Chapter 1

Introduction and Review of Literature

1. Female Reproductive System

Pregnancy is a complex, heterogeneous, biological phenomenon in which the embryo develops into a fetus. This important biological function is possible with the help of the principal organs of the female reproductive system, the most important of which are the ovaries, fallopian (uterine) tubes, uterus, and vagina as shown in Figure 1.1. *A* single ovum is expelled from an ovarian follicle into the abdominal cavity near the open fimbriated ends of the two fallopian tubes. The ovum then passes through one of the fallopian tubes into the uterus. If it is fertilized by a sperm, it implants in the uterus, where it develops into a fetus and eventually into a neonate (Hall 2010; Trounson et al. 2013).

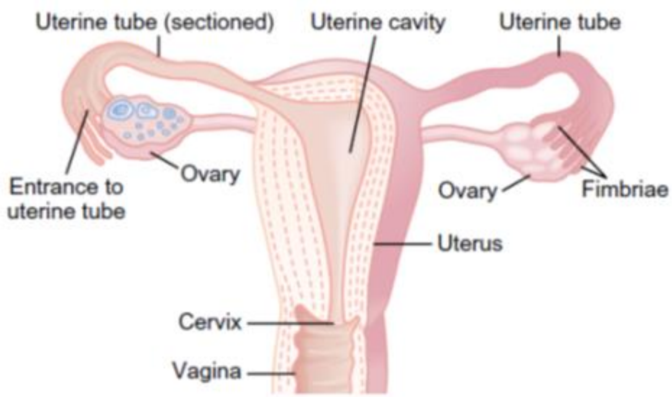


Figure 1. 1: Female reproductive organs (Hall 2010)

1.1 Ovary

The key to fertility in a female is a fully functional ovary. It produces the mature ovum and secretes reproductive hormones - progesterone, androgens, and estrogens under the influence of gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The morphology and function of ovary changes in a cyclic manner under the influence of these reproductive hormones. The two main developmental events within the ovary are follicular assembly (i.e., the formation of primordial follicles) and the primordial-to-primary follicle transition (the “initial” recruitment). The initial phase of folliculogenesis is regulated by paracrine growth factors, local concentration of steroid hormones and gonadotropins. In the developing fetus or neonate there is formation of a pool of primordial follicles (i.e ovarian follicular reserve), which when established, do not proliferate. This ovarian reserve comprises the source of female gametes for life amongst which few grow further, while others remain quiescent for months, years or decades depending upon the species (Hall 2010).

1.2 Folliculogenesis

The follicle is the functional unit of the ovary. Each follicle is comprised of mainly three somatic and one germ cell types.

Granulosa cell: It is a significant somatic cell of the ovary and is involved in the production of sex steroid hormones and a milieu of growth factors for interaction with oocyte development. Cellular growth in ovary is controlled by apoptosis of granulosa cells, a phenomenon responsible for follicular atresia (Belani et al. 2014). In granulosa cells glucose uptake and metabolism play an important part in making energy substrate available to the maturing oocyte (Rice et al. 2005). These functions make it an investigative *in vitro* reproductive cellular model to determine possible effects of several insults and other pathological situations (Beckman et al. 1991; Breckwoldt et al. 1996; Chaffkin et al. 1993; Harvey & Everett 2003; Quinn et al. 2006; Rodgers et al. 1996).

Theca cell: Once a follicle has two or more layers of granulosa cells, theca cells are formed. Theca cells are vital components of the follicle that provide structural support and are exclusive producer of ovarian androgens, which are necessary as substrates for estradiol production in the neighboring granulosa cells. During development, theca cells are the final follicular cell type to dye in those follicles that undergo atresia. In the follicles that ovulate, the theca cells undergo differentiation into luteinized theca cells of the corpus luteum (Young 2010).

Stromal cells: The stroma is abundantly supplied with blood vessels, consists of spindle-shaped cells with a small amount of ordinary connective tissue. The stroma is associated with maturing follicles and it may acquire endocrine function and secrete estrogens (Hall 2010).

Oocyte: This germ cell is surrounded by granulosa and theca cell and is the basic functional unit of the ovary. It is a haploid gamete that after fertilization forms a diploid zygote.

Thus a follicle is comprised of an outer layer of theca cells, an inner mass of granulosa cells and the oocyte, which is found in the central part of this complex structure. During embryonic development around the sixth month of gestation in humans and immediately after birth in rodents, developmentally arrested germ cells or oocytes are surrounded by a single layer of pregranulosa cells. This initial structure is known as primordial follicle. Transition from primordial to primary follicle is relatively at a constant rate throughout fetal, juvenile, prepubertal, and adult life. Once primary follicles leave the resting pool, they are committed to further development or atresia. Most become atretic, and typically only one fully developed follicle will ovulate (Figure 1.2). The development of follicles is regulated through the hypothalamic – pituitary – ovarian axis and intraovarian regulators, such as growth factors, cytokines and gonadal steroids (J.K. Findlay 2009; Baerwald 2012; Yang 2013). The decapeptide gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus is a key regulator of sexual maturation and reproductive functions. It stimulates the synthesis and release of gonadotropins, follicular stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, via specific GnRH receptor located on gonadotrope cells in a pulsatile manner (Singh 2010) (Figure 1.3). Initial effect of FSH leads to rapid proliferation of the granulosa cells giving rise to secondary stage of follicle. In the secondary follicle, the stromal cells, near the basement membrane, begin to differentiate into cell layers called theca interna and theca externa. The granulosa cells of secondary follicles acquire receptors for FSH and start producing small amounts of estrogen. The follicle continues to grow, theca layers expand, and fluid filled places or antra begin to develop around the granulosa cells. This early antral stage of follicle development is referred to as the tertiary follicular stage. As the development proceeds, pituitary FSH and the estrogens combine to promote LH receptors on the original granulosa cells, thus allowing LH stimulation to occur in addition to FSH stimulation. The follicle largens to become graafian follicle (preovulatory follicle) having a single, large, coalesced antra and the oocyte at the periphery of the follicle (Figure 1.4). Before ovulation occurs—one of the follicles begins to outgrow all the others; the remaining developing follicles involute (a process called atresia), and these follicles are said to become atretic. LH causes rapid secretion of follicular steroid hormones that contain progesterone and ultimately leading to expulsion of the ovum from the follicle, also known as ovulation. (Sahmi et al. 2004). After ovulation, the remaining granulosa and theca interna cells change rapidly into lutein cells, a process known as luteinization and the mass of cells known as corpus luteum (Figure 1.2). Thus, the ovarian processes of follicular maturation, ovulation, and formation of the corpus luteum and synthesis of the sexual hormones are under the control of the pituitary gonadotropins. Only the beginning of the first stage of follicular growth does not depend on the gonadotropic stimulation, being a process which the ovary, all by itself, sets in motion. From this stage on, in order to proceed their development up to the stage of mature follicle and produce good estrogen levels, the ovarian follicles depend on the FSH and LH stimulation. On the other hand, ovulation and formation of the corpus luteum depend on the LH ovulatory peak.

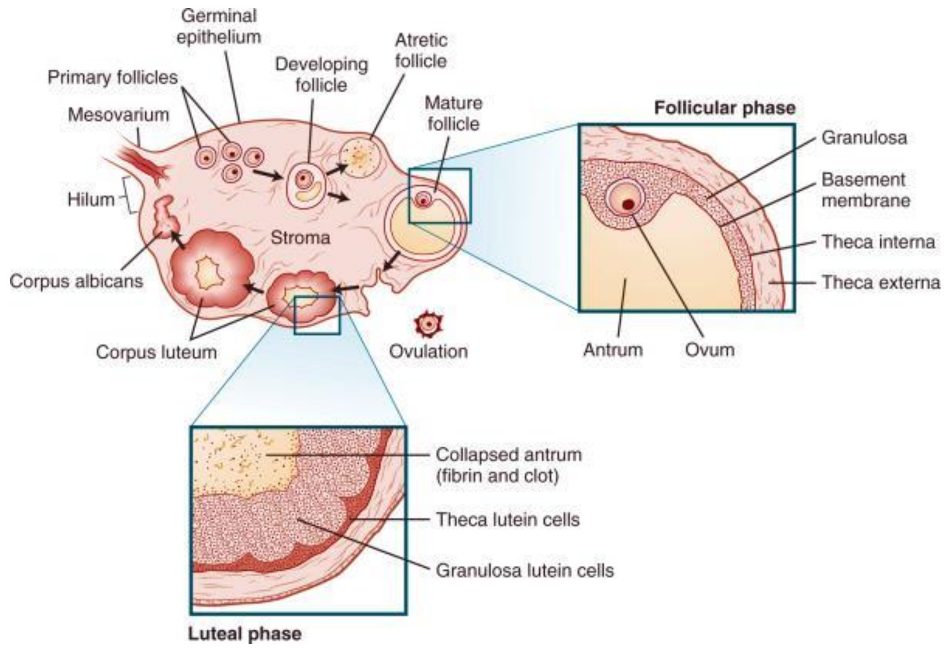
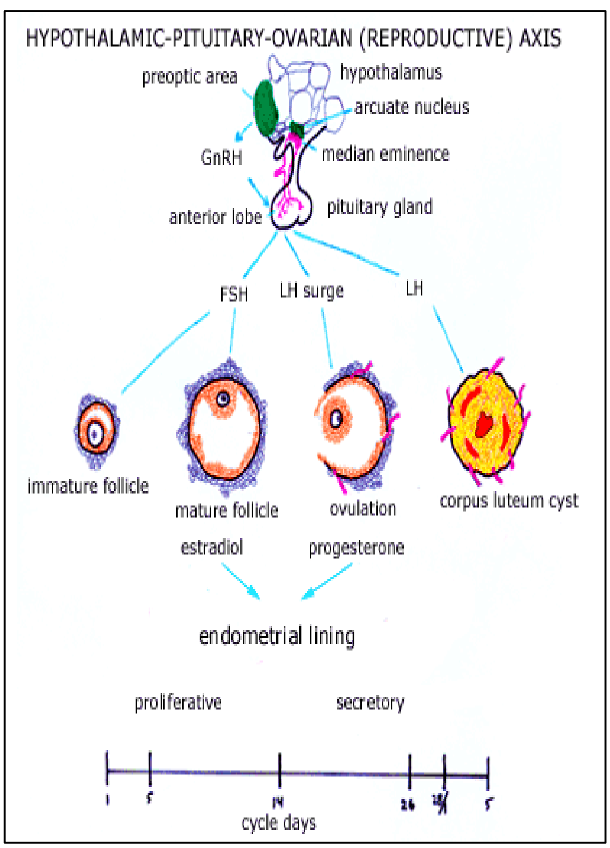


Figure 1. 2: Functional anatomy and developmental changes in adult ovary during an ovarian cycle. (Freeman 2001).



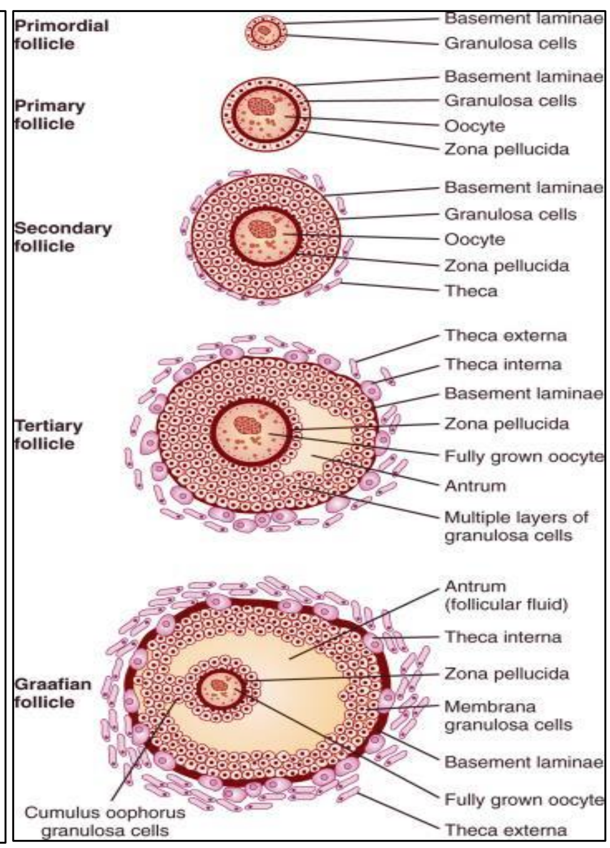


Figure 1. 3: Hypothalamus-pituitary ovarian axis (Erickson et al. 1985)

Figure 1. 4: Developmental stages of ovarian follicle. (Erickson et al. 1985)

1.3 Follicular steroidogenesis

Steroidogenesis, the process of steroid hormone production, depends on the availability of cholesterol. Cholesterol can be derived from three main sources:1) Preformed cholesterol circulating in blood in form of lipoproteins 2) cholesterol synthesized de novo within the ovary 3) cholesterol liberated from cholesterol esters stored within the lipid droplets.

The main physiologically active steroid produced by the follicles is estrogen. Granulosa cells and theca cells take part in steroidogenesis and because of following unique physiological properties neither of them can make estrogen by themselves.

1. The superficial theca cells can take up cholesterol and produce androgens but lack aromatase necessary for estrogen production. The deeper granulosa cells have aromatase but lack 17 α hydroxylase necessary for making androgens.
2. Theca cells are near blood vessels and hence are a source of LDL cholesterol. The granulosa cells, conversely being far from blood vessels are surrounded by LDL poor follicular fluid.
3. Theca cells have LH receptors and granulosa cells have both FSH and LH receptors.

LH binds to its receptors in theca cells, stimulates activity of adenylate cyclase leading to release of cyclic AMP, that serves as secondary messenger to stimulate an increase in mRNA for LDL receptor, thereby increasing binding and uptake of LDL-cholesterol and cholesterol esters (Miller and Strauss 1999). This Cholesterol is transported from the outer to the inner mitochondrial membrane using a unique protein called **steroidogenic acute regulatory protein** (StAR). StAR is a 30 Kd mitochondrial protein, which is believed to be key mediator of acute induction of steroidogenesis (Clark et al. 1995). The promoter region of StAR has a nuclear receptor protein steroidogenesis factor – 1(SF-1) which is a cis acting cognate response element and is responsible for several fold increase in the expression of the gene**.** Cholesterol is then converted to pregnenolone by cholesterol side chain cleavage enzyme (CYP11A1), a key enzyme and this step is a rate-limiting step in steroidogenesis. The sequential expression of CYP11A1 in the different follicular cells results from a gradient of gonadotropin responsiveness in the follicle.

Pregnenolone, then diffuses out of the mitochondria and enters the ER, the site of subsequent steroidogenesis. Further, steroidogenesis occurs by delta 5 pathway in theca and delta 4 pathway in granulosa cells and corpus luteum. Pregnenolone gets converted to either **progesterone by** 3β-hydroxysteroid dehydrogenase (3β-HSD) in the delta 4 pathway or to **17α-**

hydroxypregnenolone by 17α-hydroxylase in the delta 5 pathway. The primary site of 3β-HSD expression is in the thecal layer of the follicle. The expression in the granulosa layer is dependent upon the stage of folliculogenesis and species (Simard et al. 2005; Rasmussen et al. 2013). 3β-HSD expression has been localized to theca interna of the preantral, antral and atretic follicles as well as corpus luteum. However, some layers of theca interna next to the basement membrane fail to show expression of 3β-HSD. 3β-HSD mRNA and protein expression is also observed in the developing granulosa cells of the preantral and antral follicles during folliculogenesis. The subcellular localization of 3β-HSD is found in smooth endoplasmic reticulum and mitochondria (Simard et al. 2005). Further in delta 5 pathway 17α-hydroxypregnenolone gets converted to **dehydroepiandrosterone** by 17, 20-lyase which is subsequently converted to **androstenedione** by 3β-HSD. The progesterone in the delta 4 pathway gets converted to **17α-hydroxyprogesterone** by 17α-hydroxylase, which is subsequently converted to **androstenedione** and **testosterone** by 17, 20-lyase and 17β-hydroxysteroid dehydrogenase respectively. There are multiple 17β -HSD isozymes with androgen or estrogen specificity (Martel et al. 1992; Luu-The et al. 1995; Peltoketo et al. 1999). In humans, the presence of 17 beta HSD type I, II, and IV, members of the short-chain dehydrogenase/reductase superfamily, is limited to granulosa cells. Type V 17 beta HSD, a member of the aldo-keto reductase (AKR) superfamily is localized in theca and corpus luteum cells, another Type III 17 beta HSD, the testicular isoform, is not detected in either granulosa or theca cells. The immunoreactive type I 17 beta-HSD is localized in granulosa cells of developing follicles, ranging from primary, preantral and large antral follicles and syncytiotrophoblast in placenta (Sawetawan et al. 1994). In rodents, the expression of 17β –HSD type I is low in antral follicles, upregulated during maturation, highest in graffian follicles, decreases during luteinisation and is almost undetectable in corpus luteum (Ghersevich et al. 1994). 17β –HSD type II localized in corpus luteum converts estradiol to estrone. Testosterone and androstenedione in the delta 4 pathway diffuse across the basement membrane from the thecal compartment and enter the granulosa cells where they get converted in to estradiol and estrone respectively by **aromatase** (CYP19A1)**.** This enzymatic reaction is under the influence of FSH, with cAMP as a second messenger. CYP19A1 is widely expressed in many tissues besides gonads, e.g. adipocytes, breasts, central nervous system, skin and placenta and is highly concentrated in corpus luteum (Suikkari et al. 2000). mRNA for

CYP19A1 is localized in the granulosa cells and increases markedly in estrogen biosynthesis before ovulation. Figure 1.5

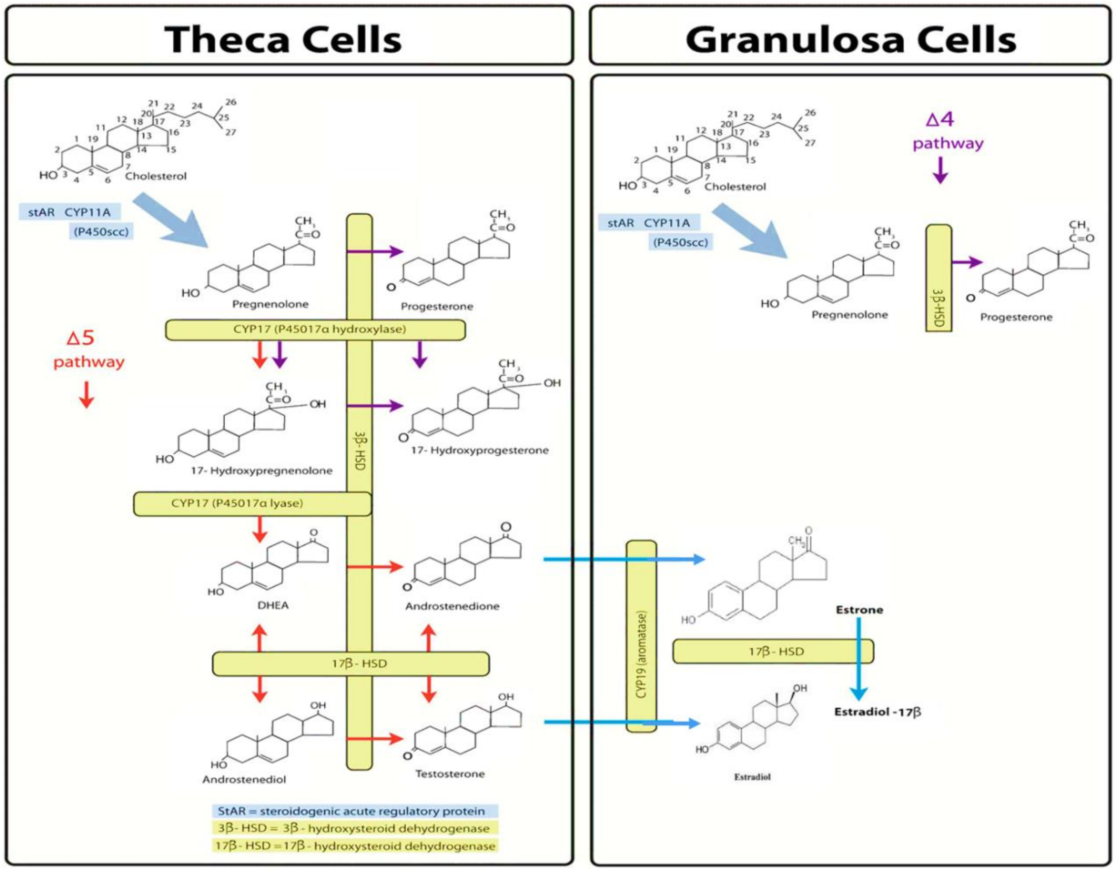


Figure 1. 5: Steroidogenic pathway in the ovary, modified from. (Leão and Esteves 2014)

1.4 Two cell two gonadotropin theory

Estradiol secretion by the follicle thus requires cooperation between granulosa and theca cells and coordination between FSH and LH giving rise to two cell-two gonadotropin theory. Main function of the LH is to encourage the production of androstenedione and testosterone – the androgens in the theca cells during the mid-late follicular phase which are then 'passed on' to the granulosa cells for conversion to estradiol. Apart from this, the finding that during the midcycle surge, as the FSH functions to express LH receptors on the granulosa cell membranes of developing follicles of >10mm diameter, then expressed LH receptors render LH capable of inducing estradiol production has been given a strong recognition in this theory (Will et al. 2001). The enzyme CYP19A1 converts the androgens passed on to granulosa cells from theca cells into estrogens, such as estrone and mainly estradiol which is the most potent form of estrogen in female reproduction. Estradiol is further metabolized into 2-hydroxyestradiol by CYP1A1/2 and CYP3A4, or to 4-hydroxyestradiol by CYP1B1 that leads to its modification thus inactivating it further damaging the cell. These modifications lead to inactivation of estradiol and further modifications are thought to lead to cell damage (Sargeant et al. 2001) (Fig. 1.6).

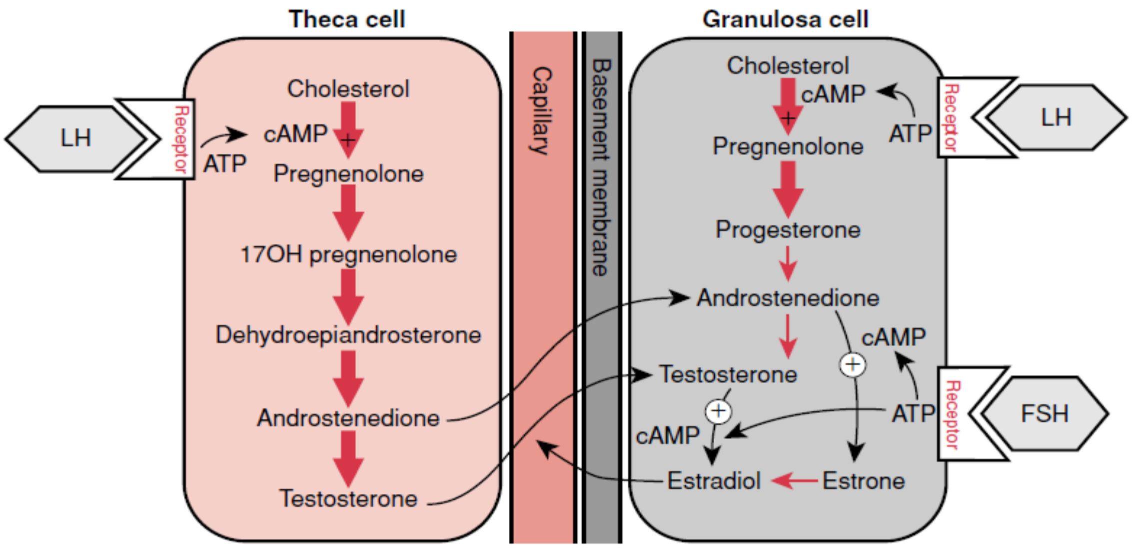


Figure 1. 6: The two cell two gonadotropin theory of ovarian steroidogenesis (Will et al. 2001; Boron and Boulpaep 2012).

1.5 Follicular Atresia

A minute fraction of ovarian follicles present in a fetal ovary complete their path to ovulation, whereas rest majority of the follicles undergo atresia, a hormonally controlled apoptotic process. Atresia has been shown to be mediated by a highly organized type of cell death called apoptosis or programmed cell death (Hsueh et al. 1994; Billig et al. 1996; Kaipia A 1997).

1.5.1 Apoptosis

In all living organisms a harmonious balance between the synthesis of new cells and the destruction of existing cells under normal conditions is maintained. The complex events that encompass these take place in response to specific and controlled signals of a physiological process known as apoptosis. The term apoptosis was coined in 1972 by Currie and colleagues to describe a common type of cell death in various tissues and cell types (Kerr et al. 1972). Apoptosis is characterized by membrane blebbing, cell shrinkage, protein fragmentation, chromatin condensation and DNA fragmentation, finally ending in the engulfment of neighboring cells, thereby avoiding an inflammatory response in surrounding tissues. The apoptotic machinery within a cell is triggered by a wide array of external signals such as irradiation-induced DNA damage, hormone addition or withdrawal and stimulation of certain "death receptors"(Billig et al. 1993; Billig H 1994; Kaipia A 1996; Hengartner 2000; Savill and Fadok 2000b).

1.5.2 Mechanisms of apoptosis

Apoptosis occurs via an extrinsic or death receptor pathway or intrinsic (mitochondrial) pathway. Both the pathways are linked and the molecules in one pathway can influence the other (Igney and Krammer 2002). An additional pathway involving T-cell mediated cytotoxicity and perforin-granzyme dependent killing can also lead to apoptosis but either via granzyme B or granzyme A. The extrinsic, intrinsic, and granzyme B pathways converge and lead to execution pathway which is initiated by the cleavage of caspase-3. The granzyme A pathway leads to caspase-independent cell death pathway via single stranded DNA damage (Martinvalet et al. 2005).

Caspase dependent pathway

Pathogens, RNA or DNA, proteins or peptides, some chemical compounds or native compounds can trigger caspase dependent apoptosis, followed by activation of Bcl2 protein. The Bcl2 family protein activation induces the mitochondria membrane change and stimulates the cytochrome c release. Some UV radiation or X rays can also depolarize mitochondria and permeabilize its membrane, leading to increase in ROS followed by cytochrome c release. Release of cytochrome c then triggers caspase-9, further activating caspase-3 ultimately leading to apoptosis (Hongmei 2012).

Caspase independent pathway

Apoptosis inducing factor (AIF) is the major caspase-independent pro-apoptosis factor that is released from the mitochondria and translocated to the cleaved DNA in the nucleus further leading to apoptosis. Compounds such as simvastatin, staurosporine and cadmium accompany with AIF production and induce cell death. Lot of ligands in a cell induce change in mitochondrial membrane potential leading to increase in ROS production followed by caspase independent apoptosis. ROS mediates poly (ADP-ribose) polymerase-1 (PARP-1) activation, which is necessary for AIF release from mitochondria. Other than AIF and ROS, Ca2+, NAD+ and ATP, protein misfolding and modification, can also trigger the caspase independent apoptosis.

Termination of apoptosis pathway

At the end cells round up and detach from the surrounding cells, there is membrane blebbing, packaging of cell content into apoptotic bodies and phagocytosis (Falcieri et al. 1994; Savill and

Fadok 2000a). In an apoptotic cell phosphatidylserine, which is normally present on the cytoplasmic side of the cell membrane, flips over to the external face of the cell membrane. This event triggers the phagocytosis reaction (Brunk and Svensson 1999; Savill and Fadok 2000b). (Figure 1.7)

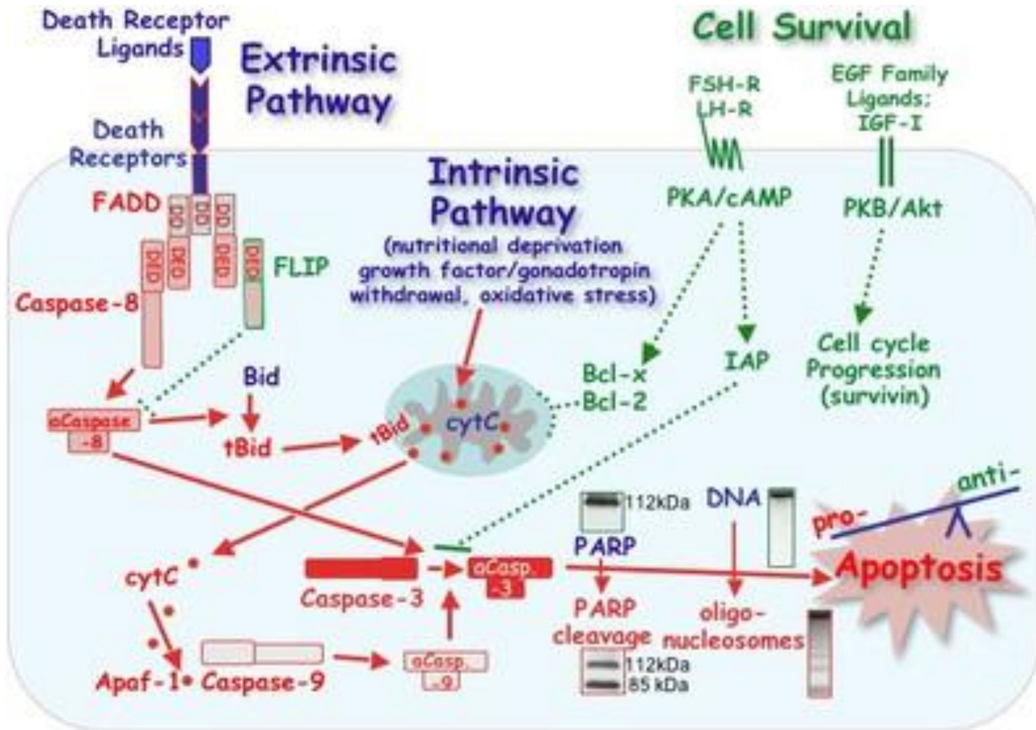


Figure 1. 7: The apoptotic pathway in a cell

1.5.3 Regulation of apoptosis in the ovary

During the course of follicle development, there is a change in the susceptibility to apoptosis as well as the regulators of follicle survival. Because of this there is a gradual reduction in the number of follicles as they differentiate and grow towards ovulation ultimately ensuring the release of only the healthiest and most viable oocytes (Markstrom et al. 2002). All the stages of follicular development can undergo apoptosis, however, the early antral follicles being the most susceptible to the atreatogenic signals are more prone to it in contrast to the pre antral and the smallest antral follicles where minimal atresia takes place (Orisaka et al. 2009). Gonadotropins, growth factors, cytokines and steroid hormone themselves have been demonstrated to regulate follicular growth. Death receptor ligands, such as TNF and FASL, interleukin 6, gonadotropin releasing hormone 1, peroxisome proliferator activated receptor-γ, clusterin, prohibitin have been observed to be the atretogenic factors leading to atresia in the ovary (Friberg 2009; Gerardo H. Vázquez-Nin 2011).

1.6 Synchrony amongst Ovary, Brain and Pituitary

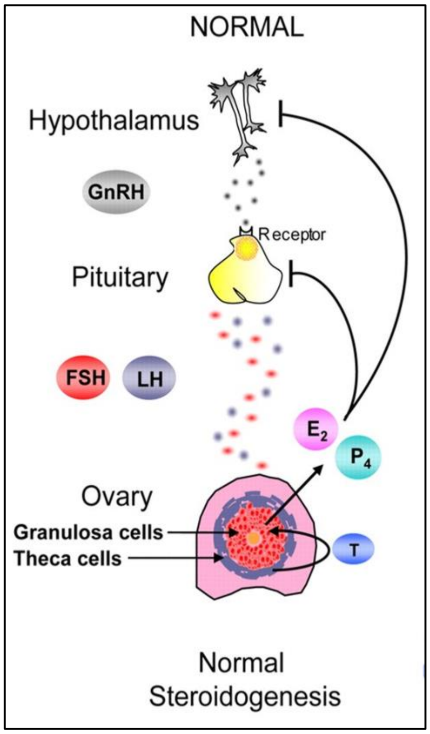


Figure 1. 8: The negative feedback along the HPO axis ensures that steroidogenic signaling is tightly controlled. (Restuccia et al. 2012)

There is an intricate functional interaction between the ovaries and the hypothalamus-pituitary system, by means of feedback mechanisms. The hypothalamus-pituitary system controls the ovarian function and vice versa. FSH and LH are the pituitary gonadotropins that control estrogen and progesterone production from the ovaries. Release of these gonadotropins by the pituitary is controlled by the hypothalamus by means of GnRH. Lower levels of oestradiol during the follicular phase provide negative feedback on the hypothalamus that leads to reduction of the amplitude of GnRH pulses. As the follicular phase progresses, a surge of GnRH release is initiated, that in combination with positive feedback oestradiol action at the pituitary and withdrawal of progesterone negative feedback of the luteal phase activates the luteinizing hormone (LH) surge and thus triggers ovulation. As a result of this switch, the pattern of GnRH release is altered from one that is strictly episodic, with little secretion in between pulses, to one that produces a continuous elevation of GnRH levels in the pituitary portal blood that lasts for many hours (Moenter et al. 2009). This results in a change in the pattern of GnRH release from the strictly episodic one to continuous elevation in the pituitary portal blood that lasts for several hours. A negative feedback action is characterized by an inhibiting effect upon some of hypothalamic neural centers while a positive feedback action produces a stimulation of other hypothalamic centers. Thus the levels of estrogen production throughout the ovarian cycle indirectly control the pituitary release of FSH and LH. On the other hand, the ovarian estrogen production depends on the FSH and LH stimulation. Progesterone is produced by the corpus luteum which is formed by the granulosa-lutein cells and the theca-lutein ones (Figure 1.8). In spite of corpus luteum's intrinsic capacity of self-regulation, it also depends on normal LH levels for its maintenance. This negative feedback mechanism of the hypothalamus-pituitary ovarian system behaves as has been utilized in medicinal concept of the hormonal contraceptive pills (estrogens and progestin's) resulting in the blockage of most of the ovarian function resulting in the suppression of ovulation. As a consequence, the growth of the ovarian follicles is interrupted in their earlier stages of development not allowing any of the follicle to reach the mature stage and thus suppressing ovulation (Messinis 2006).

1.7 Factors involved in follicular growth

Other than the steroidogenic hormones, systemic hormones play a vital role in healthy folliculogenesis by influencing gonadotropin action in the ovary which include thyroxin, growth hormone, leptin and most notably insulin and insulin like growth factors(IGF's) (Goodarzi 2011; Magon 2011). Aberrant levels of these hormones in circulation may lead to ovarian dysfunction. In this study, we will focus in particular on the role of insulin and IGF's in ovarian function.

1.7.1 Insulin and Insulin receptor (INSR):

Insulin is a peptide hormone secreted by the endocrine pancreas. It is a key hormone regulating glucose and lipid homeostasis. Insulin, under normal physiological conditions, is secreted in response to a meal. It promotes the uptake and storage of nutrients, with skeletal muscle being a major site for insulin-stimulated glucose disposal amongst fat and liver (Nandi and Poretsky 2013). Other than the metabolic pathways, insulin also regulates cell proliferation and reproduction. Insulin reaches to the follicular fluid in the ovary from the circulation by transudation. The insulin concentrations in ovarian follicular fluid (FF) range from less than 2 mU/ml to 65 mU/ml, with a mean value of approximately 16 mU/ml (Poretsky et al. 1999).

The INSR is a heterotetramer consisting of two α - (135 kDa molecular mass) and two β- (95 kDa molecular mass) subunits and the mature $\alpha 2\beta 2$ heterotetrameric form of the receptor results from dimerization. INSR α -subunits possess cysteine-rich domains that serve as insulin-binding sites and insulin receptor β -subunits have ATP-binding site and several tyrosine autophosphorylation sites. In humans and animal models, INSR are observed to be distributed throughout all ovarian compartments including granulosa, theca and stromal tissues (Poretsky et al. 1999). In primordial follicles, INSR expression is restricted only to oocyte with very moderate expression on stromal cells. It's only during the preantral follicular stage that the INSR expression becomes apparent in granulosa and theca cells. As the size of follicle increases, its density increases in oocyte, granulosa and theca cells and decreases in the stromal cells surrounding the growing follicles as compared to that of surrounding primordial follicles. Their expression is most pronounced in granulosa and theca cells of the antral follicles during mid and late follicular phase. These changes in expression of INSR in the course of follicular development is consistent with that of the steroidogenic ability which is weak during preantral stage and increases as follicle grows and matures indicating probable role of insulin in steroidogenesis. During the early luteal phase the expression of INSR rises in the luteal cells and decreases in the stromal cells surrounding the corpus luteum as compared to the late luteal phase where INSR expression is remarkably declined and becomes restricted only to the peripheral theca luteal cells. Lastly in atretic follicles, INSR expression is prominent in theca interna cells as compared to stromal cells where it is moderate and the degenerating granulosa cells where it is negligible (Samoto et al. 1993).

1.7.2 Insulin like growth factor system

The IGF’s and IGF-R’s are cycle dependent, highly compartmentalized and involved in folliculogenesis in the ovary.

1.7.2. a IGF-1

IGF-I is a single-chain polypeptides that share 70% homology with IGF-II. The major source of IGF-I is liver although it is widely expressed in most tissues, especially during postnatal development. IGF-1 is barely detectable in the human ovary and absent in granulosa cells during all stages of follicular development and its presence in theca compartment is controversial. Presence of IGF-I in FF is a result of transudation from serum. IGF-1 stimulates DNA synthesis and synergizes with gonadotropins to augment steroidogenesis in granulosa and granulosa-luteal cells (Spicer and Aad 2007). IGF-I is expressed in granulosa cells of antral and pre ovulatory follicles in rat ovary. Studies have revealed that the null mutants for IGF-1 have restricted growth in utero , have impaired ovarian steroidogenesis and are infertile (Poretsky et al. 1999).

1.7.2. b IGF-II

IGF-II is highly expressed in normal adult tissues, fetal tissues and tumors. The expression of IGF-II increases during puberty by the action of gonadotropins on granulosa cells (Livingstone 2014). It is expressed abundantly in preovulatory granulosa cells, granulosa luteal cells and corpus luteum. IGF-II functions to coordinate angiogenic processes and vessel maintenance and its loss during luteolysis affects primarily the vascular system (Silva et al. 2009). IGF-II levels in FF are higher in estrogen dominant follicle as compared to androgen dominant follicle and correlate with follicle size and cycle day indicating that IGF-II levels derive primarily from local production in granulosa cell, perhaps some from theca cell and with some additional contribution from the circulation. IGF-II is more important than IGF-I in ovary because conception and follicular development can occur in deficiency of circulating IGF-I and it stimulates embryonic growth more potently than IGF-I (Rosenfeld and Roberts 1999; Livingstone and Borai 2014).

1.7.2.c IGF-1R

IGF-1R protein is a heterotetramer consisting of two α and two β-subunits and is highly homologues to INSR. IGF-1R is found in the granulosa cells from the primary follicle to the large antral follicle with its abundance in graafian and atretic follicles (Wang and Chard 1999; Silva et al. 2009). Apart from IGF-1, insulin and IGF-II also bind to IGF-1R but with less affinity. IGF-I receptor knockout mice weigh 45% of normal at birth and die immediately afterward. Patients with mutations in IGF-I receptor gene, exhibit both intrauterine and postnatal growth restriction.

1.7.2.d IGF-IIR

IGF-IIR is a single-chain polypeptide of approximately 300kDa with a large extracellular domain containing IGF-II binding sites. IGF2R mRNA levels are greater in granulosa cells of small than in large follicles, whereas theca cell IGF2R mRNA levels are greater in large than in small follicles. IGF -IIR knockout mice die in utero whereas knockout of IGF-II and IGF-IIR at the same time lead 50% of fetuses to survive till birth (Poretsky et al. 1999). IGF-IIR is associated with decreased granulosa cell responses by acting as a type of decoy receptor for IGF-II (Juengel et al. 2010) (Fig. 1.9).

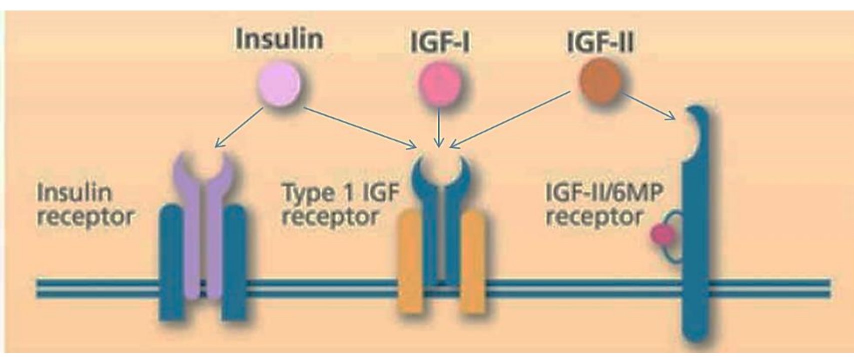


Figure 1. 9: Structure of insulin receptor, type I IGF receptor and type II IGF receptor (Livingstone 2014).

1.8 Insulin and IGF Signaling mechanism

Insulin and IGF's control metabolism, growth and survival in a wide range of mammalian tissues (Siddle 2011). In spite of their distinct physiological roles, they utilize the same signalling pathways. Their signaling begins when they bind to α-subunit and induce autophosphorylation of the receptor β-subunit on specific tyrosine residues, resulting in tyrosine phosphorylation of several substrates, including the insulin receptor substrate (IRS) 1-4, src homolog, collagen homolog (Shc) and adapter protein with a PH and homology 2 (SH2) domain (APS) (Pessin and Saltiel 2000). Phosphorylated IRS proteins act as docking sites for the SH2 domain of phosphatidylinositol 3-kinase (PI3-K) or the adaptor molecule, Nck which mediates different downstream signaling pathways. Activation of PI3-K phosphorylates membrane phospholipids and phosphatidylinositol 4, 5-bisphosphate, leads to activation of the 3-phosphoinositide-dependent protein kinases (PDK-1 and PDK-2). These kinases activate serine/threonine kinases Akt/protein kinase B (PKB), atypical protein kinase C λ and ζ (PKCλ/ζ), mammalian target of rapamycin (mTOR) and sterol regulatory element–binding protein (SREBP)-1c. The serine/threonine kinase Akt/ PKB transmits the signal by phosphorylation of its 160-kDa substrate, AS160 that is crucial for GLUT4 translocation, glycogenesis and protein synthesis. Akt/PKB also results in the serine phosphorylation and inactivation of GSK3, thus allowing glycogen synthase activity to increase, further resulting in glycogen synthesis. It also activates transcription factors such as FOXO, (SREBP)-1c and BAD responsible for regulating expression of gluconeogenesis, monounsaturated fatty acid synthesis and apoptosis respectively. In addition to PI3k activation, mitogen activated protein kinase (MAPK) is also activated by insulin/IGF receptor-mediated phosphorylation of Shc or IRS, leading to association with Grb2 and Son-of-sevenless (sos) resulting in Ras activation. This activation leads to a cascade of serine/threonine kinase resulting in the stepwise stimulation of Raf, MAPK kinase (MEK) and MAPK-ERK1/2 where ERK1/2 translocates to the nucleus and phosphorylates transcription factors to initiate cell growth and differentiation. The mitogenic pathway can be disrupted without affecting the metabolic actions of insulin and vice versa (Venkatesan et al. 2001). As mentioned, the signaling pathways result in a plethora of metabolic effects, including stimulation of DNA and protein synthesis, lipogenesis, transmembrane electrolyte transport, and effects on carbohydrate metabolism, most important being glucose uptake by GLUT-4 as shown in figure 1.10 (Siddle 2011; Diamanti-Kandarakis 2012). Further dephosphorylation of proximal signaling molecules, such as INSR by protein tyrosine phosphatase 1B (PTP1B) and lipid signaling molecules by phosphatase such as tension homolog deleted on chromosome 10 (PTEN), a lipid phosphatase, decrease PI3-K signaling. Other proteins, such as suppressor of cytokine signaling-1 (SOCS1) and SOCS3 , growth-factor-receptor bound protein 10 (Grb10) and plasma-cell-membrane glycoprotein-1 (PC1) down regulate IR function by stearically blocking its interaction with the IRS proteins, or by modifying its kinase activity (Elchebly et al. 1999; Ueki et al. 2004; Diamanti-Kandarakis 2012) (Fig. 1.10).

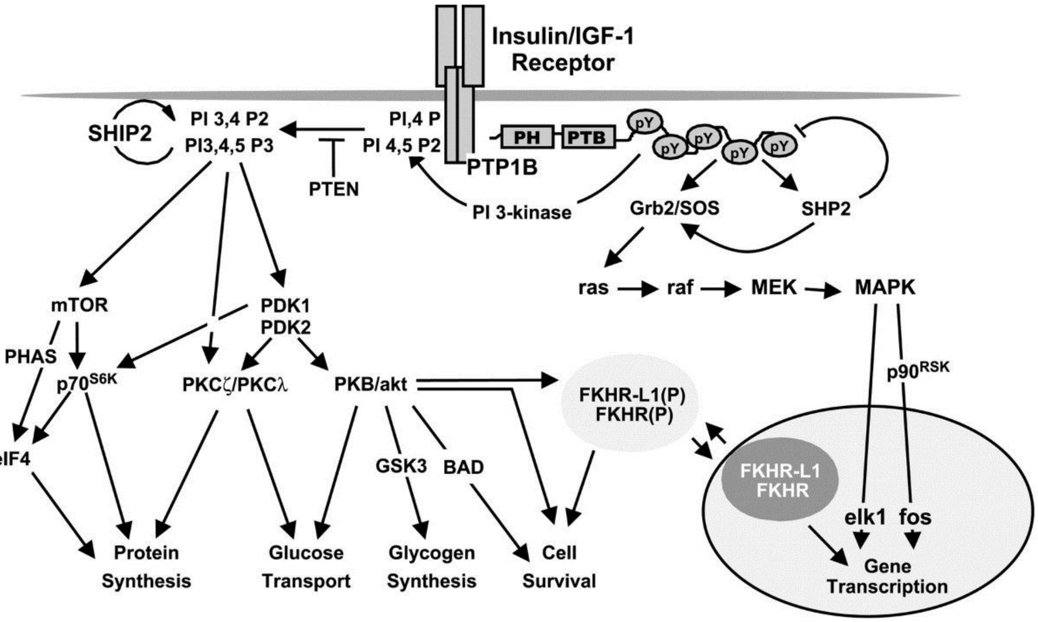


Figure 1. 10: Insulin and IGF signalling pathways. (White 2002)

1.9 Signaling in Granulosa cell

The presence of INSR and IGF-R's in granulosa cell, implicate their significance. Insulin alone or with FSH and LH can augment StAR mRNA and protein levels, CYP19A1 activity and 3 β-HSD expression ultimately stimulating estradiol (E_2) and progesterone (P_4) production in granulosa cells via cAMP, PKA, PI3K and MAPK pathways. The synergistic action of LH and insulin lead to increased expression of CYP11A1 and LDL-R. IGFs can also synergize with gonadotropins in granulosa and theca cells for inducing expression of mRNA transcripts for receptor of low-density lipoprotein (LDL-R), StAR and CYP11A1. IGFs increase the levels of gonadotropin receptors thereby helping in up regulation of genes such as hyaluronan synthases (HA), proteoglycans, inter-α-trypsin inhibitor (IαI), TNF-stimulated gene 6 (TSG-6), permtraxin-3, and cartilage link protein (Crtl1) important for the cumulus oocyte complex (COC) synthesis. (Wood and Strauss 2002). As for insulin, IGF's augment DNA synthesis, steroidogenesis and in synergy with gonadotropins, increase E_2 and P production (Poretsky et al. 1999; Wood and Strauss 2002; Rojas 2014). IGF-I and IGF-II increase CYP19A1 activity and mRNA abundance in human and rat granulosa cells. IGF-I synergizes with FSH to enhance CYP11A1, 3β-HSD and LH-R expression. (Wood and Strauss 2002) (Fig. 1.11).

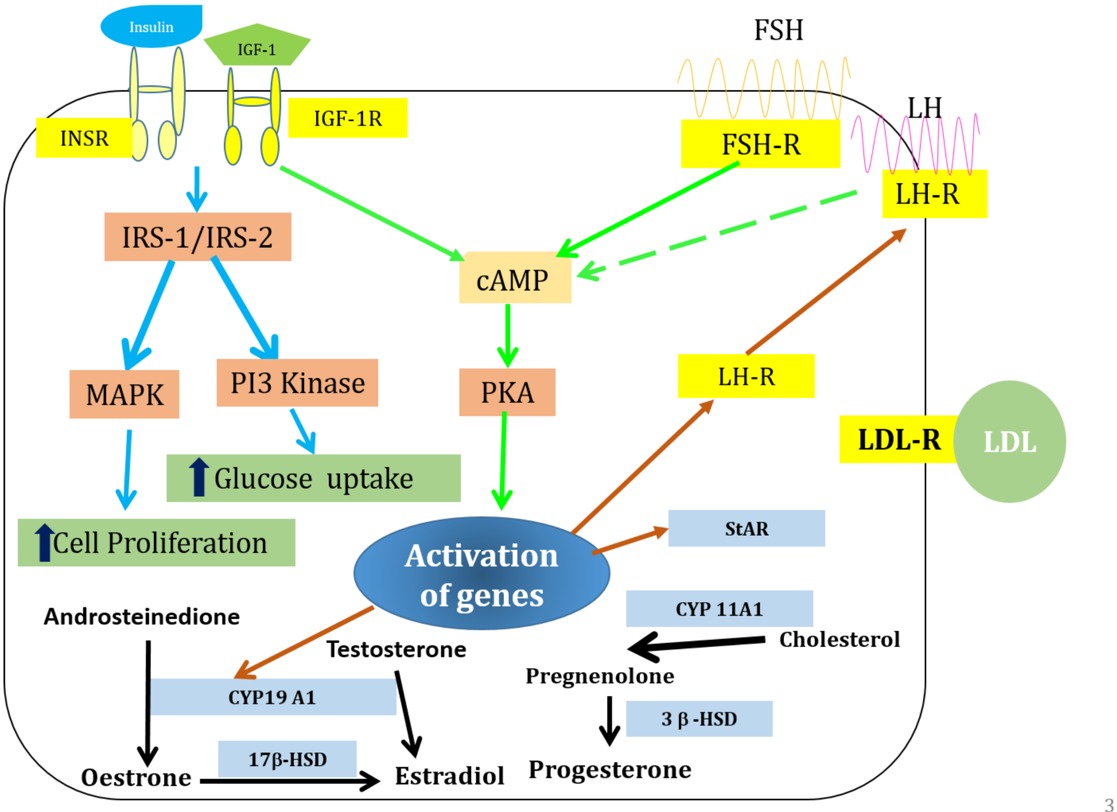


Figure 1. 11: Cross talk between gonadotropins and insulin in granulosa cells. (Leung and Adashi 2003)

1.10 Granulosa cell metabolism

Granulosa cells maintain a dynamic metabolic cooperativity with their companions oocyte (Trounson et al. 2013). The follicular growth encompassing cumulus-oocyte complex requires a range of substrates including, fatty acids, amino acids, electrolytes, purines, pyrimidines and other metabolites. The primary follicles are metabolically quiescent, but for their size they possess a relatively high level of metabolism to remain viable for extended years and for the house keeping activities. During plentiful oxygen, aerobic metabolism through glucose produces energy in the form of ATP. Alternatively during anerobic condition pyruvate is less efficiently converted to lactate through the cytosolic enzyme lactate dehydrogenase. Thus the glycolytic pathway accounts for the proportion of glucose metabolism by granulosa cells for providing pyruvate and lactate for oocytes. Under hyperglycemic condition the polyol pathway in granulosa cells oxidizes glucose to sorbitol and fructose, thus providing oocytes with an alternative energy source. The glucose metabolism is known to be regulated by gonadotropins in granulosa cells by moderate oxygen tension and GLUT's which are sensitive to insulin signaling. Folliculogenesis from early antral stage onwards depends on glycolytic breakdown of glucose for its energy requirement due to establishment of gap between oocyte and granulosa cells by the antrum (Trounson et al. 2013). Apart from carbohydrate metabolism that imparts energy, lipid metabolism plays a pivotal role for sustained reproductive function (Elis et al. 2015). Fatty acid oxidation and synthesis are important for granulosa cells to express a proliferative and steroidogenic phenotype. Variations in fatty acid levels affect the granulosa cell, folliculogenesis, oocyte quality and the conception rate (Elis et al. 2015). The expression of sterol regulatory element binding protein 1c (SREBP1c), fatty acid synthase (FAS), carnitine palmitoyltransferase-1 (CPT-1) and acetyl coA carboxylase (ACC-1) in human granulosa cells is concerned with the synthesis and uptake of cholesterol, fatty acids and other lipids for either transfer to oocyte or for the purpose of steroidogenesis (Richardson et al. 2005)(Fig. 1.12).

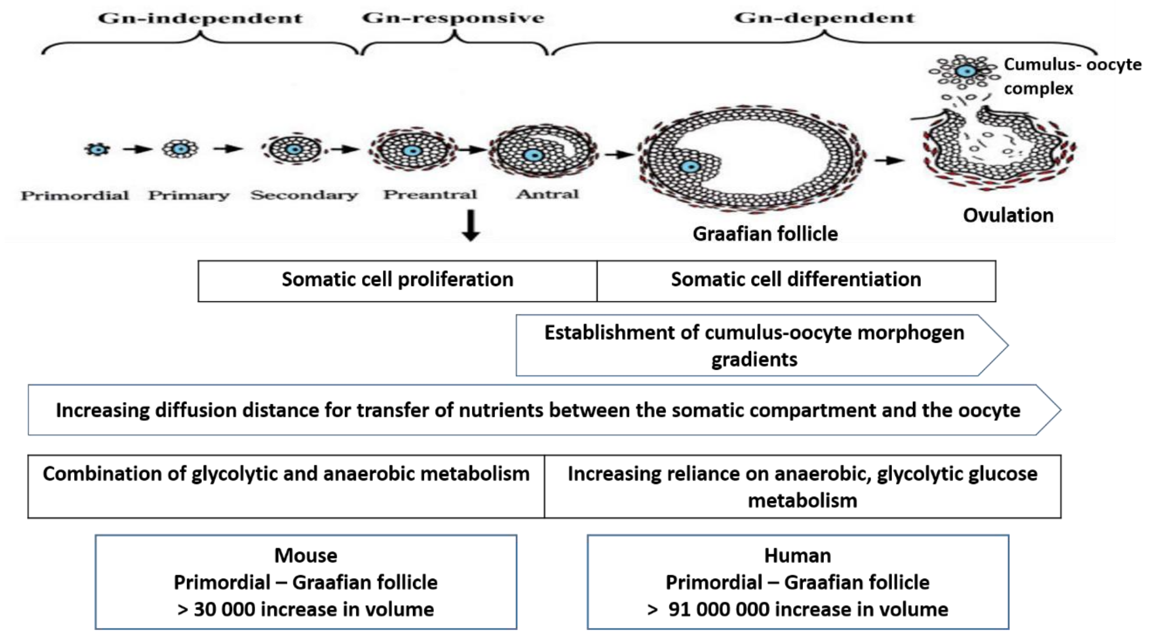


Figure 1. 12: Overview of energy metabolism during key developmental events in mammalian folliculogenesis modified from (Trounson et al. 2013).

1.11 Infertility

Any disturbances to the above signaling mechanisms would lead to malfunctions in reproductive system ultimately leading to infertility. The females nowadays either are not able to conceive or the growing embryo is unable to survive after conception ultimately leading to infertility/ pregnancy loss. Infertility as per WHO is thus defined as failure to achieve a clinical pregnancy after 12 months or more of regular unprotected relationship” which can be attributable to either male factors, female factors or both.

1.11.1 Epidemiology

Reproductive health is a priority global health area. About 10-15% of the overall population of reproductive age are infertile (Kubo 2009). The prevalence of Infertility has been observed to be highest in South Asia, Sub-Saharan Africa, North Africa/Middle East, and Central/Eastern Europe and Central Asia (Mascarenhas et al. 2012). A reliable estimate on the rate of infertility in the Indian context is deficient, as the registration of marriages, births and deaths are usually incomplete. However, multicentric study on genetic counseling carried out by the task force of Human Genetics under the auspices of the Indian Council of Medical Research (ICMR) states infertility to be topmost disorder in India (Vettriselvi 2012).

1.11.2 Etiological factors for infertility

In India, there has been no reversal in the declining birth rate and hence the tendency towards increasing potential infertility factors is a major social issue. Certain medical conditions such as pelvic inflammatory disease, endometriosis, polycystic ovarian syndrome, premature ovarian failure, uterine fibroids and damage or block to the fallopian tubes leading to infertility. Other than these certain life style factors are known to positively or negatively modulate fertility.

a. **Life style factors**: The choice of the life style has an impact on the fertility of the individual. Excessive weight gain with body mass index (BMI) greater than 27 kg/m^2 results in increase in fat cells. This state of high body fat or obesity causes increase in estrogen production which is interpreted as birth control by the body thus limiting the chances of getting pregnant. It has also been found to have effect on treatment efficacy and outcomes of assisted reproductive technique. Further, too little body fat causes insufficient estrogen production leading to menstrual irregularities with anovulatory cycle (Control and Prevention 2011). Change in eating habits and sedentary life style can lead to insulin resistance, a pathogenic condition observed in 70% of PCOS patients and further leading to type II diabetes. Late marriages seem to be another lifestyle factor contributing to infertility. Fertility in a women peaks between 18 to 24years where the ovarian reserve is 12%, begins to decline after 27 and drops at a greater rate after 35 where the ovarian reserve remains only 3%. Yet another life style factors influencing fertility are tobacco smoking and alcohol. Cigarette smoking interferes with folliculogenesis, embryo transport, endometrial receptivity, endometrial angiogenesis, uterine blood flow and the uterine myometrium, reduces the chances of IVF producing a live birth by 34% and increases the risk of an IVF pregnancy miscarrying by 30%. Alcohol intake is associated with elevated oestrogen level and this elevated oestrogen level reduces FSH secretions which then suppresses folliculogenesis and results in anovulation (Nikam and Ghorpade 2012).

b. **Environmental factors**: Individuals having direct contact with or exposure to chemicals such glues, volatile organic solvents or silicones, chemical dusts and pesticides are implicated in infertility. Other than these chlorinated hydrocarbons and fumicides are associated with the increased link of spontaneous miscarriage in women. Endocrine disrupting chemicals such as heavy metals, phthalates, bis phenol A, dioxins, organotins, parabens any many more are of particular concern for developmental effects (Nikam and Ghorpade 2012).

c. **Ovarian, Tubal and Uterine functional problem:** Absence of eggs in the ovaries, complete blockage of the ovaries, ovarian dystrophy and luteinized unruptured follicle syndrome (LUFS) result in infertility. Tubal (ectopic) and peritoneal factors include endometriosis, pelvic adhesions, pelvic inflammatory diseases usually due to *Chlamidia*, tubal occlusion and tubal dysfunction may lead to infertility by preventing transfer of egg to the tube. Uterine factors such as abnormal uterine shape, intrauterine septum, polyps, leiomyoma, Asherman's syndrome, benign fibroid and large fibroids may cause infertility by impairing the uterine lining, blocking the fallopian tube, distorting the shape of the uterine cavity or altering the position of the cervix (Nikam and Ghorpade 2012).

d. **Diseases:** Sexually transmitted diseases (STD) such as syphilis, trichomoniasis, chancroid, chlamydia, gonorrhea, herpes simplex virus, human papilloma virus, HIV, lymphogranuloma venerum are a leading cause of infertility. STDs can be transmitted vertically from mothers to children during pregnancy and childbirth (Nikam and Ghorpade 2012). Pelvic inflammatory disease (PID) caused by Chlamydia trachomatis, Gonorrhea and other bacteria's can eventually result into abscess formation, adhesions, scarring, tubal blockade, tubal damage, ectopic pregnancy and thus infertility. There are reports of spontaneous abortion in about 27% of cases during the first trimester of pregnancy by mumps (Kennedy et al. 2008).

e. **Hormonal imbalance:** Alterations in the chemical signals from the hypothalamus can affect the pituitary, ovaries, thyroid, mammary gland ultimately leading to hyperthyroidism, hypothyroidism, PCOS and hyperprolactinemia and then to anovulation. Stress is supposed to be a reason for changes in hormonal balance of the hypothalamo-pituitary-adrenal axis (HPA-axis) (Gohil et al. 2001). PCOS is also a hereditary problem where the ovaries produce high amounts of androgens, particularly testosterone and thus amenorrhea or oligomenorrhea is quite common. The increased androgen production results in high levels of LH and low levels of FSH, preventing the follicles from producing a mature egg. (Azziz et al. 2004). In hyperprolactinemia there is an increase in the release of dopamine from the hypothalamus that inhibits GnRH eventually leading to gonadal steroidogenesis followed by infertility (Nikam and Ghorpade 2012).

f. Structural obstruction: Congenital abnormalities such as during mullerian agenesis the vagina or the uterus fail to develop leading to infertility. Following pelvic surgery, postsurgical or postinfective uterine or abdominal adhesions and scarrings may occasionally result restricting the movement of ovaries and fallopian tubes ultimately causing infertility (Nikam and Ghorpade ; Nikam and Ghorpade 2012). In spite of the above proposed etiological factors, 50% of the patients still suffer from idiopathic infertility (reason is not known). It is now thought that the idiopathic infertility might be due to interaction between any two factors leading to infertility. Today's scenario exposes a female to various endocrine disruptors and changes in her life style unknowingly lead to insulin resistance condition.

1.12 Insulin resistance (IR)

Disturbances to the metabolic states in the body through various insults such as change in life style, lack of exercise and genetic problems might lead to decrease in cellular ability to respond to insulin signaling and is characterized as IR. There are increased circulating insulin levels and increased glucose load as shown in fig 1.13. IR appears to be an essential pathophysiologic mechanism in the development of all metabolic complications such as type II diabetes, obesity, and polycystic ovarian syndrome (PCOS) (Joselyn Rojas and JoséMejías 2014). It is present in approximately 50% to 70% of women suffering from PCOS and is known to progress toward the development of compensatory hyperinsulinemia and hyperandrogenemia (Mukherjee 2010; Joselyn Rojas and JoséMejías 2014).

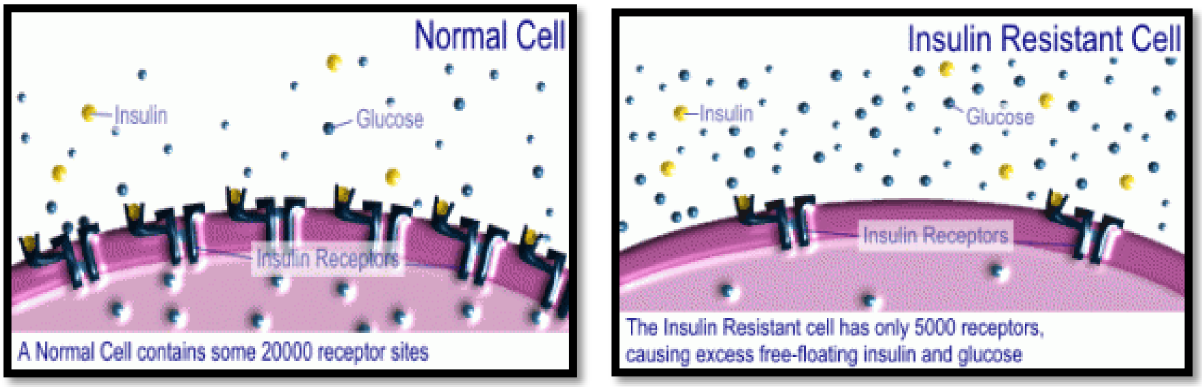


Figure 1. 13: Pictorical explanation of normal cell and insulin resistant cell. Insulin resistant cell have less insulin receptors, excess free-floating insulin and glucose.

1.13 Diagnosis of insulin resistance (IR)

As several metabolic complications related to IR, quantifying insulin sensitivity/resistance in humans and animal models is of great importance.

i. Hyperinsulinemic euglycemic glucose clamp: This technique is considered as "gold standard" for assessing metabolic IR in vivo. In this technique insulin action on whole-body glucose uptake is assessed by infusing a desired dose of insulin and maintaining euglycemia using a variable glucose infusion.
ii. Frequently sampled iv glucose tolerance test (FSIGT) with minimal model analysis: In subjects without diabetes whole-body insulin sensitivity can be accurately analyzed. The minimal model is used for determining insulin sensitivity (sensitivity index) and acute insulin response to glucose (AIRg). The product of insulin sensitivity and AIRg together give disposition index (DI) that assesses insulin secretion in the context of insulin sensitivity and is a robust parameter of pancreatic-β cell function.
iii. Mathematical models for IR: The mathematical models such as homeostasis model assessment (HOMA), the quantitative insulin sensitivity check index (QUICKI), logarithmically-transformed fasting plasma glucose (FPG) and fasting insulin resistance index (FIRI) require normal physiological dynamics of insulin and glucose for calculating insulin resistance (Singh and Saxena 2010). These models have proven to be a first-rate index of insulin resistance as they are cheaper, easy and have better precision for measuring insulin resistance in general population as compared to clamp and FSIGT models (Diamanti-Kandarakis and Dunaif 2012).

1.14 Cellular and molecular mechanisms of insulin resistance (IR)

The IR hypothesis describes defects in insulin signaling either at the ligand –receptor level or the post receptor binding thus triggering the metabolic abnormalities (Fig. 1.14).

i. In the insulin signaling cascade, important components can be decreased by either increased degradation or by decreased transcription. For example, increased stimulation of SREBP-1c can lead to a decrease in IRS-2 levels.

ii. Increase in the regulatory subunits of PI(3)kinase could act as inhibitors of the normal dimeric form of the enzyme itself.

iii. The components of insulin signaling pathway could undergo post translational modification leading to increase or decrease in their activity. Serine phosphorylation of the INSR would decrease its kinase activity. Serine phosphorylation of the IRS proteins would impairs their ability to be tyrosine phosphorylated and increase their association with the 14-3-3 proteins, removing them from the insulin signaling cascade. Serine phosphorylation of epidermal growth factor (EGF) receptor and tyrosine kinase growth factor receptor proteins by ERK, Tumor necrosis factor-α (TNF-α), JNK and IKK could terminate the signaling.

iv. Interaction of inhibitory proteins with components of the insulin signaling cascade could lead to insulin resistance. Inflammatory cytokines induce the suppressors of cytokine signaling (SOCS) proteins, which bind to the insulin receptor and block its signaling.

v. The phosphotyrosine phosphatases, e.g., PTP1b, and the PIP3 phosphatases, e.g., PTEN and SHIP are the enzymes that normally reverse insulin action. Increased activity or amount of these enzymes might lead to IR.

vi. Lowering of the GLUT4 glucose transporter and depletion of cellular adenosine could lead to decrease in inhibition of lipolysis by insulin thus contributing to IR (Kahn 2006; Saini 2010a; Saini 2010b; Laloraya 2013).

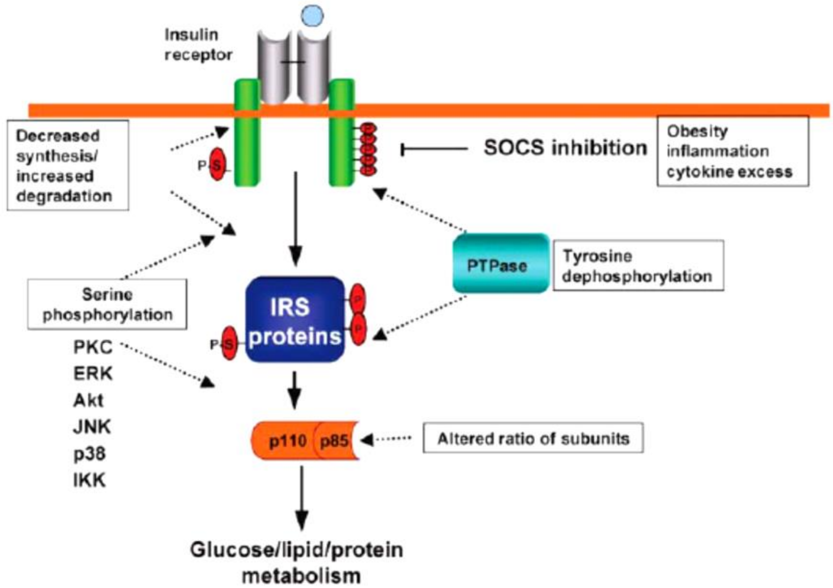


Figure 1. 14: Mechanism of insulin resistance (Biddinger and Kahn 2006)

1.15 Insulin resistance (IR) at hypothalamus and pituitary

Insulin receptor signaling has been implicated in the regulation of female reproductive function through its actions in both the central nervous system and ovaries (Irina Neganova and Stephen Franks 2007). Gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH), secreted by hypothalamus and pituitary, are under the control of insulin during the pubertal onset (H. H. Kim 2005). Studies have reported that lack of INSR in the neurons of female mice causes reduced release of LH ultimately leading to fewer antral follicles, corpora lutea followed by impaired fertility (Bruning JC and R 2000). Insulin receptor substrate (IRS) proteins mediate the effects of the insulin receptor on cellular and whole body physiology, including reproduction (DJ. 2001). A recent report by Nandi et al. demonstrated that insulin resistance in the absence of hyperglycemia affected functioning of HPG axis and altered reproductive cycle, follicular development, and early progression of gestation. The same model showed decreased embryonic weight and pathological development of the placenta during later gestation (Nandi A 2010). A correlation has been observed between elevated levels of) from granulosa cells in small antral follicles with increase in LH hormone levels in insulinresistant PCOS women ultimately inhibiting anti-mullerian hormone (AMH their progress toward primordial follicle thus playing an important role in long-term disruption of ovarian physiology and worsening fertility outcomes (A. Karkanaki 2011; Sanchez and Smitz 2012; A. Pierre 2013).

1.16 Insulin resistance (IR) at ovary

In IR condition as observed in PCOS, insulin stimulates steroidogenesis via its cognate receptor, whereas in severe insulin resistance where circulating levels of insulin are extraordinarily high, insulin can act through IGF-1 receptor (Poretsky L 1999). Hyperinsulinemia, thus, can affect steroid production in human GCs and alter the menstrual cycle and fertility. Mechanisms underlying insulin resistance – hyperinsulinemia and hyperandrogenemia – are proposed to cause serine phosphorylation of the β-subunit of INSR by protein kinase C (PKC), serine phosphorylation of the main regulatory enzyme of androgen biosynthesis, that is, P450c17, and tumor necrosis factor-α (TNF-α)-mediated serine phosphorylation of IRS-1 (Hotamisligil GS ; Muller HK and Kusser B ; Kruszynska YT 1996; Mukherjee 2010; Joselyn Rojas and JoséMejías 2014). Recent, evidences in diet-induced obese mice model suggest that differences in the proximal level of the insulin-signaling pathway (IRS-1/PI3K/Akt) between the ovary and pituitary and the energy storage tissues underlie the retained sensitivity to insulin in obese mice which suggest persistent AKT phosphorylation may be contributing to the pathologic steroidogenesis in women with PCOS (Sheng Wu 2012). In granulosa cells from PCOS ovary alterations in expression levels of both genes and proteins (implicated in insulin signaling pathway) such as INSR, PPAR-γ, GLUT-4, IRS-1/2 have been observed (Mukherjee and Maitra 2010). During insulin resistance, follicle maturation is often affected which is hypothesized in the literature to be insulin's interaction with LH that eventually leads to inappropriate advancement of granulosa cell differentiation, and hence, arrests follicle growth leading to development of cysts as shown in figure 1.15. (S. Franks 1996). Along with having varied level of aromatase activity (low or high), granulosa cells are hyper responsive to FSH and supra physiological doses of insulin for stimulation of estradiol and progesterone as shown in figure 1.16 (Erickson et al. 1990; Pierro E 1997). Next, with respect to theca cells of ovary, insulin increases the testosterone bioavailability in PCOS women by reducing hepatic production of sex hormone binding globulin (SHBG) confirming the central paradox in PCOS that ovarian theca cells remains sensitive to insulin activity and subsequent androgen production, despite of a systemic state of IR in the context of peripheral insulin resistance (Laloraya 2013). Thus, in insulin resistance condition enhanced theca cell androgen biosynthesis with exaggerated granulosa cell responsiveness to FSH alter the intra follicular microenvironment in patients with PCOS undergoing in vitro fertilization (IVF), which may increase the risks of implantation failure.

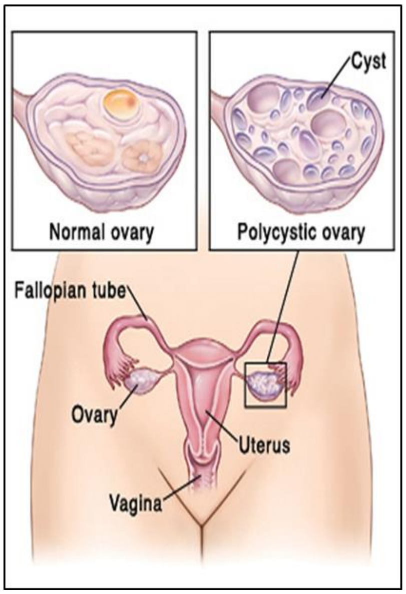


Figure 1. 15: Pictorial representation of cysts in PCOS as compared to normal ovary.

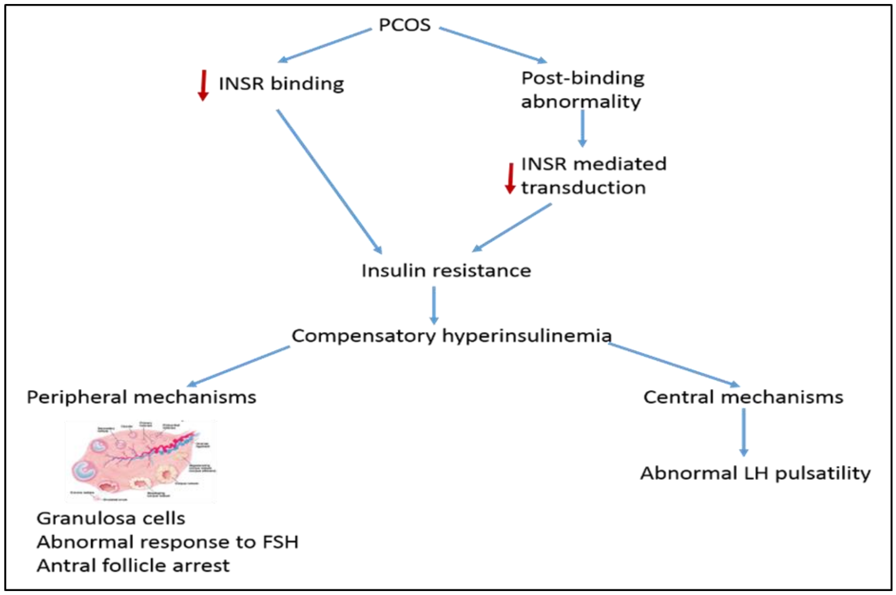


Figure 1. 16: Overall signaling relation between Insulin resistance and PCOS

1.17 Insulin resistance and conception

Insulin resistance with subsequent hyperinsulinemia plays a significant role in the pathophysiology of miscarriages in PCOS. The female germ cell is unique compared with somatic cell wherein, transcription is high during the growth phase of the oocyte and is negligible after meiotic resumption. In PCOS, the altered hormonal environment contributes to altered gene expression, that play a role in the meiotic cell cycle, ultimately disrupting the maternal-to-zygotic transition, which could contribute to developmental failures and reduced fecundity (Jennifer R. Wood 2007). Elevated serum insulin levels along with increased expression of plasminogen activator inhibitor (PAI-1) have been linked to spontaneous pregnancy loss in PCOS. Increase in ovarian androgen because of insulin resistance in PCOS is known to promote miscarriages by increasing circulating testosterone concentrations. Insulin resistance has been associated with abnormal endometrial development and endometrial defects. Decrease in follicular and luteal phase serum glycodelin and IGFBP-1 concentrations, two essential endometrial proteins, lead to miscarriages during the first trimester (Essah et al. 2004). Insulin resistance along with poor metabolic control around conception may lead to major congenital anomalies in the offspring (Tian L 2007; Jungheim ES 2008). The consequences of insulin resistance in the preimplantation embryo manifest decreased implantation rates and increased resorption rates ultimately leading to poor fetal outcome. Additionally, at embryonic day 14.5, fetuses resulting from the insulin-resistant embryos are significantly smaller than controls demonstrating that even brief exposure to a hyperinsulinemic condition in the preimplantation stage can have permanent effects on the offspring (Jungheim ES 2008).

1.18 Insulin Resistance and Cadmium

Cd exposure has been associated with elevated blood glucose levels and the development of diabetes in several animal and human studies. Animal studies with Cd exposure have demonstrated damage to pancreatic β cells accompanied by increased glucose levels thus acting as a diabetogenic agent (Schwartz et al. 2003; Edwards and Prozialeck 2009). Several human studies associating cigarette smoking and Cd exposure with impaired fasting glucose, type 2 diabetes and increased glycosylated hemoglobin in human subjects with no evidence of renal damage indicated that Cd exposure preceded the development of diabetes (Sargeant et al. 2001; Will et al. 2001; Schwartz et al. 2003; Kolachi et al. 2011). Presence of high levels of Cd in the biological samples of diabetic mother and their neonates indicate the role of Cd in the pathogenesis of diabetes mellitus and impacts on their neonates (Kolachi et al. 2011). Studies have also correlated exposure of Cd to the development of insulin resistance probably through mechanical distortion of the INSR-β (Chen et al. 2009; Satarug and Moore 2012; Bernhoft 2013; Lee and Kim 2013).

1.19 Endocrine disruptors

The functioning of endocrine system must be normal for follicle development and generation of good-quality oocytes. Several environmental pollutants have the ability to disrupt the endocrine system and lead to an array reproductive, developmental, neurological, immunological, and carcinogenic effects. According to the scientific statement of Endocrine Society, any exogenous substance either natural or synthetic that alters the function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations is termed as an endocrine disruptor (Diamanti-Kandarakis et al. 2009). Endocrine disruptors are generally referred to as ―Endocrine Disrupting Chemicals (EDCs).

EDCs can act at multiple sites through multiple mechanisms of action. They act by mimicking or antagonizing the actions of naturally occurring hormones. The consequences of exposure to EDCs are different in adults, developing fetus and infant. Moreover it may not be immediately apparent early in life but may be manifested in adulthood or during aging. It is likely that different classes of EDCs are together involved in exposing an individual ultimately resulting in additive or synergistic effect. The effect of EDCs is observed to be transgenerational (Diamanti-Kandarakis et al. 2009).

1.20 Cadmium

Cadmium (Cd) is a common environmental toxicant. The majority of general population is exposed to Cd through occupation, cigarette smoking, contaminated food, water, and household dust (M.C. Henson 2000). Up to 40% to 60% of inhaled Cd reaches the circulatory system via the lungs, whereas 5% to10% of ingested Cd is absorbed, which may lead to toxicity due to continual accumulation in a long‑term exposure. Long half-life of 15 to 30 years and low rate of excretion from the body facilitate bioaccumulation of Cd in the H-P-O axis and corroborates the likelihood for reproductive toxicity (Alexander et al. 2009; Satarug et al. 2011).

Cd is acquired by transport mechanisms developed for essential metals most likely to be calcium, zinc, copper, and iron. Cd is transported throughout the body usually bound to a sulfhydryl group-containing protein such as metallothionein. Uptake of Cd2+ in cells is mediated through L-type voltage-gated Ca2+ channels (P.M. Hinkle 1994) and receptor-mediated Ca2+ channel (M.H. Bhattacharyya 2000) because both the cations have similar radii size and charge. Cd2+ can also displace Ca2+ from its normal binding to calmodulin and protein kinase C (M.E. Blazka 1994; M.H. Bhattacharyya 2000). Calmodulin activates several enzymes of the second messenger pathways that regulate gene expression, including Ca2+/calmodulin-dependent kinase, phosphodiesterase, and the myosin light chain kinase (N. Tang 1993). The exposure is monitored by measuring levels of metals in urine and blood (Olsson IM 2005). Blood Cd levels mainly reflect current exposure, and urinary Cd levels reflect the body burden. A good correlation between blood Cd and urine Cd has been demonstrated, and either biomarker can be used for Cd estimations in biomonitoring (Jarup L 2009). Higher urinary Cd levels are associated with decreased inhibin B levels, suggesting a link to pubertal delays in adolescent girls (Gollenberg AL 2010).

1.20a Cadmium and reproductive dysfunctions

A wide spectrum of deleterious effects on the reproductive biology and the developing embryo has been described. In the context of reproductive system, several studies have evaluated the neuroendocrine toxicity by Cd specifically in hypothalamus, pituitary and gonads.

1.20b Toxic effects of Cd in the hypothalamus

Hypothalamus plays an essential role in assuring the survival of the species by controlling the expression of sexual and maternal behaviors (Paxinos 2014). Accumulation of Cd in hypothalamus modified biogenic amines (dopamine, norepinephrine and serotonin) and amino acid concentrations (glutamate, aspartate, taurine and c-aminobutyric acid or GABA) depending on the concentration and areas in rat model (Lafuente 2013). Developmental studies in humans have reported that exposure of Cd in mothers might lead to neurochemical disturbances of serotoninergic and aminoacidergic systems, with a decrease of glutamate concentration in the hypothalamus and alterations in transferring receptor gene expression which might influence cadmium transport into the neonatal brain (Antonio et al. 2010; Honda et al. 2013). Exposure of different doses of Cd in cortical neurons induced neuronal cell death through apoptosis pointing on to the toxicity evoked by Cd in hypothalamus (Yan et al. 2012). Administration of Cd in the proestrus sensitive window has been reported to suppress GnRH secretion (Eldridge and Stevens 2010).

1.20c Toxic effects of Cd in the pituitary gland

The toxic effects of Cd on the neurotransmitters in the hypothalamus might affect the regulatory mechanism responsible for the secretion of pituitary hormones. Pituitary gland is a very sensitive target organ for the Cd and its effect has been observed in many different species (Jimenez-Ortega et al. 2012). Environmentally relevant Cd levels have been associated with decreased FSH secretion among healthy, regularly menstruating women along with decreased serum inhibin B levels in peripubertal girls leading to delayed onset of puberty (Yan et al. 2012; Lafuente 2013). Alteration in pulsatile LH secretion and episodic prolactin secretion have been observed following Cd administration in both pubertal and adult male rats probably through biochemical, genomic and morphological changes in the cells (Calderoni et al. 2010). Studies with effect of Cd on pituitary membrane function demonstrate lowered membrane fluidity accompanied by alterations in receptor binding and secretory mechanisms of gonadotropin hormones (Pillai et al. 2002). All these findings indicate the toxicity of the Cd throughout the sensitive developmental window.

1.20d Toxic effects of Cd in the ovary

Cd can accumulate in the ovary and lead to several structural changes (Lubo-Palma et al. 2006). Inhibition of progesterone and estradiol secretion by Cd has been observed in several in vitro and in vivo human and rat studies (Piasek and Laskey 1994; Paksy et al. 1997) (Zhang et al. 2008; Das and Mukherjee 2013). However bi phasic responses of different concentrations of Cd at different time leading to increase or decrease of progesterone have also been demonstrated (Smida et al. 2004). Exposure of Cd in the gestational window has been reported to induce ovarian toxicity and reproductive dysfunction through increased oxidative stress (Samuel et al. 2011). Subcutaneous exposure of Cd throughout gestational and lactational window inhibited steroidogenesis by down regulating steroidogenic acute regulatory gene expression (StAR), inhibiting steroidogenic enzymes (3β-HSD and 17β-HSD) activity and altering the antioxidant system (Samuel et al. 2011). Exposure to Cd inhibits the key steroidogenic proteins responsible for secretion of progesterone and estradiol such as CYP11A1, 3β-HSD, CYP19A1 and 17β-HSD ultimately proving it detrimental for steroidogenesis (Jackson LW 2011; PollackAZ and Jones RL 2011; Ronchetti SA 2013; Hu et al. 2014). In vitro Cd exposure to rat granulosa cells demonstrated decreased LH and FSH hormone-receptor binding possibly due to co-precipitation of the metal ions and the receptors, binding to amino acid like cysteine residues of the receptor or interference with the hormone receptor complex (Priya et al. 2004). Exposure of Cd during early stage of follicular growth resulted in decreased gene expression of FSH-R and LH-R (Yang et al. 2008). Cd may also interfere with functioning of the hormones by binding indirectly through the low-density lipoprotein receptor (Stoica et al. 2000). Cd has been observed to be ovotoxic by decreasing the viability and development of oocyte in different species (Nandi et al. 2010). Production of oxidative stress by the Cd disrupts events associated with in vitro maturation of oocyte including prematuration arrest, meiotic progression, chromosomal segregation, cytoskeletal architecture and gene expression (Combelles et al. 2009). Human fetal germ cells are highly sensitive to Cd and undergo rapid apoptosis highlighted by an increase in rate of cleaved caspase-3 and caspase-9 after exposure to low concentrations similar to those occurring in the environment (Schwartz et al. 2003). Cd toxicity on different developmental stages has also been associated with failure of progression of oocyte development (Xuying Wan et al. 2010). Cd toxicity on preantral follicles leads to ovarian cycle disorder as against on antral or preovulatory follicles that leads to instant infertility. The exposure to Cd decreases placental glutathione peroxidase activity ultimately subjecting fetus to some degree of oxidative stress thus leading to spontaneous abortions and lower neonatal birth height (Masso et al. 2007; Aziza A. Saad and Amany I. Youssef 2012). Cd is associated with earlier onset of vaginal opening, increased uterine weights and enhanced mammary development in rats and menstrual cycle abnormalities among young women, endometriosis, difficulties getting pregnant among married women and preterm delivery (Jackson LW 2011).

1.21 Animal models

Several animal models of IR have been developed to facilitate opportunities to explore the adverse impact of IR on clinical outcomes in patients with PCOS.

Chemically induced model

i. Androgen induced model: Androgens (Dihydrotestosterone - (DHT)) are considered the main culprit of PCOS, and therefore, androgenization of animals is the most frequently used approach to induce symptoms that resemble PCOS. Prenatal or prepubertal androgen treatment results in anovulation, cyst-like follicles, elevated luteinizing hormone (LH) levels, increased adiposity, and insulin insensitivity (Sara 2012; van Houten EL 2014).

ii. Glucocorticoid induced model: Glucocorticoid excess leads to insulin resistance. Dexamethasone, a glucocorticoid analogue blunts insulin's action to suppress hepatic glucose production and stimulate peripheral glucose utilization. It also directly inhibits glucose-induced insulin release in the β-cells and in the peripheral tissues decreases GLUT-4 translocation to the membrane ultimately hampering glucose uptake by the cell. Dexamethasone has been previously reported to exacerbate IR in monovular animal model, such as cow, in isolated and cultured 3T3 adipocytes and in ovarian theca cells from porcine follicles (Buren et al. 2002; Qu et al. 2009; Hackbart 2013).

New Zealand obese (NZO) mice

NZO mice are obese, hyperinsulinemic and IR. These show a poor breeding performance due to decreased ovulation, increased number of primordial and atretic follicles, and ovarian size (Radavelli-Bagatini 2011).

Genetic rat models

i. Zucker fatty rats (ZFR) have a mutation in the gene coding the leptin receptor (fa/fa) that results in obesity and hyperinsulinemia. The continuous IR in this model leads to follicular atresia, reduced steroidogenesis, abnormal estrous cyclicity and abrupt changes in adiponectin levels (Honnma et al. 2010).

ii. Goto Kakizaki rats (GK) is a nonobese, diabetic strain that presents primary β-cell defects and peripheral insulin resistance in both males and females. Presence of hypothyroidism in this strain promotes LH and testosterone secretions ultimately leading to abnormal fertility (Tamura et al. 2005).

iii. Zucker diabetic fatty (ZDF) rat also carries the same mutation and, additionally, a mutation that results in spontaneous hyperglycemia. These rats show loss of ovarian estrogens.

Transgenic/knock-out diabetic models

Insulin Receptor Substrate Knockout Mice Models with specific knockout in Irs-1 causes insulin resistance but not clinical diabetes reflecting compensatory increase in insulin secretion. Knock out of Irs-2 causes insulin resistance with type 2 diabetes mellitus.Irs-2 knockout also causes obesity and infertility associating it with PCOS (Epstein 2003).

Diet induced diabetic models

Fructose: Chronic fructose consumption recapitulates some metabolic features of PCOS including hyperandrogenism and insulin resistance in rat strains like Wistar, Charles foster and SD rats (Arikawe et al. 2013). High fat diet fed to C5BL/6 mice revealed systemic metabolic dysregulation along with insulin resistance after 6 weeks.

1.22 Human models

The major source of granulosa cells for in vitro studies has been patients undergoing in vitro fertilization (IVF), a type of assisted reproductive technologies (ART). ART's are established to address problems associated with abnormal reproductive functioning in order to help couples conceive. Assisted reproductive techniques involve protocols which down-regulate the endogenous female cycle and allow exogenously administered gonadotropins to stimulate development and production of oocytes and surrounding steroidogenic cells (granulosa and theca cells) within the ovary, referred to as superovulation or hyperstimulation (Salamonsen et al. 2001). Due to hyperstimulation with gonadotropins, these cells are normally fully luteinized at the time of isolation. The granulosa cells cultured from IVF samples are termed as primary granulosa cells. Apart from these primary granulosa cells, KGN cell line, originated from a Stage III granulosa cell carcinoma removed from a 63-year-old Japanese women in 1984 also remains as a model of choice for many researchers. The cell line shows presence of steroidogenic enzymes but steroidogenic hormones estradiol and androgens are undetected (Havelock et al. 2004).

PI(3)K inhibitor in primary granulosa cells

The post receptor signaling pathways for insulin signaling include PI(3)K and MAPK leading to metabolic and mitogenic effects respectively. A defect in these pathways lead to insulin resistance (IR) and compensatory hyperinsulinemia. Wortmannin, a steroid metabolite of the fungi *Penicillium funiculosum*, leads to insulin resistance by totally abolishing the insulin action by PI(3) K phosphorylation. Wortmannin has been shown to induce IR in granulosa cells by decreasing GLUT4 expression and glucose uptake (Miaoe Yan et al. 2009).

Glucocorticoid induced insulin resistance

In numerous species, glucocorticoids result in increased serum insulin and glucose, decreased insulin-mediated glucose uptake and insulin resistance (Hackbart 2013). Dexamethasone is a glucocorticoid whose clinical use often exacerbates diabetes. Glucocorticoid-induced insulin resistance is demonstrated in clinical cases, in animal experiments and in vitro experiments using isolated or cultured cells (Hideyuki Sakoda et al. 2000; JunWei Qu 2009).

1.23 Interventions for infertility

1.23.1 Life style modifications

Lifestyles factors such as age while starting a family, nutrition, psychological stress, bariatric surgery and routine exercise are modifiable and can help in better ovulation rates and pregnancy in PCOS (Sharma 2013; Sharonjeet Kaur 2014). Routine exercise is known to improve insulin sensitivity, reduce waist circumference, regularize menstrual cycle and promote weight loss leading to improved clinical outcome in PCOS women.

1.23.2 Pharmacological interventions

Pharmacological interventions are considered as an adjunct to lifestyle modification.

i.Clomiphene citrate (CC): It is considered to be the first line approach to induce ovulation in patients with PCOS. It is anti-estrogenic and prevents the action of the endogenous estrogen on the estrogenic receptors at the hypothalamic– pituitary system thus blocking the ovarian negative feedback effect on gonadotrophin secretion (Eshre and Group 2008). This results in increase in secretion of Follicle Stimulating Hormone (FSH) and Leutinizing Hormone (LH) leading to follicle development. Its adverse effect include evidence of association of uterine cancer. Persistence anovulation after standard CC therapy (CC resistance), affects 15% to 40% of women with PCOS. Also a long half-life of CC of (2 weeks), leads to its accumulation in the body thereby affecting the endometrium, cervical mucus and leading to LH hormone hypersecretion ultimately decreasing pregnancy rates (Eshre and Group 2008).

ii. Letrozole: Letrozole is known to regulate ovulation and improve pregnancy rates in women with PCOS. However, in animal studies it is observed to be embryotoxic and fetotoxic, and there are no studies in pregnant women. CC and letrozole (Femara) have been used and studied in patients with and without PCOS (Eshre and Group 2008).

iii.Ergolin derivatives: Bromocryptine, an ergoline derivative, is a dopamine agonist used for reducing elevated levels of LH, a characteristic of PCOS. Combination of bromocryptine with CC helps in decreasing prolactin levels and improves ovulation. Another long acting ergolin derivative, ergoline D2 agonist, cabergoline, has shown a good evidence of ovarian response in hyperprolactinemic patients with PCOS (Eshre and Group 2008).

1.23.3 Insulin-sensitizing drugs (ISDs)

Antidiabetic drugs such as the biguanide, metformin, and the thiazolidinediones, pioglitazone and rosiglitazone, act by improving the sensitivity of peripheral tissues to insulin thereby decreasing circulating insulin levels. These insulin-sensitizing agents are indicated for most women with PCOS because 70% of PCOS have the insulin resistance as their pathogenesis. The positive effect of these drugs on insulin resistance, menstrual irregularities, anovulation, hirsutism, and obesity has been observed.

i.Metformin

Metformin by inhibiting hepatic glucose production, increases glucose uptake in peripheral tissues and reduces fatty acid oxidation and leads to lowering of insulin levels (Kirpichnikov et al. 2002). These pleiotropic actions occur due to the activation of 5'-AMP-activated protein kinase, mediated by action on a proximal kinase—serine-threonine protein kinase 11(Zhou et al. 2001; Shaw et al. 2005). In a meta-analysis of random clinical trial (RCTs) metformin significantly decreased circulating levels of total testosterone, androstenedione and the adrenal androgen, dehydroepiandrosterone sulfate and increased serum hormone binding globulin (SHBG) levels. Metformin is probably the first-line medication for obesity or weight reduction in patients with PCOS. Use of metformin in conjunction with gonadotropin therapy for ovulation induction is known to improve unifollicular development and conception rates, and reduces ovarian hyperstimulation syndrome (Lord et al. 2003; Falbo et al. 2005; van Santbrink EJ 2005). Metformin in combination with clomiphene was significantly more effective than clomiphene alone when assessed for clinical pregnancy rates than ovulation rates in the meta-analysis of ISDs. In PCO women having history of increased risk for spontaneous abortion, the continued use of metformin during pregnancy led to reduce the spontaneous abortions. Metformin during pregnancy reduced the risk of GDM in women with PCOS. Metformin in combination with life style modification such as a hypo-caloric diet led to significant reductions in visceral fat mass compared with the hypocaloric di*et al*one (Pasquali et al. 2000; Gambineri et al. 2004; Gambineri A1 2006). Studies have revealed that metformin therapy decreased systolic blood pressure and LDL-cholesterol levels and improves endothelial dysfunction in PCOS (Diamanti-Kandarakis et al. 2005; Gambineri A1 2006 ; Gambineri A 2006). Apart from its beneficial effects metformin has some adverse effects also that include significant increase in nausea, vomiting and gastrointestinal distress in women with PCOS (Lord et al. 2003; Richard S. Legro 2007). Administration of metformin to normo insulinemic PCOS women decreased the basal insulin levels along with improving the steroidogenesis (Önalan et al. 2005).Treatment with metformin till date is not known for increasing any live birth rate (Nestler 2008).

ii.Thiazolidinediones

These are ligands for PPARγ, which is part of the superfamily of nuclear receptors (Yki-Jarvinen 2004). Thiazolidinediones increase peripheral glucose uptake also decrease hepatic glucose production but to a lesser degree than metformin (Inzucchi et al. 1998; Maggs et al. 1998). Thiazolidinediones such as pioglitazone and rosiglitazone can reduce androgen levels, particularly free testosterone levels to ameliorate hirsutism in women with PCOS. Thiazolidinediones are also known to improve endothelial dysfunctions and fibrinolysis in PCOS (David A. Ehrmann 2005). Thiazolidinediones, being PPARγ ligands are, associated with weight gain and fluid retention that could result in edema and dilutional anemia, sleep disorders, headache, stomach pain and could worsen congestive heart (Brettenthaler et al. 2004).

iii.D-chiro-Inositol

It is a naturally occurring substance that is postulated to improve insulin sensitivity by an alternative pathway mediated by inositolphosphoglycans (Nestler et al. 1999; Yki-Jarvinen 2004). The signaling mechanism of insulin sometimes involves low molecular-weight inositol phosphoglycan mediators. These mediators are generated by hydrolysis of glycosylphosphatidylinositol lipids located at the outer leaflet of the cell membrane, when insulin binds to its receptor. Amongst the different molecules that have been identified, an inositol phosphoglycan molecule containing D-chiro-Inositol and galactosamine is known to have a role in activating key enzymes that control the oxidative and nonoxidative metabolism of glucose. The deficiency of this molecule may result in resistance to insulin. Administration of D-chiro-Inositol has been observed to improve glucose tolerance, decrease serum androgens and improve ovulation in PCOS (Nestler et al. 1999).

iv.N-acetyl cysteine (NAC)

It is the acetylated precursor of both the amino acid L-cysteine and reduced glutathione. It is characterized as an antioxidant, mucolytic and functions for insulin secretion in pancreatic cells and regulates insulin receptor in human erythrocytes. Studies have illustrated that Treatment of PCOS patients having hyperinsulinemia with NAC improves insulin sensitivity and declines plasma testosterone and lipid levels. Combination of CC and NAC increases ovulation and pregnancy rates in CC-resistant PCOS patients. The adverse effects of NAC are still lacking (Rizk et al. 2005).

v.Melatonin

Reactive oxygen species and apoptosis are involved in a number of reproductive events which includes folliculogenesis, follicular atresia, ovulation, oocyte maturation, and corpus luteum (CL) formation. Melatonin has magical properties of being a powerful antioxidant and receptor independent free radical scavenger, which bestow its direct effect on ovarian function in pathophysiology of PCOS (Tamura et al. 2009).

vi.Acarbose

It is used for reducing the postprandial rise in both serum glucose and insulin levels by inhibiting α-glucosidase, an enzyme responsible for the intestinal absorption of carbohydrates in type 2 diabetes. Acarbose has been demonstrated to improve various clinical manifestations of PCOS such as insulin resistance, hyperandrogenism, menstrual irregularity, obesity and infertility. Widely reported adverse effect of treatment with acarbose are related to gastrointestinal adverse effects (SÃ¶nmez et al. 2005).

1.23.4 Herbal medicine in treatment of Insulin resistance

Owing to the fact that all drugs are associated with a significantly higher incidence of disturbances, their use for ameliorating insulin resistance appears to be limited. Moreover in recent years, there has been renewed interest in the treatment of insulin resistance using herbal drugs, as World Health Organization (WHO) has recommended evaluation of the effectiveness of plants due to side effects of modern drugs (Joseph 2011). Ethnopharmacological studies have noted that many traditional remedies have relatively fewer side-effects, better patient tolerance in diverse cultural contexts, less toxicity, and lower costs compared to modern drugs for DM treatment (Ma 2014). In India medicinal plants are being used since 1000 years in herbal preparations (Kayarohanam 2015). Medicinal plants such as *Allium sativum, Aloe vera, Acacia Arabica, Aegle marmelos, Brassica nigra, Cinnamon zeylaniucm, Caffeine, Enicostemma littorale, , Trigonella foenum-graecum, Canna indica, Artemisia dracunculus, Momordica charantia* and *Dang Gui Bu Xue Tang* to name a few have been used as therapy for their insulin mimetic property (Patel 2012). These herbs function by affecting the downstream signaling of insulin, especially the PI3 K and IRS-1 activity leading to decreased insulin resistance in the peripheral tissues (Purintrapiban et al. 2006; Govorko et al. 2007; Klein et al. 2007; Sridhar et al. 2008; Mao et al. 2009). However, searching for new drugs from natural plants for ameliorating insulin resistance is still attractive because they might contain potential molecules which might demonstrate alternative and safe effects on insulin resistance.

i.Swertiamarin – An active lead from *Enicostemma Littorale*

This chemical compound is secoiridoid glycoside in nature. It is a major constituent of various plants belonging to Gentianaceae family like *Enicostemma Allixare, Swertia Chirata Clarke, S. Japonica, S. Angustifolia.* It is used as complementary and alternative medicine for many of human disease. There are many evidences describing multifactorial effects of this compound.

Hypoglycemic and anti-diabetic activity:

Swertiamarin an active lead compound from *Enicostemma littorale* markedly decreased hyperglycemia in vivo in experimental non-insulin dependent diabetes mellitus. It regulated gene expression by targeting PPAR-γ & improved insulin sensitivity thereby restoring glucose and lipid metabolism in peripheral tissues like liver and adipose tissues (Patel 2015). In vitro studies with swertiamarin on 3T3-L1 showed marked reduction in the levels of expressions of PPAR-γ, GLUT-4 and adiponectin (Vaidya 2012). Swertiamarin treatment increased key insulin signaling proteins with reduced expression of the stress kinases P38 MAPK and ERK1/2 proving that it potentially improved skeletal muscle insulin resistance and ameliorated glucose and glycogen metabolism in TNF-α mediated insulin resistance in L6 myocytes (Patel 2015).

Hypolipidemic activity: Swertiamarin inhibited adipogenesis with decreased expression of the key transcriptional factors and the downstream genes involved in lipogenesis and maturation of adipocytes(Patel 2015). Significant reduction was observed in serum triglycerides, cholesterol and LDL indicated hypolipidemic activity of swertiamarin in diabetic rats when compared to the control animals (Vaidya et al. 2012). Intraperitoneal administration of swertiamarin administered to hyperlipidemic and hypercholesterolemic rats reduced the levels of total cholesterol and serum LDL/HDL cholesterol ratio and 3-hydroxy 3-methyl glutaryl CoA (HMG-Co A) confirming swertiamarin as a potent lipid lowering compound (Vaidya et al. 2009).

Anti-inflammatory activity: Swertiamarin administration in IL-1b induced fibroblast-like synoviocytes depicted dose dependent reduction in proliferation of cells along with concomitant lowered levels of nitric oxide. The lowered inflammatory response was attributed to decrease in gene and protein expressions of apoptotic markers, osteoclastogenic mediators, various proinflammatory mediators and p38 MAPK and its release (Saravanan et al. 2014).

Curcumin- Active compound from Turmeric

It is diferuloylmethane and the most active component of turmeric. The spice turmeric, is derived from the root of the plant Curcuma longa. Curcumin is a potential therapeutic agent for treating and its complications because it is effective in reducing glycemia and hyperlipidemia.

Hypoglycemic activity: Oral administration of various doses of curcumin reduces the levels of glucose, hemoglobin (Hb), and glycosylated hemoglobin (HbA1C) in blood of alloxan, streptozotocin and streptozotocin-niacinamide induced rat models. In HFD induced insulin resistance and type 2 diabetes rat model curcumin showed antihyperglycemic effect and improved insulin sensitivity (Arun and Nalini 2002).

Reduction of peripheral insulin resistance: In insulin-resistant ob/ob mice with steatosis curcumin improved peripheral insulin resistance by reducing NF- κB/RelA DNA-binding activity, decreasing mRNA level of TNF and IL-6, and enhancing IL-4 production in hepatic TNF/iNOS-producing dendritic cells and adipose tissue macrophages (Yekollu et al. 2013).

Anti-inflammatory activity: Administration of curcumin restored transmembrane potential and stiffened membrane fluidity, limiting the release of proinflammatory factors, such as MCP- 1 from endothelial and immune cells in human umbilical vein endothelial cells and Jurkat T lymphoblasts in the presence of high glucose or increased concentrations of AGEs. Curcumin suppressed the activities of immune cells, release of proinflammatory cytokines and histone acetylation (Zhang et al. 2013).

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