1.0. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) has become commonplace in today’s world. PCOS must be considered a serious issue because of its implication on long term health regardless of a woman’s age. It needs to be seen as a lifelong condition, not one tied only to pregnancy. Polycystic ovary syndrome (PCOS) is a very common and complex female endocrine disorder. It affects women in their reproductive years with an estimated prevalence of 4–8 % (Azziz et al., 2004). PCOS is the most common (Giallauria et al., 2008) cause of oligo-anovulatory infertility which is characterized by insulin resistance (IR), while hyperinsulinemia is found in 50–70 % of women diagnosed with PCOS. Women with PCOS are at increased risk for diabetes, dyslipidemia, (Carmina et al., 2006) atherosclerosis (Carmina et al., 2006; Giallauria et al., 2008; F. Orio et al., 2006) as well as endometrial carcinoma (De Franca et al., 2010). Furthermore it is suggested that women with PCOS are at an increased risk for miscarriages, gestational diabetes, preeclampsia and preterm labor (Boomsma et al., 2006). Due to the clinical and biochemical heterogeneity of PCOS, several studies have focused on the aspects of hormonal, genetic and environmental factors involved in the development of the syndrome.

A study of PCOS subjects representing three different ethnic groups revealed that obesity and hirsutism varied with genetic and environmental factors. At the same time, the prevalence of adrenal androgen excess and insulin resistance among these subjects appeared fairly uniform (Miles et al., 1992). More recently, De Ugarte observed that ethnicity and PCOS were associated with independent and additive
defects of insulin action in Caribbean-Hispanic PCOS women (DeUgarte, Bartolucci, & Azziz, 2005).

However, women with PCOS undergo several interrelated features including ovarian hyperandrogenism, chronic anovulation, polycystic ovaries; these are coupled with anomalous androgen and insulin-related parameters irrespective of other standard reproductive factors (Adams, Taylor, Crowley, & Hall, 2004). The genetic basis of the disease is not clearly known, which is largely due to the difficulties in determining the inheritability of PCOS. The genes that regulate insulin secretion and action, ovarian and adrenal steroidogenesis and energy regulation act as candidate genes which determine the expression of several integral phenotypes of PCOS. The present study concentrates on the polymorphisms in the genes that are involved in insulin secretion and action.

1. 1. History of PCOS

1. 1. 1. PCOS: The past

The syndrome was first described by the American gynecologists Irving F. Stein and Michael L. Leventhal in 1935, as accumulation of incompletely developed follicles in the ovaries hence the syndrome was named after Stein and Leventhal as Stein-Leventhal syndrome. They found an association between the presence of polycystic ovaries and signs of hirsutism andamenorrhea (I. Stein & Leventhal, 1935). After the diagnosis of women with this syndrome the physicians underwent successful wedge resection of the ovaries. Post treatment, menstrual cycles became regular and they were able to conceive (IF Stein, 1964). As a corollary, primary
ovarian defect was thought to be the main culprit, and the disorder came to be known as polycystic ovarian disease. For many years, these factors were used as the diagnostic criteria of the syndrome. In a while, it was realized that anovulation and an elevated level of androgens, not ovarian cysts, were the more correct diagnostic criteria, since it is the combination of these factors that results from or is symptomatic of the other characteristics of the syndrome. Despite the fact that the cause of the syndrome remains unknown, evidence suggests that the syndrome is complex, involving multiple physiological systems.

1.2. Proposed diagnostic criteria’s for polycystic ovarian syndrome

1.2.1. Multiple classification system on PCOS: Criteria for diagnosis

Several diagnostic criteria’s have been proposed to diagnose PCOS. The criteria’s proposed include menstrual irregularity, polycystic ovaries, hyperandrogenism and exclusion of other disorders in varying combinations. The diagnostic criteria for defining the PCOS are as heterogeneous as the disease itself and have been amended in recent years. Initially the criterion was proposed in the year 1990 in a PCOS conference by the National Institute of Health which did not require the presence of polycystic ovaries to be diagnosed for PCOS (Zawadski & Dunaif, 1992). In 2003 the criteria’s were re-proposed by the European society of human reproduction and embryology/American society of reproductive medicine in a consensus workshop held in Rotterdam which required presence of polycystic ovaries confirmed by ultrasound imaging ("Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS)," 2004).
followed by AES criteria by the Androgen Excess (AE) and PCOS Society in the year 2006 (Azziz, Carmina, & Dewailly, 2006). These multiple classification system makes clinicians difficult to diagnose. In order to overcome these confusions The National Institutes of Health Evidence-based Methodology Workshop on PCOS held in December 2012 concluded that the Rotterdam criteria should be adopted for now because it is the most inclusive.

Table 1.1. Diagnostic criterion for polycystic ovary syndrome

<table>
<thead>
<tr>
<th>NIH consensus criteria 1990 (all required)</th>
<th>Rotterdam criteria 2003* (two out of three required)</th>
<th>AES definition 2008 (all required)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual irregularity due to oligo- or anovulation</td>
<td>Oligo- or anovulation</td>
<td>Clinical and/or biochemical signs of hyperandrogenism</td>
</tr>
<tr>
<td>Clinical and/or biochemical signs of hyperandrogenism</td>
<td>Clinical and/or biochemical signs of hyperandrogenism</td>
<td>Ovarian dysfunction – oligo-anovulation and/or polycystic ovaries on ultrasound</td>
</tr>
<tr>
<td>Exclusion of other disorders: NCCAH, androgen-secreting tumors</td>
<td>Polycystic ovaries (by ultrasound)</td>
<td>Exclusion of other androgen excess or ovulatory disorders</td>
</tr>
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1.3. Prevalence of PCOS

1.3.1. Global

Polycystic ovary syndrome affects women of all races and nationalities. Indeed, this heterogeneous condition affects 7–10% of women worldwide (Asuncion et al., 2000; Azziz et al., 2004) irrespective of their ethnic background (M. A. Goodarzi & Firoozabadi, 2005). The prevalence statistic is much higher and increasing universally. Across globe, the prevalence of PCOS was highest in Turkey (Anakara), Pakistan and Australia with 19.9%, 17.6% and 15.3% while Srilanka, Turkey (Alabama), USA, Spain, Greece and Mexico showed prevalence in the range 6-7%. The lowest prevalence was seen in Oman with 2.8%. An estimated prevalence of 20 % of the normal female population has polycystic ovaries (K. F. Michelmore, Balen, Dunger, & Vessey, 1999). Prevalence values assessed using Rotterdam criteria were twice over those with NIH criteria (Broekmans et al., 2006). Prevalence of 4 and 8% using NIH criteria (Asuncion et al., 2000; Azziz et al., 2004; Diamanti-Kandarakis et al., 1999; Knochenhauer et al., 1998; March et al., 2010; K. F. Michelmore et al., 1999; C. Moran et al., 2010; L. J. Moran, Misso, Wild, & Norman, 2010) between 2.4 and 11.9% using Rotterdam criteria (X. Chen et al., 2008; Kumarapeli, Seneviratne Rde, Wijeyaratne, Yapa, & Dodampahala, 2008; March et al., 2010; C. Moran et al., 2010; L. J. Moran et al., 2010) and between 2.2 and 10.2% using AE-PCOS Society criteria (X. Chen et al., 2008; March et al., 2010) were observed from studies from different populations. Studies have reported that 5-10% of Chinese women, 6.5% of Caucasian women from Spain and 6% in Mexican women were diagnosed with PCOS (Asuncion et al., 2000; R. Li et al., 2000; Azziz et al., 2004; Diamanti-Kandarakis et al., 1999; Knochenhauer et al., 1998; March et al., 2010; C. Moran et al., 2010; L. J. Moran et al., 2010).
Therefore, estimating an absolute value for prevalence is difficult. Inconsistency even among the studies using the same diagnostic criteria might be due to differences in background study populations, difficulties in phenotypic definition and design limitations including biased sampling.

1.3.2. India

The above alarming prevalence is in rise in the Indian population as well. A study conducted at a residential college in Anantapur, South India showed a PCOS prevalence rate of 9.13% in Indian adolescents (Nidhi, Padmalatha, Nagarathna, & Amritanshu, 2011). Yet another study conducted at Lucknow, North India, shows an estimated prevalence of PCOS in women between the ages of 18-25 years (subject population of 200) as 3.7% (Gill, Tiwari, & Dabadghao, 2012). So an average prevalence rate of 6.45% (4-9%) was observed. However, a study conducted by Balaji et al from vellore, Tamil Nadu showcased the highest prevalence of PCOS with 18% (Balaji et al., 2015). Along with overall prevalence clinical presentations has also found to fluctuate among different ethnic and racial groups within India. A study with 460 students of a college in Andhra Pradesh 6.30% presented Oligomenorrhea, 2.39% had hirsutism (Nidhi et al., 2011). In another study with 120 newly diagnosed, post-pubertal women with PCOS in Western Maharashtra, 65% of women affected presented with Oligomenorrhea, 44.68% reported infertility, and 44.16% presented with hirsutism and were positive for Acanthosis nigricans (Ramanand et al., 2013). The prevalence of PCOS among medical undergraduate young women in a teaching hospital in Pondicherry reported 11.76% (Vijaya & Bharatwaj, 2014). The above statistical data suggests that there is no definite value
for the prevalence of PCOS in India. Nevertheless, it is found to range between 2% - 26% (Nidhi et al., 2011).

1.4. Pathophysiology of PCOS

1.4.1. Menstrual and Ovarian physiology

Interaction between the hypothalamus, pituitary and ovaries regulate the phases of menstrual cycle (MC) and ovulation. Normal reproductive function engross monthly follicular development, ovulation and prepare the endometrium for implantation. The hypotalamus secretes gonadotropin releasing hormone (GnRH) in a pulsatile fashion. GnRH activity is first evident at puberty. Release of GnRH is modulated by external neural signals. The hypothalamus exerts slow pulse frequency releasing GnRH. This surge initiates anterior pituitary to produce follicle stimulating hormone (FSH) which is the initial part of the menstrual cycle. The increase in FSH enables growth of follicles (follicular development). Two types of cells are mainly found in the developing follicle, the granulose cells (smaller cells on the inside of the follicle), and the theca cells (cells on outside of the follicle). FSH stimulates the secretion of inhibin B from granulose cells. Luteinizing hormone (LH) stimulates the theca cells in the ovarian follicle. On stimulation, the theca cells convert cholesterol into androgen. Androgen diffuses from theca cells into the adjacent granulose cells. FSH stimulates granulose cells in the ovarian follicle. On stimulation, the granulose cells convert androgen into estrogen. Part of estrogen is secreted into the blood, where it exerts systemic effect and part of the estrogen remains within the follicle and contributes to antral formation. Increase in estrogen sends a ‘negative feedback’ signal to the hypothalamus and anterior pituitary to stop
producing GnRH- FSH. At some point increasing estrogen reaches its threshold and switches to positive feedback.

Hypothalamus secretes more GnRH – particularly LhRH at this point. This LH surge initiates luteinization of granulose cells and synthesis of progesterone. Subsequently, corpus luteum is formed. Corpus luteum produces progesterone, inhibin A and estrogen. Elevated progesterone levels inhibit LH secretion via negative feedback loops at the hypothalamus-pituitary axis. Estrogen and progesterone causes changes in reproductive organs. Estrogen stimulates growth of cells in lining of the uterus for fertilization. In early pregnancy, human chorionic gonadotropin (hcG) rescues the corpus luteum. However, when a pregnancy does occur, LH level fall giving rise to the demise of corpus luteum. This results in a decrease in progesterone, inhibin A and estrogen levels. Decrease in inhibin A eliminates a suppressing influence on FSH secretion in the pituitary. The decrease in estrogen and progesterone results in an increase in GnRH pulse frequency. This increase in combination with the removal of the suppressing influence on the pituitary leads to a new cohort follicles to be recruited. The primary and small antral follicles of granulosa cells produce Anti Mullerian Hormone (AMH). Early recruitment of primordial follicles is repressed by AMH and weakens follicle sensitivity to FSH, thus affecting folliculogenesis (Durlinger, Visser, & Themmen, 2002). As follicles mature the AMH production starts to decline. This deterioration seems to be an essential requirement in selection of the dominant follicle and progression to ovulation (Pellatt, Rice, & Mason, 2010). Both the ovaries and the adrenal glands contribute towards androgen production in women. Dehydroepiandrosterone sulfate (DHEAS) is almost exclusively produced by the
adrenal gland. DHEAS is converted to dehydroepiandrosterone (DHEA), which serves as a precursor for biologically active androgens and estrogens (Labrie, 1991). One among the biologically active androgens is testosterone. Ovaries are in control of half of the circulating testosterone while the remaining is taken control by the adrenal gland. In circulation, testosterone is bound to albumin and sex-hormone binding globulin (SHBG). Only around 1% of testosterone circulates freely and is biologically active in women.

1.4.2. Hypothalamus-pituitary ovarian axis dysfunction

The increased pulse frequency of hypothalamic gonadotropin-releasing hormone (GnRH) (Rebar et al., 1976) increased the production of the β-subunit of LH compared to FSH (Haisenleder, Dalkin, Ortolano, Marshall, & Shupnik, 1991). Also, alterations in the GnRh pulsatility causes an increase in LH secretion in two third of PCOS patients which in turn causes a three-fold rise in circulating LH over FSH levels (Dumesic, Abbott, & Padmanabhan, 2007). The low concentration of FSH compared to LH in anovulatory PCOS women may attribute towards disturbances in the plasma inhibin and androstenedione concentrations which are thought to be correlated. Women with PCOS show elevated levels of serum inhibin-B. Inhibin stimulates androgen production, and in turn, androgen stimulates inhibin secretion (Anderson, Groome, & Baird, 1998). Studies have also reported central hypothalamic-pituitary disturbances were determined to be secondary to peripheral ovarian factors (Balen, 2004). Pre-pubertal hyperandrogenism tend to have preliminary role in reduced hypothalamic negative feedback with rapid GnRh pulsatility (Chhabra et al., 2005).
1. 4. 3. Adrenal androgen production

Besides the ovaries, adrenal cortex synthesizes three most important androgens (dehydroepiandrosterone sulfate (DHEAS), androstenedione and testosterone) which is the other major site of female androgen production. DHEAS is mostly (97-99%) produced by the adrenal cortex while androstenedione is produced both by the adrenal gland and the ovaries. However, only 25% of testosterone is synthesized by the adrenal gland and 25% by the ovaries, the remaining part being produced through peripheral conversion from androstenedione in liver, adipose tissue and skin (Burger et al., 2002). About 60-80% of PCOS women have high concentrations of circulating testosterone (Chang et al., 2005).

1. 4. 4. Hyperandrogenism and PCOS

Hyperandrogenism (HA) or excess androgen can be considered as a biochemical marker in determining PCOS. A study with more than 1700 women reported a third of patients with PCOS had risen up levels of serum testosterone (Balen, 2004). Hyperandrogenism causes an increased production of the cytotoxic T lymphocytes which in turn might contribute to oxidative stress in the ovarian tissue (Luchetti et al., 2004). Studies have reported the association of mutation in tumor necrosis factor (TNF) receptor with hyperandrogenism (Peral, San Millan, Castello, Moghetti, & Escobar-Morreale, 2002). Pre-pubertal hyperandrogenism increases serum tumor necrosis factor alpha (TNFα) levels (Luchetti et al., 2004). Iranian women with PCOS and revealed a strong relationship between hyperandrogenism, IR and metabolic syndrome (Mehrabian, Khani, Kelishadi, & Kermani, 2011). Hyperandrogenemia is thought to have compound
effects on different tissues. Hyperandrogenism provokes local pro-inflammatory phases in the ovaries and the endometrium. Apart from an increase in the T lymphocyte (CD8+/CD4+) cell ratio in the ovaries, increased CD4+ T lymphocytes and a parallel increase of tissue apoptosis was described in the endometrium (Motta, 2010). An increased PGF2α levels, enhanced expression of cyclooxygenase-2 (COX2), nitric oxide synthase (NOS), and decreased levels of PGE, catalase and superoxide dismutase were reported in the ovaries during the pro-inflammatory stage (Elia, Vighi, Lombardi, & Motta, 2009). HA induce morphological changes similar to pre-cancerous endometrial structures (Motta, 2010) and these alterations of endometrial tissue are prospective in link with infertility and recurrent miscarriage in PCOS.

1.5. PCOS: Animal studies

Prenatal and postnatal studies have ascertained using several animal models PCOS and PCOS like symptoms. Prenatal androgenization or prenatal androgen exposures in models such as rhesus monkey and sheep developed PCOS postnatally (Dumesic et al., 2002; Eisner, Dumesic, Kemnitz, & Abbott, 2000; Recabarren et al., 2005), The same in precocial mammals like sheep, pig and rhesus monkey display ovulatory dysfunctions (Abbott, Zhou, Bird, Dumesic, & Conley, 2008). Testosterone propionate treatment study in rats and mice resulted in PCOS with persistent anovulation (Walters, Allan, & Handelsman, 2012). Subsequently, chronic treatment with testosterone propionate for 35 consecutive days, developed insulin resistance in rats (Beloosesky et al., 2004). Ayclicity, polycystic ovaries, anovulation and hyperandrogenism similar to human PCOS were observed in
postnatal dihydroepiandosterone (DHEA) murine models (Walters et al., 2012). Treatment of adolescent female rats with dihydrotestosterone (DHT) induces PCOS-like condition with early deterioration of carbohydrate metabolism. Also, PCOS ovarian features with similarities to human and metabolic alterations such as IR, increased body fat and weight, increased leptin, cholesterol level and hypertension was observed when DHT treatment was done continuously for 11-13 weeks in adolescence (after the 21.postnatal day) (Mannerås-Holm et al., 2007; Yanes et al., 2011). Letrozole (aromatase inhibitor) administration led to acyclicity and anovulation with ovarian morphology similar to human PCOS (Mannerås-Holm et al., 2007). Usage of parallel insulin and 0.3 IU hCG daily for 22 days induced hyperandrogenism in female Wister rats. Consequently, increased activation of insulin in turn activated IRS1-P13 kinase-Akt pathway in the ovaries which led to significant elevated androstenedione levels.

These animals displayed an altered ovarian morphology with numerous fluid-filled cysts resembling to human PCOS and LH induced animal models (Lima et al., 2006). The over-expression of luteinizing hormone (LH) in transgenic mice [Tg(Cga-LHB/CGB)94Jhn/J] lead to continuous elevated levels of LH, testosterone and estrogen levels with polycystic ovaries, infertility and anovulation (Risma et al., 1995). On the other hand, the higher incidence of ovarian tumor and multiple corpora lutea exposed that impairment in LH secretion alone was not enough to induce PCOS (Risma et al., 1995; Walters et al., 2012).

LH and plasminogen activator inhibitor 1 were found to be associated with the development of PCOS (Balen, 2004). Transgenic overexpression of plasminogen activator inhibitor-1 (Tg-Serpine1) in mice presented hyperandrogenism,
anovulation, infertility and polycystic ovary morphology (Devin et al., 2007). Studies from the New Zealand obese (NZO) mice displayed IR, dyslipidemia and hypertension (Ortlepp et al., 2000). The New Zealand obese (NZO/HILt) mice and the JCR:LA-cp (cp/cp) rat, with leptin- (ob/ob) and leptin receptor-deficient (db/db), presented with altered leptin signaling that contributes to severe metabolic disorders like hyperinsulinemia, IGT, elevated testosterone, and decreased FSH levels (Walters et al., 2012).

1. 6. Insulin

All of the food we eat — fats, proteins and carbohydrates — is broken down during digestion into proteins, micronutrients and glucose. The glucose level rises in the blood stream. The cells in our body need sugar for energy. However sugar cannot get into most of our cells directly. Insulin is a hormone produced by the β cells in the pancreas. These β cells are positioned in clusters in the pancreas well-known as the “islets of Langerhans”. When sugar level rises in blood β cells the pancreas are signaled to release insulin into the blood stream and signals cells to absorb this sugar and use it for energy.

1. 6. 1. Insulin Structure

Insulin hormone is not synthesized as an active protein; insulin mRNA is initially translated into a single chain precursor called pre-proinsulin. The pre-proinsulin is 110 amino acids long and made up of a signal peptide, the A, B and C chains. The pre-proinsulin enters the endoplasmic reticulum and loses its signal peptide and converts into proinsulin which is 86 amino acids long. Later, the
proinsulin is exposed to several specific endopeptidases and further loses the C chain; it is thus left with only the A and B chains, which is considered as insulin hormone [Figure 1.1].

1.6.2. Insulin signaling pathway

Insulin regulates both metabolism and gene expression. The insulin signal passes from the plasma membrane receptor to insulin-sensitive metabolic enzymes and then reaches the nucleus where it stimulates the transcription of certain genes. The insulin receptor (INSR) is a heterodimeric complex consisting of 2 extracellular α-subunits and 2 transmembrane β-subunits linked together by disulfide bonds. The α-subunit contains the insulin binding domain. The binding of insulin to the -α- subunit induces a conformational change and activates the tyrosine kinase activity of β subunit to trans phosphorylate one another (Van Obberghen et al., 2001). This allows association of insulin receptor substrates, such as IRS-1 and IRS-2 a cascade of intracellular signaling proteins to the regulatory subunit of P13k kinase (Lizcano & Alessi, 2002; Saltiel & Kahn, 2001). The activated P13k further phosphorylates the membrane phospholipids and produces the phosphatidylinositol- 3, 4, 5 triphosphate (PIP3). PI3K also activates 3-phosphoinositide-dependent protein kinase 1 (PDK1), which in turn activates Akt, a serine kinase. AKT enters the cytoplasm where it leads to the phosphorylation and deactivates glycogen synthase kinase 3 (GSK-3), leading to activation of glycogen synthase (GYS) and thus glycogen synthesis. Phosphorylation of glycogen synthase by GSK3 inhibits glycogen synthesis; therefore the inactivation of GSK3 by AKT promotes glucose storage as glycogen.
Figure 1.1. Process in the synthesis of Insulin

**Figure explains the synthesis of hormone insulin from insulin mRNA.** Pre-proinsulin is synthesized from the mRNA which has A, B & C chains and a signal peptide. Pre-proinsulin loses its signal peptide and converted to pro insulin. Pro insulin exposed to endopeptidases and loses its C chain to form insulin.

Activation of Akt also results in the translocation of GLUT4 vesicles from their intracellular pool to the plasma membrane, where GLUT4 transporters are inserted and become available for transporting glucose into the cell (Ablooglu & Kohanski, 2001; Cushman & Wardzala, 1980). Any defects in the signaling to Glut4 vesicle will interrupt glucose uptake. In addition to upholding glucose storage, insulin also inhibits the construction and discharge of glucose by the liver by blocking gluconeogenesis and glycogenolysis (Saltiel & Kahn, 2001).
The translocation of GLUT4 protein is also elicited through the CAP:Cbl:CrkII:TC10 complex pathway, once Cbl is phosphorylated by INSR. Other signal transduction proteins interact with IRS including GRB2. GRB2 is part of the cascade including SOS, RAS, RAF and MEK that leads to activation of mitogen-activated protein kinase (MAPK) and mitogenic responses in the form of gene transcription. SHC is another substrate of INSR. When tyrosine phosphorylated, SHC associates with GRB2 and can thus activate the RAS/MAPK pathway independently of IRS-1. Insulin signaling also promotes fatty acid synthesis through activation of SREBP-1C, USF1, and LXR. A negative feedback signal, emanating from Akt/PKB, PKCζ, p70 S6K, and the MAPK cascades results in serine phosphorylation and inactivation of IRS signaling (Wong & Sul, 2010) [Figure 1.2].

1.6.3 Insulin Resistance

Insulin resistance refers to a state, in which circulating insulin does not bind to the insulin receptors on the cell, or it does bind but its effects are deficient, thus giving a less than normal reduction of glucose to a given amount of insulin (Ciaraldi, 2000). The pancreas then continues to secrete more insulin, leading to higher levels in the blood and ensuring normal glucose tolerance (Georgia & Bhushan, 2006). Because insulin is one of the “major” hormones, it’s also impossible for your body to balance its “minor” hormones (estrogen, progesterone and testosterone among them) until your insulin metabolism is balanced first. Women who are insulin resistant are at much greater risk of obesity, diabetes, hypertension (high blood pressure), heart disease, high cholesterol, breast cancer and polycystic ovarian syndrome (PCOS).
Introduction

Implication of Genetic Factors Contributing to Insulin Resistance in the Pathogenesis of Polycystic Ovary Syndrome

Figure 1.2. Insulin signaling pathway

The figure labels the process involved in uptake of glucose via insulin signaling and activation of different molecules and the pathways involved.

Glucose builds up in the blood instead of being absorbed by the cells, leading to type2 diabetes or pre-diabetes.

1. 6. 4. Insulin resistance and PCOS

PCOS and insulin resistance are interlinked, as approximately 40 % of women with PCOS have been found to be insulin resistant (Sawathiparnich, Weerakulwattana, Santiprabhob, & Likitmaskul, 2005; Vigouroux, 2010b;
Wijeyaratne, Balen, Barth, & Belchetz, 2002). Insulin resistance is a common feature in both polycystic ovary syndrome (PCOS) and non-insulin dependent diabetes mellitus (NIDDM); however, persistent reproductive disturbances were limited to the PCOS, suggesting that insulin resistance in the ovary itself may be responsible for this susceptibility (X. K. Wu et al., 2003). The association between insulin resistance and PCOS has provided significant insight into the pathogenesis of PCOS (Skov et al., 2007). Several studies indicated altered insulin levels which can directly stimulate ovarian androgen production in PCOS (Rosenfield & Bordini, 2010; Seto-Young et al., 2005).

Hyperinsulinemia leads to hyperandrogenemia by stimulating ovarian androgen production (DeClue, Shah, Marchese, & Malone, 1991; M. O. Goodarzi, Erickson, Port, Jennrich, & Korenman, 2005). Insulin can also stimulate adrenal steroidogenesis by enhancing sensitivity to adrenocorticotropic hormone (ACTH) and can increase pituitary LH release (Dunaif, Scott, Finegood, Quintana, & Whitcomb, 1996; Tosi et al., 2011). Increased androgen levels lead to menstrual disturbances, development of ovarian cysts, hirsutism and other related disorders (Carmina et al., 2006; Giallauria et al., 2008; F. Orio et al., 2006). Important physiological processes including cellular glucose uptake (Chang, Chiang, & Saltiel, 2004; Saltiel & Kahn, 2001), metabolism (Plum, Belgardt, & Bruning, 2006; Saltiel & Kahn, 2001) and gene expression (Mounier & Posner, 2006) are regulated by insulin. Specific abnormalities of insulin metabolism have been identified in PCOS. These include reduction in secretion, reduced hepatic extraction (O'Meara et al., 1993), impaired suppression of hepatic gluconeogenesis (Dunaif & Graf, 1989) and abnormalities in insulin receptor signaling (Dunaif, 1997).
Androgen synthesis by insulin in the ovaries can be proposed in two ways; Insulin activates testosterone biosynthesis via its receptor at physiological concentrations in cultured polycystic ovary theca (Nestler et al., 1998) and granulosa cells (Willis & Franks, 1995). Secondly, androgen excess due to insulin occurs via insulin growth factor1 (IGF1) receptor activation because insulin acts through IGF1 only when the circulating levels of insulin are extremely high (Poretsky, Cataldo, Rosenwaks, & Guidice, 1990). About 50-70% of PCOS women have insulin resistance independent of obesity (Mukherjee & Maitra, 2010). Insulin increases steroidogenesis in the ovaries and in turn reduces sex hormone binding globulin (SHBG) production by the liver directly or through elevated levels of LH (Bremer & Miller, 2008; Poretsky et al., 1990). Insulin fuels glucose uptake in tissues such as skeletal, cardiac muscle and adipocytes and suppresses glucose production by the liver (Bergman, 2007; R. A. DeFronzo & Lilly, 1988). Insulin declines lipolysis and by this means decreases the level of circulating free fatty acids (FFA) in the body (Groop et al., 1992). In healthy lean individuals, skeletal muscle accounts for 85% of insulin-mediated glucose disposal (IMGD) (R. A. DeFronzo, Tobin, & Andres, 1979). An increase in fat masses lead to a decrease in the skeletal muscle insulin sensitivity (R. A. DeFronzo & Tripathy, 2009). A significant decrease in IMGD in PCOS women (35% to 40%) was observed compared to controls (Dunaif, Segal, Futterweit, & Dobrjansky, 1989). Lean PCOS women with normal glucose tolerance showed a decrease in IMGD (uptake into muscle). Cultured fibroblasts from PCOS women show decrease in insulin receptor binding or auto-phosphorylation which might reflect mutations of genes regulating these pathways (Cheatham & Kahn, 1995). Visceral fat adipocytes may perhaps contribute
to IR since the visceral adipocytes are more metabolically active (Barber, McCarthy, Wass, & Franks, 2006). The incidence of IR is up to 20% in lean women with PCOS and can be greater than 40% in the obese PCOS population (Bhathena, 2011; Dunaif et al., 1989).

1.7. Lifestyle modifications and other factors influence on PCOS

1.7.1. Obesity

A high prevalence of obesity is found to be observed in women with PCOS (Yildiz, Knochenhauer, & Azziz, 2008). Obesity is found to worsen metabolic indices and amplify the clinical manifestations manifold (Pasquali et al., 2011). Clinical features exacerbated by obesity include hyperandrogenism, reduced levels of SHBG, severe hirsutism, presence of Acanthosis nigricans, hyperinsulinemia, and supra normal estrogen production. The role of obesity in PCOS was unraveled when PCOS was studied among sisters (Dunaif et al., 1989). It was found that while sisters who exhibited hyperandrogenemia and menstrual irregularities were heavy, their normal counterparts showed low body weight. The pattern of body fat distribution plays a major role in regulating the various levels of hormones (Diamanti-Kandarakis et al., 2006). A study conducted by Pasquali et al. examined 97 hyper androgenic women with PCOS. They were grouped into three on the basis of waist to hip ratio. It was observed that compared to women with peripheral fat, women with central adiposity had higher levels of LH, estrone and/or stenedione, higher concentrations of fasting glucose, a higher prevalence of hirsutism, acanthosis nigricans and a moreatherogenic lipid profile. Several studies have reported that an increase in metabolism of visceral fat leads to the accumulation of pro-inflammatory cytokines (due to the increased production of free fatty acids that
Obesity is found to worsen metabolic indices and amplify the clinical manifestations manifold which includes hyperandrogenism, reduced levels of SHBG, severe hirsutism, presence of Acanthosis nigricans, hyperinsulinemia, and supra normal estrogen production. Metabolism of abdominal fat leads to the development of abnormal atherogenic and metabolic profile, which in turn triggers weight gain. Increased insulin level leads to elevated levels of estrogen due to an increase in aromatase activity. Furthermore, hyperinsulinemia is postulated to play a major role in causing hyperandrogenism that is frequently observed. Women consuming a vegetarian-rich and fibre-rich diet may be at a slight advantage over their Western counterparts as they are found to decrease androgen levels in blood. Furthermore, calorie restricted diets are found to improve metabolic indices in PCOS women who are otherwise susceptible to type 2 diabetes mellitus three times more as compared to the general population. Not only is the quantity of food consumed but the quality and type of nutrition also play a significant role. The standard diet recommended in most countries is rich in carbohydrates, has moderate levels of protein and low levels of fat. Thankfully, appropriate diet with concomitant exercise leads to an appreciable decrease in weight.
accumulate in non-adipose tissues thus casing lipotoxicity), which in turn, lead to insulin resistance.

Compensatory hyperinsulinemia and insulin resistance are closely associated to obesity although the former is proved to occur independently. Hepatic insulin resistance is exclusive to obese women with PCOS (Sam, 2007). Structural defects of the insulin receptor are absent in PCOS patients and hence it can be safe to conclude that post receptor defects must be involved in the development of insulin resistance (Bremer, 2010). Serine phosphorylation of IRβ subunit of the insulin receptor leading to subsequent inhibition of tyrosine auto-phosphorylation thus constituting a block in the signaling pathway is observed in most PCOS patients as the cause of insulin resistance. Early studies have clearly demonstrated that infertility and anovulation observed in women with PCOS can be attributed to hyperinsulinemia (Kovacs & Norman, 2007). However, several studies have demonstrated the administration of insulin reducing agents have led to a reduction in the effects of increased androgen levels while androgen therapy did not have any effect on insulin resistance and consequently it is still unclear as to whether hyperinsulinemia causes androgenism or vice – versa (Farrell & Antoni, 2010). In general, a modest decrease of 5% or greater in weight of obese women led to changes in the levels of insulin, Insulin like Growth Factor (IGF), and SHBG; induced menstrual regulation, improved hirsutism, decreased the chances of acquiring type 2 diabetes mellitus and cardiovascular disease and offered protection from the harmful effects of hyperlipidemia, in addition to increasing the chances of becoming pregnant (Kovacs & Norman, 2007). Furthermore, it is recommended that women with PCOS undergo moderately intense exercise for 30 minutes 3 times a week in order to achieve
long term weight management. The contributions of obesity to PCOS and the pathways involved are depicted in **Figure 1.3**.

1. 7. 2. **Endocrine disrupting chemicals**

According to the US Environment Protection Agency, an endocrine disruptor is “an exogenous agent that interferes with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are required for the maintenance of homeostasis, reproduction, development and/or normal behavior” (Birkett & Lester, 2003). A vast ocean of studies has been conducted to investigate the role played by these chemicals in disrupting the endocrine system.

1. 7. 2. 1. **Mechanism of action**

Endocrine Disrupting Chemicals (EDCs) are observed to act in three ways: 1) by directly binding to Nuclear Receptors (NR) like Steroid Receptors, Androgen Receptors, non-nuclear steroid hormone receptors, non-steroid receptors (like dopamine receptor), orphan receptors (aryl hydrocarbon receptor), 2) by competing with the original ligand for co-activators that would enable the activated receptor to transcribe the appropriate genes, 3) by increasing both hormone catabolism and production. Various mechanisms have been demonstrated to play a major role in throwing the endocrine system off balance. Most notable of all EDCs is Bisphenol A (BPA). A 228Da estrogenic monomer used in the manufacturing of polycarbonate plastic, plastic water bottles, toys and as an additive in manufacturing polyvinylchloride (used in medical tubing), BPA is one of the most ubiquitous environmental pollutant associated with the pathogenesis of PCOS. Being one of the potent ligands for Estrogen Receptor (ER), it has been demonstrated that BPA interacts in a different manner than estradiol, has a higher affinity for ERβ.
than ERα and activates its co-regulators differently (Crain et al., 2008; De Coster & van Larebeke, 2012; Schug, Janesick, Blumberg, & Heindel, 2011; Swedenborg, Ruegg, Makela, & Pongratz, 2009; Welshons, Nagel, & vom Saal, 2006). Apart from its estrogen mimetic properties, many studies have reported an increase in androgen levels. Increased androgen levels have found to down regulate uridinediphosphate-glucuronosyltransferase activity, thereby leading to its decreased metabolic clearance. Furthermore, it is a potent ligand for Sex Hormone Binding Globulin (SHBG) and consequently results in an increase in the free androgen levels (Kandaraki et al., 2011). Additionally, BPA is found to inhibit the release of adipokines from adipose tissues, thereby conferring an increased susceptibility to metabolic syndrome (Welshons et al., 2006).

Organochlorines are potent EDCs owing to the absence of detoxification mechanisms and subsequent accumulation in lipid secreting cells. They have a strong carbon-chlorine bond which is resistant to cleavage by normal metabolic pathways. They are major components of pesticides and contaminate nearby water bodies, which upon ingestion, leads to hormonal imbalances. 2, 3, 7, 8-tetrachlorodibenzod-p-dioxin (TCDD) is a commonly investigated organochlorine. It is postulated to act via the aromatic hydrocarbon receptor (De Coster & van Larebeke, 2012) and induce the expression of cytochrome P450, leading to its anti-estrogenic properties. In addition, in vitro studies have shed light on disruption of major pathways. Administration of human chorionic gonadotrophin and TCDD in immature female rats have been found to alter levels of estradiol, FSH and LH but no decrease in the estradiol levels were observed. In cynomolgus monkeys treated with TCDD, decreased concentrations of estradiol,
Endocrine disrupting chemicals (EDCs) are exogenous agents that act by competing with the original ligand, or directly binding to the nuclear receptor or by increasing both hormone catabolism and production. EDC’s interferes with the synthesis, secretion, transport, binding action or elimination of natural hormones in the body that are required for the maintenance of homeostasis, reproduction, development and/or normal behavior”. There is a consequent imbalance in the levels of hormones as the hypothalamo-pituitary-gonadal axis is affected. This leads to the various clinical features observed in PCOS patients.

progesterone and chorionic gonadotrophin was observed. Hexachlorobenzene (HCB) is a worldwide organic pollutant. When a pregnant woman is exposed to HCB, it is observed to be transmitted to the fetus by crossing the placenta prenatally and via the lactating milk after birth. Cynomologus monkeys exposed to a concentration of 10mg/kg of body weight per day for three menstrual cycles decreased the levels of
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estradiol. Studies on rats indicate that HCB indirectly targets steroidogenesis. Studies demonstrating the effect of other organochlorines have been performed. Estrous irregularities were observed in rats treated with organochlorine pesticides like atrapine, hexachlor and methoxychlor. Women who consumed fish from Lake Ontario had a reduced menstrual length indicating a possible relationship between fish consumption and polychlorinated biphenyls (PCB) exposure. Many studies have demonstrated a decreased fecundity with exposure to different PCBs (Chalupka & Chalupka, 2010; Nicolopoulou-Stamati & Pitsos, 2001). PCBs disrupt follicular steroidogenesis by altering pattern of hormone synthesis, altering enzyme secretion, changing hormone affinities and mimicking natural hormones as an agonist (Gregoraszczuk & Ptak, 2013).

Polychlorinated naphthalenes (PCH) have toxicological characteristics similar to that of PCB. It is postulated to be due to the inhibitory activity of the enzyme required for the conversion of testosterone to estradiol. Geinstein is an isoflavonoid phytoestrogen which can be found in soy products, which are lactose-free substrate. An increase in anogenital distance (masculinization), accelerated puberty, and irregular estrous cycles in adult CD-1 mice was observed with neonatal administration of 0.5-50mg/kg of Geinstein. Batesman and Paul demonstrated that a short 2-day exposure of Geinstein caused defeminization of Hypothalamus Pituitary Gonad (HPG) axis (Zama & Uzumcu, 2010). Endocrine disruptors have an effect on estrogen and steroid receptors and, in line, directly influence PCOS development. Studies have reported a positive association of BPA with PCOS. Women with PCOS showed higher levels of BPA and are strongly associated with metabolic and hormonal disturbances (T. Takeuchi, Tsutsumi, Ikezuki, Takai, & Taketani, 2004).
And also neonatal exposure to BPA results in PCOS development (Fernández, Bourguignon, Lux-Lantos, & Libertun, 2010). Studies also confirmed that excess fetal androgen exposure in female nonhuman primates may develop PCOS like symptoms in adulthood (N. Xu, Kwon, Abbott, Geller, & Dumesic, 2011). Thus, EDCs are environment pollutants which interfere with the functioning of normal metabolic pathways, culminating in hormonal imbalances and PCOS. The contributions of EDCs to PCOS and the pathways involved are depicted in Figure 1.4.

1.7.3. Vitamin D

Many studies have been conducted to evaluate the relationship between vitamin D levels and PCOS (Hahn et al., 2006; Pal, Shu, Zeitlian, & Hickmon, 2008; Panidis et al., 2005; Wehr et al., 2009; Yildizhan et al., 2009). In tandem, a study involving 206 women with PCOS observed that 72.8% of them had insufficient vitamin D levels and demonstrated that almost three out of every four PCOS women may have vitamin D deficiencies (Wehr et al., 2009). About 60 – 75% of women with PCOS are found to have vitamin D levels of less than 20ng/ml (Thomson, Spedding, & Buckley, 2012). Deficiency of vitamin D is found to exacerbate the symptoms of PCOS through genetic and cellular mechanisms which can be explained as follows: nuclear vitamin D receptors are found in a number of tissues like the parathyroid gland. These receptors are found to regulate the transcription of genes that are involved in the maintenance of LH, SHBG, and testosterone levels. So, a decrease in vitamin D levels leads to the improper transcription of the above mentioned genes which consequently affect their plasma levels, leading to observable symptoms.
Figure 1.5. The role played by vitamin D in the maintenance of normal hormonal levels

The sources of Vitamin D are from Sun i.e. exposure to sunlight, diet or through pharmaceutical supplements. Vitamin D exists in two main forms, vitamin D3 (VD3, cholecalciferol) and vitamin D2 (VD2, ergocalciferol), differing in their side chain structure. The primary form of vitamin D in blood is calcidiol which is converted to 25-hydroxy vitamin D and further converted to 1, 25-dihydroxy vitamin D which is an active hormone. Deficiency of vitamin D is found to exacerbate the symptoms of PCOS through genetic and cellular mechanisms which can be explained as follows: Vitamin D binds to nuclear receptors in the parathyroid gland, thereby regulating the transcription of genes involved in the maintenance of hormones like LH, SHBG, and Testosterone. This consequently affects estrogen production from the ovary. Furthermore, calcium is observed to regulate functions like egg activation, oocyte maturation, follicular development and mammalian ovary development and consequently a decrease in calcium levels leads to its improper control. Also, vitamin D deficiency in PCOS women is associated with increased estrogen production from ovary due to its control over the aromatase gene. The presence of VDR in ovary, uterus, and placenta suggest a regulatory role of vitamin D in reproductive physiology. Vitamin D binding protein (VDBP) and its salient actions in reproduction include folliculogenesis, steroidogenesis, endometriosis, gametogenesis, implantation, fetal growth and maternal wellbeing. Hence, sufficient amount of vitamin D must be consumed for proper regulation of genes involved in the synthesis of hormones. VDR- vitamin D receptor, VDBP- vitamin D binding protein.
Furthermore, calcium is observed to regulate functions like egg activation, oocyte maturation, follicular development and mammalian ovary development and consequently a decrease in calcium levels leads to its improper control (Bagheri, Abdi Rad, Hosseini Jazani, & Nanbakhsh, 2013). The authors of a study involving only 13 women with PCOS (conducted by the Columbia University) hypothesize that a positive correlation exists between vitamin D levels and follicular development. Of the 13 subjects, five of them were found to have obvious vitamin D deficiency and three of them showed borderline deficiency. They were administered with vitamin D2 at a dose of 50,000 IU once or twice a week and also received an additional 1500mg of supplemental calcium per day. After the treatment period, seven subjects experienced a regular cycle within two months and two of them became pregnant (Thys-Jacobs, Donovan, Papadopoulos, Sarrel, & Bilezikian, 1999). In another study, observational studies show lower 25OHD levels were associated with insulin resistance; ovulatory and menstrual irregularities lower pregnancy success, hirsutism, hyperandrogenism, obesity and elevated cardiovascular disease risk factors (Thomson et al., 2012). Hence, it is evident from a number of studies that administration of adequate doses of vitamin D is beneficial to the management of PCOS (Brzozowska & Karowicz-Bilinska, 2013). Also, vitamin D deficiency in PCOS women is associated with increased estrogen production from ovary due to its control over the aromatase gene.

Many studies have expressed the confounding role of insulin resistance, which is one of the most commonly observed phenotypes in women with PCOS (Thomson et al., 2012). However, recent studies indicate that individuals with increased vitamin D levels are 40% less likely to develop diabetes as this vitamin is found to increase insulin secretion, and reduce systemic inflammation (Ozfirat & Chowdhury, 2010). Furthermore, a
deficiency of vitamin D in PCOS women has found to be correlated with increased cardiovascular risk while a marked decrease in symptoms of depression has been observed in 50% of the patients when supplemented with vitamin D (More research is required in this area as the exact mechanism is not established) (Gloth, Alam, & Hollis, 1999; Jorde, Sneve, Figenschau, Svartberg, & Waterloo, 2008). Thus, vitamin D deficiency has a negative correlation with metabolic risk factors (insulin resistance, BMI, waist to hip ratio) and is found to aggravate symptoms of PCOS like hirsutism, hyperandrogenism, menstrual irregularities, obesity and increased risk for cardiovascular disease (Grundmann & von Versen-Hoynck, 2011). The contributions of vitamin D to PCOS and the pathways involved are depicted in Figure 1.5.

1.7.4. Advanced glycation end products

Advanced glycation end products (AGEs) are produced when reducing sugars react non-enzymatically with the amino groups of protein (like lysine or arginine) to produce Schiff Bases and Amadori products, which consequently undergo reactions like rearrangement, dehydration and condensation to become heterogeneously linked AGEs (Yamagishi & Matsui, 2010). They are involved in crosslinking of collagen by reducing enzyme activity and changing the biophysical properties to alter interactions with other enzymes. Immuno histochemical techniques have demonstrated the selective accumulation of AGEs in the ovaries of PCOS women (Diamanti-Kandarakis et al., 2006). The stroma of ovary is made up of collagen. So, both endogenous and exogenous AGEs (whose sources include smoking and improper cooking of food substances, consumption of pre-cooked fast food meals) can lead to dysregulation in the production of hormones and reproductive functions. Additionally, AGEs and their receptor RAGE
have been localized in the granulosa layer of the ovarian tissue (Kandaraki et al., 2011). Furthermore, it has been observed that AGEs perpetuate insulin resistance by increasing the levels of protein kinase C. However, Phosphatidylinositol 3 kinase has been found to sequester AGEs through the Macrophage Scavenger Pathway (MSP) (Pasquali et al., 2011). Consequently small tweaks in one’s diet can reduce the contribution of AGEs in exacerbation of the symptoms. The contributions of AGEs to PCOS and the pathways involved are depicted in Figure 1.6.

1.7.5. Smoking

The physiological and genetic aspects of PCOS are extensively researched, however, very little has been done to study the psychological effects. It is observed that PCOS affected women are more prone to depression and anxiety, have a low self-esteem, and a negative body image and feminine identity (Farrell & Antoni, 2010). However, smoking only deteriorates their quality of life. Women with PCOS become susceptible to cardiovascular risk. In a study by Morotti et al., 81 women were divided into three groups on the basis of the number of cigarette packets they smoked. It was demonstrated that the group that smoked more than 10 packets had a higher diastolic blood pressure, atherogenic index plasma and insulin sensitivity (Morotti et al., 2014; Wild et al., 2010). Furthermore, smoking has profound negative effects on the hypothalamus – gonad axis that is found to operate by various mechanisms. Additionally, women with PCOS who smoke are susceptible to metabolic syndrome (Pau, Keefe, & Welt, 2013). Also, many studies have demonstrated a positive correlation between smoking, delay in time of conception and decreased fecundity.
(Norman, Davies, Lord, & Moran, 2002). Hence, smoking by PCOS women must be avoided in order to improve the quality of life.

1.8. Environmental factors and PCOS

Many studies have concentrated on possible environmental factors that contribute to the development or progression of PCOS. Several environmental factors are known to unveil genetically programmed susceptibility to PCOS and contribute to its phenotypic expression. These factors interact chiefly with early stages of human development and convert a predisposed genotype to the phenotypic expression of PCOS. Low-birth-weight infants show an increased incidence of precocious puberty, hyperinsulinemia, and hyperandrogenism compared to normal-weight infants (Ibanez, Potau, Francois, & de Zegher, 1998). A nutritional surplus with the consumption of high-calorie diets lead to obesity and induces the development or progression of the clinical spectrum of PCOS (L. Moran & Teede, 2009; Pasquali et al., 2011). Furthermore, environmental determinants may influence the clinical severity of PCOS, ranging from a less-severe phenotype to themature phenotype of classic PCOS. The exposure of pregnant non-human primates and sheep to excess androgens can cause the development of a syndrome similar to PCOS, indicating that the exposure to androgen-like chemicals absorbed by the human body can lead to PCOS (Abbott, Dumesic, & Franks, 2002; Robinson, Forsdike, & Taylor, 1999). A retrospective study demonstrated that disposable plastic drinking cups, cooking oil fumes and indoor decorations made of plastic increased the PCOS risk, indicating that environmental endocrine-disrupting chemicals are associated with the risk of PCOS (W. J. Huang, Liu, & Li, 2007).
1.6. The complex network of metabolism of Advanced Glycation End products (AGEs) – their production and mechanism of causing disruption in signaling pathways, thus culminating in PCOS

Advanced glycation end products (AGEs) are formed naturally inside the body when proteins or fats combine with sugars non-enzymatically to produce Schiff base and amadori products. These products undergo rearrangement like the oxidation, reduction and hydrolysis to become heterogeneously linked AGE’s. They are involved in crosslinking of collagen by reducing enzyme activity and changing the biophysical properties to alter interactions with other enzymes. The other pathways involved in the production of AGE’s include oxidation of glucose, peroxidation of lipids and polyol pathway. There are two forms of AGE’s endogenous (blood stream) and exogenous AGEs (whose sources include smoking and improper cooking of food substances, consumption of pre-cooked fast food meals). High levels of AGEs in the stroma of the ovary due to improper cooking of food has been implicated to perpetuate insulin resistance that is observed in PCOS through increased levels of protein kinase C. Also, this leads to improper secretion of hormones and consequent dysregulation of reproductive functions. However, AGE’s are sequestered through the Macrophage Scavenger Pathway.

Bisphenol A (BPA), a known hormone disrupter which is present in our environment, food, and consumer products, is elevated and associated with higher
levels of male hormones in the blood of women and results in a deviation from normal homeostasis or reproduction. Studies using experimental animals have demonstrated that neonatal exposure to BPA leads to PCOS development (Fernandez, Bourguignon, Lux-Lantos, & Libertun, 2010). Moreover, serum BPA levels were positively associated with serum androgen levels and insulin resistance indices in both lean and obese PCOS women (Kandaraki et al., 2011).

1.9. Therapeutic approaches

The cumulative effects of various environmental factors outlined above leads to the development of PCOS, which can be treated by adopting any one of the following approaches:

1.9.1. Pharmacological approach

Administration of certain drugs helps in reducing the activity of metabolic and endocrine features of PCOS. This, in conjunction with regular exercise is found to ameliorate the various clinical manifestations of PCOS. The following drugs (which act via different pathways) are administered with the goal of minimizing the symptoms of PCOS:

1.9.1.1. Antidiabetic drugs

1.9.1.1.1. Metformin

Velasquez was the first scientist to have introduced metformin with the purpose of demonstrating the role of insulin resistance in the pathogenesis of PCOS. Since then, numerous studies have supported the hypothesis that metformin decreases
hyperinsulinemia, hyperandrogenism and resulted in weight loss (Badawy & Elnashar, 2011; Geller, Pacaud, Gordon, & Misra, 2011; Legro, 2012; Traub, 2011). By reducing the activity of gluconeogenic enzymes, decreasing hepatic uptake of alanine and lactate, promoting the conversion of pyruvate to alanine, inhibiting glucogenic output, modulating AMP-activated protein kinase effects, increasing the uptake of glucose by cells and decreasing intestinal absorption, metformin results in lowering of blood glucose level. However, it has been proved that metformin has no effect on super obese patients and patients with severe hyperinsulinemia. Additionally, the dose used in many studies was less than the dose at which clinical efficacy is observed. Initially, clomiphene citrate was used for ovulation induction and was successful in 75% of the cases. However, studies have demonstrated that a combination therapy of the already mentioned drugs is found to be more efficacious than monotherapy with either of the drugs. An increase in pregnancy rates is observed after administration of metformin. It is also found to ameliorate the harmful complications associated with such pregnancies such as spontaneous abortions and gestational diabetes. Metformin is classified as a class ‘B’ drug for its safety has yet to be assessed.

1.9.1.1.2. Thiazolidindiones

This is an insulin sensitizing agent that is found to decrease hyperinsulinemia, hyperandrogenism, menstrual abnormalities and anovulation in a dose-dependent manner. Unlike metformin, thiazolidindiones like rosiglitazone and pioglitazone produce redistribution of fat and do not result in weight loss. Studies have shown that these drugs are superior to metformin therapy. However, more studies are required to demonstrate the benefits over metformin (Hwang et al., 2013).
1.9.1.2. Incretin mimetics therapy

Incretins are a group of gastrointestinal hormones that stimulate the production of insulin by stimulating the pancreatic β cells through the production of Glucagon-like Peptide -1 (GLP-1) by the L cells of the small intestine. In combination with metformin, they proved to be superior to monotherapy with either of the agents. In addition, they exhibit a dose dependent weight-loss. They are not licensed and are generally well tolerated (Kahal, Atkin, & Sathyapalan, 2011).

1.9.1.3. Acarbose

Acarbose is a α-glucosidase inhibitor that decreases the digestion and absorption of polysaccharides by the intestine and thus helps in the reduction of blood glucose levels. There are many studies with different views on its efficacy and hence this is not widely used (Duleba, 2012).

1.9.1.4. Oral Contraceptive Pills (OCPs)

This is the perfect treatment option for women who do not wish to get pregnant. It is a combination of an estrogenic component like ethinyl estriol and a progestin with variable potency and androgenecity. There are many mechanisms by which menstrual regularity are induced: estrogen increases SHBG levels while progestins protect from developing endometrial hyperplasia. They are, however, not preferred over metformin for factor V Leiden mutations increase the risk of thromboembolitic episodes with use of estrogen (Geller et al., 2011).
1.9.1.5. Antiandrogens

In order to decrease the symptoms that are caused by an increase in the levels of androgens like hirsutism and acne, antiandrogens like Spironolactone and flutamide are used (Ndefo, Eaton, & Green, 2013).

1.9.1.6. Gonadotrophins

They can be used in patients who are resistant to Clomiphene citrate and as an alternative to metformin therapy. Controlled administration of Follicle Stimulating Hormone (FSH) leads to the induction of ovulation and maintenance of follicles for the process of fertilization. However, some of the drawbacks associated with it include the fact that it may lead to ovarian hyperstimulation syndrome (OHSS) (due to production of multiple follicles), time consuming, expensive and requires constant monitoring (Badawy & Elnashar, 2011).

1.9.1.7. Others

Aromatase inhibitors are being extensively researched pharmacological entity. They act on the hypothalamus – pituitary axis, stimulate the production of FSH and Gonadotrophin Releasing Hormone (GnRH) and induce ovulation. Letrozole and anastrozole are the most commonly used aromatase inhibitors (Elkind-Hirsch, Marrioneaux, Bhushan, Vernor, & Bhushan, 2008). Topical administration of eflomithine (an inhibitor of L-ornithine decarboxylase) for a period of 7 weeks results in the improvement of facial hirsutism (Escobar-Morreale et al., 2012). Glucocorticoids like prednisone and dexamethasone have been used to induce ovulation, but their use over time has been discouraged (Badawy & Elnashar, 2011). Statins, HMG-CoA Reductase inhibitors
reductase inhibitors are generally used to reduce cholesterol biosynthesis; however their anti-inflammatory role has led to its use in the treatment of PCOS (Kodaman & Duleba, 2008).

1.9.2. Non pharmacological approaches

1.9.2.1. Diet

There are only a handful of studies that delineate the composition of diet and there is no accurate composition just yet. Women consuming a vegetarian-rich and fiber-rich diet may be at a slight advantage over their western counterparts as they are found to decrease androgen levels in blood. Furthermore, calorie restricted diets are found to improve metabolic indices in PCOS women who are otherwise susceptible to type 2 diabetes mellitus three times more as compared to the general population. Not only is the quantity of food consumed but the quality and type of nutrition also play a significant role. A study by Carmina et al. demonstrated that even with similar calorie intake and dietary constituents (except for higher levels of saturated fat in the diet of USA women); Sicily women were less obese than women from Pennsylvania, USA. Adolescents diagnosed with PCOS must refrain from ingesting drinks with high sugar content (Bremer, 2010). Since, lipid accumulation in androgen secreting cells is found in many PCOS patients, diet that is rich in Poly Unsaturated Fatty Acids over a 3 month period found to improve endocrine profile. As a rule of thumb, a low carbohydrate, high protein diet is found to improve metabolic index and is currently recommended. This essentially is aimed at reducing weight on a long – term basis that is accompanied with strict exercise routine. Thus, obesity in PCOS women precipitates clinical manifestations which make it harder to treat them. Thanks to increasing awareness
about the harmful role played by obesity in various disorders, it is now possible to keep the causative factors at bay.

1. 9. 2. 2. Bariatric surgery

This is the most suitable strategy for morbidly obese patients who are not able to achieve weight loss through a combined diet and exercise regimen. There are many approaches like restrictive and malabsorptive procedures, adjustable gastric banding and Roux-en-Y gastric bypass that are frequently used (Badawy & Elnashar, 2011). Studies have reported significant weight loss, however individuals who undergo bariatrics surgery are at a risk of nutritional deficiencies and so the pros and cons must be weighed carefully.