Pathophysiology of Polycystic Ovary Syndrome (PCOS): Lessons from Animal Studies

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Polycystic ovary syndrome is a complex and heterogeneous disorder mainly associated with increased circulating level of androgen. Morphological, endocrine, metabolic and clinical features are well defined. Genetic basis are currently under intense investigation. Genes related to the steroidogenic enzymes, insulin secretion and action and adiposity are currently under intense investigation. To understand the pathogenesis of PCOS, two recently developed models (Rhesus monkey and Bats) are studied in more detail. Studies on these animal models suggest that excess androgen level during prenatal or peri-pubertal period may be responsible for development of PCOS-like characteristics in adults. Animal studies further showed that excess androgen production during development might be responsible for abnormal pituitary function and enhanced visceral fat distribution, which in turn causes insulin resistance, hyperinsulinemia and abnormal follicular development. Additional animal studies are needed to establish the primary pathophysiologic mechanisms underlying this disorder.

Key Words: Polycystic ovary syndrome, Hyperandrogenism, Hyperinsulinemia, Animal model, Insulin resistance, Anovulation, Obesity

Introduction
PCOS is among the most common endocrinological disorders of women. It affects up to 10% women of reproductive age and over 70% of all cases of anovulatory infertility. Patients with PCOS have clinical and biochemical features of hyperandrogenism (HA), subfertility and recurrent miscarriage (Meenakumari et al. 2004). Despite its prevalence little is known about its etiology. Physicians have recently recognized the potential long-term consequences of PCOS, which include type 2 diabetes mellitus, hypertension and cardiovascular disease. In addition, endometrial hyperplasia and endometrial cancer also occur more frequently in women with PCOS. Therefore, PCOS is a major issue for women's health with ramifications well beyond the reproductive age.

Studies on PCOS had incited more controversy than the definition. Causes of PCOS may be due to a primary hypothalamic defect, ovarian steroidogenic defect and/or defect in the insulin secretion and action. There are now increasing evidences to support genetic basis for PCOS. Notwithstanding the high prevalence and significant morbidity of this disorder, little is known about the developmental origin of PCOS. This is primarily due to the lack of relevant animal models for studies on PCOS. Although cystic ovaries and anovulation can be produced in the laboratory and domestic animal species with various pharmacological manipulations, but many of these animal models failed to replicate the human disorder. Typical PCOS-like conditions are induced experimentally in rhesus monkey (Abbott et al. 1998) as well as in the sheep (Padmanabhan et al. 1998, Robinson et al. 1999). Studies from our laboratory also noted a typical PCOS-like feature in the bat, Scotophilus heathi, naturally during a specific phase of the reproductive

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cycle (Abhilasha & Krishna 1996). The bat exhibits a unique phenomenon of delayed ovulation. The bat during the period of delayed ovulation showed a number of features, such as hyperandrogenism (HA), hyper-insulinemia (HI), presence of a number of antral follicles, anovulation, hyperthecosis, obesity etc., similar to the women with PCOS. The studies on the bat are specially described here in more detail and its usefulness for studying PCOS is also discussed. Occurrence of PCOS-like condition naturally during the reproductive cycle is also noted in the spotted hyenas (Yalcinkaya et al. 1993). The aim of the present review is to summarize our present knowledge on the hormonal, clinical and metabolic features of this very prevalent syndrome and to discuss how the present knowledge on animal studies explains about the pathogenesis of PCOS.

PCOS: A Syndrome of Unresolved Etiology

PCOS is a complex, multifunctional syndrome with heterogeneous clinical features. The complexity of PCOS pathophysiology is of such magnitude that its etiology remains unknown, its treatment is unresolved and its definition remained unclear (Zawadzki & Dunaif 1992). Based on current understanding, PCOS may be defined as HA accompanied by anovulation, without underlying adrenal or pituitary disorders or ovarian tumors. It has been established that, PCOS also exhibits a unique form of HI and insulin resistance (IR) (Dunaif et al. 1995) associated with or without obesity. Women with the PCOS have some aberration in gonadotropin secretion as compared with normal women (Ehrmann 2005). Since gonadotropin concentrations vary over the menstrual cycles and are released in a pulsatile fashion into the circulation, a single measurement of luteinizing hormone (LH) and follicle stimulating hormone (FSH) provides little diagnostic sensitivity.

PCOS has a heterogenous etiology, involving a variety of combination of reproductive, metabolic and genetic determinants (Table 1 & 3). No single etiology has so far been found to particular predictive power in explaining the occurrence of PCOS. Furthermore, no single etiological gene or inheritance pattern has been found for PCOS. Clearly, improvements in our understanding of the developmental processes causing PCOS are necessary to clarify the relative importance of the multifunctional components in PCOS development and to focus clinical initiative in prevention, diagnosis and treatment. A recent workshop held in 2005 under the auspices of the American Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine proposed a revision of the criteria for the diagnosis of PCOS, of which two of the following three would be needed: 1) oligo- and/or anovulation; 2) clinical and/or biochemical signs of HA; and 3) polycystic ovaries, together with the exclusion of other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors, or cushing’s syndrome (Escobar-Morreale et al. 2005).

Characteristic Features of PCOS

Polycystic ovary syndrome is now recognized as an important reproductive and metabolic disorder. The HA and anovulation are two most common reproductive disorders of PCOS. Many patients with PCOS also develop obesity, dyslipidemia and IR suggesting metabolic disorders (Apter 1998). Majority of women with PCOS have elevated serum androgen levels or a biological expression of HA (acne or hirsutism). In a recent study about 90% of PCOS women of Indian sub-continent showed HA (figure 1) (Chanda 2002). The source of androgen is primarily ovarian though adrenal gland may also contribute to HA. Increased serum testosterone level is the best marker for ovarian HA, whereas dehydroepiandrosterone (DHEA) is the best marker for adrenal HA (Auchus et al. 1998). All measures that reduce androgen like ovarian wedge resection, follicle drilling and antiandrogen treatment restore ovulation. The mechanism involved in excess ovarian androgen production remains uncertain and may be multifactorial (Gilling-Smith et al. 1997, Escobar-Morreale et al. 2005). Some of the most important factors, include increased stimulation and sensitivity to circulating gonadotropins and/or increased ovarian stimulation by insulin, growth factors etc. (Marshall et al. 1999, Tsilikorchidou et al. 2004). The genetic mechanism underlying HA in PCOS is also extensively studied and is recently described in detail (Escobar-Morreale et al. 2005).

Within ovary, thecal cells are major sites of androgen synthesis (Gilling-Smith et al. 1997). Both in vivo and in vitro studies of thecal cell function show an intrinsic abnormality of ovarian steroidogenesis (Rosenfield et al. 1990, Auchus et al. 1998). Theca cells from polycystic ovaries produce 20 times more androstenedione than similar cells from normal ovaries (Gilling-Smith et al. 1994). Further studies confirmed that theca cells from PCOS show increased activity of steroidogenic enzymes resulting in raised androgen production both basally and in response to LH stimulation (Franks et al. 1999, Nelson et al. 2001). Nelson et al. (2001) reported P450 c 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme activities were increased by more than 500% and 1000%, respectively, in PCOS theca cells compared with controls, whereas 17β-hydroxysteroid dehydro-
Table 1. Characteristic Features of PCOS

<table>
<thead>
<tr>
<th>Reproductive features</th>
<th>Polycystic ovaries (a number of antral follicles located on periphery)</th>
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<td>Hyperthecosis</td>
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<td>Hyperandrogenism</td>
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<td>Increased frequency and amplitude of LH secretion</td>
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<td>Normal to low FSH</td>
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<td>Increased GnRH pulse frequency</td>
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<td>Metabolic features</td>
<td>Hyperinsulinemia</td>
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<td>Insulin resistance</td>
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<td>Obesity (abdominal adiposity)</td>
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<td>Hyperlipidemia (Elevated level of cholesterol, triglycerides and LDL and low level of HDL and apolipoprotein A)</td>
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<td>Impaired pancreatic β-cell function</td>
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<td>Clinical features</td>
<td>Menstrual disturbances (amenorrhea, oligomenorrhea)</td>
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<td>Hirsutism, Acne</td>
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<td>Alopecia</td>
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<td>Anovulatory infertility</td>
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<td>Recurrent miscarriage</td>
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<td>Obstructive sleep apnea</td>
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<td>Acanthosis nigricans</td>
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<td>Long term risk consequences</td>
<td>Endometrial cancer</td>
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<td>Endometrial hyperplasia</td>
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<td>Impaired glucose tolerance and diabetes</td>
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<td>Increased risk factors for Cardiovascular Diseases (Hypertension, Myocardial infarction)</td>
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<td>Impaired fibrinolysis (risk for vascular lesions)</td>
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<td>Gestational diabetes</td>
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genase (17β-HSD) enzyme activity was unaffected.

Women with PCOS show an accelerated LH pulse frequency and amplitude (Kazar et al. 1987), whereas FSH levels remain normal to low (Marshall & Eagleson 1999). These studies have led to the use of an elevated LH to FSH ratio as a marker for PCOS. Ratio of 2 to 3:1 is commonly used to indicate abnormal gonadotropin secretion. The predominant reason for high serum LH is because of chronic absence of progesterone in PCOS due to anovulation. The frequency of gonadotropin releasing hormone (GnRH) release is increased secondary to decreased sensitivity of GnRH pulse generator to the negative feedback effect of estradiol and progesterone. This increased GnRH pulse frequency increases LH release (Robinson et al. 1999, Marshall & Eagleson 1999). The raised LH level enhances theca androgen production and because of arrested folliculogenesis the androgens are incompletely aromatized to estrogen. The so called vicious cycle of PCOS is created, in which abnormal gonadotropins secretion begets increased ovarian androgen production, which in turn alter gonadal steroid feedback, perpetuating abnormal gonadotropins release (Sam & Dunaif 2003). Abnormalities in neuroendocrine modulators, such as the endogenous opioids, dopamine and leptin, have also been proposed as determinants of altered gonadotropin secretion in PCOS (Poretsky et al. 1999). Endogenous opioid excess may sensitize the gonadotropes to GnRH particularly in association with HI (Lanzone et al. 1995). It is not clear whether the accelerated pulse frequency is due to an intrinsic abnormality in the GnRH pulse generator or is caused by the relatively low progesterone resulting from infrequent ovulatory events.

PCOS women are mostly chronically anovulatory, showing either oligomenorrhea or amenorrhea. Nevertheless, a history of regular menses is also found in some PCOS. Some women with normal menses may also be anovulatory. Anovulatory women with PCOS are generally hyperinsulinemic and more insulin resistant (IR) than weight match control subjects.

Table 2. Obesity factors: affecting IR in PCOS

<table>
<thead>
<tr>
<th>Insulin sensitizer</th>
<th>Leptin</th>
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<td>Insulin antagonists</td>
<td>Adiponectin</td>
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<td></td>
<td>TNF-α</td>
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<td>Resistin</td>
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<td></td>
<td>IL-6</td>
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<tr>
<td>Gene</td>
<td>Encoding protein</td>
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<tr>
<td>INS Gene VNTR</td>
<td>Variable number tandem repeats upstream of insulin gene which might regulate its expression</td>
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<tr>
<td>FST Gene</td>
<td>Follistatin</td>
</tr>
<tr>
<td>IRS-1 and 2 gene</td>
<td>Insulin receptor substrates-1 and 2</td>
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<tr>
<td>TNF-α receptor 2 gene</td>
<td>Serum soluble TNF-α receptor 2</td>
</tr>
<tr>
<td>RETN gene</td>
<td>Resistin</td>
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<tr>
<td>CYP 1 A 1</td>
<td>Cytochrome P 450 enzyme</td>
</tr>
<tr>
<td>11β HSD and H 6 PD</td>
<td>11β Hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase enzyme</td>
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(Dunaif 1997). Although IR is usually associated with obesity, anovulation is also found in normal weight women with PCOS (Carmina et al. 1999, Morales et al. 1996). Obesity and PCOS have an additive deleterious effect on IR (Dunaif 1999). It has recently been found that all the women with PCOS may have evidence of IR, it is more pronounced in those with chronic anovulation than in those who have ovulatory cycle. The pathogenesis of IR in PCOS is currently under intense investigation.

It has been reported that IR may be related with excessive serine phosphorylation of the insulin receptor in at least 50% of women with PCOS (Dunaif et al. 1985). Serine phosphorylation may also increase the activity of P450c 17α hydroxylase (CYP17), the rate-limiting enzyme for androsterone biosynthesis (Franks et al. 1998). It is possible that the same serine kinase phosphorylates the insulin receptor, producing IR, and phosphorylated P450c 17α hydroxylase producing HA. Cytokines and free fatty acid (FFA) can activate intracellular serine kinases and might play a role in the pathogenesis of PCOS. It is also possible that the serine kinase phosphorylates downstream signaling molecules, such as insulin receptor substrate (IRS) 1 or 2, further compromising insulin signaling. Martens et al. (2000) have further investigated the unified hypothesis of increased serine kinase activity for pathogenesis of PCOS by transfecting P450c 17α hydroxylase into skin fibroblasts, but failed to detect increase in serine phosphorylation of the enzyme in PCOS fibroblasts. This study only fails to support rather than disprove the serine kinase hypothesis.

Growth hormone (GH) may be involved in the genesis and maintenance of HA, chronic anovulation and polycystic ovarian morphology, at least in the subgroup of lean PCOS women in which it is hypersecreted (Morales et al. 1996). GH may modify ovarian steroidogenic responses to trophic hormones either directly or via local insulin-like growth factor-1 (IGF-1). GH action on growth factors such as IGF-1
and their binding proteins are known to be similar to those of insulin (Conway et al. 1990). IGF stimulate ovarian cell proliferation and steroidogenesis, inhibit apoptosis and may be related to the development of HA in PCOS (Giudice 1999).

More than 65 percent of women with PCOS are obese (Dunaif 1992). The fat distribution often is abdominal/visceral, similar to that frequently associated with metabolic abnormalities (e.g. hypertension, dyslipidemia, IR, glucose intolerance). Women with PCOS have many abnormalities in lipid and lipoprotein profiles, including elevated levels of cholesterol, triglyceride, and low density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I (Lobo & Carmina 2000) and increased concentration of atherogenic LDL-III (Dejoger et al. 2001). Body fat distribution is a major determinant of insulin sensitivity in PCOS. It is well documented that HA in PCOS has a profound effect on body fat distribution, with a proclivity to abdominal adiposity, thus predisposing to IR. Obesity is likely to facilitate the metabolic abnormalities of PCOS, as evident by the reduction in IR and restoration of cyclic menses following weight loss (Kiddy et al. 1992). An earlier study showed that a 10-15 percent weight reduction had resulted in spontaneous conception in more than 75 percent of obese patients with PCOS (Andersen et al. 1995, Huber-Buchholz et al. 1999).

Adipose tissue is known to secrete a large number of factors with significant effect on IR (Table 2) as well as many of these factors are also shown to affect ovary-pituitary-hypothalamic axis (Pittas et al. 2004). Recent studies have implicated the cytokine, Tumor necrosis factor-alpha (TNF-α) as a contributor to IR in obesity (Chanda et al. 2003). In Native American Pimas, in whom IR and obesity are highly prevalent, and in whom oligomenorrhea is common, a mutation closely linked to TNF-α has been associated with IR (Norman et al. 1995). A recent study showed that the methionine 196 arginine polymorphism in exon 6 of the TNF-α receptor 2 gene is associated with the PCOS and HA (Peral et al. 2002). TNF-α also stimulates interleukines-6 (IL-6) secretion by adipocytes, evidences suggest that IL-6 is also implicated in IR and associated syndromes (Fernandez-Real et al. 2000). IL-6 concentrations are increased in peritoneal fluid in anovulatory patients, suggesting a role in the pathogenesis of HA disorders (Omu et al. 2003). Leptin has been shown to potentiate the GnRH secretion (Burcelin et al. 2003). Moreover, in rodents mutation causing leptin deficiency or leptin receptor defects cause obesity and infertility associated with hypogonadotropic anovulation (Zhang et al. 1994).

Most studies, however, report that leptin levels in women with PCOS do not differ significantly from controls, regardless of their body weight (Rouru et al. 1997). In addition there is no evidence for mutations of leptin or leptin receptor genes in women with PCOS (Oksanen et al. 2000). Resistin is a recently discovered adipocyte-secreted polypeptide that has been implicated in the development of IR (Pittas et al. 2004). The relationships between resistin and IR in patients with PCOS are recently studied and the results suggest overexpression of the resistin gene in PCOS (Seow et al. 2004). The mechanisms by which these factors (TNF-α, leptin, resistin, etc.) from adipocytes promote IR are complex and our understanding incomplete. Additional human studies are needed to understand their role in pathogenesis of PCOS.

HI is known to play a central role in promoting ovarian androgen production in PCOS. Insulin receptors are widely distributed throughout all ovarian compartments, and insulin exerts several important effects on ovaries including: (1) stimulation of ovarian production and secretion of androgens by stimulating P450c 17-α hydroxylase activity, (2) potentiation of the ovarian steroidogenic response to LH and FSH, (3) enhancing the amplitude of LH pulses (Nestler, 1997), and (4) suppression of apoptosis in ovarian follicles which could reduce the rates of atresia and lead to cyst formation (Poretsky et al. 1999). Thus, insulin actions on the ovaries, which are mediated through ovarian insulin receptors, may explain the characteristic findings in PCOS including HA, anovulation, and ovarian cyst formation. Reversal of IR in PCOS constitutes the fundamental approach in the management of hyperandrogenic anovulatory infertility and in the prevention of long-term consequences. It is now well demonstrated that the treatments of PCOS patients with insulin sensitizers (e.g. metformin), which reduce serum insulin levels, often induce ovulation and pregnancy (Poretsky et al. 1999, Moghetti et al. 2000, Meenakumari et al. 2004). Previous studies have shown either normal or decreased insulin receptor expression in the ovaries of PCOS as compared with the controls (Poretsky et al. 1999). Analyses of insulin receptor gene in PCOS showed mutation in the tyrosine kinase domain of the insulin receptor gene in a few patients with severe IR (Moller & Flier 1988, Cama et al. 1991), but no sign of mutations of insulin receptor gene in many PCOS patients (Conway et al. 1994, Talbot et al. 1996).

Majority of studies on PCOS were conducted on women from North American subcontinent or from Europe. Only limited studies on PCOS women are available from Indian subcontinent (Rodin et al. 1998, Zargar et al. 1997, Maitra et al 1994, Norman et al.
Studies on Animal Model

Various animal models used from time to time to investigate the pathogenesis of PCOS are briefly described. Most of the earlier models are developed keeping primarily morphological features (polycystic ovary) in consideration. Only recently, some models have been developed, in which both morphological (polycystic ovary) and biochemical characteristics of PCOS (HA, HI etc) are attained.

Rat Models

Rats treated with estradiol valerate (EV), dehydroepiandrosterone (DHEA), anti-progesterone, insulin, and human chorionic gonadotropin (hCG) and testosterone neonatally or exposed to constant illumination showed morphological features in the ovary similar to the polycystic ovary in human.

Brawer et al. (1986) observed that the adult female rat treated with estradiol valerate had showed cystic follicles along with degenerating granulosa cells and hypertrophy of the theca cells within 8-9 weeks. However, the circulating concentration of DHEA, androstenedione and testosterone showed no significant change as compared with the normal female rats during proestrus (Mahajan 1988). Therefore this model was not pursued further for studies related to PCOS.

Rat treated with DHEA is one of the most thoroughly studied animal model for investigating changes in the ovarian morphology during follicular cyst development. Continued treatment with DHEA (3mg/100gm body weight) for 30 days to immature female rats affects follicular maturation and ovulation leading to formation of polycystic ovary. Rats treated with DHEA also showed increased concentration of androstenedione and estradiol and caused alteration in LH:FSH ratio. The DHEA model can be useful for the study of changes in hypothalamo-pituitary-oestrogenic axis during development of cystic ovary (Krishna et al. 2001). However, observation of heterogeneity in the ovarian histology and hormonal profiles in different rats treated with DHEA make this model unreliable for studies on PCOS. Also, not all the DHEA treated rats show PCO and therefore has limited use.

When rats are exposed to light continuously for more than 4 weeks, the ovary develops a number of cystic follicles as found in the PCOS (Brownman 1937, Crichlow 1963). The ovaries of these rats contain a number of large antral follicles with no sign of ovulation. It is probably due to irreversible synthesis and release of cyclic LHRH. However, the levels of circulating androgen are not increased in these rats. This model may be used for the study of abnormalities in synthesis and release of gonadotropin as also found in PCOS.

1995). The study of Rodin et al. (1998) suggests high prevalence of polycystic ovary among the Indian women. Norman et al. (1995) showed that the Indian women with PCOS had higher insulin response than the White women thus suggesting the importance of ethnic background. Interestingly, Asian ethnic groups have substantial HA without any significant skin manifestation (Carmine et al. 1995).

Genetics of PCOS

Several lines of evidence suggest that there is an underlying genetic cause for PCOS (Table 3). Familial clustering of PCOS and PCOS mediated phenotypes suggest that genetic factors are involved in the etiology of the disorder (Legro et al. 2002, Strauss, 1999). Linkage and association studies using affected sib pair analysis and the transmission/ disequilibrium test to explore candidate genes point a finger at a region on chromosomes 19p13.3. The putative PCOS gene lying in this region has yet to be identified. However, existing data suggest that it is probably involved in signal transduction mechanism leading to altered expression of a suite of genes that affect ovarian steroidogenic enzymes activities as well as the metabolic phenotypes of other cell types, including muscle and fat. Whether there are only one or several genetic defects causing reproductive as well as metabolic abnormalities, characteristics of PCOS, is not yet clear. In a recent study on women from the south India showed a strong association between mutation in CYP1A1 and increased susceptibility to polycystic ovary (Babu et al. 2004).
The administration of a single injection of testosterone to female mice (Barraclough 1955) or rat (Barraclough 1961) on day 2-5 neonatally causes the development of polycystic ovary and anovulation during adult period. These females showed no significant increase in serum concentrations of testosterone and estradiol. The ovaries of neonatally androgenized rats were found to be insensitive to FSH and required further investigation.

Female rats treated daily with anti-progesterone (RU 486) result in ovarian enlargement with follicular cysts (Sanchez-Criado et al. 1993). Administration of RU 486 in the rat results in an increase of the basal level of LH and prolactin, but a decrease in the FSH level. This increased LH level stimulates the production of androgen. Further, RU486 suppresses preovulatory LH surge and therefore causes anovulation. Thus, the RU 486 shows anovulation, an increased LH/FSH ratio and an increased androgen as shown in PCOS women (Ruiz et al. 1996).

The rats stimulated with low doses of hCG induces ovarian follicular cysts. Insulin alone fails to induce polycystic ovaries in rats. But simultaneous treatment of rats with both hCG and insulin induces anovulation, enlargement of the ovaries with multiple cystic follicles, high androgen levels and hyperinsulinemia as found in PCOS women (Poretsky et al. 1992, Bogovich et al. 1999).

Rhesus Monkey model
Based on the concept that chronically elevated circulating concentration of androgen and/or estrogen may be the primary cause for anovulation in PCOS, Billiar et al. (1985) and Mahajan (1988) developed a monkey model by increasing the circulating concentrations of androstenedione and estrone using sialistic capsule. The increased concentrations of androstenedione and testosterone caused changes in the ovaries that resembled with polycystic ovary. However, unlike PCOS patients, androstenedione implanted monkey remained ovulatory. The estrone treated monkey showed anovulation but had low circulating LH concentration, suggesting a negative feedback effect. Moreover, chronically elevated androgen neither affects serum insulin level nor insulin resistance (Billiar et al. 1987).

Abbott et al. (1998) developed an animal model for PCOS studies by prenatal androgenization of female rhesus monkey. These prenatally androgenized monkeys show enlarged ovaries with multiple medium-sized follicles and relatively high serum testosterone levels quite similar to those of women with PCOS (Dumesic et al. 1997). Most of these monkeys showed elevated level of serum LH but low serum FSH level. This imbalance in circulating gonadotropins may be responsible for the slow rate of follicular development and maturation, but high rate of testosterone production. These neonatally androgenized monkeys also showed hyperinsulinemia and insulin resistance, which is associated with increased adiposity in a manner similar to that of PCOS (Kemnitz et al. 1989, Eisner et al. 2002). Although, some prenatally androgenized females have body mass index and fasting serum insulin level comparable with those of control, whereas other hyperinsulinemic monkeys are more prone to anovulation than control. This suggests that insulin may have important role in the anovulation in prenatally androgenized monkey. So adult female rhesus monkey, prenatally androgenized, shows many clinical and biochemical features of PCOS. HA seems to be the core functional disorder in both women with PCOS and adult female rhesus monkey, androgenized prenatally (Abbott et al. 2002).

Studies on Sheep
Adult female sheep exposed in utero to large doses of testosterone shows enlarged ovaries with multiple, medium sized antral follicles, abnormal ovarian cycle and increased LH secretion as found in PCOS (Padmanabhan et al. 1998, Robinson et al. 1999). This model is used extensively to investigate the mechanism by which high androgen during neonatal period affects the regulation of GnRH secretory activity in the adult animal.

Studies on Bat (Scotophilus heathi)
Bat showing PCOS-like features during the period of delayed ovulation: Studies from our laboratory have demonstrated that Scotophilus heathi, a seasonally monoeestrous tropical Vespertilionid bat (Order: Chiroptera), show many large antral follicles during the months of November but ovulation is delayed until early March (Krisha & Singh 1992). This unique feature is commonly known as delayed ovulation. All attempts to induce ovulation during this period were unsuccessful (Singh & Krishna 1992). Further studies from our laboratory suggested that increased androstenedione produced by the ovary from November to February might be responsible for delayed ovulation in the bat (S. heathi) (Abhilasha & Krishna 1996, 1997). A similar association between HA and chronic anovulation is also known to exist in patients with PCOS. Recently more data have been accumulated suggesting a close similarity between PCOS and ovarian morphological and endocrinological features in the bat during the period of delayed ovulation (Chanda et al. 2003). Thus, bats provide a unique animal model, which is having naturally occurring symptoms of PCOS.
Morphological features of the ovary of bat (S. heathii): The ovary of the bat is relatively large in November and December. Ovarian follicles both healthy and atretic, of different sizes are peripherally located, and separated by extensive stroma (figure 2). A major portion of the stroma contained large and highly vacuolated hypertrophied interstitial cells, similar to luteinized stromal cells. Histologically, both thecal and interstitial cells looked alike. These luteinized thecal interstitial cells are full of lipid droplets and exhibit histological features similar to hyperthecosis of human ovaries during PCOS (figure 3)(Abhilasha & Krishna 1997).

High circulating androgen and active androgen synthesis in the ovary of bat (S. heathii): Female bat during the period of delayed ovulation display concentration of androstenedione that are unusually high compared with the values reported for most mammals (Feder 1985, Glickman et al. 1992). The concentrations of circulating testosterone are also high. The in vitro studies show that the ovary is the major source of both androstenedione and testosterone in the bat. Immunocytochemical localization of P450 side chain cleavage enzyme (scc) and P450 17α hydroxylase in the ovary suggests that the thecal interstitial cells are the major site of steroidogenic activity and androgen secretion. Overproduction of androgen is therefore attributed to hypertrophied thecal-interstitial cells of the bat ovary.

Figure 2 Bat ovary showing polycystic ovarian morphology. Note the presence of antral follicles (AF) in the periphery and stroma area filled with interstitial cells (IC).

Figure 3 Fig 2 enlarged to show the presence of interstitial cells (IC) in the ovarian stroma of bat ovary. Note the presence of hypertrophied interstitial cells (arrow).

Figure 4 Scotophilus heathi showing deposition of subcutaneous white adipose tissue during November and little or no deposition of subcutaneous adipose tissue during February

High androstenedione concentration correlated with increased body weight in the bat (S. heathii): The bat exhibit a remarkable seasonal fat deposition (October to January) as an adaptation for low insect availability during winter (figure 4)(Krishna 1982). The period of fat deposition in the bat is preceded by a period of increased insect availability (August to October) and consumption. The circulating androstenedione concentration in the bat is also increased during the period of increased food intake. Increased androstenedione production and high calorie intake may be responsible for the upper body fat deposition in the bat, as it is suggested for obese women (Barbieri & Hornstein 1988). The fat deposition chiefly account for the increase in body weight (obesity) during winter dormancy, which also coincide with the increased androstenedione concentration in the bat (figure 5)(Abhilasha & Krishna 1997).

Ovarian androstenedione production enhanced by insulin in the bat (S. heathii): Both insulin and androstenedione concentration increase during the
period of delayed ovulation and decline significantly during the preovulatory period suggesting a significant correlation between circulating insulin and androstenedione concentration in this bat (Doval & Krishna 1998). Exposure of the bat ovaries in vitro to a supraphysiological dose of human insulin during November significantly augmented the production of androstenedione. During this period a supraphysiological dose of human insulin also significantly potentiated hCG induced androstenedione production by the ovary. These findings support the hypothesis that insulin amplifies the LH dependent mechanism that regulates ovarian androgen secretion (Harnandez et al. 1988). Demonstration of insulin binding sites in the ovary of the bat suggests a direct action of insulin on the ovarian tissues. Binding sites for insulin in the ovary of the bat were found mainly in the thecal and stromal cells. The extensive stromal luteinization has been shown in the ovary of the bat during November, which coincides with a high concentration of circulating insulin. It is suggested that the insulin induced increase in androstenedione synthesis may be partly mediated through stromal luteinization in the bat ovaries. Recent study on the ovary showed a linear relationship between adiposity (body weight), insulin and androstenedione in the bat as reported in women with PCOS. Bats during the period of delayed ovulation thus exhibit features such as obesity, HA, HI and ovarian hyperthecosis, which bear similarities to those seen in women with PCOS. Bat during the period of delayed ovulation represent one of the severest cases of PCOS because they exhibit extensive hyperthecosis. The bat ovaries during the period of delayed ovulation remain unresponsive to various stimuli that induce ovulation. It is also an ‘experiment in nature’ that should help to guide our research into interrelationship between obesity, HI and causes of HA.

Studies on Spotted hyenas (Crocuta crocuta)

Female spotted hyenas, known for their highly masculinized genitalia, are naturally exposed to high androgen level neonatally in utero. High testosterone level during sexual differentiation results from the placental conversion of androstenedione, which is secreted by the maternal ovary in significant amounts (Yalcinkaya et al. 1993). Wynn and Amoroso (1964) describe the ovaries of the spotted hyena as being histologically similar to those of women with PCOS specifically with regard to the hyperthecosis and rarity of follicles. The ovaries in reproductively active spotted hyenas have a striking paucity of mature antral follicles and an unusual abundance of androgen-secreting theca-interstitial tissue (Matthews 1939). However, female spotted hyenas show no evidence of losing their capacity to ovulate, either in wild or in the captivity, despite their exposure to naturally high levels of testosterone during gestation.

Inferences Derived from Animal Studies:

1. Animals exposed neonatally to elevated levels of androgen either naturally (Bat, Hyena) or experimentally (rhesus monkey) develop polycystic ovarian morphology, such as unusual abundance of androgen secreting theca-interstitial tissue (Matthews 1939, Abhilasha & Krishna 1996) and premature arrest of follicular development (Abhilasha & Krishna 1998). The patients with congenital adrenal HA due to 21-hydroxylase deficiencies also showed evidence of polycystic ovary (PCO) (Barnes et al. 1994). Thus studies on human supports animal studies that either prenatal or prepubertal HA leads to development of PCO.

2. Animals showing polycystic ovarian morphology are hyperandrogenic. Therefore, it may be suggested that HA is a biochemical manifestation of PCO. Although, Norman et al. (1995) failed to demonstrate HA in the patients with PCOS.

3. The major source of excess androgen synthesis in the animals showing polycystic ovarian morphology is usually ovary. Both in vivo and in vitro studies of theca cell function in bats show an intrinsic abnormality of ovarian steroidogenesis (Abhilasha & Krishna 1996, 1998). A pronounced ovarian androgen production in response to exogenous hCG was observed in adult female rhesus monkey androgenized prenatally (Eisner et al. 2002). These studies on animal models support the previous findings on cultured human thecal cells from PCOS producing 20 times more androgen than the similar cells from normal ovaries (Gilling-Smith et al. 1994).

4. It is observed that the HA during prenatal or prepubertal period has a profound effect on body fat distribution, with a proclivity to abdominal adiposity (Abbott et al. 1998, Abhilasha & Krishna 1998, Chanda et al. 2003). This supports the hypothesis of androgen dependent body fat accumulation in PCOS women.
5. Animal exposed to androgen excess in utero (rhesus monkey model) or in prepubertal period (Bat) exhibit a specific impairment of insulin secretion or insulin action (Abbott et al. 1998, Doval & Krishna 1998). It has been suggested that the increased adiposity associated with IR and consequently HI in prenatally androgenized monkeys and bats during the period of delayed (Doval & Krishna 1998) are similar to that of PCOS women (Kemnitz et al. 1989). HI secondary to IR, plays an important role in the pathogenesis of PCOS by amplifying androgen production (Dunaif 1997).

6. HI may contribute to the mechanism of anovulation in prenatally androgenized female rhesus monkey as well as in the bat as found in PCOS women. Studies on the bats show that the ovarian steroidogenesis is spared from the effects of IR and therefore is responsive to the high circulating concentration of insulin. Insulin synergistically interacts with LH to augment steroidogenesis and to induce premature arrest of follicular development in the bat and rhesus monkey (Doval & Krishna, 1998, Willis et al. 1998).

7. Hypersecretion of LH is demonstrated in prenatally androgenized female rhesus monkey (Steiner et al. 1976) and sheep (Robinson et al. 1999). The mechanism for LH hypersecretion in PCOS is not entirely known but recent studies on sheep suggest that it involve the decreased sensitivity of the GnRH pulse generator to gonadal steroid negative feedback as a consequence of raised circulating androgen levels.

8. Anovulatory condition (during the period of delayed ovulation) in the bat coincides closely with the adiposity and ovulation in these bats is achieved only when body weight declines due to resorption of deposited fat (Abhulsha & Krishna 1997). Since the treatment of obesity is now a major focus of preventive health care for women with PCOS, further studies on bat model may be useful in this regard.

A Hypothetical Model about the Pathogenesis of PCOS

Based on the information noted from the animal studies supported with clinical data and genetic studies following hypothetical model about the pathogenesis of PCOS is proposed (figure 6).

Synopsis and Conclusion

A comparison between adult female rhesus monkeys androgenized prenatally, female bat, S. heathii during the period of delayed ovulation, and women with PCOS showed striking similarities and results are outlined in Table 4. Since animals achieve HA during de-

![Figure 6 A hypothetical model about the pathogenesis of PCOS](image)

Table 4. Comparison of PCOS, bat & monkey model

<table>
<thead>
<tr>
<th>Signs/Symptoms</th>
<th>PCOS</th>
<th>Scotophilus heathii during delayed ovulation</th>
<th>Rhesus monkey neonatally androgenized</th>
</tr>
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<tbody>
<tr>
<td>Anovulation</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Enlarged ovaries with multiple small follicles</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hyperthecosis</td>
<td>+</td>
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<tr>
<td>Hyperandrogenism</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hyperinsulinemia from insulin resistance</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LH hypersecretion</td>
<td>+</td>
<td>*</td>
<td>+</td>
</tr>
<tr>
<td>FSH insufficiency</td>
<td>+</td>
<td>*</td>
<td>+</td>
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<tr>
<td>Increased abdominal adiposity</td>
<td>+</td>
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</tbody>
</table>
Pathophysiology of Polycystic Ovary Syndrome

velopment either experimentally (for examples, rhesus monkey and sheep) or naturally (for examples bat and hyena) exhibit a close phenotypic mimic of PCOS; these studies thus suggest that androgen excess during development may be a basic requirement for the differentiation of the metabolic and reproductive features of the syndrome. Although, the perimenarchal onset of HA and PCOS-like condition has earlier been described in women with PCOS (Noble & DeWailly 1992, Apter 1998). The age of initial androgen excess might be one of the causes of heterogeneity among PCOS. Exposed to excess androgen to the adult animals however, produced only moderate features of PCOS.

The ovaries of spotted hyena and the bat have a striking paucity of mature follicles and an unusual abundance of androgen sensitive theca-interstitial tissue. Interestingly, these highly androgenized females do not lose their capacity to ovulate. During the ovulatory period in the bat, the PCOS-like conditions are reverted. What causes the reversal in the metabolic and reproductive features during the ovulatory period is an intriguing question and answer to this might help to develop a suitable therapy for anovulatory PCOS women. Although studies on animal models strongly support the hypothesis that abnormalities of PCOS are initiated prenatally, this has been examined recently in human being (Tsilchorozidou et al. 2004). Cresswell et al. (1997) and Mrchelmore et al. (2001) have noted a strong association between polycystic ovary and large birth weight. However, Ibanez et al. (1999) found a close association of low birth weight in girls with precocious pubarche and HI-HA. The understanding of the pathogenesis of PCOS has advanced significantly. These achievements are to some extent the result of studies on animal models. However, a fully convincing animal model for study of PCOS as whole has not been established.

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