Neuroendocrinology involves the study of anatomical and functional interactions between the nervous and the endocrine systems for regulation of various physiological processes. It encompasses the functions of neurons, neurochemicals, hormones, and endocrine glands that work in an integrated manner to register, transduce and interpret important signals from internal and external environment to maintain a physiological state (Levine, 2011). Research on the neuroendocrine system gained momentum when the concept of neurosecretion emerged. Scharrer and Scharrer (1945) demonstrated that neurons produce and secrete neurohormones which can be termed as neurosecretion. Neurosecretory cells are mainly present in the hypothalamus that connects to the pituitary forming a hypothamic-pituitary complex which is also known as the “command centre” of endocrine system. These two tissues therefore lie at the centre of almost all neuroendocrine research.

ANATOMY OF NEUROENDOCRINE SYSTEM

Hypothalamus
The hypothalamus is located at the base of forebrain, adjacent to limbic and cortical structures and brainstem. This position allows it to communicate with endocrine signals, as well as neural signals from sensory systems, emotion- and memory-processing circuitries and autonomic centres. There are various hypothalamic nuclei formed by bilaterally similar groups of neuronal cell bodies and their neuropils. These nuclei generally function as “centres” for the control of specific functions such as feeding, stress, water balance, and
sexual behaviour. All these nuclei interact with each other as well as with different areas of the brain to perform specific functions. Several of the hypothalamic nuclei contain well-characterised neurohormones and neurotransmitter-producing cell groups. For example, paraventricular nucleus (PVN) expresses corticotrophin-releasing hormone (CRH) and thereby regulates stress responses; the preoptic area (POA) contains neurons that produces gonadotropin-releasing hormone (GnRH), which is essential for reproductive function (Levine, 2011).

**Pituitary**

The pituitary gland (or hypophysis) is a bean-sized organ suspended from the hypothalamus by a stem called the infundibulum (or pituitary stalk). The pituitary gland consists of two lobes that arise from distinct parts of embryonic tissue: the posterior pituitary (neurohypophysis) is neural tissue, whereas the anterior pituitary (adenohypophysis) is glandular tissue. The posterior pituitary receives signals from the hypothalamus via neurosecretory cells to release hormones. In contrast, the anterior pituitary is regulated by releasing or release-inhibiting hormones which are sent into hypophyseal portal system by hypothalamus (Levine, 2011).

The hypothalamus-pituitary complex together with target organs forms regulatory-endocrine axes which include: hypothalamic-pituitary-gonadal axis, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-thyroid axis, among which our main focus is the hypothalamic-pituitary-ovarian axis (Female reproductive axis) and its regulators.

![Hypothalamic-pituitary unit](image)

Figure 1.1 Hypothalamic-pituitary unit

*Introduction*
HYPOTHALAMIC-PITUITARY-OVARIAN (HPO) AXIS

Normal reproductive function in women involves repetitive cycles of follicle development, ovulation and preparation of the endometrium for implantation. This pattern of regular ovulatory cycles is achieved through precise functional and temporal integration of stimulatory and inhibitory signals from the hypothalamus, the pituitary and the ovary. The reproductive system functions in a classic endocrine mode initiated by pulsatile secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus into the pituitary portal venous system. GnRH regulates the synthesis and subsequent release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary into the circulation. FSH and LH stimulate ovarian follicular development, ovulation, and corpus luteum formation and the coordinated secretion of estradiol, progesterone, inhibin A, and inhibin B. A key component of this system is the modulatory effect of ovarian steroids and inhibins on gonadotropin secretion, acting either directly at the pituitary level or through alterations in the amplitude or frequency of GnRH secretion making a feedback loop (Strauss III & Barbieri, 2014; Hall, 2015).

GnRH pulse generator is the central player for cyclic changes in gonadotropin levels which is of absolute necessity for the ovarian follicular development cycle, better known as the menstrual cycle in humans.

Menstrual cycle

The reproductive system of women, unlike that of men, shows regular cyclic changes that may be regarded as periodic preparations for fertilization and pregnancy. In humans and other primates, the cycle is known as menstrual cycle, and its most conspicuous feature is the periodic vaginal bleeding that occurs with the shedding of the uterine mucosa (menstruation).
The length of the cycle is highly variable in women ranging from 25 to 35 days, but typically averaging at 28 days.

**Follicular phase/ Proliferative phase**

The proliferative phase begins at the onset of menses until ovulation takes place. Folliculogenesis takes place during this phase of the menstrual cycle. A dominant follicle is selected from a pool of growing follicles that will be destined to ovulate. The growth of follicles in this stage will depend on pituitary hormones such as FSH. The growth of the follicle also leads to production of estradiol from the layers of granulosa cells, which is responsible for the proliferation of the endometrial lining of the uterus (Beshay & Carr, 2013).

**Ovulation**

Ovulation happens at the peak of follicular growth in response to LH surge (Cahill et al., 1998). Prior to ovulation, follicles grow to sizes greater than 20 mm in average diameter. LH is then released in a positive-feedback mechanism from the anterior pituitary due to prolonged exposure to estradiol. Approximately 12 h after the LH peak, the oocyte is released. In order for the oocyte to release from the follicle, several proteolytic enzymes and prostaglandins are activated, leading to the digestion of the follicle wall collagen (Espey, 1994). Once an oocyte is released, the fallopian tube is responsible for picking it up where it will await fertilization (Beshay & Carr, 2013).

![Figure 1.3: Ovulatory cycle in humans](image)
Luteal phase/Secretory phase

The secretory phase starts after ovulation. During this phase, the remaining granulosa cells that are not released with the oocyte during the ovulation process enlarge and acquire lutein (carotenoids), which is yellow in color. These granulosa cells are now called the corpus luteum and predominantly secrete progesterone. The progesterone level peaks after one week of ovulation which is required to convert the endometrial lining of the uterus from a proliferative one into a secretory endometrium in preparation for embryo implantation. The life span of the corpus luteum and, hence, progesterone production will depend on continued LH support from the anterior pituitary. If a pregnancy takes place, hCG of pregnancy will maintain the corpus luteum. However, if a pregnancy fails to happen, luteolysis takes place and the corpus luteum is converted to a white scar called the corpus albicans. The loss of the corpus luteum and the subsequent loss of progesterone leads to the instability of the endometrium and the sloughing of the endometrium, signaling a new menstrual cycle (Beshay & Carr, 2013).

Ovarian Hormones

Ovaries release several hormones upon stimulation by pituitary gonadotropin which, in turn, mediate the feedback regulation of HPO axis. The ovarian hormone includes steroid hormones like estrogens, progesterones, androgens and peptide hormones like inhibin, activin and anti-mullerian hormone (AMH).

Estrogens

Estrogens are 18-carbon steroid hormones that include Estrone (E1), Estradiol (E2), and Estriol (E3) among which, Estradiol is the most potent estrogen primarily produced by granulosa cells of the ovary. Estrone is mainly the product of peripheral androstenedione conversion and it is also generated in the liver via 17β-hydroxysteroid dehydrogenase conversion of estradiol. Estriol is the principal estrogen formed by the placenta during pregnancy (Beshay & Carr, 2013).

Serum estradiol levels rise during the follicular phase of the menstrual cycle and are in parallel to the growth of the follicle. Estradiol is mainly found bound in the bloodstream to carrier proteins. Albumin carries approximately 60% of estradiol, while sex hormone-binding globulin (SHBG) binds 38% of estradiol with 2% remaining as free in the bloodstream. This free hormone is active and capable of entering target cells. There are two known estrogen receptors – ERα and ERβ (Kuiper et al., 1996; Mosselman et al., 1996).
Both receptors contain DNA-binding and hormone-binding domains, a hinge region and a transcriptional activation function (TAF) domain. Once estrogen binds to its receptor, activation of gene transcription takes place (Beshay & Carr, 2013). Estrogen receptors are widespread the body tissues like uterus, ovary, mammary gland, prostate, lung and brain (Heldring et al., 2007). In ovary, ERα is predominantly found on theca cells where it aids in cell proliferation upon LH stimulation whereas ERβ, present on granulosa cells, helps in FSH-induced differentiation of antral follicle (Couse et al., 2005; Woodruff & Mayo, 2005). Also, presence of ERα and ERβ has been observed on hypothalamic GnRH neurons and pituitary gonadotrophs, mainly assisting estrogen mediated feedback response to HPG axis. In addition to reproductive function, estrogen receptors in brain help in learning and memory, cognition and defining mood (Heldring et al., 2007).

**Progesterones**

Progesterone is a 21-carbon steroid molecule which is the key hormone of corpus luteum. In the follicular phase, progesterone level remains low but with the progression of luteal phase, progesterone level rises, reaching its peak during mid-luteal phase. The majority of progesterone in the bloodstream is bound to albumin (80%), corticosteroid-binding globulin (18%) and SHBG (0.5%). The remaining progesterone is free in the circulation (Beshay & Carr, 2013).

Similar to estrogen, there are several progesterone receptors – PR-A, PR-B and PR-C. PR-B is the positive regulator of progesterone effects, while PR-A and PR-C antagonize progesterone action mediated by PR-B (Beshay & Carr, 2013).

**Androgens**

Androgens, 19-carbon steroids, are the major products of theca cells that include androstenedione, testosterone and dehydroepiandrosterone (DHEA). The principal secreted androgen by theca cells is androstenedione. Most of the testosterone is produced by peripheral conversion of androstenedione through the actions of 17β-hydroxysteroid dehydrogenase. The androgen receptor exists in a full-length B form and a shorter A form (Wilson & McPhaul, 1994). Androgens and progestins can cross-react to their receptor but only when present in high concentration (Beshay & Carr, 2013). Androgen receptors are present on granulosa and theca cells of ovary wherein they help in steroidogenesis and cell proliferation (Chen et al., 2013)
Inhibin, Activin and Anti-mullerian hormone (AMH)

Inhibin, activin and AMH all belong to the transforming growth factor-β (TGFβ) superfamily of ligands. They are peptide hormones of the ovary and play a role in the regulation of menstrual cycle. Inhibin is a polypeptide mainly secreted by granulosa cells, but has also been found in pituitary gonadotropes (Blumenfeld, 2001). Inhibin is released by granulosa cells in response to FSH and selectively inhibits FSH secretion from the anterior pituitary, thus creating a negative-feedback loop (Rivier et al., 1986; Beshay & Carr, 2013). In contrast, activin, which is also secreted by the granulosa cells, augments the secretion of FSH by enhancing GnRH receptor formation (Norwitz et al., 2002). AMH is a product of the granulosa cells of small antral and pre-antral follicles and is reflective of their quantity (Durlinger et al., 2002). Although the role of AMH has been well described for causing Müllerian duct regression in the male fetus, its role in females in the post-fetal life period has not been well defined. It is believed that AMH, through a paracrine effect in the ovary, inhibits FSH-stimulated follicle growth, contributing to the emergence of the dominant follicle (Speroff and Fritz, 2005; Beshay & Carr, 2013).

Ovarian Steroidogenesis

Steroidogenesis is the process by which steroid hormones are synthesized through enzymatic reaction from the precursor cholesterol molecule (Wickenheisser et al., 2006; Miller, 2008). Most of the enzymes of steroidogenesis are members of the cytochrome P450 family, which add oxygen to the sterol ring structure. Steroidogenic enzymes are located within mitochondria or smooth endoplasmic reticulum (SER), so the steroids must move to these organelles to be acted on by the enzymes. The substrate cholesterol can come from four different sources: i) de novo synthesis in the ER; ii) cholesterol stored in lipid droplets as cholesterol esters; iii) uptake of circulating HDL via scavenger receptor B1 (SR-B1); and iv) uptake of LDL via receptor-mediated endocytosis (Miller & Bose, 2011). Once, cholesterol is available, it can be utilized for steroidogenesis. The first (and rate-limiting) step is transport of cholesterol from the outer to the inner mitochondrial membrane, a function provided by a protein called StAR (steroid acute regulatory protein) (Clark et al., 1995). Cholesterol is then converted to pregnenolone by cholesterol side-chain cleavage cytochrome P450 enzyme. Steroidogenesis within the ovary can then follow one of two pathways: In the Δ⁵ pathway, which occurs predominantly in theca cells of large tertiary follicles, pregnenolone is converted to 17-hydroxypregnenolone, followed by dehydroepiandrosterone (DHEA) and then to androstenedione. The first two reactions of Δ⁵ pathway are catalyzed by the same
enzyme 17α-hydroxylase (also known as 17,20-lyase) which possess hydroxylase and lyase activity. Several factors affect follicular development through influencing 17α-hydroxylase activity which include FSH, LH, insulin, IGF-1 & 2, inhibin (stimulators of enzyme activity) and EGF, FGF, GDF-9 and activin which inhibits 17α-hydroxylase function (Andersen & Ezcurra, 2014).
In the second (Δ⁴) pathway which mainly occurs in corpus luteum, pregnenolone is converted to progesterone by the enzyme 3β hydroxysteroid dehydrogenase (3βHSD). Further, the progesterone is converted to 17-hydroxyprogesterone, which is then changed to androstenedione (Andersen & Ezcurra, 2014). From here on, both the Δ⁵ and the Δ⁴ pathways can produce estrogens. Androstenedione is converted to testosterone (a potent androgen) and then to estradiol, the major estrogenic hormone secreted by ovarian follicles and the corpus luteum (Andersen & Ezcurra, 2014). The rate-limiting step in estradiol formation is catalyzed by the enzyme aromatase which is stimulated by FSH, cyclic AMP and protein kinase A (PKA) phosphorylation (Zhao et al., 2016).

Each step in the steroidogenic pathway is catalysed by a specific enzyme, and a cell can convert one steroid to another only if it has the appropriate enzyme needed for the conversion step. In the growing ovarian follicle, no single cell type expresses all of the enzymes in the steroidogenic pathway. During the late secondary or early tertiary stage, the theca cells synthesize androstenedione (Smyth et al., 1993), which then diffuses into the granulosa cells and converted into estradiol by aromatase enzyme (Whitelaw et al., 1992). Thus, estrogen synthesis in the follicle depends on the coordinated biochemical activity of theca and granulosa cells which is known as the two-cell model of ovarian steroidogenesis (Jones & Lopez, 2014).

Regulation of Hypothalamic-pituitary-Ovarian (HPO) axis
Reproductive axis is regulated by various intra and extra ovarian factors. Intra ovarian factors mainly comprises of gonadal steroids and peptide hormones that control HPO axis via feedback mechanism. Neurotransmitters, neuropeptides and opioids are the extra ovarian factors that modulate GnRH and gonadotropin secretion.

Steroid-mediated feedback regulation of HPO axis
During the early follicular phase, inhibin B is low and FSH is modestly elevated, fostering follicular growth. LH secretion is low due to the negative feedback effect of the rising plasma estrogen level. At 36-48 h before ovulation, the estrogen feedback effect becomes positive, and this initiates the burst of LH secretion (LH surge) that produces ovulation. Ovulation occurs about 9 h after the LH peak. FSH secretion also peaks, despite a small rise in inhibin, probably because of the strong stimulation of gonadotropes by GnRH. During the luteal phase, the secretion of LH and FSH is low because of the elevated levels of estrogen,
progesterone, and inhibin. It should be emphasized that a moderate, constant level of circulating estrogen exerts a negative feedback effect on LH secretion, whereas during the cycle, an elevated estrogen level exerts a positive feedback effect and stimulates LH secretion. In monkeys, it has been demonstrated that estrogens must also be elevated for a minimum time to produce positive feedback. When circulating estrogen was increased about 300% for 24 h, only negative feedback was seen; but when it was increased about 300% for 36 h or more, a brief decline in secretion was followed by a burst of LH secretion that resembled the midcycle surge. When circulating levels of progesterone were high, the positive feedback effect of estrogen was inhibited. In primates, there is evidence that both the negative and the positive feedback effects of estrogen are exerted in the mediobasal hypothalamus, but exactly how negative feedback is switched to positive feedback and then back to negative feedback in the luteal phase remains unknown (Barret et al., 2016).

**Neuromodulators of GnRH release**

![Neuromodulators of GnRH](image)

Figure 1.6: Neuromodulators of GnRH

In addition to classic steroid feedback regulation, GnRH axis is influenced by several factors including neuropeptides, neurotransmitters, opioids, stress, etc. Kisspeptin, a product of KISS1 gene is the most potent regulator of GnRH release, mediating its action through binding to its receptor-GPR54. In brain, kisspeptin neurons are located mainly in two subpopulations: Anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) of...
hypothalamus. In the ARC, kisspeptin is coexpressed with two other neuropeptides, namely, neurokinin B (NKB) and dynorphin (Dyn) and thus this population is known as KNDy neurons. Estrogen mediated negative feedback to GnRH/LH release is attributed by KNDy neurons of ARC whereas kisspeptin neurons of AVPV are associated with estrogen mediated-positive feedback during the pre-ovulatory phase (Pinilla et al., 2012). Neuropeptide Y (NPY) and opioids are also found in the vicinity of GnRH neurons. NPY stimulate GnRH release while inhibition of GnRH release was observed in the presence of opioids (Bakker & Baum, 2000). Corticotropin releasing hormone (CRH), a neuropeptide responsible for culminating stress response, also affects GnRH system. CRH directly suppresses GnRH release as well as it inhibits GnRH gene expression in hypothalamus (Ciechanowska et al., 2011).

In addition to neuropeptides, neurotransmitter from various regions of brain forms synapses with GnRH neurons in hypothalamus and directly or indirectly influences GnRH release. Norepinephrine has a stimulatory effect on GnRH release and it can influence the amplitude as well as frequency of LH release (Smith & Jennes, 2001). The role of serotonin in GnRH release is contradictory but recent study has described the stimulatory effect of serotonin on GnRH is through 5HT2A receptor whereas 5HT1A receptor is responsible for serotonin mediated GnRH inhibition (Bhattarai et al., 2014). Glutamate is the fast acting neurotransmitter and it can directly excite GnRH neuron activity whereas dopamine and GABA (γ-amino butyric acid) are the most potent inhibitors of GnRH release (Liu & Herbison, 2013; Watanabe et al., 2014; Kanasaki et al., 2017).

Along with neuromodulators, several metabolic signals also influence reproduction. Insulin is the major factor, maintaining energy balance of the body. Insulin mRNA as well as insulin receptor expression was found to be present on GnRH neurons and it has demonstrated to directly stimulate GnRH release (Kim et al., 2005). Leptin is a cytokine-like protein mainly functioning as satiety signal. Although leptin was not localized with GnRH neurons, it acts as a GnRH stimulator through activating NPY pathway (Pralong, 2010).

In summary, proper reproductive function requires a remarkable coordination between all components of the hypothalamic-pituitary-gonadal axis and other target tissues. A synchronized activity of GnRH pulse generator results in typical gonadotropin release which promote normal ovulatory menstrual cycle. The ovarian steroids, through their positive and negative feedback loops, play a crucial role by regulating gonadotropin levels. However, any
abnormality that prevent or interfere with the function of this axis may reflect in reproductive endocrine disorder such as polycystic ovarian syndrome.

**Polycystic Ovarian Syndrome (PCOS)**

Polycystic ovarian syndrome is a most common endocrine disorder of women of reproductive age. It is characterized by excess androgen, ovulatory dysfunction and polycystic ovaries. In addition to reproductive anomalies, PCOS is also linked with several metabolic dysfunctions including type 2 diabetes mellitus, obesity, cardiovascular disorders and Psychological comorbidity namely, anxiety, depression, mood disorders (Azziz et al., 2016). PCOS is also known as Stein-Leventhal syndrome because, they were the first to describe the association of menstrual disturbances, hirsutism (male like hair pattern), and polycystic ovaries in a group of women (Stein and Leventhal, 1935). Currently, three different criteria for defining PCOS exist. According to 1990 US National Institute of Health (NIH), the major criteria for PCOS should include hyperandrogenism and/or hyperandrogenemia, oligo- or anovulation and exclusion of other known disorders. A consensus held in Rotterdam (Eshre and ASRM-Sponsored PCOS Consensus Workshop Group, 2004), included two out of three criteria for diagnosis of PCOS: oligo-/anovulation, clinical or biochemical hyperandrogenism and/or polycystic ovaries on ultrasound, excluding other endocrinopathies. In 2006, the androgen excess-PCOS society recommended that PCOS be defined by clinical and/or biochemical hyperandrogenism with either oligo-/anovulation and/or polycystic ovarian morphology, excluding related disorders (Goodarzi et al., 2011). Both the Rotterdam consensus and androgen excess-PCOS society suggested presence of polycystic ovarian morphology on ultrasound as one of the diagnostic criteria to define PCOS. However, the morphological evaluation of ovaries by ultrasound is highly variable due to difference in the sonographic techniques. Also, visualization of ovaries through abdominal ultrasonography in obese patient is difficult (Sahmay et al., 2014). To overcome these limitations, assessment of anti-mullerian hormone (AMH) for PCOS diagnosis is emerging. AMH is secreted by the granulosa cells of small antral and pre-antral follicles for the regulation of early follicular development. Elevated serum AMH levels are found in PCOS patient suggesting it as an important tool for PCOS diagnosis (Cassar et al., 2014; Lauritsen et al., 2014; Sahmay et al., 2014).
### Prevalence of PCOS

PCOS is the most widespread disorder across the globe affecting 2.2% to 26% of women in their reproductive age (Diamanti-Kandarakis et al., 1999; Asuncion et al., 2000; Azziz et al., 2004b; Joshi et al., 2014). The variation in the prevalence is mainly due to different diagnostic criteria. Studies based on Rotterdam criteria have demonstrated PCOS prevalence in China (2-7.5%) (Chen et al., 2008; Li et al., 2013) and in Sri Lanka (6.3%) (Kumarapeli et al., 2008) whereas using NIH criteria, PCOS was found to be prevalent in 5-8% of Caucasian women (Asuncion et al., 2000; Azziz et al., 2004b; Joshi et al., 2014). In an Australian study, PCOS prevalence was about 12% using Rotterdam criteria which increased to 18% when imputed data was included (March et al., 2009). In India prevalence of PCOS ranges from 3.7-22.5%. The prevalence of PCOS in adolescents and young girls was found to be 3.7% in Lucknow, Uttar Pradesh (Gill et al., 2012), 9.13% in Andhra Pradesh (Nidhi et al., 2011), 18% in Tamil Nadu (Balaji et al., 2015) and 22.5% in Mumbai, Maharashtra (Joshi et al., 2014).
Reproductive features of PCOS

Menstrual disorders in PCOS

In PCOS, menstrual irregularities are very common and it can be characterized by oligomenorrhea (infrequent menstruation, less than 8 cycles in a year) and amenorrhea (absence of menstruation for more than three months). Typically, this pattern of bleeding is an extension of postmenarchal irregularity and monthly menstrual cyclicity is never established. In some women, the onset of chronic anovulation emerges beyond adolescence, but this is unusual (Sirmans & Pate, 2013). Prolonged heavy bleeding should raise consideration of abnormal endometrial hyperplasia and even endometrial adenocarcinoma. In approximately 70-80% of PCOS women oligomenorrhea is observed while 20% of PCOS women, there is complete absence of menses, whereas 5-10% of cases demonstrate regular ovulatory function (Strauss III & Barbieri, 2014; Jalilian et al., 2015).

Hyperandrogenism / Androgen excess and PCOS

Androgen excess is the most common characteristic of PCOS and yet only 80-85% of women with clinical hyperandrogenism have PCOS (Azziz et al., 2004a; 2009; Goodarzi et al., 2011). Clinical hyperandrogenism can be defined by presence of hirsutism (male pattern hair), acne and androgenic alopecia (male pattern baldness). In PCOS, excessive hair is
mostly observed on the side of the face, upper lip, and chin extending down to the neck region. According to clinical studies, hirsutism is observed in 65-75% of white and black women with PCOS but its occurrence is very less in east Asian women with PCOS (Goodarzi et al., 2011). The prevalence of acne in PCOS varies with ethnicity and age group; however it is still present in 15-25% of PCOS women (Azziz et al., 2009; 2016).

In addition to clinical features of hyperandrogenism, biochemical evaluation of androgens may also help in PCOS diagnosis. Circulating total and free testosterone and dehydroepiandrosterone sulphate (DHEAS) levels are elevated in 50-75% of women with PCOS (Huang et al., 2010; Goodarzi et al., 2011).

Polycystic ovarian morphology and PCOS
Rotterdam consensus and androgen excess society, both have included polycystic ovarian morphology for PCOS diagnosis. Polycystic ovaries can be defined by the presence of 12 or more follicles of 2-9mm in diameter, arranged on each ovarian periphery and/or an increased ovarian volume of greater than 10 cm$^3$ in at least one ovary (Eshre and ASRM-Sponsored PCOS Consensus Workshop Group, 2004; Azziz et al., 2006). Various studies have detected the presence of polycystic ovaries by transvaginal sonography in 75% of clinically diagnosed PCOS women (Amer et al., 2002; Jonard et al., 2003; Carmina et al., 2005; Hahn et al., 2005; Azziz et al., 2016).

Infertility and PCOS
PCOS is the most common cause of anovulatory infertility. It accounts for 90% to 95% of women attending infertility clinics with anovulation. However, 60% of women with PCOS are fertile (defined as the ability to conceive within 12 months), although time to conceive is often increased (Brassard et al., 2008; Teede et al., 2010). In addition to anovulation, a subgroup of PCOS women also exhibit impaired oocyte development competence (ability of the oocyte to undergo fertilization, embryogenesis and development) which may hinder the pregnancy (Azziz et al., 2016). Also, infertility is more prevalent in PCOS women with obesity and insulin resistance than PCOS alone (Clark et al., 1998; Tian et al., 2007; Goodarzi et al., 2011).

Obstetrical complications and PCOS
Patients with PCOS are more prone to experiencing complications during pregnancy. A population based study indicated that pregnancies in women with PCOS had significantly higher rates of pre-eclampsia, very preterm birth (defined as less than 32 weeks of gestation),
gestational diabetes mellitus and pregnancy induced-hypertension (Roos et al., 2011; Azziz et al., 2016). Also, infants born to PCOS women had a higher risk of being large for gestational age and they have a greater risk of perinatal mortality (Goodarzi et al., 2011; Yu et al., 2016).

**Metabolic features of PCOS**

In addition to reproductive abnormalities, PCOS is linked with several metabolic complications which will be discussed in following section:

**Insulin resistance**

The prevalence of insulin resistance in PCOS ranges from 50-70%. Most women with PCOS develop compensatory hyperinsulinemia from insulin resistance, with impaired glucose tolerance (Goodarzi et al., 2011). Also, the prevalence of glucose intolerance and subsequent diabetes has been reported to be as high as 31% and 7.5% respectively (Strauss III & Barbieri, 2014). Commonly, hyperglycemia is not evident in PCOS women; instead defect in postprandial glucose uptake results into peripheral insulin resistance. Most women with PCOS have normal or exaggerated insulin secretory response whereas women with a family history of type 2 diabetes mellitus, show impaired β-cell function (Legro et al., 1999; Ehrmann et al., 2005; Goodarzi et al., 2011). Besides the increased risk for diabetes, insulin resistance may exacerbate the clinical manifestation of PCOS. Insulin resistance may also contribute to metabolic dysfunction in PCOS, including an increased likelihood of lipid abnormalities (Strauss III & Barbieri, 2014).

**Dyslipidaemia**

An increased risk of dyslipidemia has been demonstrated in PCOS. Lipid abnormalities include reduced high-density lipoprotein-cholesterol (HDL-C), increased triglycerides, and increased low density lipoprotein-cholesterol (LDL-C) (Wild, 1995; Meyer et al., 2005; Sirmans & Pate, 2014). The dyslipidaemia occurs independent of body mass index (BMI), however there is a synergistic deleterious effect of obesity and insulin resistance in PCOS analogous to that seen in type 2 diabetes mellitus (Teede et al., 2010). The causes of dyslipidaemia in PCOS are again multifactorial. Insulin resistance appears to have a pivotal role mediated in part by stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase (Teede et al., 2010).
**Obesity**
There exists a complex two-way relationship between PCOS and obesity, and strong evidence of an association is currently lacking. Although PCOS occurs in obese and lean women, a recent systematic review and meta-analysis concluded that obesity was present in around 50% of PCOS women (Lim et al., 2012; Azziz et al., 2016). Furthermore, menstrual irregularities and anovulation appear to be more prevalent and severe in obese women with PCOS than in their non-obese counterparts, and weight loss of at least 5% tends to be associated with improvement of these conditions (Pasquali et al., 2006; Rojas et al., 2014).

**Cardiovascular disease risk**
Alongside insulin resistance, metabolic syndrome, glucose intolerance and type 2 diabetes mellitus, women with PCOS also have increased cardiovascular risk factors (inflammation, oxidative stress and impaired fibrinolysis) (Moran & Teede, 2009). Also, increased early clinical and subclinical markers of atherosclerosis seen in PCOS which include endothelial dysfunction, increased carotid intima media wall thickness, presence of carotid plaque and increased coronary artery calcification (Teede et al, 2010; Carmina, 2014). Also, the risk of venous thromboembolisms is increased in PCOS women compared to BMI-matched controls (Anderson et al., 2014; Azziz et al., 2016).

**Psychological complications in PCOS**
The clinical spectrum of PCOS encompasses hirsutism, acne, obesity, male pattern alopecia, and infertility as a result of ovulatory disturbance. These clinical features and health implications of PCOS may predispose to an impaired quality of life (QoL), leading to a loss of self-esteem, poor body image, and psychological morbidity (Ching et al., 2007; Sharma, 2015). It was also found that women with PCOS had a significantly poorer quality of life when compared with age-matched population. This decreased QoL observed in PCOS, combined with poor coping strategies, can result in comorbid psychiatric conditions such as depression and anxiety (Azziz et al., 2016). Studies have reported that women with PCOS have an increased prevalence of mood disorders, including depression (26–40%), anxiety (11.6%) and binge-eating (23.3%) (Pasch et al., 2008; Kerchner et al., 2009; Goodarzi et al., 2011). Although the clinical features of PCOS increases the risk of psychological complications, the causal factors underpinning the mood disturbance in PCOS remain unclear.
Pathophysiology/Mechanism of PCOS

The pathophysiology of PCOS is complex and reflects the interactions between genetic, metabolic, fetal and environmental factors. The relative importance of these factors may vary in individual affected women. Among these factors, disordered gonadotropin secretion, hyperandrogenism, insulin resistance, ovarian dysfunction, and follicular arrest are prominent. The potential role of these factors and their actions are summarized in this section.

Genetic factors

In search of the genetics of PCOS, most studies to date have focused on identifying candidate genes that are linked to recognized abnormalities of steroid hormone production and action, carbohydrate and fuel metabolism, and gonadotropin secretion (Strauss III & Barbieri, 2014). Studies in women with PCOS identified a specific genetic association for LHCGR (Luteinizing hormone/ Choriogonadotropin receptor) and INSR (Insulin receptor) with anovulation, and THADA (Thyroid adenoma-associated protein) and DENND1A (DENN domain-containing protein 1A) with polycystic ovaries. C9orf3 (Chromosome 9 open reading frame 3) and rs4385527 conferred a particular risk for all three of the definitive manifestations of PCOS, which suggests their fundamental role in the aetiology of the disorder (Cui et al., 2015; Azziz et al., 2016). According to in silico pathway analysis, INS, GNAQ (Guanine nucleotide-binding protein G(q) subunit alpha), PLCB3 (Phospholipase C, beta 3), PLCB2 (Phospholipase C, beta 3), PLCZ1 (Phospholipase C, zeta 1), STXBPI (Syntaxin binding protein 1) and SMC3 (Structural maintenance of chromosomes protein 3) are significantly associated with oocyte meiosis and the regulation of insulin secretion (Shim et al., 2015). A recent study measured DNA methylation and gene expression of 11 Chinese GWAS (genome-wide association studies) risk loci in subcutaneous adipose tissue of patients with PCOS. This study found that the genetic variants in LHCGR and INSR might have changed the expression level via modification on methylation. Hypomethylation of LHCGR was concordant with LHCGR overexpression in non-obese patients, but not in the obese ones, whereas hypermethylation of INSR was not associated with different gene expression between obese and non-obese women with PCOS (Jones et al., 2015; Azziz et al., 2016). Despite the vast progress in the identification of PCOS loci, the quantitative traits associated with the disorder and the underlying mechanisms are still largely unknown.

Recent studies have also identified association of various microRNA (miRNA) in PCOS. miRNA are small non-coding RNA molecules which modulate gene expression by post-
transcriptional modifications (Bartel, 2004). When PCOS patients were compared with healthy controls serum miRNA-21, miRNA-27b and miRNA-103 found to be associated with PCOS as well as metabolic features, such as obesity, type 2 diabetes mellitus, inflammation and adipogenesis (Sirotkin et al., 2009; Sorensen et al., 2014). Also, association of miRNA-9, miRNA-18b, miRNA-132, and miRNA-224 was observed in follicular fluid of PCOS women suggesting the role of miRNA in PCOS pathogenesis (Sirotkin et al., 2009; Murri et al., 2013a).

**Environmental factors**

Lifestyle profoundly affects the phenotypic expression of PCOS. Weight gain worsens metabolic and reproductive abnormalities of PCOS, as evidenced by increased total and abdominal obesity as well as insulin resistance, menstrual irregularity and hyperandrogenism in women with the most severe PCOS phenotype (Carmina et al., 2009; Moran & Teede, 2009; Goodarzi et al., 2011). A sedentary lifestyle alone also contributes to metabolic dysfunction in PCOS because moderate-intensity exercise without weight loss improves insulin resistance and decreases body adipose tissue (Bruner et al., 2006). Poor dietary choices, such as relying on energy dense foods instead of unprocessed grains, fruits, and vegetables, and larger portion sizes, have been implicated as contributors to the obesity and increases risk of PCOS (Diamanti-Kandarakis et al., 2006).

Environmental endocrine disrupting chemicals also might disrupt ovarian and metabolic function, causing PCOS-like abnormalities. Bisphenol A (BPA), a widely used estrogenic industrial plasticizer, is one such endocrine disrupting chemical that is detectable in most individuals (Vandenberg et al., 2009). Rodent studies indicate that BPA enhances ovarian androgen production *in vitro* and induces insulin resistance *in vivo* (Alosolo-Magdalena et al., 2006; Zhou et al, 2008). BPA accumulates to an increased level in women with PCOS owing to the decreased hepatic clearance which might exaggerate the severity of the PCOS phenotype (Diamanti-Kandarakis et al., 2009a). Another example of an environmental substance implicated in the development of a PCOS phenotype is valproic acid, which is widely used to treat epilepsy and bipolar disorders as well as migraines and generalized mood disorders. There are studies to suggest that women treated with valproic acid may develop symptoms of PCOS, including polycystic ovaries, hyperandrogenism, obesity, and anovulation, and that these stigmata may reverse with discontinuation of the medication (Franks et al., 1997; 2001). Although this is a highly contentious area and there may be clear
ethnic differences in susceptibility, recent studies suggest that weight gain on this medication is essential for the development of the full PCOS phenotype (Diamanti-Kandarakis et al., 2006).

**Intrauterine environment/ Epigenetic modulations**

Epigenetic changes in fetal life are implicated in the developmental origins of PCOS (Dumesic et al., 2007; Goodarzi et al., 2011). Experimentally, excess fetal testosterone (T) induces PCOS-like reproductive and metabolic traits in female mammals, from rodents to primates (Dumesic et al, 2015). Sheep models of PCOS have provided useful information for a better understanding of hormonal regulation. Sheep exposed to prenatal testosterone had increased LH pulsatility and impaired estrogen/progesterone feedback mechanisms, which resulted in altered ovulatory and follicular dynamics, loss of estrus cycles, polycystic ovarian morphology and insulin resistance (Padmanabhan & Veiga-Lopez, 2013). Similar results were also observed in female rhesus monkey exposed to in utero testosterone. After puberty, the offspring of T-exposed monkey exhibit LH hypersecretion, ovulatory dysfunction, hyperandrogenism, and insulin resistance; in addition, roughly 50% of the offspring have enlarged ovaries with increased follicle counts (Abbott et al., 2005). Some of these changes appear developmentally programmed in utero because second generation female offspring also manifest elevated LH pulsatility from reduced hypothalamic steroid negative feedback, exaggerated T responses to chorionic gonadotropin, diminished ovarian reserve, excess adrenal androgen production, and altered abdominal adipose characteristics (Keller et al., 2014; Dumesic et al., 2015).

Evidence for the in utero effects of excess androgen exposure in humans is less convincing. Earlier work had documented the occurrence of cystic ovaries and PCOS-like symptoms in girls with congenital adrenal hyperplasia with 21-hydroxylase deficiency (Barnes et al, 1994., Dumesic et al, 2015). Also, maternal T during pregnancy is elevated in women with PCOS (Sir-Petermann et al., 2002), but whether this results in increased fetal exposure is unclear, given the markedly increased levels of SHBG and abundant placental aromatase activity during pregnancy (Dmitrovic et al., 2011; Dumesic et al., 2015). These observations have led to the hypothesis that the clinical phenotype of PCOS may be the result of intrauterine androgen exposure during pregnancy.
Gonadotropic abnormalities

In normal circumstances, immature oocytes mature under the influence of several hormones-most notably follicle stimulating hormone (FSH), and ovulation as well as final maturation occur upon luteinizing hormone (LH) stimulation. A neuroendocrine abnormality in PCOS includes increased gonadotropin-releasing hormone (GnRH) pulse frequency, which increases the frequency and pulse amplitude of LH over FSH production culminating into increased circulating LH/FSH ratio (Banaszewska et al., 2003; Azziz et al., 2016). Increased LH pulse frequency in PCOS, occurs due to reduced steroid hormone negative feedback on LH secretion because of androgen excess (Blank et al., 2006). This neuroendocrine abnormality occurs in adolescent girls with PCOS (Marshall & Eagleson, 1999; Chhabra et al., 2005) and is ameliorated with the androgen receptor blocker flutamide, which suggests that androgen excess reduces hypothalamic feedback inhibition that causes increased GnRH pulsatility during puberty (Eagleson et al., 2000; Goodarzi et al., 2011). However, not all adolescent girls with PCOS exhibit reduced hypothalamic feedback inhibition from androgen excess, which may be because the presence of this defect requires a genetic component or depends on the duration of androgen excess (Chhabra et al., 2005). Other neuroendocrine abnormalities in women with PCOS include exaggerated LH responsiveness to GnRH (Lobo, 1991). It has been observed that pattern of LH release in PCOS resembles more with that of men or women with congenital adrenal virilizing disorder than of women without PCOS (Goodarzi et al., 2011). Abnormalities in the circulating LH:FSH ratio are primarily observed in thin women with PCOS, as obesity lowers LH pulse amplitude and alters LH pharmacokinetic structure, which contributes to reduced serum LH levels with increased percent body adipose tissue (Pagan et al., 2006; Srouji et al., 2007; Goodarzi et al., 2011). Although gonadotropin derangement in PCOS is known for decades, the exact mechanism underlying this phenotype has yet to be discovered.

Ovarian follicular arrest

During ovarian follicular development, primordial follicles are recruited into a group of growing follicles, from which one antral follicle is selected to ovulate. These events require coordinated reproductive, metabolic and intraovarian interactions. In PCOS, ovarian hyper-androgenism, hyperinsulinemia from insulin resistance and altered intraovarian paracrine signalling can disrupt follicle growth. The consequent follicular arrest in PCOS is accompanied by menstrual irregularity, anovulatory subfertility and the accumulation of
small antral follicles within the periphery of the ovary, giving it a polycystic morphology (Jonard and Dewaily, 2004; Goodarzi et al., 2011).

The ovulatory dysfunction in PCOS is characterized by increased follicular activation, but the growth of these follicles is arrested before they mature (Jonard & Dewaily, 2004). The arrested follicle development can possibly be explained by the normal but relatively low circulating FSH levels (in reference to LH levels) in women with PCOS (Franks et al., 2008). LH hypersecretion is also detrimental for follicular growth and ovulation in women with PCOS and may cause the premature luteinization of granulosa cells by decreasing FSH sensitivity (Qiao & Feng, 2011; Dumesic et al., 2015). The frequent occurrence of associated hyperinsulinemia in PCOS further exacerbates ovarian follicular arrest, which promotes ovarian hyper androgenism by stimulation of 17α-hydroxylase activity in theca cells (Moggetti et al., 2000). Hyperinsulinemia also amplifies LH-stimulated and insulin-like growth factor 1 (IGF-1)-stimulated androgen production, elevates serum free testosterone levels through decreased hepatic SHBG production and enhances serum IGF-1 bio activity through suppressed IGF-binding protein production (Bergh et al., 1993; Balen et al., 2005).

Figure 1.8: Pathophysiology of PCOS (Azziz et al., 2016)
Insulin excess also promotes premature follicle luteinization through enhanced follicle-stimulating hormone (FSH)-induced granulosa cell differentiation, which arrests granulosa cell proliferation and subsequent follicle growth (Goodarzi et al., 2011).

Changes in intraovarian factors involving follicular recruitment and growth such as members of the TGFβ family (anti-Mullarian hormone (AMH), inhibins, activins, bone morphogenic proteins, and growth differentiation factors (GDFs), other growth factors, and cytokines (Diamanti-Kandarakis, 2008; Raja-Khan et al., 2014) may also contribute to the abnormal follicle development and function in PCOS (Qiao & Feng, 2011). Gene expression of GDF9, an oocyte-derived growth factor affecting theca cell layer formation (Young & McNeilly, 2010), is reduced in ovaries of anovulatory PCOS women (Teixeira Filho et al., 2002), linking dysregulated oocyte GDF9 gene expression with altered folliculogenesis. AMH, a potential PCOS marker (Dewailly et al., 2010) produced by increased numbers of preantral and small antral follicles (Pellatt et al., 2010), may enhance theca cell androgen activity by inhibiting FSH and follicular development (Visser et al., 2012; Dumesic et al., 2015). In addition, decreased inhibin A and B levels occur in some small PCOS follicles despite normal amounts of activin and follistatin unbound to activin (Welt et al., 2005; Dumesic et al., 2015). Although inhibins, activins, follistatin and IGF1 all have a crucial role in folliculogenesis, their possible permissive role in the pathophysiology of ovarian dysfunction in women with PCOS remains to be demonstrated (Dumesic et al., 2015; Azziz et al., 2016)

**Insulin resistance and hyperinsulinemia**

Insulin resistance and its compensatory hyperinsulinemia are hallmarks of PCOS, and this puts women with PCOS at an increased risk of impaired glucose tolerance and type 2 diabetes mellitus (Dumesic et al., 2015). Insulin resistance in PCOS is characterized by reduced sensitivity and responsiveness to insulin-mediated glucose utilization primarily in skeletal muscle and adipose tissue (Ciaraldi et al., 2009; Rosenfield and Ehrmann, 2016). In muscle, serine phosphorylation of the insulin receptor and of insulin receptor substrate 1 (IRS1) is increased, resulting in impaired insulin signalling and in constitutive activation of mitogen-activated protein kinase kinase (MEK1) and MEK2 (MEK1/2) in PCOS (Dunaif et al., 1992; Corbould et al., 2006; Rajkhowa et al., 2009). The PCOS-associated insulin resistance is selective, affecting metabolic, but not mitogenic, signalling pathways, which might explain the paradox of the persistent reproductive actions of insulin in the face of systemic insulin resistance (Azziz et al., 2016).
The role of insulin resistance and hyperinsulinemia in the development of PCOS has been thoroughly explored, and it is generally accepted to play an important role in the molecular mechanisms implicated in the androgenic hypersecretion typical of this pathology (Diamanti-Kandarakis & Dunaif, 2012; Rojas et al., 2014). Insulin acts through multiple sites to increase endogeneous androgen levels. Increased peripheral insulin resistance results in a higher serum insulin concentration. Excess insulin binds to the IGF-1 receptors and enhances the theca cells androgen production in response to LH stimulation (Balen, 2004). Hyperinsulinemia also decreases the synthesis of SHBG by the liver (Dumesic et al., 2015). There is therefore an increase in the concentration of free testosterone in the serum and consequent peripheral androgen action. In addition, hyperinsulinemia inhibits the hepatic secretion of IGF binding protein-1 (IGFBP-1), leading to increased bioavailability of IGF-1 and IGF-2, the important regulators of ovarian follicular maturation and steroidogenesis (De Leo et al., 2000; Balen, 2004). Together with more IGF-2 secretion from the theca cells, IGF-1 and IGF-2 further augment ovarian androgen production by acting on IGF-1 receptors (Balen, 2004). Insulin also increases endogenous androgen concentrations by increased cytochrome P450c17α enzyme activity, which is important for ovarian and adrenal steroid hormone biosynthesis (la Marca et al., 2000; Balen, 2004).

**Hyperandrogenemia**

Hyperandrogenemia may be among the inciting factors of metabolic aberrations in PCOS. Effect of hyperandrogenemia on insulin sensitivity may be mediated by upregulation of β3 adrenergic receptors and hormone-sensitive lipase expression in visceral adipose tissue.
(VAT) through testosterone or DHEAS signalling, modifying lipolytic activity and favouring release of free fatty acid (FFA) into circulation (de Pergola, 2000; Rojas et al., 2014). This increase in FFA availability causes functional and structural changes in hepatocytes and skeletal myocytes, with the accumulation of metabolites from the long-chain FFA reesterification pathway, including Acyl-CoA and diacyl glycerol. In turn, these molecules can activate PKC, a serine/threonine kinase which is widely accepted as key player for the mechanisms underlying insulin resistance, particularly through serine phosphorylation of IRS-1 (Boden, 2011). In PCOS, androgens also appear to modify metabolic architecture and functionality in skeletal muscle, by decreasing the amount of type-I muscle fibers (insulin sensitive), and increasing type-II fibers (less sensitive for insulin), as well as decreasing expression of glycogen synthase (Giallauria et al., 2009). However, further mechanism of androgen-induced cytokine secretion from VAT and interference of insulin signalling through androgen is not well understood (Rojas et al., 2014).

In addition to reproductive and metabolic anomalies, one of the consequences of hyperandrogenism is hirsutism. Androgens, primarily testosterone and dihydrotestosterone, through their effect on the androgen receptor, stimulate ornithine decarboxylase synthesis in the hair follicle, which in turn stimulates polyamine production. Polyamines are multifunctional cationic amines that are indispensable for cellular proliferation, including hair growth in the hair follicle (Azziz et al., 2016).

**Adipose tissue dysfunction**

Although women with PCOS can show little difference in fat distribution and possibly in overall BMI, strong evidence supports that adipocytes and adipocyte function are aberrant in PCOS, favouring insulin resistance and subclinical inflammation. The peripheral insulin resistance observed in PCOS might be the result, at least in part, of adipocyte dysfunction. For example, inflammatory cytokines (such as tumour necrosis factor and IL-6) suppress insulin-mediated glucose transport more in adipocytes derived from patients with PCOS than in adipocytes derived from matched controls (Chazenbalk et al., 2010). Women with PCOS seem to have larger adipocytes, lower lipoprotein lipase activity and impaired catecholamine-induced lipolysis compared with matched controls (Ek et al., 1997; Manneras-Holm et al., 2011; Azziz et al., 2016). Inflammatory cytokines also suppress adiponectin secretion to a greater degree in adipocytes derived from patients with PCOS than in matched controls, favouring the development of a more pro-inflammatory, insulin-resistant environment.
Glucose transporter 4 (GLUT4) protein expression is decreased in adipocytes in PCOS, similar to levels observed in adipocytes derived from patients with type 2 diabetes mellitus (Carvalho et al., 2001; Carlson et al., 2003). Overall, adipocyte functioning, including the stimulation of glucose transport, GLUT4 production, and insulin-stimulated inhibition of lipolysis, are defective in PCOS (Ciaraldi, 2000; Chen et al., 2013; Azziz et al., 2016). Epigenetic dysregulation of adipocyte function has been observed in PCOS, primarily of microRNA-93 (miR-93) and miR-223, which seem to have a role in suppressing GLUT4 content and altering glucose transport (Chen et al., 2013; Chuang et al., 2015; Azziz et al., 2016).

**Inflammation and Oxidative stress**
Recent evidence describes a central role for certain proinflammatory mediators in the pathophysiology of PCOS, posing a new focus on the etiological considerations for PCOS (Escobar-Morreale et al., 2011; Gonzalez, 2012). Reports demonstrate that women with PCOS, both with obesity and normal weight, exhibit elevated serum TNF\(\alpha\), C-reactive protein (CRP), monocyte and lymphocyte circulating levels, and inflammatory infiltration in ovarian tissue (Xiong et al., 2011). It has been suggested that polymorphisms of genes encoding proinflammatory cytokines-TNF\(\alpha\), TNF receptor, IL-6 and IL-10 may result in increased inflammation seen in PCOS (Vural et al., 2010). Remarkably, greater expression of the CD11c gene is associated with greater proinflammatory macrophage infiltration in subcutaneous and visceral adipose tissue, favoring a transition to decreased secretion of adiponectin and increased TNF\(\alpha\) and leptin secretion from adipocytes (Tao et al., 2012; Rojas et al., 2014). The role of TNF\(\alpha\) is especially important in the setting of insulin resistance and PCOS. The deteriorating effect of TNF\(\alpha\) on insulin sensitivity is mediated through serine phosphorylation of IRS-1 by PKC (Rojas et al., 2008). This cytokine also stimulates steroidogenesis and proliferation of theca cells, contributing to hyperandrogenemia (Spaczynski et al., 1999; Rojas et al., 2014). Furthermore, hyperglycemia may contribute to inflammation in PCOS. Circulating mononuclear cells utilize glucose as their main redox substrate, with part of its metabolites going into the pentose-phosphate pathway to yield NADPH (Piotrowski et al., 2005; Rojas et al., 2014). Oxidation of this molecule leads to the production of reactive oxygen species (ROS), which induce oxidative stress, with the subsequent activation of NF-kB, a transcription factor involved in the expression of proinflammatory mediators such as TNF\(\alpha\) and IL-6 (Gambineri et al, 2002). Hence, hyperglycemia may result in increased ROS production. Additionally, oxidative stress...
appears to induce key steroidogenic molecules in theca cells, namely, CYP11A1, CYP17A1, 3β-HSD, and StAR, favoring hyperandrogenemia (Duleba & Dokras, 2012). The cross-talk between insulin resistance and chronic inflammation generates an environment, which further amplifies the complications for the overall health of women with PCOS (Moran et al., 2010; Rojas et al., 2014).

**Adrenal androgen excess**

Although the ovaries are the main source of hyperandrogenism in PCOS, between 20-30% of patients also show adrenal androgen excess suggesting adrenocortical hyperfunction (Kumar et al., 2005; Azziz et al., 2016). The mechanism for adrenal hyperandrogenemia may arise from either altered adrenal responsiveness to adreno-corticotropic hormone (ACTH) or abnormal adrenal stimulation by factor(s) other than ACTH. Increased 17-hydroxyprogesterone (17-OHP) responses to ACTH, following dexamethasone, have been observed in women with PCOS, which suggested dysregulation of P450c17 (Ehrmann et al., 1992; Strauss III & Barbieri, 2014). However, other studies have not been able to confirm these results (Azziz et al., 1995; Ditkoff et al., 1995). In PCOS women, in vitro and in vivo studies have shown that serum 17-hydroxyprogesterone and androstenedione responses to ACTH were significantly greater in the presence of hyperinsulinemia compared to those in the absence of elevated insulin levels (l’Allemand et al., 1996; Lanzone et al., 1992). In addition, it has been demonstrated that in individuals with PCOS, ACTH administration during insulin infusion was associated with a significantly higher 17-hydroxypregnenolone and 17-hydroxyprogesterone responses than those measured during saline infusion (Moghetti et al, 1996; Strauss III & Barbieri, 2014). This facilitatory effect of insulin appeared to result in a relative lowering of 17-20 lyase activity as indicated by higher 17-hydroxypregnenolone to DHEA and 17-hydroxyprogesterone to androstenedione ratios. These findings are consistent with reports that demonstrate a reduction in serum DHEA-S in women during administration of insulin infusion or a glucose tolerance test (Falcone et al., 1990; Strauss III & Barbieri, 2014). However, adrenal hyperandrogenemia is contributory to the pathogenesis of PCOS warrants further investigation.

In summary, PCOS is a multifaceted disorder, affecting a large population of women in their reproductive age. In addition to reproductive anomalies, it is associated with metabolic complications like insulin resistance, obesity, and psychological co-morbidities including anxiety, depression, etc. Though the prevalence and discomfort caused by PCOS is very high, the etiology remains elusive. The characteristics and developmental pathogenesis of PCOS
can be understood by mainly two approaches – *in vitro* and *in vivo* studies. *In vitro* PCOS models may help in evaluation of cellular abnormalities – mainly steroidogenesis. Two well-known cellular models to study steroidogenesis are the ovarian cell line (KGN cell line) and adrenal cell line (NCI-H295R cell line) (Indran et al., 2016). Although these models are useful in examining the regulation of steroidogenesis, and are useful tools for drug development, they are unable to imitate the actual conditions found in PCOS women. In contrast to *in vitro* study, *in vivo* study has potential to offer complete insight about the disease progression. Most of the studies in PCOS have been carried out with women using blood and follicular fluid (Nestler et al., 1991; Sieminska et al., 2004; Das et al., 2008; Sang et al., 2013). As PCOS is a complex endocrinopathy involving dysfunction of multiple organ systems, logistic and ethical limitations on human patients demand the need of animal models for better understanding of pathogenesis.

**Animal models of PCOS**

Till date many animal models of PCOS have been developed using wide variety of species including rhesus monkeys, sheep, rats and mice (Abbot et al., 2006; Walters et al., 2012; Padmanabhan & Veiga-Lopez, 2013; Indran et al., 2016). Despite the many similarities to the human PCOS condition, long development time and high cost of husbandry limits the feasibility of monkeys and sheep models for extensive use in PCOS research. Due to these facts, rodents are the most widely used animals in research on PCOS, with benefits relating to their smaller size, short lifespan and high reproduction index (Singh, 2005; Shi & Vine, 2012).

**Estrogen-induced rodent model of PCOS**

Estradiol valerate (EV) is a long acting estrogen and on administration causes hypothalamic-pituitary dysregulation of GnRH, resulting in improper release of gonadotropins (Shi & Vine 2012). A single dose of EV to the adult female rats is able to generate polycystic ovaries and anovulation within 8 weeks of treatment. EV-induced rats have smaller ovaries with large cystic follicles compared to control animals (Brawer et al., 1986; Shi & Vine, 2012). EV-induced rats demonstrated decrease in serum LH, FSH, testosterone and estradiol concentration when compared to control animals (Brawer et al., 1978, 1986; Shi & Vine, 2012). Although EV treatment results in ovarian dysfunction, it is unable to mimic hormonal and metabolic profile of PCOS condition.
Androgen-induced rodent models of PCOS

Hyperandrogenism is the primary manifestation of PCOS and hence, many of the PCOS models have been developed by exposure of androgens during different developmental time-points.

i) Prenatally androgenised (PNA) PCOS model

Androgen exposure during gestation (d16-19) results into reproductive and metabolic dysfunctions. Prenatal testosterone treatment of female rats causes disturbance in estrus cycle with presence of ovarian cysts. Also, these animals demonstrate elevated LH and testosterone levels with reduced FSH levels (Wu et al., 2010). In addition, prenatal androgen exposure causes hyperinsulinemia and dyslipidemia (Demmissie et al., 2008; Roland et al, 2010). Similar results are also observed in prenatally androgenised mice (Moore et al., 2012). This model helps in understanding the role of in utero androgen exposure in PCOS development.

ii) Dehydroepiandrosterone (DHEA)-induced PCOS model

DHEA treatment to prepubertal (approximately 21 days of age) female rats for 20-27 days produces multiple cysts in the ovary with increased ovarian weight. DHEA-induced rats displayed increased serum DHEA, testosterone, estradiol, FSH and LH concentration (Knudsen & Mahesh, 1975; Lee et al., 1991), whereas other groups did not find any change in FSH and LH concentration between DHEA-induced and control animals (Anderson et al., 1997; T. Endo et al., 2001; Shi & Vine, 2012). DHEA-induced rats demonstrated increased fasting glucose and insulin levels when compared to control animals (Yuxia et al., 2004). Although DHEA is able to induce reproductive and metabolic dysfunctions of PCOS, further studies to confirm gonadotropin levels in this model are needed.

iii) Dihydrotestosterone (DHT)-induced PCOS model

DHT is a nonaromatizable androgen and it has the potential of generating reproductive and metabolic abnormalities of PCOS in rodents. When 21-day old female rats were implanted with continuous-release pellet of DHT for 90 days, they developed polycystic ovaries and irregular estrus cyclicity (Manneras et al., 2007). DHT-induced rats showed no difference in plasma concentrations of testosterone and estradiol. DHT-induced animals exhibited insulin resistance, dyslipidemia and obesity (Manneras et al., 2007; Shi & Vine, 2012; Indran et al., 2016). DHT treatment in rodents is able to induce ovarian characteristic of PCOS with metabolic aberration but it fails to develop hyperandrogenic condition which is a prominent feature in PCOS women.
iv) **Testosterone propionate (TP)-induced PCOS model**

Prepubertal female rats (21-day old) injected with TP for 35 days develop multiple cystic follicles and absence of corpora lutea (Beloosesky et al., 2004). TP-induced rats have increased serum testosterone, LH and prolactin levels whereas estradiol, progesterone and FSH levels were decreased compared to control animals (Ota et al., 1983; Beloosesky et al., 2004). Also, TP-induction resulted into increased serum insulin level, suggesting development of hyperinsulinemia (Beloosesky et al., 2004; Shi & Vine, 2012). Thus, indicating the potential of TP treatment to rats in developing reproductive and metabolic traits of human PCOS condition.

Although steroid induced animal models are able to develop human PCOS like condition, steroids – estogens and androgens – can directly modulate various body functions and thus may not be able to suffice in simulating exact PCOS pathology. Thereby, a better PCOS model could be one that does not utilize steroids for PCOS induction. One such as letrozole.

**Letrozole-induced PCOS model**

Letrozole is a non-steroidal aromatase inhibitor which reduces the conversion of androgens to estrogens. Female rats (6 weeks old) treated orally with letrozole for 21 days demonstrated irregular estrus cyclicity, many ovarian cysts and decreased corpora lutea (Kafali et al., 2004). Hormone profile of these animals exhibited significantly elevated testosterone and LH levels along with reduced estradiol and progesterone levels (Kafali et al., 2004; Shi & Vine, 2012). Moreover, letrozole treatment was able to induce dyslipidemia, hyperinsulinemia and insulin resistance in female rats (Desai et al., 2012; Radha and Laxmipriya, 2016). Thus, letrozole-induced PCOS model possesses reproductive and metabolic characteristics of human PCOS condition, making it a favourable model for use in PCOS research.

**Rationale**

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting 6-10% of reproductive aged women. It is characterised by oligo-/anovulation, hyperandrogenism and polycystic ovarian morphology (Azziz et al., 2016). In addition to reproductive anomalies, PCOS is associated with metabolic aberrations like hyperinsulinemia, dyslipidemia, insulin resistance and obesity and psychological co-morbidities-anxiety, depression, mood disorders (Goodarzi et al., 2011). Although the prevalence of PCOS is very high, very little is known about its clear etiology.
In this line, the literature surveyed has pointed out the role of gonadotropin abnormalities as a key player underpinning this pathology. PCOS women show increased GnRH pulsatility leading to elevated LH/FSH ratio and increased ovarian androgen production, which culminates into follicular arrest followed by cyst formation in ovary. Furthermore, dysfunctional feedback regulation to HPO axis has also been reported in prenatally androgenised (PNA) female mice (Moore et al., 2012). PNA mice demonstrated inhibition of progesterone mediated negative feedback to GnRH release, leading to increased GnRH pulsatility, typical of this disorder. As mentioned earlier, steroid feedback regulation is quite complex and is influenced by several inputs from various regions of the brain which include neuropeptides, neurotransmitters and other factors including stress. Stress response in the body is executed by Hypothalamic-Pituitary-Adrenal (HPA) axis and the components of this axis can directly modulate reproductive function via interacting with GnRH, gonadotropins as well as ovarian steroidogenesis. Corticotropin-releasing hormone (CRH) and cortisol can alter GnRH secretion and function. Further, various theories have been postulated to indicate the role of HPA axis in the pathophysiology of PCOS (Tsilchorozidou et al., 2003; Goodarzi et al., 2015). However, none of these completely explicates the neuroendocrine alterations of PCOS condition.

In addition to classical endocrine regulations, the HPO axis is regulated by higher centers of brain. Neurons from various areas of brain form synapses with GnRH neurons in hypothalamus and they influence GnRH release via several neuropeptides amongst which neurotransmitters are the key players (Bhattarai et al., 2014; Kalil et al., 2016). The involvement of neurotransmitters in PCOS has scarcely been reported. The relative deficiency in dopamine has been proposed to be associated with the increased GnRH secretion in PCOS (Shi et al., 2011). Also, few evidences for altered catecholamine metabolism (Moro et al., 2009) and brain opioid activity were found in PCOS (Eyvazzadeh et al., 2009). Yet, the status of GnRH regulatory neurotransmitter has not been conclusively studied in PCOS.

It is evident that PCOS is the multifactorial disorder affecting various organ systems further resulting into metabolic syndrome. One of the hypotheses indicates that increased oxidative stress and inflammation contribute to pathogenesis of PCOS (Gonzalez, 2012; Zuo et al., 2015). In this regard, studies on PCOS patients have demonstrated increased oxidative stress in serum and follicular fluid (Murri et al., 2013; Piomboni et al., 2014). Also, elevated inflammatory cytokines IL-1, IL-2, IL-6 have been associated with PCOS condition (Gonzalez, 2012; Ebejer & Calleja-Agius, 2013). However, the redox status as well as the
condition of inflammatory markers in the GnRH regulatory brain regions has not been studied in PCOS pathology.

With reference to the above understanding, regulation of GnRH release is very important for proper reproductive functions. Moreover, the key molecule GnRH is influenced by several factors including steroids, neurotransmitters, stress and therefore, any alteration in them may precipitate into several metabolic and psychological complications in PCOS pathology. However, crosstalk between a range of intra- and extra-ovarian factors such as neurosteroids, neurotransmitter, stress and their effect on GnRH pulse generation in PCOS has not been studied extensively. Consequently, the overall aim of the present study was to understand the status of various regulatory molecules of reproductive axis to decipher the neuroendocrine pathology in PCOS, using rodent model. The major objectives of the present study were:

**Objectives:**

1. Assessment of Hypothalamic-Pituitary-Ovarian axis in PCOS rat model

2. Evaluation of GnRH regulatory molecules in PCOS rat model
   a. Assessment of Hypothalamic-pituitary-adrenal axis in PCOS rat model
   b. Evaluation of Neurotransmitter status in PCOS rat model

3. To study the oxidative stress and inflammatory markers in PCOS rat model