight had a free androgen index of less than 5. Only one fulfilled the criteria of PCOS. Although this is a relatively small observational study, the changes demonstrated support the hypothesis that obesity itself mediates the androgen abnormalities and the clinical consequences of these in severely obese women with PCOS.

Table 19.1. Paired baseline and 1-year weight, anthropometric and biochemical parameters (paired Student t-test, mean ± standard deviation) for 42 pre-menopausal women following laparoscopic adjustable gastric band surgery (LAGB)

	Pre-surgery	1 year	*p* value
Percent excess weight loss (EWL)[a]		43 ± 17	
BMI (kg/m^2)	45.3 ± 7.3	36.4 ± 6.8	<0.001
Weight (kg)	124 ± 21	100 ± 20	<0.001
Neck (cm)	41.0 ± 2.7	37.5 ± 3.4	<0.001
Waist (cm)	120 ± 14	103 ± 14	<0.001
SHBG[b] (nmol/l)	29 ± 21	42 ± 35	<0.001
Serum testosterone (nmol/l)	1.43 ± 0.60	1.11 ± 0.83	0.022
Free androgen index[c]	5.58 ± 4.0	2.9 ± 2.3	<0.001
Fasting plasma glucose (mmol/l)	5.4 ± 1.5	4.8 ± 0.9	0.03
Insulin* (mU/l)	18.7 ± 10.4	8.9 ± 6.8	<0.001

Notes:

[a] Calculated as [weight loss/(initial weight − ideal weight)] × 100; ideal weight obtained from Metropolitan Life Foundation (1983).

[b] Wilcoxon signed rank test, median ± interquartile range.

[c] Calculated using the testosterone concentration (nmol/l) × 100, divided by SHBG concentration (nmol/l), normal value for pre-menopausal women <5.

Source: Dixon and O'Brien (2002c).

Obesity surgery and pregnancy

Eighty-five percent of patients presenting for obesity surgery are women and with mean age around 40 years many patients are in their childbearing years (O'Brien *et al.* 2002). Many of these are suffering from obesity related infertility and are keen to conceive soon after surgery. Severely obese women are at greater risk of obstetric complications such as pregnancy induced hypertension and gestational diabetes, and have poorer neonatal outcomes. However, reproduction for women who have had obesity surgery confronts us with a new series of questions and concerns for both mother and offspring. Nutrition for fetal growth and development is important. It requires adequate vitamin and other micronutrient levels, and a balance of macronutrients between sufficient maternal weight gain to allow normal fetal growth, development, and metabolic programming, and excessive weight gain leading to increased obstetric risk, macrosomia, and post pregnancy weight retention. It is critical that nutrition is considered throughout pregnancy.

Reports for pregnancy outcomes in women who have previously had obesity surgery are very limited and usually involve a retrospective collection of data. Outcomes following surgery where there is significant malabsorption or gastrointestinal diversion have indicated problems related to both macronutrient and micronutrient inadequacy, and resulted in lower mean birthweights (Ingardia and Fischer 1978, Knudsen and Kallen 1986a, b, Marceau *et al.* 2004, Matielli *et al.* 2004). While maternal and fetal nutrition is of major concern it is reassuring to find that several authors report reductions in obesity related complications of pregnancy such as pregnancy induced hypertension and gestational diabetes (Wittgrove *et al.* 1998, Skull *et al.* 2004), and in general pregnancy following obesity surgery has not been associated with adverse perinatal outcomes (Sheiner *et al.* 2004).

For women desiring pregnancy following obesity surgery LAGB provides the most appealing procedure. The risk of nutritional deficiency is low as there is no gastrointestinal diversion and the adjustability of the band provides an ability to adapt the surgery to the changing nutritional needs of pregnancy. We have collected pregnancy details prospectively and reported the results of the first 79 first pregnancies following LAGB surgery and compared these with these patient's penultimate pregnancies, severely obese controls, and local state birth outcomes. Briefly, mean birthweights, incidence of small for gestational age, macrosomia, and preterm births are consistent with community levels. The incidence of pregnancy induced hypertension and gestational diabetes was significantly lower than found in severely obese subjects and consistent with community normal values. We demonstrate that active management of the band and advice regarding nutritional supplementation enhances outcomes. A multidisciplinary approach involving co-operation between both the bariatric surgery and obstetric services is advised.

The diversion associated with RYGB greatly increases the risk of iron deficiency as gastric acid increases the ferrous form and iron is best absorbed in the duodenum and upper jejunum (Brolin *et al.* 1991, 1998). Adequate monitoring of iron status is essential and supplementation may be needed in premenopausal women following RYGB. Refractory anemia has been reported (Gurewitsch *et al.* 1996). The RYGB diversion also predisposes to calcium and vitamin B_{12} malabsorption. Nutritional problems are of greater concern following BPD which provides true macronutrient and micronutrient malabsorption predisposing to a broad range of nutritional deficiencies.

Surgery is not without risk

Complications associated with obesity surgery vary greatly and in general are specific to the type of procedure performed, making it hard to compare directly the incidence and severity of complications, and need for revisional surgery.

Technically LAGB is the easiest procedure, being least invasive, and has proven to be the safest. There are few post-operative problems and low peri-operative mortality at 0.05% (Chapman *et al.* 2004). As there is no gastrointestinal diversion the risk of nutritional problems are low. Late complications requiring surgical intervention have been reported; these include band slippage or the development of an abnormally large gastric pouch above the band, erosion of the band into the stomach, and leakage of saline from the system. Improvements to the method of placing and fixing the band have reduced the risk of slippage and erosion and technical modifications to the reservoir used for adjustment have reduced the risk of leak (Fielding and Allen 2002). Today, approximately 10% of patients will need some revisional surgery in the first 5 years.

The RYGB technique is a more complex procedure and carries greater risk of early death, in the range of 0.5% to 1.9%, and serious complications (Chapman *et al.* 2004, Flum and Dellinger 2004). It involves the creation of major change in the anatomy of the gut, is non-adjustable and essentially irreversible. Diversion of most of the stomach and upper small bowel leads to iron, vitamin B_{12}, and calcium malabsorption, and a high risk of deficiency if supplementation is inadequate.

There is also major change in anatomy of the gut with BPD and its variant, the duodenal switch, which act both by malabsorption and restriction with significant side effects and long-term nutritional risk. It is the most effective procedure for weight loss, an effect which appears to be durable. However, because of its severe metabolic effect and higher peri-operative mortality, it has limited appeal to both the patient and surgeon.

In summary, obesity surgery provides significant sustained weight loss; in association with weight loss there is improvement in the hormonal imbalance of the PCOS, and improved ovulation and fertility. Perhaps one of the most attractive features of this therapy is the concomitant improvement in insulin sensitivity and reduced risk associated with the metabolic syndrome and of developing type 2 diabetes, and greatly improved quality of life.

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20

Nutritional aspects of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting women of reproductive age, with varying signs and symptoms. Its various manifestations will bring PCOS patients to healthcare providers from different backgrounds, for differing reasons. The metabolic, endocrine, and reproductive aspects of PCOS interact and intertwine, and all may be influenced by nutrition and alternative metabolic pathways. These have been highlighted by research in recent years, which will be reviewed in this chapter. Treatment of PCOS may be enhanced by this evolving knowledge, in all aspects of the syndrome, including short-term problems such as acne or infertility, and long-term problems such as obesity, diabetes mellitus, atherosclerosis, and even possibly breast cancer (Kaaks 1996).

Pathogenesis of PCOS and nutrition

Polycystic ovary syndrome is a syndrome whose appearance depends on a combination of genetic and environmental factors. Environmental/nutritional factors may come into play even before birth, as birthweight depends both on genetic factors and maternal nutrition and uteroplacental function (Armitage *et al.* 2004). A positive correlation has been found between birthweight and subsequent presentation of polycystic ovaries (Michelmore *et al.* 2001). Conversely, large population-based studies in the United Kingdom clearly correlated low birthweight to subsequent markers of metabolic syndrome (Godfrey and Barker 2000). Rapid early postnatal weight gain (possibly following maternal–uterine restraint) strongly predicts later childhood obesity and insulin resistance (Ong and Dunger 2004). It is becoming clear that nutrition in early life has an immense impact on adult health. The insulin gene (*INS*) VNTR (variable number of tandem repeats)

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is associated with birthweight and fetal serum insulin-like growth factor-2 (IGF-2) levels. In postnatal life, the type III/III genotype is associated with body size, body mass index (BMI), waist circumference, and measures of insulin resistance in girls. Teleologically, *INS* VNTR type III genes may have conferred a genetic advantage for survival by conferring larger size at birth (Dunger *et al.* 1998). Similarly, insulin resistance associated with subclinical PCOS may confer metabolic advantages during life in periods of nutritional shortage (Holte 1998). But if this genetic constitution is exposed to unlimited access to nutrition and a sedentary lifestyle, full-blown PCOS may appear. Thus, we recognize the importance of studying genetic markers of PCOS, such as *INS* VNTR, paraoxonase variants, and IGF-2 variants (San Millan *et al.* 2004), and applying targeted early (as early as fetal life) nutritional interventions to minimize adult insulin resistance and PCOS.

PCOS and insulin resistance

Insulin resistance is a major metabolic feature of PCOS and is probably central to many of its manifestations (this is discussed in detail in Chapter 13). Insulin resistance has been demonstrated in the vast majority (65–90%) of obese patients with PCOS, and 25–45% of lean patients with PCOS, depending on the definition (or test used) for insulin resistance (Dunaif *et al.* 1992, Gennarelli *et al.* 2000). In brief, insulin resistance comprises a situation in which a receptor or post-receptor defect leads to a diminished peripheral cell (skeletal muscle, fibroblasts, adipocytes) insulin response. Negative feedback then determines a higher insulin–glucose set-point, in effect leading to compensatory hyperinsulinemia. These higher levels of insulin then have secondary effects on intracellular pathways in different cell types and tissues. Among others, two crucial cell types that are affected are vascular endothelia and ovarian theca cells. Beta cell function is another important aspect of insulin resistance in PCOS; compensatory hyperinsulinemia was greater in insulin resistant PCOS women than that in non-PCOS insulin resistant women, and was correlated to hyperandrogenemia (Goodarzi *et al.* 2005). This suggests that both insulin resistance and pancreatic function should be addressed when nutritional management is considered.

Nutritional modification of insulin resistance

Insulin resistance can be modified by a number of measures. Attaining optimal body weight, proportional lean/fat ratio, and minimal visceral body fat have all been described to improve insulin resistance measures in PCOS women (Huber-Buchholz *et al.* 1999, Moran and Norman 2004). Ghrelin, a hormone implicated in appetite regulation, was found to be lower in PCOS women than in controls; it appears that appetite and satiety are primarily altered in women with

PCOS (Moran *et al.* 2004). Visceral fat is closely linked to insulin resistance and PCOS, even in lean PCOS women. Serum triglycerides and fasting insulin were correlated to visceral fat but not subcutaneous fat in lean PCOS, as well as higher low-density lipoproteins (LDL) and lower high-density lipoproteins (HDL) (Yildirim *et al.* 2003). Another study demonstrated defects in primary lipolysis in visceral fat from women with PCOS (Ek *et al.* 2002). Visceral fat cells incorporate free fatty acids (FFA) less readily than peripheral fat, leading to high postprandial FFA levels in insulin resistant women (McCarty 2003a). Logistic regression analysis in a cohort of PCOS patients showed that measuring fasting insulin levels and measuring abdominal circumference predicted insulin resistance with the same accuracy as a full euglycemic clamp intravenous insulin glucose tolerance test (IVIGTT) (Gennarelli *et al.* 2000). These findings emphasize the importance of minimizing visceral fat, and of minimizing postprandial FFA influx in PCOS patients (McCarty 2003b).

Diet

In a very general sense, weight gain or loss depends on the difference between energy intake and energy expenditure. Thus, hypocaloric dieting will almost universally cause weight loss, although loss of fat, especially visceral fat, has more significant effects on insulin resistance and ovarian function than overall weight loss. Energy needs can be calculated using the Harris–Benedict equation adjusting for obesity and activity levels. In an average-sized adult woman, a 1000 kcal/day energy deficit will usually lead to the loss of approximately 0.8–1 kg body weight per week. A high protein diet (30% protein or more) has been popularly associated with excellent weight loss results in insulin resistant or type 2 diabetics (Mathers and Daly 1998, Skov *et al.* 1999), although a systematic study with PCOS women (Stamets *et al.* 2004) comparing a high protein (30% protein and 40% carbohydrate) with a high carbohydrate (15% protein and 55% carbohydrate) diet did not reveal any substantial difference between the two in terms of insulin resistance parameters or effect on ovarian function, although both in fact led to weight loss, improvement in insulin resistance, and a degree of normalization of menstrual function.

The type of carbohydrate consumed is equally important as the amount consumed, and many studies have demonstrated the advantage of substituting simple carbohydrates with a high glycemic index with complex carbohydrates (Hung *et al.* 2003), addition of fiber to the diet, especially guar gum, and separation of carbohydrate intake from protein intake (Landin *et al.* 1992, Kelly 2000, Vuksan *et al.* 2000). Acarbose may have a similar effect by interfering with glucose uptake in the intestine (Sonmez *et al.* 2005).

Fat intake also is of cardinal importance in insulin resistant states, including PCOS. High fat intake leads to high plasma levels of FFA, which compounds insulin resistance due to activation of hepatic gluconeogenesis, and decreasing muscle cell insulin sensitivity because of decreased muscle phosphatidylinositol-3 (PI-3) kinase activity and triglyceride deposition. Nutritional intervention in this respect can include switching saturated fat intake to polyunsaturated fat or monounsaturated fat. N-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) have been studied in regard to insulin resistance in both animal and human studies, and have been found to have a protective effect resulting from effects on PI-3 kinase, GLUT4, lipogenesis enzymes, and FFA oxidation pathways (Delarue *et al.* 2004). Specifically in PCOS women, a PUFA-enriched diet (utilizing walnuts as a source of linoleic acid and α-linoleic acid) reduced FFA, increased plasma linoleic acids, and increased urinary pregnanediol-3G (Kasim-Karakas *et al.* 2004). N-3 fatty acid intake was also found to be associated with a significantly improved vascular inflammatory marker profile (namely reduced C-reactive protein, interleukin-6, E selectin, soluble intracellular adhesion molecule, and soluble vascular adhesion molecule levels), indicating a possible long-term protective effect of these fatty acids on cardiovascular health (Lopez-Garcia *et al.* 2004). Multicenter studies demonstrated a clear improvement of insulin resistance parameters and upper body fat loss when a high monounsaturated fat diet replaced a high saturated fat diet (Walker *et al.* 1996); however, this beneficial effect disappeared when total fat intake exceeded 38% of total caloric intake (Riccardi and Rivellese 2000). Plasminogen activator inhibitor-1 (PAI-1) increases in PCOS women may also be lowered by low fat diets, or diets that decrease FFA influx (McCarty 2005a). Examples of foods rich in monounsaturated or polyunsaturated fat include liquid oils from olives, canola, soybeans, corn, flaxseed, sunflower, and peanuts. Also, fats derived from nuts, seeds, and deep-sea fish are from the same category.

The "mediterranean-style" (MS) diet consists of additional fruits, vegetables, and nuts, with an increased whole grain and olive oil intake. This diet has been studied in a number of clinical situations. Esposito and colleagues (2004) studied a large group of patients with clearly defined metabolic syndrome and insulin resistance who were instructed in regard to diet, with and without MS intake for 2 years. The patients on the MS diet lost significantly more weight, had significantly higher insulin sensitivity, and had significantly lower vascular endothelial inflammation markers than those on the regular diet. After the study period, 50/90 of the MS diet patients were no longer classified as having metabolic syndrome, whereas only 12/90 of the control group could be reclassified. These differences were also seen in a cross-sectional study of American and Italian PCOS women (Carmina *et al.* 2003). Despite similar caloric intake and carbohydrate/protein

ratio, the Italian group exhibited lower BMI, lower triglycerides, higher HDL, and less insulin resistance than their American counterparts. This was in part attributed to a lower saturated fat intake in the Italian group, and might also be due to a MS diet profile in the Italian group.

Soybeans and inositol

Whole soybeans also improve lipid abnormalities in patients with insulin resistance due to their phytoestrogen content, including genistein, daidzein, and glycetin and high soluble fiber content (Merritt 2004), thus lowering LDL and increasing HDL. Diets rich in soybean content can improve glycemic control in diabetic (type 2) patients by improving insulin resistance parameters. This was documented by treating diabetic patients with soybean-derived pinitol (methyl-D-chiro-inositol) in a randomized study in Korea (Kim *et al.* 2004). D-Chiro-inositol has been hypothesized to play a part in post-insulin-receptor events in cells, being a phosphoglycan that can mediate the action of insulin. Supplementation of D-chiro-inositol to obese PCOS women significantly lowered the area under the curve (AUC) insulin and free testosterone when compared to placebo (Nestler *et al.* 1999). Dietary supplementation of inositol was evaluated in a double-blind, placebo controlled study in 281 women with PCOS (Gerli *et al.* 2003). The treatment group lost weight and had more ovulations during the study period than control patients, although no significant differences in insulin parameters were found between the groups. It is possible that other alpha galactosides or galactosyl-pinitols produced from soybean leaves or vetch seeds (Szczecinski *et al.* 2000) may have similar effects, and these are currently being examined. The mechanism of their action probably includes activation of the intracellular phosphoglycan pathway, which may prove to be another way of bypassing insulin resistance, and dietary manipulations could enable these effects (Ostlund *et al.* 1996).

B vitamins, folic acid, and insulin resistance: the homocysteine connection

Homocysteine (Hcy) is an intermediate formed during the breakdown of the amino acid methionine, and may undergo remethylation to methionine, or trans-sulfuration to cystathione and cysteine. Folic acid and vitamin B_{12} are essential cofactors in the remethylation pathway. Classic homocysteinemia has been characterized as the accumulation of Hcy due to defects in enzymatic pathways, most commonly methyltetrahydrofolate reductase, or vitamin deficiencies, most commonly folic acid and vitamin B_{12} deficiency (Fig. 20.1). Recent research has pointed to many non-enzymatic factors which may influence Hcy levels, the most relevant involving increased insulin levels. Insulin levels have been

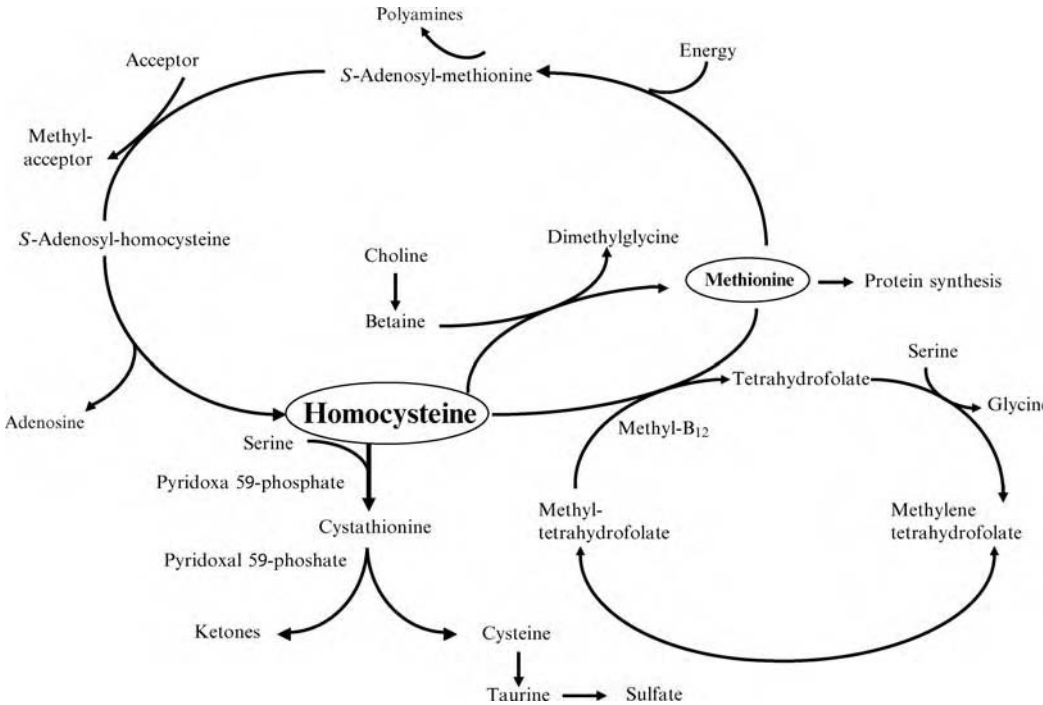

Fig. 20.1 Metabolic pathways of homocysteine (Hcy).

implicated as a modulating factor of Hcy, in that insulin inhibits hepatic cystathione β synthase activity (House *et al.* 1999, McCarty 2000). Increased levels of Hcy have been positively associated with insulin levels in a number of clinical situations, including type 2 diabetes, metabolic syndrome with hypertension, and nephropathy, and in women with pre-eclampsia (Giltay *et al.* 1998, Laivuori *et al.* 1999, Meigs *et al.* 2001).

Several studies demonstrated increased Hcy levels in PCOS patients. Yarali and colleagues (2001) demonstrated significantly higher Hcy levels in insulin resistant PCOS patients, along with non-restrictive type diastolic dysfunction, in the presence of normal folic acid and vitamin B_{12} concentrations. Schachter and colleagues (2003) found significant correlations between insulin resistance parameters and Hcy in PCOS patients, regardless of BMI. Bayraktar *et al.* (2004) found clear correlations between Hcy and insulin resistance, but not to androgen levels in PCOS women. Although one study did not find increased Hcy in PCOS women, insulin resistance parameters were not addressed in this study (Orio *et al.* 2003).

Elevated levels of Hcy are associated with vascular inflammation, atherosclerosis, increased rates of miscarriage, and poor reproductive performance. Therefore

reducing elevated Hcy could improve these parameters. Strategies for reducing Hcy may include folic acid and vitamin B_{12} supplementation, supplying additional sources of methyl donors to improve the conversion of Hcy to methionine, and reduction of insulin levels.

Betaine (trimethylglycine)

Betaine (trimethylglycine) occurs naturally in most tissues, and is generated in liver and kidney from choline. It acts as an osmolyte and serves as a methyl donor of the zinc methalloenzyme betaine-homocysteine methyltransferase (BHMT). Betaine is formed in cells as an oxidation product of choline and can be obtained from spinach or beets. Several studies have shown that elevated Hcy levels in insulin resistant patients may be reduced by betaine supplementation (Schwab *et al.* 2002). Folic acid (as folate), by itself, and in conjunction with vitamin B_{12}, has consistently shown Hcy-lowering effects in many studies. Betaine acts via a different route in Hcy metabolism, and may enhance Hcy metabolism even when folic acid levels are insufficient, in patients with methylenetetrahydrofolate reductase deficiency (Sakura *et al.* 1998) and in B_6 non-responsive homocystinuria (Singh *et al.* 2004). Recently, Setola *et al.* (2004) demonstrated a reduction in Hcy, improved endothelial function parameters, and lowered insulin levels, after 1 month of folic acid (5 mg/day) and vitamin B_{12} (0.5 mg/day).

Studies in adolescent hypertension and metabolic syndrome also confirmed inverse correlations between low levels of folate and vitamin B_{12}, and high levels of Hcy and insulin resistance parameters (Kahleova *et al.* 2002).

Our own studies in 96 insulin resistant PCOS women with a combination of vitamin B_6 50 mg, vitamin B_{12} (as cobalamin) 0.5 mg, folic acid 0.4 mg, and trimethyglycine (betaine) 1 g per day (Homocysteine Modulators, Solgar, NJ, USA) for three infertility treatment cycles revealed significant reductions in Hcy levels (Table 20.1). Interestingly, metformin treatment (1700 mg/day) or a combination of the two treatments in the same women reduced Hcy to a lesser (not statistically different) degree (Table 20.1). These data support the notion that one of the factors determining Hcy levels is the degree of insulin resistance (with compensatory hyperinsulinemia), and that improving insulin sensitivity will lower Hcy levels. When the study population was divided into two major groups, those treated with homocysteine modulators (HM) ($n = 51$) and those not (no-HM) ($n = 45$), irrespective of metformin treatment, the mean reduction in Hcy in the HM group was 33%, and mean reduction in the no-HM group was 18% ($p = 0.005$). Cumulative pregnancy rates after three cycles of treatment in the no-HM group was 63.8% (30/47) as opposed to a cumulative rate of 77.5% (38/49) in the HM group; pregnancy rates were 14% higher in the vitamin group, but this did not reach statistical significance ($p = 0.2$). It is possible that larger

Table 20.1. Reduction in plasma homocysteine (Hcy) by vitamin and/or metformin protocols in insulin resistant PCOS patients

Group	$n = 96$	BMI (kg/m^2) (mean)	LogHOMA (norm<0.3)	Pre-Hcy (mol/l) (mean)	Post-Hcy (mol/l) (mean)	Reduction of Hcy[a]
Control	$n = 21$	26.7	0.59	10.8	9.6	6%*
Metformin	$n = 26$	27.7	0.7	13.4	8.5	28%
Vitamins	$n = 24$	27.9	0.6	12.7	7.3	39%
Combination	$n = 25$	29.4	0.76	13.2	9.3	24%
Significance		NS	NS	NS	NS	$p < 0.01$*

Note:

[a] *Hcy reduction in control group significantly less than other groups.

studies will confirm improvement in pregnancy and live birth rates with Hcy-lowering protocols such as these.

Niacin (vitamin B_3), or its other forms (nicotinic acid or nicotinamide), has been documented as an effective nutritional supplement for the treatment of the dyslipidemia of metabolic syndrome. PCOS is associated with insulin resistance and male-pattern upper body fat accumulation. This obesity pattern is associated with elevated non-esterified fatty acids, and niacin and its analogs alleviate these high levels by binding to a high-affinity G-protein coupled receptor. Nicotinic acid might also be active via a mechanism which is anti-inflammatory, and by mobilizing cholesterol from macrophages, thus contributing to the regression of atherosclerotic plaque. Niacin–statin supplementation is one of the protocols being examined for the long-term treatment of atherosclerosis in patients with documented coronary disease and dyslipidemia, and was found to result in a low restenosis rate (Brown *et al.* 2001).

PPAR-gamma, rexinoids, and post-insulin-receptor signaling

Rexinoids and thiazolidinediones (TZDs) are two classes of nuclear receptor ligands that induce insulin sensitization in mammalian cells. The TZDs are activators of peroxisome proliferator-activated receptor-gamma (PPAR-γ), whereas retinoids and rexinoids are selective ligands for the retinoid X receptors (RXRs). Activated PPAR-γ activates gene expression in adipose tissue, altering fatty acid metabolism and fatty acid blood levels and increasing skeletal muscle sensitivity. Activated PPAR-γ also increases adiponectin and suppresses tumor necrosis factor-alpha (TNF-α) and resistin. Activation of RXRs by rexinoids leads to

improved insulin sensitivity in the whole organism by improving muscle insulin sensitivity (increasing muscle glucose disposal). Many nuclear respiratory factors are under the transcriptional control of PPAR-γ coactivator 1 alpha, which acts as a mitochondrial "master switch," and its activation can greatly improve insulin sensitivity. The fuel-sensing enzyme 5′-AMP-activated protein kinase (AMPK) also plays a role in the regulation of cellular lipid and protein metabolism in response to stimuli such as exercise, changes in fuel availability, and the adipocyte-derived hormones leptin and adiponectin. Insulin resistance may also be the result of dysregulation of AMPK and malonyl-CoA, a closely related molecule (Luo *et al.* 2005). Insulin resistance is also associated with increased insulin-like growth factor-1 IGF-1, while suppressing IGF binding protein-3. The latter is another natural ligand for RXR, so that reductions in this factor might influence transcription of antiproliferative genes normally activated by the body's endogenous retinoids or dietary retinoids. Most importantly, PPAR-γ activation in ovarian theca cells decreased luteinizing hormone (LH) and/or insulin driven androgen production by impairing the ability of cytochrome CYP17 to synthesize androstenedione from available progestins. This pathway may prove to be the intracellular link between insulin resistance, hyperinsulinemia, and theca cell androgen overproduction (Schoppee *et al.* 2002). The understanding of intracellular post-insulin-receptor pathways is crucial to achieving the best results in treatment of insulin resistance, both with classical drugs, and with nutritional manipulations (Cordain *et al.* 2003, McCarty 2005b).

Nutrition and nuclear receptors

Retinoic acid, the acid form of vitamin A, has been identified as a signal that inhibits the expression of resistin in adipocytes. Its effects were reproduced by selective agonists of retinoic acid receptors and rexinoid receptors. Vitamin A reduced body weight and improved glucose tolerance in mice (Felipe *et al.* 2004). Diets higher in vitamin A showed an inverse relationship with insulin resistance (Facchini *et al.* 1996), hence the importance of verifying normal vitamin A status in PCOS women.

Phytanic acid, a branched-chain fatty acid found in omnivore diets, can activate PPAR, and thus has a potential in respect to its impact on insulin sensitivity.

Green tea polyphenols, especially the catechin epigallocatechin gallate (EGCG) have known effects on endocrine systems (Kao *et al.* 2000); these are probably effected via their binding to nuclear receptors (Roy *et al.* 2003). Epigallocatechin gallate was found to reduce food intake, body weight, testosterone, LH, and IGF-1. It was also found to suppress endothelial responsiveness to vascular endothelial growth factor (VEGF), thus reducing the risk for ovarian hyperstimulation

syndrome (OHSS) in PCOS patients (McCarty 2003a). Green tea polyphenols were also found to reduce LDL and increase HDL in a rat model (Yokozawa *et al.* 2002), and to inhibit LDL peroxidation. These polyphenols also enable α-tocoperoxyl radical to be regenerated to α-tocopherol, demonstrating their value in preventing long-term damage and atherogenesis in insulin resistant patients, by virtue of their antioxidant properties and by increasing HDL cholesterol levels (Yokozawa *et al.* 2002). Insulin resistance may also be reversed by inhibition of the signalsome inhibitor of Kappa B kinase (IKK beta), which catalyzes nuclear factor-kappa B activation. This may be aided by polyphenols such as EGCG and milk-thistle extract silymarin (McCarty 2003c). Three natural polyphenols – quercetin, myricetin, and catechingallate – were found to interact directly with the GLU4 glucose transporter in adipocytes and muscle cells; these may impact on insulin resistance through glucose uptake in cells (Strobel *et al.* 2005). Natural sources of polyphenols include green tea, grape skin extracts (especially red grapes, and red wine), olive oil, flaxseed, apples, onions, garlic, and soybeans.

Cinnamon

Cinnamon contains α-phenyl cinnamic acid which was found to mimic the actions of PPAR-γ. These compounds inhibit cytochrome P450c17 activated enzyme synthesis and improve insulin sensitivity, and may be of potential use in PCOS hypersecretion of androgens. Cinnamon also contains methylhydroxy-chalcone polymer. This compound has been examined in an animal fructose diet model, and was found to prevent insulin resistance. Cinnamon administration prevented the development of insulin resistance at least in part by enhancing insulin signaling and possibly via the NO pathway in skeletal muscle (Qin *et al.* 2004).

PCOS and minerals

Magnesium

Available research suggests an association between magnesium deficiency and insulin resistance. Magnesium deficiency was a relatively common finding in both overweight adults and patients with Type 2 diabetes (Lima *et al.* 1998). Intracellular magnesium depletion was found to be a characteristic feature of insulin resistance in hypertensive patients; in fact experimentally increased insulin levels reduced cells' ability to accumulate magnesium (Paolisso and Ravussin 1995). A study conducted in PCOS women looked at the status of serum calcium and magnesium, and their ratio (Muneyyirci-Delale *et al.* 2001). Significantly lower serum magnesium ion and total body magnesium, and higher serum Ca^{2+}/Mg^{2+} ratios were found in PCOS women as compared to controls, irrespective of steroid hormone

concentrations. Another aspect of mineral metabolism in women with PCOS was examined by Thys-Jacobs and colleagues (1999). They found relatively low levels of vitamin D and relatively high parathyroid hormone, with normocalcemia. Treatment with vitamin D and calcium normalized cycles in 7/13 women. Calcium supplementation (1500 mg/day for 6 weeks) improved insulin sensitivity in diabetic, insulin resistant patients (Sanchez *et al.* 1997); these patients did not have PCOS. Vitamin D may have an anti-inflammatory role, as its deficiency is associated with elevated C-reactive protein, and its supplementation has been shown to lower TNF-α, and have other anti-inflammatory effects (Bubanovic 2004). It appears that some patients with insulin resistance have non-optimal calcium and magnesium metabolism; thus efforts should be made to investigate divalent cation status and to correct deficiencies or imbalances, if found.

Chromium

Evidence exists linking low levels of chromium and insulin resistance; this may be either primary, or secondary loss of chromium to the urine due to high insulin levels (Anderson *et al.* 1991, Morris *et al.* 1993). Nonetheless, chromium's therapeutic role in insulin resistance remains equivocal. Studies that support the use of chromium–nicotinic acid complex, chromium-rich brewer's yeast, Chelavite (chromium–niacin–amino-acid chelate form), or chromium picolinate have been published (Anderson *et al.* 1997, Kelly 2000, Rabinovitz *et al.* 2004) which among other effects, improved the lipid profile, and yet others have found no advantage to chromium (Joseph *et al.* 1999).

PCOS and antioxidants

Evidence has been gathered which supports the concept that PCOS and insulin resistance are associated with a state of increased oxidative stress and endothelial inflammation. The pathophysiological focus of this association resides most likely within the vascular endothelial cell. Increased intracellular deposition of lipoproteins leads to modifications in intracellular oxidation pathways, and the oxidation–reduction status is predisposed to an oxidative state; this is associated with increased lymphocyte and macrophage activation, thus perpetuating the inflammatory–oxidative stress situation. Increased oxidative stress and decreased antioxidant capacity in women with PCOS could be a contributing factor to the increased risk of cardiovascular disease in addition to classic risk factors as insulin resistance, hypertension, obesity, and dyslipidemia. These principles are well founded on clinical data as well as basic scientific findings. The Third National Health and Nutrition study in the US (Chen *et al.* 2004) found significant ($p < 0.01$) associations between insulin resistance parameters and ferritin, uric acid, fibrinogen,

and especially C-reactive protein. Other studies found similar associations between insulin resistance and platelet aggregation and interleukins 6 and 18. Direct (protein carbonyls, malondialdehyde concentrations) and indirect (total antioxidant status) measurement of oxidative status was measured in PCOS patients and correlated with insulin resistance parameters, glucose intolerance parameters, and ratios of LH to follicle stimulating hormone (FSH), and correlated inversely with HDL levels (Sabuncu *et al.* 2001, Fenkci *et al.* 2003). Similar findings were found in several patient groups – starting as early as childhood (Molnar *et al.* 2004), adolescence (Kahleova *et al.* 2002), and adulthood, in all patients with metabolic syndrome, and in the metabolic syndrome subtype of PCOS. Endothelial function was studied by flow-mediated dilation of the brachial artery, and arterial structure was evaluated by intima media thickness measurement using Doppler ultrasound of both common carotid arteries in a group of young women with PCOS; both were found to be impaired much before overt signs of vascular endothelial damage could be diagnosed (Paradisi *et al.* 2001, Kelly *et al.* 2002, Orio *et al.* 2004). Other studies demonstrate that women with PCOS, obese and non-obese, have elevated endothelin-1 levels (Diamanti-Kandarakis *et al.* 2001). A study using an in vitro ovarian theca–interstitial cell culture demonstrated a dose–response effect between oxidative stress (induced by hypoxanthine and xanthine oxidase) and theca cell proliferation (Duleba *et al.* 2004). These studies emphasize the importance of considering PCOS as a generalized systemic disease state, with insulin resistance and oxidative stress being central axes in the pathophysiology of both vascular endothelial and ovarian aspects of the syndrome. As such, nutritional balance in terms of oxidation–reduction is extremely important for both short-term results of infertility treatment and long-term health consequences.

Vitamin E (α-tocopherol) and beta-carotene status was found to be inversely related with plasma insulin levels in children with multimetabolic syndrome (Molnar *et al.* 2004), and in adults with insulin resistance (Facchini *et al.* 2000). Animal models have also found a correlation between lipid peroxidation and other oxidative stress parameters and insulin resistance (Thirunavukkarasu *et al.* 2004). Elevated Hcy is also associated with oxidative stress, and, as previously mentioned, is correlated with insulin resistance in PCOS patients. These studies form a logical theoretical basis for the supplementation of antioxidants to the diet of patients with insulin resistant PCOS.

Fish oil is rich in omega-3 essential fatty acids; two active forms are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Several studies have shown improved insulin sensitivity, decreased inflammatory markers, and improved blood lipids after EPA/DHA supplementation (Rivellese *et al.* 1996). Gamma linolenic acid (GLA), from borage oil or evening primrose oil, is an omega-6 fatty

acid normally synthesized from linolenic acid. This fatty acid is more efficacious when administered with EPA/DHA than on its own. Gamma linolenic acid has been found to have anti-inflammatory effects, and, importantly for PCOS patients, can inhibit 5-alpha reductase, thus reducing skin dihydrotestosterone. Lopez-Garcia *et al.* (2004) examined the effect of n-3 fatty acid consumption on inflammation biomarkers in women from the Nurses Health Study. Intake of EPA/DHA was inversely associated with soluble intracellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), C-reactive protein, interleukin-6, and E-selectin, which might explain in part the effect of these fatty acids in preventing cardiovascular disease.

Alpha-lipoic acid was examined in animal and clinical studies for its ability to improve insulin sensitivity. Using a high-fructose diet model in rats, α-lipoic acid improved both insulin sensitivity and decreased oxidation parameters such as lipid peroxidation and diene conjugate levels (Thirunavukkarasu *et al.* 2004). In clinical studies in humans, both intravenous and oral administration of α-lipoic acid increased insulin stimulated glucose disposal (mean improvement 27% in 74 patients treated with 600–1800 mg/day for 4 weeks) (Kelly 2000).

N-acetyl cysteine (NAC) is the acetylated form of the amino acid L-cysteine. Administration of this compound improves insulin sensitivity and inhibits VEGF, and can increase glutathione; thus it acts as an antioxidant, which also reduces plasma homocysteine. A prospective study investigating the effect of NAC on hormone and lipid profile and Hcy levels in insulin resistant PCOS women showed that NAC may reduce Hcy levels and improve lipid profiles making it an alternative to other treatments (Kilic-Okman and Kucuk 2004). Another study with NAC in insulin resistant PCOS women demonstrated a significant reduction in insulin resistance, testosterone levels, and in free androgen index, when 37 PCOS patients were treated with 1800 mg/day of NAC (Fulghesu *et al.* 2002). Rizk *et al.* (2005) recently looked at the contribution of NAC to ovulation induction in clomiphene resistant PCOS patients. The clomiphene–NAC treated group had a significantly higher ovulation rate than the clomiphene–placebo group. These studies highlight the potential of NAC in treating PCOS; further studies are certainly forthcoming.

Kris-Etherton and colleagues (2004) summarized the controlled clinical evidence regarding the effect of antioxidant vitamin and mineral supplements on cardiovascular risk. Collectively, for the most part, clinical trials failed to demonstrate a consistent beneficial effect of antioxidant supplements on cardiovascular morbidity. These studies dealt with supplementation of vitamins C, E, beta carotene, selenium, and/or niacin. Conversely, supplementation of B group vitamins and especially folic acid has been shown in specific patient populations (such as smokers, diabetics, and thrombophilic patients) to lower cardiovascular risk

and to improve reproductive performance, especially in hyperhomocysteinemia (Lee *et al.* 2003, Setola *et al.* 2004).

Summary

Polycystic ovary syndrome is a complicated endocrinological–metabolic syndrome which has insulin resistance as central to its multisystem manifestations. Nutrition/metabolic status has a critical effect on the appearance and probably the short- and long-term effects of the syndrome. Utilizing dietary recommendations and lifestyle modifications to optimize body weight and visceral fat status, both short-term fertility goals and long-term vascular health may be enhanced. Dietary recommendations should include consideration of the total caloric intake, distribution of carbohydrate/fat/protein intake, and type of fat consumed, especially unsaturated fats; those derived from nuts, seeds, olives, and fish are to be encouraged. Other dietary recommendations may include use of soybean products, fiber, and fresh leafy vegetables and whole grains as sources of folic acid, calcium, and B vitamins to reduce elevations of Hcy observed in insulin resistant PCOS. Foods rich in polyphenols, such as green tea, grape and soy products, milk thistle, and vitamin A, can be recommended as part of the strategy to improve nuclear receptor activation thus improving insulin sensitivity. Magnesium, calcium, and vitamin D status may also impact on insulin sensitivity. Finally, antioxidant status should be optimized, which may improve general vascular endothelial health in the long term. Vitamin E, N-acetyl-cysteine, and α-lipoic acid are supplements which seem to hold promise in this regard.

Both reproductive performance and cardiovascular/endothelial health are significantly affected by nutritional/metabolic status, especially insulin levels and insulin resistance parameters. A paramount effort should be made by all clinicians and healthcare providers to identify and address insulin resistance, obesity, and nutritional deficiencies in women with PCOS.

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