1 Introduction

Human infertility is an individual and communal health issue affecting millions of people worldwide. The escalating prevalence of infertility, specifically in women with the polycystic ovarian syndrome (PCOS) during reproductive age has created a psycho-social burden to have their "*babies*". In the resources restricted communities, this burden causes health and economic challenges, as seen in India. One of the numerous obstacles is the increased frequency of infertility like polycystic ovarian syndrome in emerging countries, which has forced the creation of health policies and global intervention programs. These efforts will undoubtedly require a thorough etiological understanding of the factors that contribute to and promote the development of infertility, thus, demanding assisted reproduction and health-giving strategies to reverse the trends.

1.1 Polycystic Ovary Syndrome

Polycystic Ovary Syndrome (PCOS) is a heterogenous endocrinopathy in reproductive-aged women with discomposure to endocrine, reproductive and metabolic functions (Balen, 2004a). PCOS was first recounted by Stein and Leventhal as the syndrome of oligo-amenorrhea, hirsutism (male-like hair pattern), and polycystic changes in the ovaries (Stein & Leventhal, 1935; Azziz et al., 2016). Women with PCOS are characterized by ovulatory dysfunction, polycystic ovaries, and hyperandrogenism accompanied by several metabolic abnormalities including cardiovascular disorders, obesity, insulin resistance, glucose metabolism impairment, and type 2 diabetes mellitus (Dumesic et al., 2015; Azziz et al., 2016). Also, PCOS women are at high risk for pregnancy-related complications compared to healthy women (Palomba, 2021). Owing to the multi-etiological pathology of PCOS, the characteristic features of the syndrome have been greatly discussed. The following three definitions are valid currently to diagnose PCOS:

1. According to the **1990** US **National Institute of Health (NIH),** the presence of clinical and/or biochemical signs of high androgen and oligo-or anovulation in patients with PCOS.
2. Group of European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproduction Medicine (ASRM) in **Rotterdam (2003)** propose that the clinical diagnosis of PCOS in women requires the presence of two of

the three following criteria: i) oligo-/anovulation, ii) clinical and/or biochemical signs of androgen excess, and iii) polycystic ovarian morphology (PCOM).

3. In **2006**, The **Androgen Excess and PCOS Society** (AE-PCOS) recommended that PCOS be defined by biochemical signs of hyperandrogenism, ovarian dysfunction including oligo-/anovulation, and polycystic ovarian morphology.

Amongst all the above-defined criteria the Rotterdam definition is the most acceptable for PCOS characterization, and it is widely supported by the scientific community and health administrations (Legro et al., 2013).

1.1.1 Prevalence of PCOS

The prevalence of PCOS is remarkably identical globally (Azziz & Adashi, 2016), affecting 4 – 26% of women in their reproductive age (Azziz et al., 2006; Joshi et al., 2014; Diamanti-Kandarakis et al., 1999). The variation in the prevalence is mainly due to the use of different diagnostic criteria. Studies based on the NIH criteria demonstrated a clinical prevalence of PCOS in Europe, Asia, and Australia ranging between 5 - 9 % whereas using 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). In India, PCOS becoming more common, with rates ranging from 3.7 to 22.5 % (Deswal et al., 2020). PCOS was reported to be present in 3.7 % of adolescents and young girls in Lucknow (Gill et al., 2012), 9.13 % in Andhra Pradesh (Nidhi et al., 2011), 15% in Kerala (Vijayan et al., 2013), 18% in Tamil Nadu (Balaji et al., 2015) and 22.5% in Mumbai, Maharashtra (Srabani et al., 2014).

1.1.2 Pathophysiology of PCOS

The complex aetiologies and intrinsic processes underlying PCOS pathology are complicated. The interconnected mechanism between them causes and maintains the clinical characteristic of PCOS, like ovulatory dysfunction, excess androgen, and polycystic ovarian morphology. Moreover, understating the pathophysiology of the syndrome is central to the management of the syndrome. Several possible mechanisms of PCOS and its endocrinology have been discussed below.

1.1.2.1 Role of the ovary in PCOS

The appearance of enlarged peripheral polycystic ovaries indicates that the ovary is the principal site of endocrinopathy in PCOS. The ovarian morphological characteristics can be

recognized on the ultrasound examination, comprised of stroma enlargement and numerous small follicles (2-8 mm in diameter) arrayed either around the periphery or dispersed through the stroma (Kurobe et al., 2012).

1.1.2.2 Ovarian follicles development in PCOS

In normal conditions, the follicle development and the production of mature oocytes are under the influence of several hormones, mainly FSH and LH which influences ovulation and ultimate oocyte maturation (Azziz et al., 2016). In PCOS, ovarian follicular maturation is interrupted, resulting in a premature halt in follicle growth (**Figure 1.1**) leading to failure of ovarian maturation. The hyperstimulation of LH is deleterious to follicular development and has a stimulatory effect on the theca cells causing early luteinization (Balen, 2004b). Women with PCOS have a tendency to have many antral follicles that grow prematurely and arrest at an antral stage, gaining up to 2 to 5 mm in diameter, which is bigger than that seen in normal ovaries (Carvalho et al., 2018). Further, the intraovarian factor, anti-Mullerian hormone (AMH) is known to moderate ovarian folliculogenesis. PCOS patients have increased levels of AMH in the circulation and follicular fluid has been reported (Chang & Cook-Andersen, 2013). It reduces the sensitivity of the ovarian follicles to the FSH, inhibiting the recruitment of primordial follicles and altering the development of the prenatal and antral follicles (Xu et al., 2016). The AMH is generated by the granulosa cells and works on the primordial follicle in healthy women, limiting the recruitment of numerous follicles which decreases the effect of the FSH on the growing follicles. Elevated levels of AMH in the PCOS cause follicle resistance to FSH, which affect follicle development (Carvalho et al., 2018), and block the conversation of androgens to estrogens, thereby contributing to the excess ovarian androgen/hyperandrogenism in the PCOS phenotype (Azziz et al., 2016). Because serum AMH levels are related to the ovarian follicles/cysts and are found to be high in PCOS (as a symptom of PCOS), thus it can be a possible biomarker for this pathology (Dumont et al., 2015; Quinn et al., 2017; Singh & Singh, 2015).

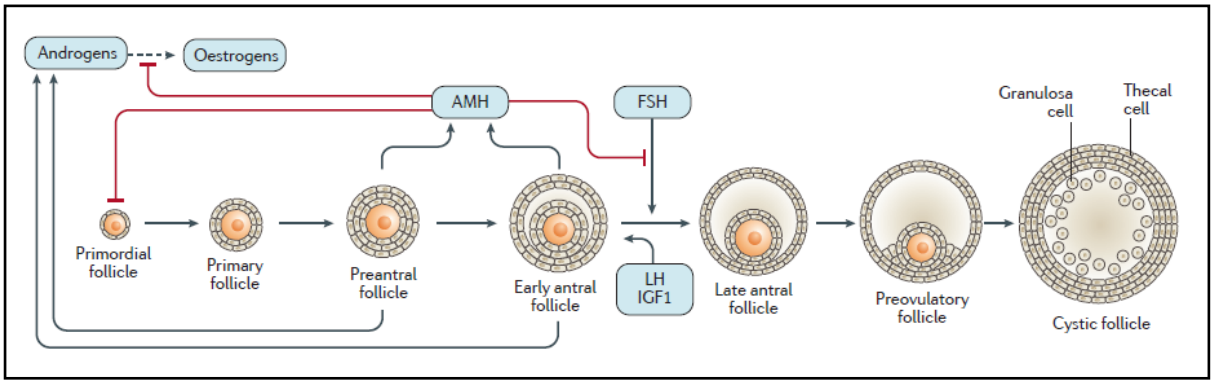

Figure 1.1 Ovarian follicle maturation arrest in PCOS (Adapted from Azziz et al., 2016).

1.1.2.3 Ovarian Steroidogenesis in PCOS

Disturbed ovarian folliculogenesis is closely connected to the alteration in ovarian steroid synthesis. The production of steroids in the ovary is bicompartmental, occurring in the two major cells of the ovary, granulosa, and theca cells (The pathway of steroid synthesis and the enzymes involved in the biosynthesis have been provided in **figure 1.2**). However, these cells of polycystic ovaries exhibit higher GnRH pulsatile activity, hypersecretion of LH, hyperactivity of theca stromal cell, and abnormal granulosa cell activity.

1.1.2.4 Theca and Granulosa cell function

Many reports have demonstrated the activities of enzymes involved in steroid hormone synthesis in PCOS. The genetic foundation of receptors in polycystic ovaries-granulosa and theca cells, with increased mRNA expression of *Lhr, Star, Cyp11a1,* and *Cyp17a1*. In stimulated ovaries of women with PCOS, (Catteau-Jonard et al., 2008) found dysregulation of granulosa cells, resulting in hyperandrogenism and elevated expression of FSH receptor (FSHR) and androgen receptor (AR). Prenatal androgens impact the hypersecretion of androgens in animals by changing LH sensitivity, leading to the up-regulation of steroidogenic genes in theca cells, causing them to generate excessive quantities of androgens. *Lhr* and *Cyp11a1*, but not *Star,* mRNA expression in granulosa cells were greater in PCOS follicles than in normal follicles, indicating that granulosa cells in PCOS had enhanced LH responsiveness, which may contribute to delayed follicle growth. Theca cell's inherent deficiency in steroid production is due to specific changes in steroidogenic enzyme expression. Also, *Cyp17a1* expression is increased in the ovaries of women with PCOS, and it is partly responsible for the altered steroidogenesis (Comim et al., 2013) at both transcriptional and post-transcriptional levels, demonstrating four-fold greater CYP17A1

promoter activity in theca internal cells of human polycystic ovaries (Wickenheisser et al., 2005). *Cyp17a1* gene expression in PCOS theca cells is cAMP-dependent, and *Cyp17a1* mRNA degradation is slower. It implies that the deregulation of *Cyp17a1* transcription mechanisms in PCOS theca cells may account for some of the increased ovarian androgen production. Furthermore, the aromatase enzyme, which catalyses the synthesis of estrogens (estrone and E2) from androgens (androstenedione and T) during steroidogenesis, is downregulated in PCOS and has been linked to steroidogenesis abnormalities (Kirilovas et al., 2006). P450arom dysregulation has been seen in women with PCOS in several investigations. In females with PCOS, the Estradiol/Testosterone (E2/T) ratio is thought to be a direct indicator of Aromatase activity. Reduced E2/T, FSH/LH production with an increase in LH, and Testosterone in PCOS patients. Although follicles are resistant to FSH, their size remains modest.

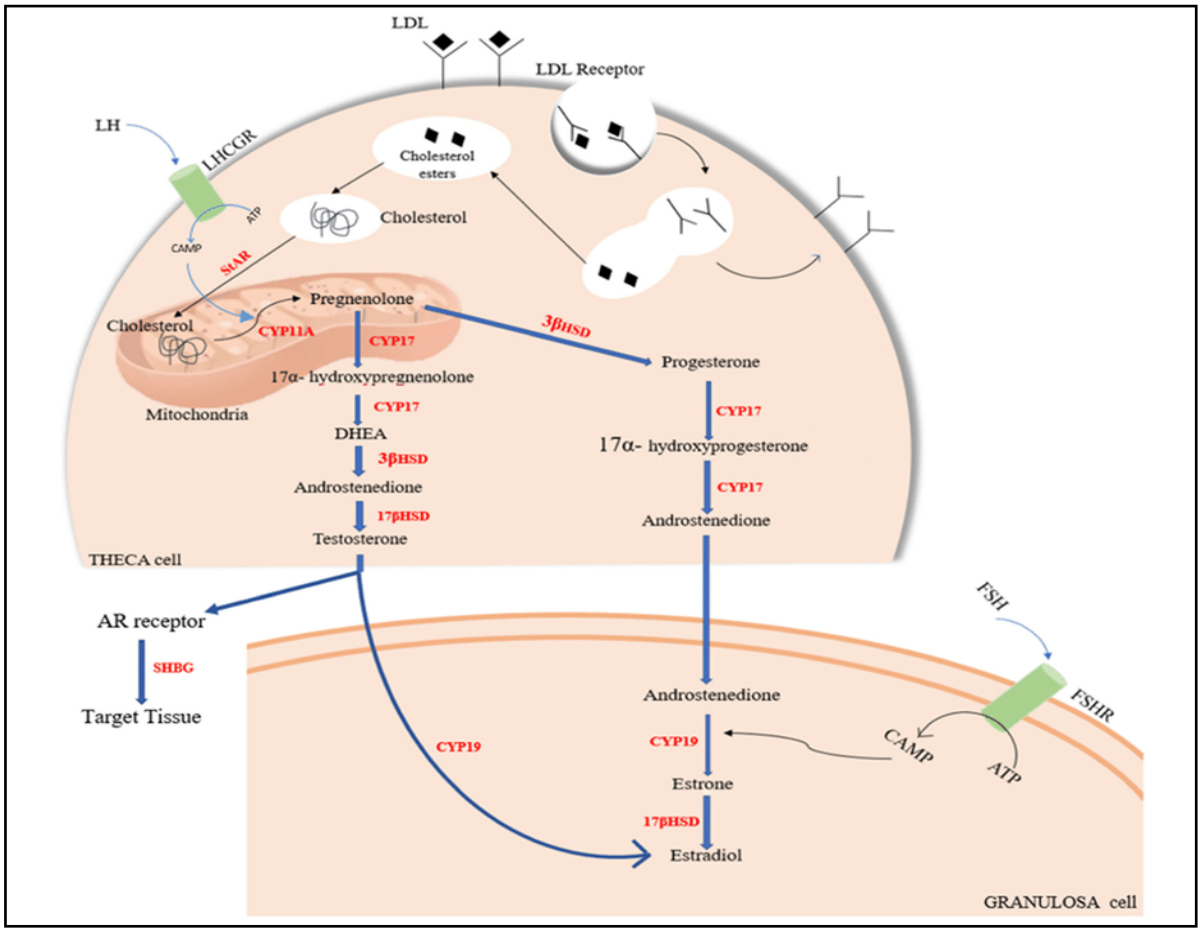

Figure 1.2 Schematic diagram of the Steroidogenesis pathway in the ovary (Adapted from Chaudhary et al., 2021). LHCGR: luteinizing hormone/choriogonadotropin receptor, LDL: low-density lipoprotein, LDL receptor: low-density lipoprotein receptor, 3βHSD: 3β-

hydroxysteroid dehydrogenase; StAR: steroidogenic acute regulatory protein, cAMP: cyclic adenosine monophosphate, ATP: adenosine triphosphate, FSH: follicle-stimulating hormone, FSHR: follicle-stimulating hormone receptor, LH: luteinizing hormone, CYP: Cytochrome P450, 17βHSD: 17β-hydroxysteroid dehydrogenase, DHEA: dehydroepiandrosterone, AR: androgen receptor, SHBG: sex hormone-binding globulin.

1.1.3 Hyperandrogenism

The major clinical feature of women with PCOS is hyperandrogenism, the primary indicator of excess androgen is the appearance of hirsutism (Balen, 2004a). The ovaries and adrenal are the main sources of excess androgen. The genetic and clinical variability associated with hyperandrogenic conditions suggests that anomalies in the steroid production pathway may be involved (Reddy et al., 2014). In the theca cells of the ovary, CYP17A1, the enzyme for androgen synthesis is found to be overexpressed via an overactivated PI3K/AKT pathway, leading to excess androgen production and ovarian abnormalities in the PCOS ovaries (Ye et al., 2021). Also, the enhanced hypothalamic GnRH surge favors the production of LH over FSH resulting in the classical hallmark of elevated LH/FSH ratio in PCOS leading to subsequent abnormalities in ovarian androgen production and ovulatory function (Catteau-Jonard & Dewailly, 2012). The follicles of the ovaries become arrested as a result of enhanced LH stimulation, causing the hyperplasia of theca cells and subsequent accumulation of follicular fluid, forming cyst-like structures along the ovary's periphery, giving it a string of pearls-like appearance (Abbott et al., 2009). An excess of androgens is produced as a consequence of an increase in the number of follicles and the expression of key enzymes involved in androgen production. Furthermore, excess insulin levels could also be considered as a factor for androgen excess wherein increased insulin secretion may imitate the tropic effect of luteinizing hormone on ovarian theca cells, leading to a rise in androgens (Wu et al., 2014). This is further supported by the fact that improving insulin resistance in PCOS women lowers hyperandrogenism levels (Baillargeon et al., 2004). Insulin also increases the flow towards excess androgen synthesis by promoting Serine phosphorylation of the multi-functional enzyme – C-17,20 Lyase/ 17α hydroxylase (Pergamon et al., 1989). Recent research suggests that the hyperandrogenic phenotype in PCOS is inherited from the mother, indicating the involvement of genetic factors, notably genes controlling steroid hormone production (Prapas et al., 2009). Furthermore, it is known that the altered expression of genes involved in androgen production in PCOS women affects the amount of androgen exposure *in utero* (Xita & Tsatsoulis, 2006). It is reported that prenatal exposure to high levels of

androgen causes hypersecretion of luteinizing hormone, changes in the differentiating process of thecal cells, and male-type fat distribution in female children (Xita et al., 2010).

1.1.4 Insulin resistance and hyperinsulinemia

In normal conditions, insulin action is known to stimulate a feature of human reproduction (Codner & Escobar-Morreale, 2007). It increases follicular development and ovarian steroidogenesis in granulosa cells via insulin receptors. Additionally, it has been discovered to produce more steroids in response to FSH stimulation (Sirotkin, 2011). Because of its gonadotropic effects on folliculogenesis, it also encourages recruitment and pre-ovary follicular development. Furthermore, the link between excess insulin (Hyperinsulinemia), insulin resistance, and androgen excess have been widely investigated in the progression of PCOS pathology. Insulin acts via multiple sites:

- Hyper insulinemic milieu has been shown to stimulate the LH response and increase androgen production at the level of P450c17 in the ovary (Rosenfield & Ehrmann, 2016).
- In response to LH stimulation, excess insulin binds to the IGF-1 and magnifies the theca cells' androgen synthesis (Bergh et al., 1993)
- High insulin levels also reduced the SHBG, resulting in increased androgen bioavailability (Wallace et al., 2013).
- Insulin has been shown to repress IGFBP1 (Insulin-like growth factor-1 binding protein) synthesis in both liver and ovaries in a direct and complete manner, allowing for IGF-1 availability, which in turn boosts the insulin activity not only in the liver-contributing to lower SHBG levels but also in the ovaries, reinforcing the PCOS pathology (Mounier et al., 2006).
- Insulin appears to increase the expression of StAR, CYP1A1, CYP17a1, and CYP19a1 (key enzymes of steroidogenesis pathway Figure 1.2), leading to an increase in progesterone, 17-hydroxyprogesterone and testosterone in the polycystic ovaries compared to the normal ovaries.

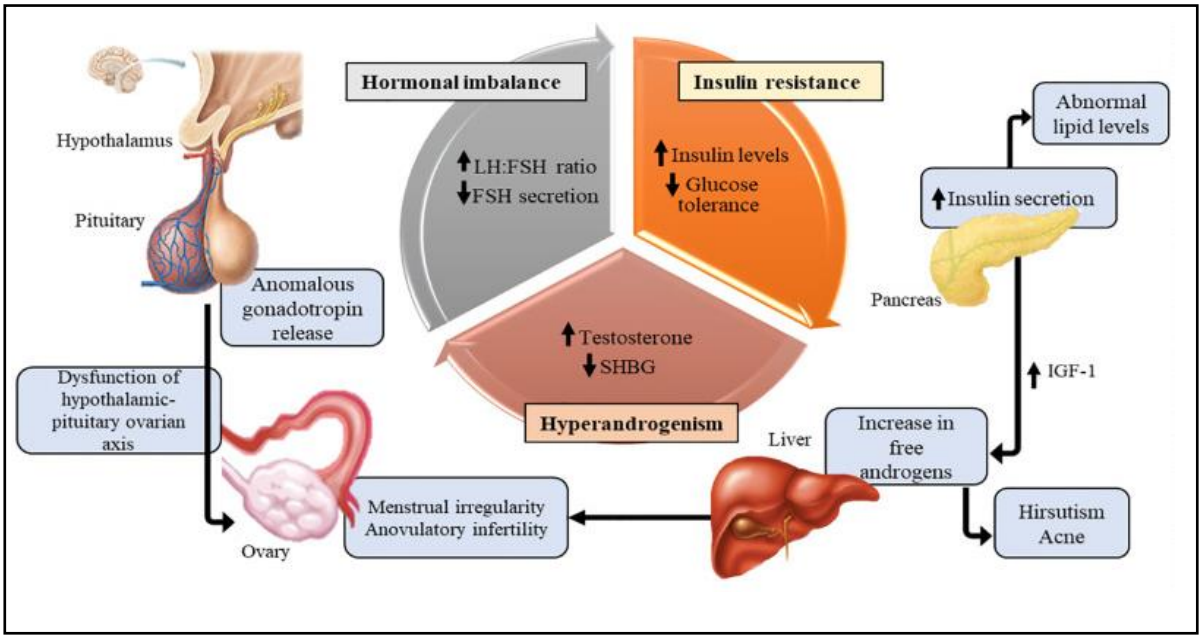

Figure 1.3 Schematic diagram for Pathophysiology of Polycystic ovary syndrome (Adapted from Bulsara et al., 2021. Abbreviations, IGF1: Insulin-like growth factor, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone.

Anovulation-infertility of PCOS is a frequent presenting complaint in patients with this pathology. Thus, improvement in ovarian function is the foremost concern for both researchers and clinicians. Restoration of the ovulation has been carried out using different pharmacological approaches (Elnashar & Aboul-Enein, 2011; Flyckt & Goldberg, 2011). Many studies have been carried out to reveal the mechanism underlying the abnormalities in PCOS ovulation that leads to infertility. In contrast, restricted studies have been reported on the endometrium of women with PCOS. In fact, increasing pieces of evidence have suggested a significant association of PCOS with pregnancy outcomes (Boomsma et al., 2006). Patients with PCOS exhibit an elevated rate of spontaneous miscarriage and implantation failure, even after the ovulation is restored. The primary and secondary abnormalities in the endometrium related to the PCOS features are still under discussion. However, all the experimental and clinical data indicated that PCOS and its associated co-morbidities are related to early and late pregnancy complications through impaired endometrium and placental function, (Shang et al., 2012a) impacting the well-being of the offspring (Hart & Doherty, 2015). The process of becoming pregnant is intricate, irreversible, and is preliminary controlled by endometrial functions. The following sections outline the normal physiological function of endometrium and events that occur throughout early pregnancy.

1.2 Endometrium function

The endometrium is dynamic tissue consisting of columnar epithelium which is connected to the underlying stromal cells. The epithelial glands are surrounded by stroma, thus allowing the interaction between these two cell compartments (Piltonen, 2016). Under the normal ovulatory cycle, the endometrium undergoes cyclic-dependent changes in response to ovarian steroid hormones (progestogens, androgens, and estrogens), dividing the endometrium's monthly cycle into proliferative and secretory phases (Snijders et al., 1992). During the proliferative phase, enhanced estrogen and its receptors (ERα/β) levels, a result of ovarian growth, encourage the proliferation of the endometrium tissue. The study has denoted this phase committed to DNA replication, tissue remodelling, and cell proliferation (Talbi et al., 2006). Following ovulation, the endometrium goes through changes, wherein the proliferative effect of estrogen is inhibited by progesterone, and endometrium cells start to differentiate. The estrogen receptors in the epithelium are down-regulated and the enzymes responsible for estrogen metabolism (sulfotransferase and 17b-hydroxysteroid dehydrogenase type 2) are induced which minimizes the function of estrogen in the endometrium (Falany & Falany, 1996; Talbi et al., 2006). Eight to ten days after ovulation, in the presence of progesterone endometrial stromal cells undergo decidualization to prepare for embryonic implantation and the endometrium becomes receptive to embryo implantation for the short period known as the "window of implantation". In the absence of implantation, evacuate estrogen and progesterone, especially progesterone leads to the shedding of the endometrium tissue causing menstruation (Giudice, 2006).

1.3 Implantation window

The window of implantation represents the most critical step in the reproductive procedure and can be classified into three phases, apposition, adhesion, and invasion (**Figure 1.5**). In the first step, the floating blastocyst interacts with the receptive state of the endometrium surface under the influence of ovarian hormones and many growth factors (Tabibzadeh & Babaknia, 1995). During the apposition, the trophoblast cells attached to the receptive epithelium by establishing contact with pinopods present on the surface of the uterine epithelium (Lopata et al. 2002). Resulting in the stable interaction of blastocyst to the endometrial basal lamina and stromal extracellular matrix (ECM). Stronger adhesion is accompanied by the paracrine signal between implanting embryo and the uterus. The indication of the interaction is the increased stromal vascular permeability at the attachment

site of the blastocyst (Sharkey & Smith, 2003). In the invasion step of implantation, the penetration of the embryo luminal epithelium into the stroma, therefore, initiates a vascular connection with the mother. In the presence of progesterone, the stromal cells and ECM undergo decidualization (changes in the endometrium cells which are required for the establishment of pregnancy). The completion of each event and decidualization (Singh et al., 2011) gives rise to a functional unit, the placenta which provides an interface between fetal and maternal circulation (Aplin, 2000; Guzeloglu-Kayisli et al., 2009). If the coordination between them is disturbed, implantation becomes inoperative or implantation fails but the ethical restriction and lack of mechanistic studies have excluded the study on the interaction of embryo-endometrium in humans, thereby, the information obtained from the mouse models have assisted to recognize the molecular basis of the implantation and early pregnancy events in humans. The common characteristic between mice and human is that both have hemochorial placentation wherein trophoblasts are direct in connection with maternal blood (Cha et al., 2019).

1.3.1 Molecular signatures of early pregnancy

Estrogen and progesterone are the key regulators of pregnancy outcomes in both rodents and humans (Cha et al., 2019), and hormonal conditions during early pregnancy are similar in both mice and humans (**Figure 1.4).** In mice, the changes in estrogen and progesterone levels differentially regulate the uterine cells. On the day after mating and ovulation, the vaginal plug is observed in the early morning which is defined as the day 1st of the pregnancy. Under the control of preovulatory estrogen, epithelial cells of the endometrium undergo proliferation and on day 2, apoptosis takes place with declining in estrogen levels. On day 3, the newly formed corpus lutea, start to release the progesterone. The progesterone becomes a completely controlling factor by day 4 which controls the proliferation of the stromal cell. This process is called pre-decidualization, and this phenomenon is identical to humans. Simultaneously, the luminal epithelium comes to end and differentiates for the blastocyst adhesion. Also, on day 4, the small estrogen surge caused the apposition of the blastocyst to the epithelium followed by the strong interaction of the blastocyst and uterus. After the interaction, stromal cells surrounding the implanting blastocyst begin to differentiate and change their morphology, a procedure called decidualization. Further, embryos invade the endometrium, and implantation of the embryo is accomplished (Cha et al., 2012; Egashira & Hirota, 2013).

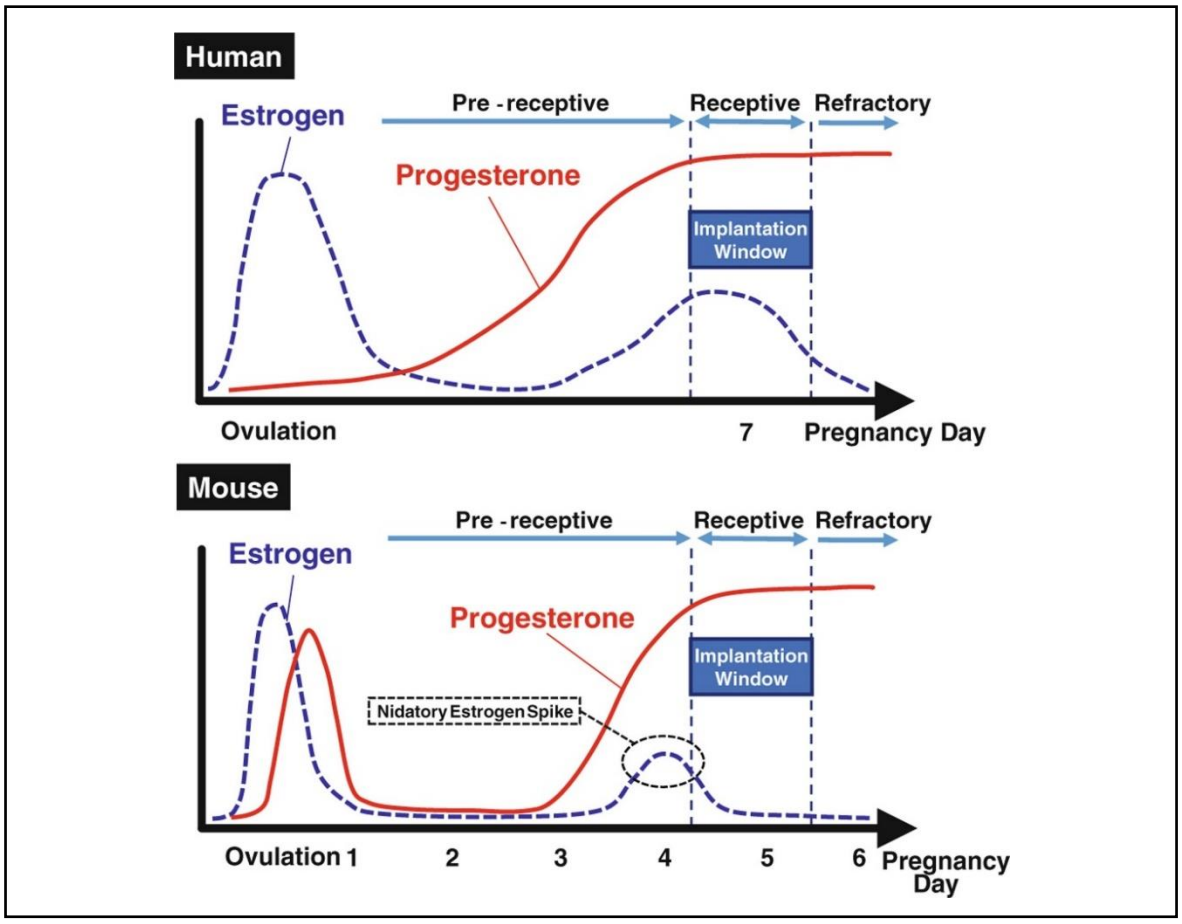

Figure 1.4. The hormonal status during early pregnancy in human and mouse (Adapted from Egashira & Hirota, 2013).

1.3.2 Steroid hormone receptors during early pregnancy

It has been known that estrogen, progesterone, and androgen act via their receptors, estrogen (ERα/β), progesterone (PRα/β), and androgen receptors (AR) respectively. They regulate cell differentiation, proliferation, and secretory protein production in the uterus (Critchley & Saunders, 2009).

1.3.2.1 Role of Estrogen receptor

Estrogen plays a critical role in the uterus during early pregnancy (explained earlier). The genetically induced knockout mice model of ERα and ERβ knockout demonstrated that the ERα is required for uterine receptivity (Buchanan et al., 1999; Cooke et al., 1997; Lubahn et al., 1993), particularly in the estrogen-induced growth of the uterine tissue. During the initial day (Gestation days 1 and 2), the estrogen receptor α is confined in the luminal and glandular epithelium while on gestation days 3 and 4, ERα is localized in the stroma of the uterus. This

study indicates the specific compartment-dependent regulation of the estrogen receptor seems to determine the window of implantation (Tan et al., 1999). In the uterine epithelial cells, deletion of the ERα causes infertility, although, it does not block the estrogen-induced epithelial cell proliferation (Winuthayanon et al., 2010). In the same direction, studies using tissue recombination have demonstrated that the ERα activity in the stromal cells induces the proliferation of the epithelium through paracrine action (Cooke et al., 1997). Additionally, it is reported that uterine decidualization is under the control of ERα/β via epithelial-stromal cross-talk during early pregnancy (Pawar et al., 2014).

1.3.2.2 Role of Progesterone receptor

The "hormone of pregnancy", Progesterone mediates its effect on the uterus via its receptor and is necessary for decidualization and embryo implantation (Lydon et al., 1995). The study using PR null mouse strains has exhibited that uterine stromal cells are the intermediates of progesterone repressive effects on the estrogen-activated proliferative response of the uterine epithelium (Kurita et al., 1998). Progesterone receptor has two isoforms (PR-A and PR-B), PR-A is reported to have a critical function during early pregnancy, especially in the switching of the endometrium proliferation and differentiation. On the other side, systemic deletion of the PR-B did not cause any deleterious effect on the pregnancy outcomes (Lydon et al., 1995; Conneely et al., 2002), thus suggesting that isoform PR-A signalling have a crucial role in pregnancy role in endometrium function.

Steroid hormones and their signalling coordinate uterine function in a spatiotemporal manner via a variety of paracrine, juxtacrine, and autocrine components. The downstream signalling pathways involved in the formation of a healthy pregnancy are discussed further below.

1.3.3 Adhesion phase of implantation

Adhesive signalling systems are accurately thought to be required for the attachment of the embryonic trophoblast to the receptive endometrium. During embryonic development, cells engage in interactions with one another and their surroundings, creating cell junctions and interactions with the extracellular matrix (ECM), respectively. One of the most common classes of ECM receptors is integrin protein which mediates cell-cell and cell-matrix interactions, permitting cell and tissue formation (Darribére et al., 2000). Twenty years ago, it was discovered that the endometrium expresses integrin αvβ3 in a menstrual cycle-dependent manner. Maximum levels were reported in the mid-secretory phase, implying that it may

function as an embryo adhesion receptor during implantation (Lessey, 1992a). Integrins are made up of two distinct, non-covalently coupled subunits and. i.e, α and β, which can be found in the epithelium and stroma, and have been identified as one of the markers of endometrial receptivity (Lessey et al., 1995). A transmembrane region, an intracellular domain, and an extracellular domain make up each subunit. Integrins are heterodimers that create specified combinations, around 24 distinct integrin molecules are formed by the combination of 18 α and 8 β subunits (Hynes, 2002). Also, three integrin isoforms exist: α1β1, α4β1 and αvβ3 (Thomas et al., 2002). Among these, αvβ3 integrin has been shown to interact with trophoblasts throughout the uterine receptivity period (Apparao et al., 2001). Due to the presence of αvβ3 ligands in both the human embryo and endometrial epithelium, integrin αvβ3 is well-studied integrin in terms of implantation (Casals et al., 2012).

There is evidence to link lower or delayed epithelial expression of αvβ3 with decreased fertility. Since then, numerous clinical investigations have assessed αvβ3 expression and its timing in infertile or assisted reproduction (Lessey et al., 1995; Lessey et al., 1992). Further, it was observed that embryos derived from integrin β3 mutant mice fail to implant, because of their inability to adhere to or infiltrate the endometrium's basement membrane (Brakebusch et al., 1997). Moreover, it has been reported that the effectiveness of embryo implantation can be decreased by administering blocking antibodies or arginine-glycine-aspartate (RGD) peptides that target the ligand recognition site of αvβ3 to the uterine cavity of mice and rabbits (Kang et al. 2014), suggesting the importance of the integrin signalling during receptivity phase. The regulation of the integrins throughout the endometrium cycle is mediated by ovarian steroid transmission wherein integrin αvβ3 in the human endometrium demonstrates an increase in expression during the mid-secretory phase (Daftary et al., 2002). Indeed, αvβ3 is up-regulated in immature ovariectomized rats in response to exogenous progesterone or a combination of oestrogen and progesterone, demonstrating that maternal hormones, particularly progesterone, influence αvβ3 epithelial expression (Srinivasan et al., 2009). All of these findings suggest that the adhesion mechanism represents the first phase during early pregnancy and is regulated by ovarian steroid production.

1.3.4 Decidualization during the establishment of pregnancy

Following the embryo-uterine contact, decidual cells are specialized cell types that develop from the substantial proliferation and differentiation of the stromal cells that surround the implanting blastocyst, eventually, the embryo is embedded in the antimesometrial stromal

bed. This process is known as decidualization (Cha et al., 2012a). Before the development of a functional placenta, the decidua supports the developing embryo's nutritional needs. This process begins in humans in the middle of the secretory phase of each menstrual cycle due to result of high progesterone levels. If pregnancy occurs, the increased levels of progesterone will keep the decidua intact to ensure that the pregnancy will continue (Coulam, 2016; Zhang & Liao, 2010). During decidualization, numerous signalling molecules, including cytokines, homeobox transcription factors, cell-cycle molecules, extracellular matrix remodelling factors, and lipid mediators, are expressed in the endometrium and are critical to the process (Dey et al., 2004). In mice, the blastocyst often serves as the stimulation for decidualization (Cha et al., 2012a), the two Abdominal-B-like Homeobox genes Hoxa10 and Hoxa11 (evolutionarily conserved transcriptional regulators), are expressed in uterine stromal cells during decidualization. They play a crucial role in this process, and the majority of $Hoxa10^{-/-}$ females are sterile, because of lower stromal cell proliferation and subsequent failure to decidualize (Hsieh-Li et al., 1995; Lim et al., 1999). In the human uterus, Hoxa10 and Hoxa11 are both increased during the secretory phase, suggesting that they may play a role in uterine receptivity (Lim et al., 1999). In terms of the pathophysiology of HOX genes in human implantation, expression of HOXA10 and HOXA11 in the endometrium increases dramatically during the mid-luteal phase, when the uterus is receptive to embryo attachment (Gui et al., 1999) and is markedly decreased in infertile women (Fischer et al., 2011; Matsuzaki et al., 2010).

Steroid hormones are the central player in decidualization wherein PR-mediated ovarian progesterone signalling is required for uterine development and decidualization (Conneely et al., 2002). Additionally, research demonstrates that inhibiting P450 aromatase, a crucial decidual enzyme that transforms testosterone into estrogen, suppresses decidualization, indicating that normal decidualization requires de novo-produced estrogen in the uterus (Simpson et al., 1994; Das et al., 2009). Thus, it can be noted that the ovarian steroid-stimulated procedure of decidualization is vital in the maintenance of pregnancy.

1.3.5 Invasion of an embryo during early pregnancy

The successful implantation of the embryo is dependent on the synchronised development of both the embryo's invasiveness and the endometrium's receptivity. This process is accompanied by extensive degradation and remodelling of the extracellular matrix (ECM). ECM breakdown is mediated by three enzyme families: plasminogen activators (PAs),

cathepsin, and matrix metalloproteinases (MMPs) (Zhang et al., 2004). Recent research has identified MMPs, specifically MMP-2 and MMP-9, as the primary rate-limiting enzymes in ECM remodelling following implantation (Fata et al., 2000). The tissue inhibitors of MMPs (TIMPs), which are MMPs endogenous inhibitors, strictly regulated the activities of MMPs. The intricate balance between MMP activation and TIMP inhibition is critical for embryo implantation regulation (Leco et al., 1996). During mouse embryo implantation, there is also a unique and cell-specific pattern of MMP-2, 9, and TIMP-1, 2, 3 have been observed. In particular, MMP-9 mRNA is specifically found in many trophoblast large cells and invades the uterine stroma on days 6.5–8.5 of pregnancy while TIMP-1 and 2 mRNA are not seen in the embryo, trophoblast, or decidua, however, TIMP-3 mRNA is strongly abundant in maternal cells in the region of the decidual reaction surrounding the day 5.5 - 6, and this reduces considerably by day 7.5 (Zhao et al., 2002). Also, it has been reported that endometrial extracts from early pregnant rats demonstrate a coordinated rise in MMP2 activity and demonstrate that this activity is predominantly located at the sites of implantation or uterine mucosal stromal cells outside the decidualized area (Novaro et al., 2002). The significance of this type of tightly expressed patterns of proteases and their inhibitors gives fine-tuning to the embryo invasion at the materno-fetal interface. The importance of the MMPs has been demonstrated in one of the studies in which the administration of the MMP inhibitor to rats alters numerous aspects of placental formation, including the width and length of the decidual zones and embryo implantation displacement (Rechtman et al., 1999). For the establishment of implantation, regulation of the local expression and activity of MMPs may be essential. In this direction, at the transcriptional level, inflammatory cytokines including interleukin-1 and tumor necrosis factor-α promote the production of MMPs whereas progesterone inhibits its expression. Tissue inhibitors of metalloproteases (TIMPs) are produced locally and specifically inhibit active forms of MMPs in the extracellular space. Progesterone was found to stimulate TIMP-3 expression in the early stages of pregnancy indicating that TIMP-3 may play a key role in regulating the trophoblast invasion (Rawdanowicz et al., 1994; Curry & Osteen, 2003; Higuchi & Fujita, 1995). Although, it is interesting to note that molecular control of the proteases/TIMPs during early pregnancy remains unclear.

1.3.6 LIF-STAT signalling during early pregnancy

The interplay of different transcription factors, cytokines, and growth factors regulates embryo implantation. Spatial and temporal expression studies have shown that these factors

are expressed in several uterine compartments, including luminal epithelium, glandular epithelium, and stroma, overlapping the window of implantation (Bazer et al., 2009; Aghajanova et al., 2008). Leukemia inhibitory factor (LIF), is an IL-6 family pleiotropic cytokine that is crucial for embryo implantation and is abundantly expressed in the mouse uterus during the receptivity phase (Stewart, 1992). LIF controls a number of cellular processes by interacting with both gp130 and the membrane-bound LIF receptor (LIFR). When LIF binds to LIFR, it recruits gp130 to form a high-affinity functional receptor complex, which activates downstream signal transduction pathways such as signal transducer and activator of transcription (STAT) (Mathieu et al., 2012; Sun et al., 2013). The failure of blastocysts to implant has been reported in mice lacking the LIF gene. Additionally, mice with both a gp130 mutation and a STAT-binding site deletion are sterile, demonstrating the importance of both gp130 and STAT in controlling LIF function (Sun et al., 2013). In mice, uterine LIF has a biphasic expression pattern, with the first peak occurring in the glands in preparation for uterine receptivity and the second peak appearing in the stroma surrounding the implanting blastocyst during the attachment reaction. Also, throughout the implantation period, the luminal epithelia and stroma express LIFR and gp130 simultaneously. This emphasises the importance of LIF in the early events of embryo implantation (Song & Lim, 2006). Ovarian steroids are thought to play a significant role in regulating LIF, LIFR, and gp130 expression in the uterus during early pregnancy. During the establishment of pregnancy in mice, obligatory estrogen can cause endometrial LIF secretion while exogenous estrogen and progesterone treatment to ovariectomized mice increased gp130 expression in the uterine glands. However, a study found that luteal estrogen was not necessary to start the implantation process in humans (Wakitani et al., 2008; Shuya et al., 2011). There is currently less information available about the control of LIF, LIFR, and gp130 expression in humans. Several lines of clinical data suggested that the analysis of uterine luminal fluid revealed that the endometrium of infertile women secretes considerably less LIF and gp130 than normal fertile women between luteal day (LH) 6 and 13, which corresponds to the implantation window (Tawfeek et al., 2012). Cumulatively, the role of LIF and its signalling has been demonstrated to mediate multiple steps of early pregnancy, including blastocyst development and growth, uterine preparation for implantation, decidualization, and embryo-endometrial interaction/fusion.

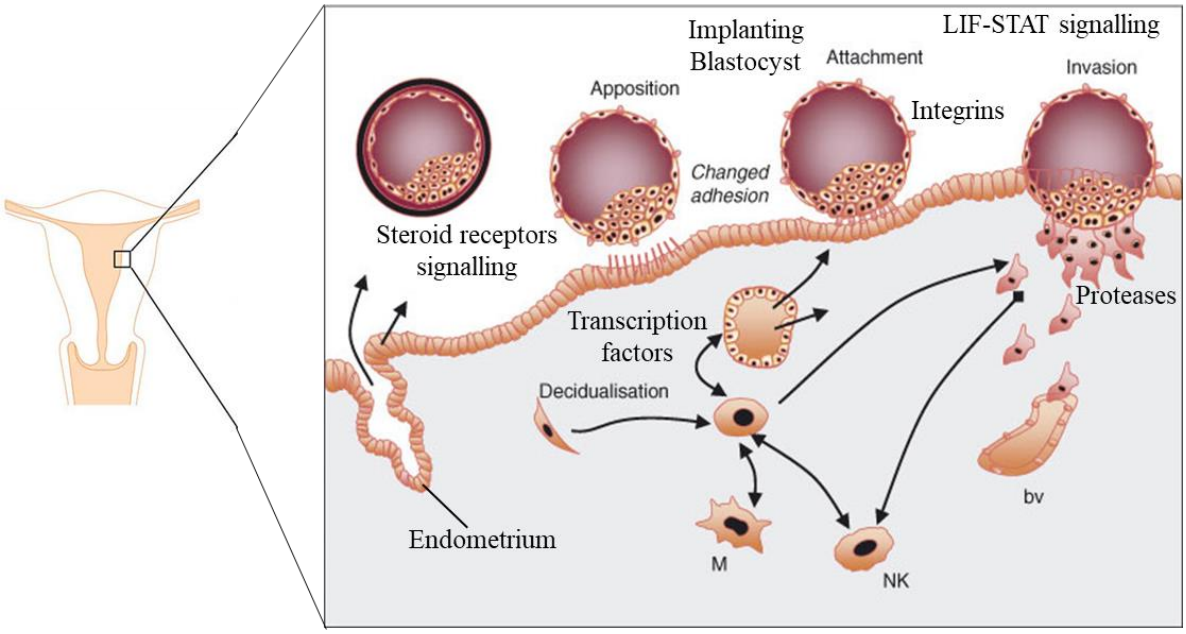

Figure 1.5. Early pregnancy stages and molecular signals involved in each stage.

The triad for reproduction success is the embryo, the endometrium, and their interactions (**Figure 1.5**) and the success of each event is essential to advance toward the next stage, but failure to achieve, which is common in PCOS, could lead to aberrant pregnancy outcomes. the complications associated with PCOS pregnancy are explained in the following section.

1.4 Pregnancy complications in Polycystic Ovary Syndrome

The experimental findings indicated that even after conception is achieved, women with PCOS are at risk of late pregnancy complications, compromising pregnancy-induced hypertension, gestational diabetes, pre-eclampsia, preterm delivery, and fetal growth abnormalities (Palomba et al., 2015a). In addition, they are also at risk of early pregnancy complications, including miscarriage, and early pregnancy loss, when compared to women without PCOS (Khomami et al., 2022; Bu et al 2020). A high prevalence of Early pregnancy loss is found in women with PCOS both after spontaneous and induced ovulation (Spuy & Dyer, 2004). From the clinical point of view, the risk elements connected to pregnancy complications in PCOS are hyperandrogenism, metabolic cofactors like obesity, hyperinsulinemia/insulin resistance, and other factors of endometrium functions associated with infertility (Palomba et al., 2015a).

1.4.1 Pathogenesis of early pregnancy loss in PCOS

The success of the establishment of pregnancy requires the interaction of the embryo with the endometrium. However, the difference in oocyte development and altered endometrium function could hamper embryo-endometrium cross-talk, which is observed in women with PCOS (**Figure 1.6**).

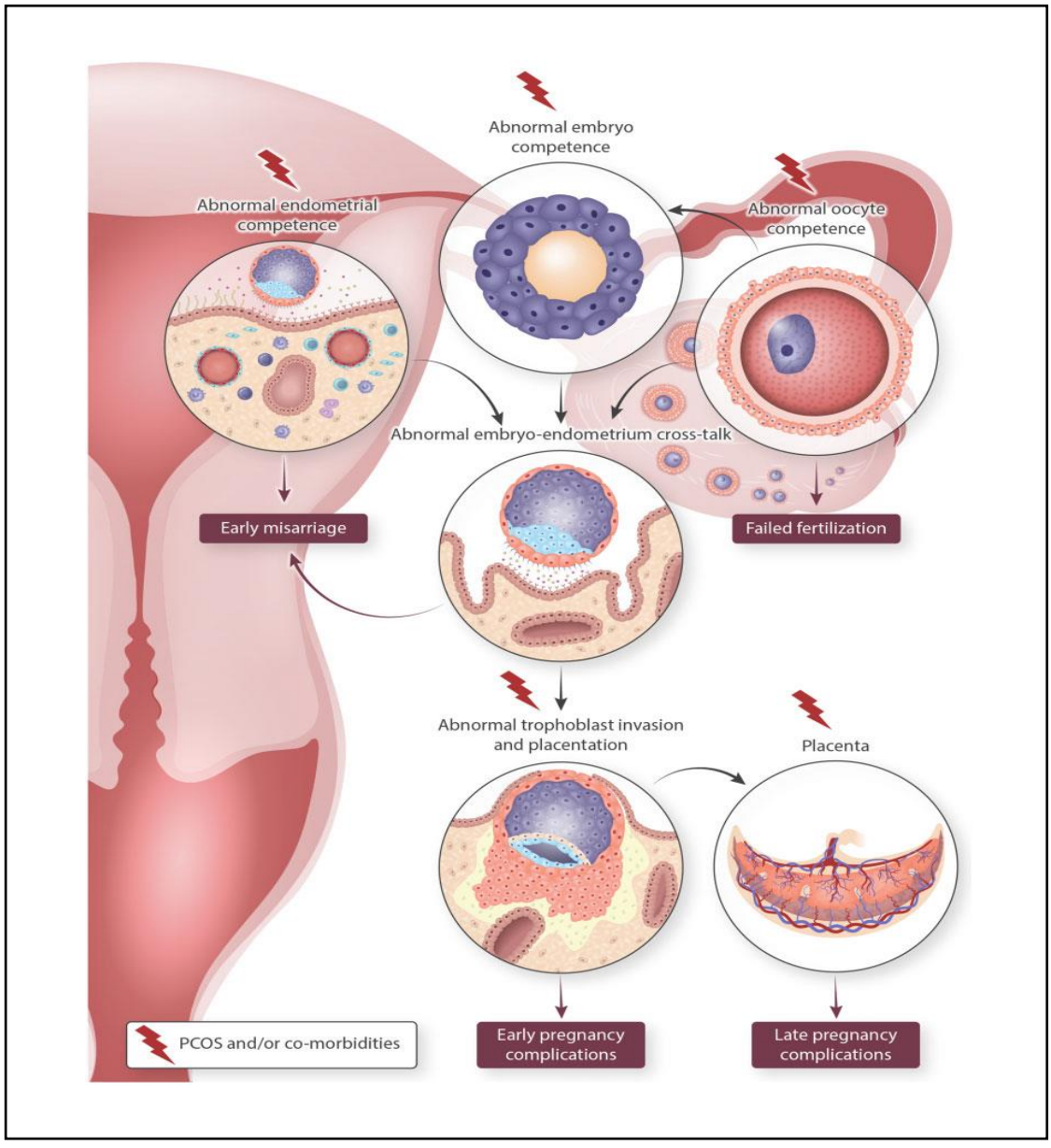

Figure 1.6 Diagram representing the PCOS co-morbidities and pregnancy complications (Adapted from Palomba et al.,2021).

1.5 Endometrium abnormalities in PCOS

Anovulation and oligo-ovulation are in high proportion in PCOS women (Azziz et al., 2006; Franks, 2006). Therefore, the endometrium in the patient with PCOS is discrete from the healthy women. An increase in estrogen, androgen circulating levels and withdrawal of progesterone content have resulted from chronic anovulation/oligo-ovulation is suboptimal or absent in the endometrium. Thus, the endometrium is exposed to the prolonged stimulatory reaction of estrogen without suppression of the progesterone effect (Shang et al., 2012a). On days 6 to 10 of the menstrual cycle, the ultrasound of the endometrium tissue demonstrated that the thickness of the endometrium in PCOS patients is significantly higher compared to the women without PCOS (Iatrakis et al., 2006). Thus, in PCOS, growth, and differentiation of the endometrium are promoted by higher estrogen and androgen, in the absence of progesterone opposition. Due to the unopposed effect of progesterone, the endometrium did not experience the secretory transformation and is constantly exposed to the mitogenic effects of estrogen causing endometrial overgrowth (Lopes et al., 2011).

1.5.1 Primary endometrial function in PCOS

Both ovulatory and anovulatory PCOS women have an abnormal expression of proteins involved in cell cycle regulation, cellular transport and signalling, DNA repair, apoptotic processes, and mitochondrial metabolism, indicating a disruption of several cellular processes leading to compromised endometrial receptivity (Rashid et al., 2020), demonstrating that primary abnormalities are independent to ovulatory function.

1.5.1.1 Steroid receptors function in PCOS-endometrium

In general, the endometrium of PCOS women is more sensitive to estrogen. This is owing to the existence of high levels of ER-α in anovulatory PCOS women as opposed to proliferative endometrium in non-PCOS women. However, recent research has found that the expression of ER-α and β is lower in the secretory endometrium of ovulatory PCOS women than in controls (Wang et al., 2011; Villavicencio et al., 2006). Increased sensitivity to estrogen is also owing to the increased intracrine synthesis of estrogenic compounds, such as estrone (E1) converted to estradiol (E2), or estrogenic molecules, such as dehydroepiandrosterone (DHEA) converted to androstenediol (Wang et al., 2011). These are caused by the downregulation of 17- β hydroxysteroid dehydrogenase (17β-HSD) and the overexpression of HSD type 1 (Plaza et al., 2010). Moreover, Endometrial dysfunction is caused by increased

ER-a activation mostly due to infrequent or absent apoptosis and concurrent enhanced cell proliferation in the mid-secretory endometrium. The effects of estrogen are likewise linked to altered progesterone effects in PCOS endometrium (Quezada et al., 2006) is explained below.

The expression of progesterone receptor mRNA is altered in the endometrium of women with PCOS, more in the epithelia than in the stroma (Quezada et al., 2006). PR-α expression is lower in the proliferative endometrium of PCOS women, although PR-β expression is higher when compared to healthy women with regular ovulatory cycles (Paulson et al., 2017). This could be due to the above-mentioned extended action of estrogen on the PCOS endometrium. The endometrium of women with PCOS exhibits progesterone resistance, a complex mechanism linked to the altered quantitative and qualitative expression of progesterone receptors (Paulson et al., 2017). In particular, the effects of hyperandrogenism on the expression and function of PR suggest and support the concept that progesterone resistance in women with PCOS is attributable to inactive or less active isoforms of PR resulting in aberrant endometrium function during pregnancy (Babayev et al., 2017; Young, 2018; Su et al., 2012).

Further, hyperandrogenic women with PCOS have increased AR mRNA and protein expression in the epithelial cells of the endometrium compared to healthy controls. The increased AR expression could be attributed to the synergistic action of estrogen and higher serum androgens. Poor female fertility has been linked to high serum androgen levels and the presence of AR in the endometrium (Shang et al., 2012a). Thus, elevated androgen levels in women with PCOS may thus have a deleterious effect on endometrial function by leading to suspected deficiencies in uterine receptivity and contributing to infertility. In summary, the endometrium of PCOS women is characterized by a predominance of E/ER and A/AR action and a compromised P/PR action. This may result in a disturbance of the tissue's regular, cyclical hormonal response, which primes it for embryo implantation.

1.5.2 Secondary endometrial function related to PCOS characteristics

The endometrium in PCOS women can be altered by PCOS traits such as hyperandrogenism, menstrual abnormalities, polycystic ovaries, and by clinical and biochemical changes associated with the disease such as obesity and insulin resistance.

1.5.2.1 Hyperandrogenism

Experiment results confirm a link between high levels of androgen, particularly testosterone and androstenedione, and recurrent miscarriages in PCOS (Rahman et al., 2018). Furthermore, PCOS individuals with complicated pregnancies had significantly higher blood androgen concentrations and insulin sensitivity indexes than participants without any pregnancy and/or neonatal issues (Falbo et al., 2010; Palomba et al., 2015a). Women with PCOS who are hyperandrogenic exhibit aberrant endometrial growth wherein high serum levels of testosterone can downregulate endometrial homeobox A10 (HOXA10), a homeobox transcription factor necessary for developmental regulation of endometrial differentiation. (Daftary & Taylor, 2006; Cermik et al., 2003). Data on pregnant mice treated with DHEA as a PCOS model also demonstrated a compromised LIF-signal transducer and activator of the transcription 3 (STAT3) pathway and showed that its downregulation is directly related to implantation failure (Li et al., 2016). In addition, endometrial pinopodes, which are protrusions of the endometrial apical plasma membrane linked with endometrial receptivity, are affected by high androgen levels (Palomba, 2021). High serum androgen could also affect the decidualization process during embryo implantation and trophoblast invasion, by impairing the oxidative stress response and influencing the expression of genes (such as prolactin (PRL), Forkhead box protein O (FOXO)-1, and SOD-2) involved in oxidative stress resistance in decidualized endometrial cells, (Kajihara et al., 2012). Also, experiment results show that hyperandrogenism can impair endometrial function, either directly or indirectly through hyperinsulinemia wherein high levels of testosterone and DHT alter the endometrial expression and/or phosphorylation of proteins involved in the insulin signalling system. These findings exhibited impaired glucose transport and metabolism (Lee et al., 2019; Rivero et al., 2012). However, whether it has direct or indirect effects are still unknown.

1.5.2.2 Polycystic ovarian morphology

Polycystic ovarian morphology (PCOM) is correlated with high estrogen concentrations produced by a large number of antral follicles and the consequences of hyper estrogen on the endometrium, including direct and indirect effects (through upregulation of AR and enhanced progesterone resistance), have already been discussed above. PCOM is also associated with high levels of anti-Mullerian hormone (AMH), a protein mostly produced by ovarian granulosa cells (Dewailly et al., 2014). Women with PCOS have 2- to 3-fold greater circulating AMH levels than non-PCOS women (Piltonen et al., 2005). The expression of the

AMH receptor (AMHR) and AMH itself in the endometrium suggests that AMH works on endometrial tissue in endocrine, autocrine, and paracrine ways (Dewailly et al., 2014). In this direction, it has been reported that high AMH concentrations in the PCOS endometrium may impair endometrial cell survival via pro-apoptotic actions (Signorile et al., 2014). Surprisingly, new research from PCOS populations revealed a higher risk of premature birth in women with high AMH concentrations. Also still there is a debate going on regarding this evidence (Hsu et al., 2018; Hu et al., 2020).

1.5.2.3 Obesity/Insulin resistance (IR)

Because 50-70% of women with PCOS are overweight or obese, adiposity has a significant role in PCOS pathologies, including endometrial function (Dumesic et al., 2015). Obesity induces IR and hyperinsulinemia, hyperandrogenism, low-grade inflammation, and oxidative stress, compromising decidualization and the implantation process, due to altered MAPK/ERK pathway activation (Hu et al., 2020). Obesity, on the other hand, can impact and enhance insulin signalling and glucose metabolism in the PCOS endometrium independently of hyperinsulinemia (Mozzanega et al., 2004). Furthermore, free fatty acid accumulation is harmful to the endometrium, and palmitic acid has been found to directly affect the decidualization of human endometrial stromal cells (Rhee et al., 2016). In the endometrium, GLUT-4 mediates insulin sensitivity and glucose homeostasis. Insulin binds to its receptor, activates InRS, and then promotes glucose absorption via GLUT-4. Hyperandrogenism impairs endometrial function by influencing insulin and glucose actions. T decreases glucose tolerance by triggering IR and lowering the expression of InRS-1 and GLUT-4 in uterine glandular epithelial cells (Zhang & Liao, 2010). This is most likely related to decreased phosphoinositide 3-kinase (PI3K)/Akt pathway activation (Ormazabal et al., 2013). To corroborate these findings, (Fornes et al., 2010) discovered that overall InRS-1 levels are lower in PCOS endometrium, with a decrease in its active (pTyr) phosphorylated form and an increase in its inactive (pSer) phosphorylated form (Oróstica et al., 2020). Taking into account the possibility that endometrial functions and implantation in PCOS could have been negatively impacted by insulin signalling and glucose homeostasis.

In addition to the endometrial dysfunction, and due to the impact of hyperandrogenism and hyperinsulinemia on the intrafollicular milieu of the developing ovarian follicles and on the oocyte, these conditions may potentially contribute to miscarriage and implantation problems.

1.5.2.4 Oocyte quality

Genomic data show differences in global transcriptomic signatures in oocytes from PCOS women that are relevant to changing meiotic processes, intra-follicular oxidative stress, glucose, and lipid metabolism regulation, and cross-talk between developing oocytes and surrounding somatic cells during folliculogenesis (Palomba et al., 2017). In actuality, PCOS-afflicted women exhibit aberrant expression of a number of biomolecules with regulatory roles in oocyte development, such as growth factors, cytokines, deaminases, metalloproteinase, hormones, adipokines, and lipidases (Qiao & Feng, 2011a; Ambekar et al., 2015). The most convincing evidence for PCOS oocyte quality/competence in achieving pregnancy has come from the donor oocyte model, which produced results that were comparable with those of oocytes from women without PCOS. In PCOS and non-PCOS oocyte donor cycles with embryo transfers (ETs) to women with premature ovarian insufficiency, no differences in fertilization rates (11% vs 13%), implantation rates (69% vs 67%) or clinical pregnancy rates (28% vs 26%) were observed with embryos from PCOS versus non-PCOS women (Vaz et al., 2016), indicating that the lower clinical pregnancy rates seen in PCOS cannot be caused by oocyte quality issues. Although, the evidences regarding aberrant oocyte quality/competence cannot be excluded.

However, it is still unclear whether the decreased pregnancy rates are caused by defective embryos that do not implant or by endometrial changes that prevent implantation. Therefore, it should be highlighted that PCOS women may experience a decreased conception rate as a result of anomalies in their uterus and/or embryos. However, there aren't enough pieces of information regarding molecular changes in the embryo-containing uterine milieu. In this line, different approaches have been attempted to alleviate these problems in PCOS. However, ethical constraints and overlapping expression patterns during early pregnancy make it challenging to mimic their stage-specific role in humans and *in vitro*. Thus, understanding the pathophysiology of early pregnancy complications in PCOS can be addressed by using *in vivo* study. Thereby, the present study attempted to understand molecular signatures of early pregnancy using *in vivo* PCOS mouse model.

1.6 Letrozole-induced PCOS model

Letrozole is a non-steroidal aromatase inhibitor that 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). The hormone profile of these animals demonstrated significantly elevated testosterone and LH levels along with reduced estradiol and progesterone levels (Kafali et al., 2004; Shi & Vine, 2012). Additionally, a previous lab study reported that female rats and mice treated with letrozole (given orally for 21 days) developed dyslipidemia, hyperinsulinemia, and insulin resistance (Dey et al., 2022; Radha & Laxmipriya, 2016a; Desai et al., 2012a). Thus, the letrozole-induced PCOS model possesses reproductive and metabolic characteristics of the human PCOS condition, making it a desirable model for use in PCOS research.

Based upon the previous discussion on etiopathology of the impaired endometrial-embryo cross-talk in the PCOS phenotype, many medical and nonmedical methods targeting the endometrium have been investigated, to further enhance the reproductive outcome in PCOS. However, the majority of the clinical data are based on interventions toward better ovarian function, ovulation, and oocyte quality. In order to ascertain the "pure" impact of any intervention on endometrial function, more experimental results are required. The following sections will discuss the intervention strategies for management of the pregnancy complications in PCOS.

1.7 Lifestyle change initiatives

The lifestyle modification initiatives primarily consist of a hypocaloric diet and/or regular physical activity. These results can effectively help in maintaining symptoms to a certain extent, but it is unlikely to have a direct effect on the endometrium because sex hormone-independent endometrial indicators of implantation are unaffected by this intervention (Kataoka et al., 2019). Apart from lifestyle changes, several drugs have been prescribed for the management of this pathology.

1.8 Drugs

1.8.1 Insulin-sensitizing drugs

Many insulin-sensitising drugs (ISDs) have been used to treat PCOS in women (Checa et al., 2005). ISDs like metformin, rosiglitazone, troglitazone, pioglitazone, inositol, and irisin may be helpful in PCOS patients because they have positive effects on metabolic levels or directly on peripheral targets such as endometrium. But it is interesting to point out that more research is needed before these drugs can be used clinically in the management of endometrial

receptivity and safety in PCOS. Although, out of the all listed ISDs, metformin has been widely researched in PCOS women (Palomba et al., 2009) and has beneficial effects on endometrial function (Palomba, 2021).

1.8.1.1 Metformin

Metformin hydrochloride is the active ingredient in this medication, and it has anti-hyperglycemic properties (Lashen, 2010). It is known to enhance ovulation and menstruation frequency in anovulatory PCOS patients when compared to placebo, however, the benefit of metformin in addition to ovulation induction medications is still unclear (Palomba et al., 2009; Morley et al., 2017; Sharpe et al., 2019). Also, it is debatable whether metformin should be continued during pregnancy and when it should be started. Despite the fact that the research suggests that metformin is safe to use during pregnancy (Cassina et al., 2014; Panchaud et al., 2018; Scherneck et al., 2018). Further, clinical evidence suggests that metformin may be used to minimize the risk of miscarriage in the presence of hyperandrogenism (Jakubowicz et al., 2001; Palomba et al., 2009). Indeed, the greatest benefit has been found when metformin is provided before to pregnancy/prior to implantation, emphasising metformin's effects on the endometrial environment. In this direction, some of the direct or indirect benefits in the endometrium of PCOS patients have been provided in **figure 1.7.**

1.8.2 Ovulation induction agents

Clomiphene citrate is commonly used as a first-line treatment for ovulation induction with gonadotropins as a second-line approach (Wang et al., 2017; Palomba et al., 2015b). Besides, Clomiphene citrate treatment, even when accompanied by normal ovulatory function, causes a significant prevalence of endometrial side effects due to its anti-estrogenic qualities (Homburg et al., 2006). In the large Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) trial, the treatment of ovulating agents was compared in patients with PCOS exhibiting the efficacy of all treatments (ovulation) with particular regard for gonadotropins. However, the probability of multiple pregnancies was higher with gonadotropins than with ovulating agents (Diamond et al., 2015).

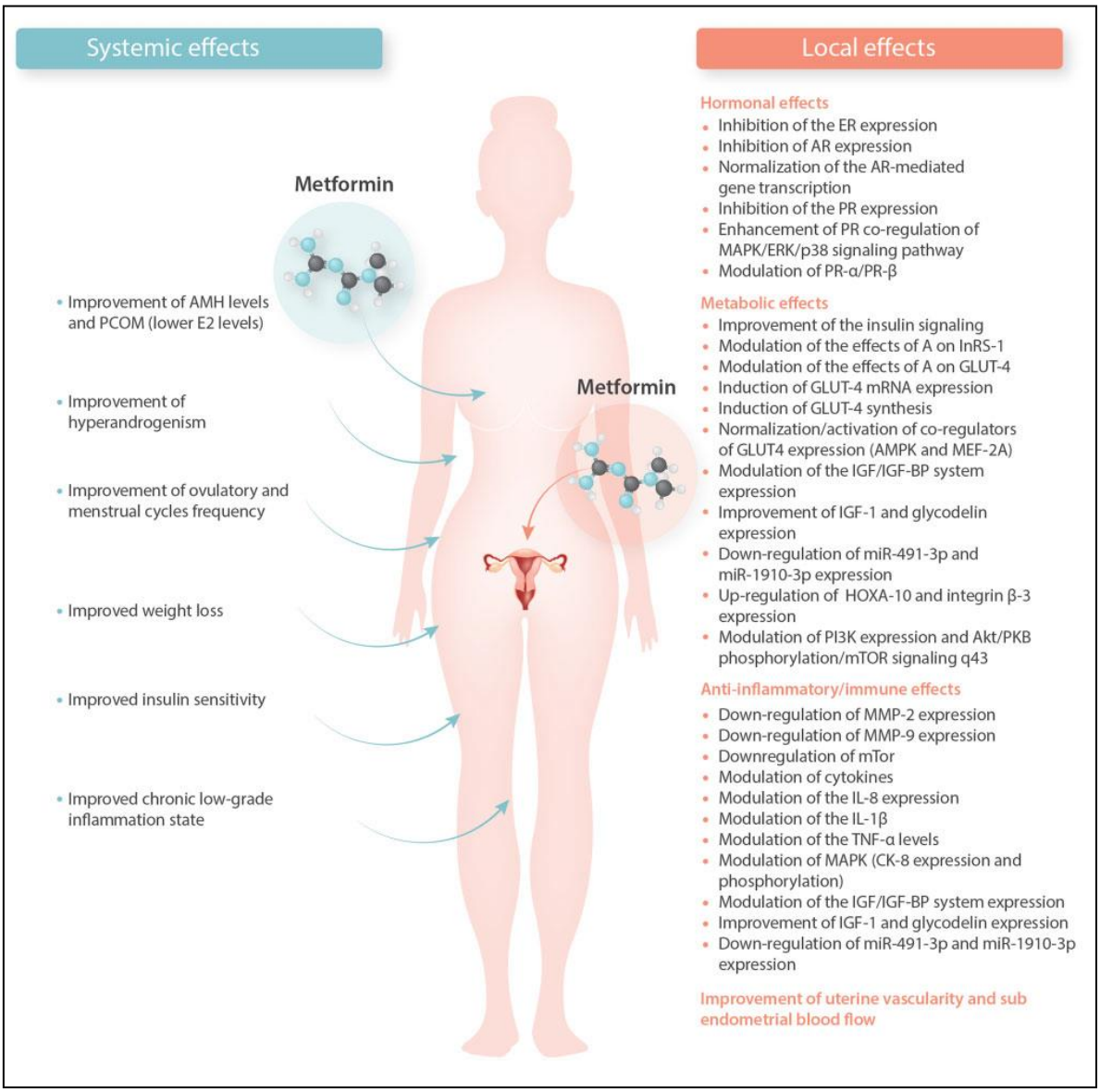

Figure 1.7. Metformin's indirect (systemic) and direct (local) actions on the endometrium in women with PCOS (Adapted from Palomba et al., 2021).

1.8.3 Anti-androgens

In view of the detrimental effects of hyperandrogenism in the endometrium of PCOS women, anti-Androgen therapy may be useful in restoring normal endometrial function in hyperandrogenic women with PCOS. A more recent mouse study found that flutamide (a nonsteroidal Androgen receptor blocker) reduces the harmful effect of high testosterone on the endometrium in terms of reabsorbed embryos (Gong et al., 2019). A decrease in the endometrium total AR protein could be the mechanism underlying this impact. However,

flutamide's effects on the endometrium may possibly manifest through more intricate pathways (Apparao et al., 2002). Due to its anti-progestin effect, preliminary investigations showed that flutamide can negatively impact decidualization and trophoblast invasion when administered in the early stages of pregnancy (Chandrasekhar et al., 1990; Dukes et al., 2000). In addition to the above facts, all anti-androgens are teratogenic medications with negative effects on a male fetus and so cannot be used in pregnant women (Goodman et al., 2015).

From all of the above-discussed strategies for the improvement of implantation and pregnancy rates in PCOS, it can be noted that the mode of action or efficacy underlying these observations have yet to be elucidated. Furthermore, it has been observed that the prolonged usage of these drugs shows adverse effects such as rosiglitazone can cause skin flushing, alopecia, heartburn, liver failure, edema, chest pain, and hypoglycaemic shock leading to coma (Sepilian & Nagamani, 2005), long-term use of clomiphene can result in to increase in ovarian size and cyst, due to its vast list of negative effects, this medicine is currently prohibited and should not be used. Additionally, an increased risk of vascular thromboembolism and digestive complications such as nausea, diarrhea, dizziness, and vitamin B12 deficiency was observed upon prolonged usage of metformin (Saha et al., 2012; Lashen, 2010). Moreover, the above section has already covered the negative effects of these medications on endometrial function and pregnancy outcomes. As a result, there is a need to find a more potential healthcare therapy that is safer, more tolerated, efficient, and economical than the present pharmaceutical approaches.

In this context, herbs have been utilised as ayurvedic remedies since the beginning of time (Sasikala et al.,2010). Nutraceuticals are one such beneficial and quickly emerging medicine (Herbal remedies). Herbal medicines are recognised to contain pharmacologically active components with physiological effects on female endocrinology (Arentz et al., 2014). Therefore, herbal remedies are currently being researched scientifically for their application in multi-etiology metabolic endocrinopathies such as PCOS.

1.9 Herbal therapy for PCOS

Numerous therapeutic herbs have been researched in relation to PCOS. **Table 1** highlights a few of the potential medicinal plants utilized in Ayurveda for the treatment of PCOS.

Table 1. Plant extracts used in the treatment of PCOS in rodent models.

Sr no.	Plant	Effects in PCOS	Reference
1	*Trigonella foenumgraecum* (Fenugreek)	Improved steroid hormone levels	(Lodhi et al., 2021)
2	*Withania somnifera* (Ashwagandha)	Increase in FSH levels and decrease in LH levels	(Saiyed et al., 2016)
3	*Cinnamomum zeylanicum* (Cinnamon)	Hypolipidemic impact	(Khare et al., 2016)
4	*Ocimum tenuflorum L* (Holy Basil)	Anti-androgenic properties	(Satapathy et al., 2017)
5	*Taraxacum officinale* (Dandelion Root)	Improve menstrual irregularities	(Wang et al., 2018)
6	*Tinospora cordifolia* (Guduchi)	Lowering insulin resistance	(Chandrasekaran et al., 2012)
7	*Vitex negundo L* (Chinese chaste tree)	Reduced testosterone and glucose	(Shetty et al., 2015)
8	*Alium cepa* (Onion)	Antioxidant capacity and reduced number of ovarian cysts	(Ghasemzadeh et al., 2013)
9	*Commiphora wightii* (Gugal)	Increase in hormone profile and decrease in glucose levels.	(Kavitha et al., 2016)
10	*Asparagus Racemosus* (Shatavari)	Normal development of female ovarian follicles	(Kumar & Dang, 2015)
11	***Aloe barbadensis Miller* (Aloe vera)**	Hypoglycemic, anti-dyslipidemic, antioxidant, and anti-inflammatory properties, improve ovarian-structure function	(Misawa et al., 2012; Desai et al., 2012b; Maharjan et al., 2010a; Radha et al., 2014a)

From the above-reported plant extracts in the treatment of PCOS (Table 1), it can be noted that the results shown in the studies are only been hinted at in preliminary investigations. Also, the endometrium's function, receptivity for embryo implantation, placentation, and pregnancy outcomes have not been highlighted in this research, which should be one of the focuses as the majority of PCOS patients are associated with pregnancy complications. In this line, only a few studies have reported the efficacy of herbs on the endometrium function wherein Zhuyun recipe (Traditional Chinese medicine) has beneficial effects on the pinopods expression on the surface of the endometrium in mice with embryo implantation dysfunction and enhances the number of implantation sites and pregnancy rate (Yu et al., 2015). Also, Chinese herbal medicine has been shown to have an improvement in oocyte quality, increase endometrial blood flow, and support early embryonic development (Cao et al., 2013). However, more research is needed to elucidate the mechanisms of herbal medication in improving embryo-uterine cross-talk in PCOS without any adverse effects.

In this context, ***Aloe barbadensis Miller*** commonly known as *Aloe vera* has been explored in the current study. *Aloe vera* is a succulent perennial herb of the Liliaceae family that is extensively distributed throughout the world's tropical and subtropical regions.

1.9.1 Classification of Aloe vera

Kingdom	Plantae
Division	Angiospermae
Order	Asparagales
Family	Asphodelaceae/ Liliaceae
Genus	Aloe
Species	*Aloe vera*

1.9.2 Traditional Uses of Aloe vera

Aloe vera was first used to treat ailments common in young females, including acne and menstrual problems (Nadkarni, 1976; Risvan et al., 2017). *Aloe vera* is one of the few botanical remedies that are widely used in Western culture and it has found significant use in the cosmetic, pharmaceutical, and food industries (Foster et al., 2011). There are two distinct exudates secreted by *Aloe* leaves. One is a highly cutinized reddish-yellow juice that is bitter and found in the pericyclic cells beneath the epidermis of the leaves. This juice has

historically been used in dried form and as a laxative. As the other exudate, the thin-walled tubular cells in the leaf's parenchyma produce a clear, slippery mucilage or gel. Clinical research has shown that the gel and rind of *Aloe vera* leaves contain the majority of the pharmacologically active substances. The functional compounds of *Aloe vera* have analgesic, antioxidant, anticancer properties, and anti-inflammatory. Also, it helps with burns, allergic reactions, diabetes, rheumatoid arthritis, rheumatic fever, acid reflux, ulcers, skin disorders, dysentery, diarrhea, piles, and inflammatory illnesses of the gastrointestinal tract and other internal organs, including the liver, stomach, kidney, small intestine, and pancreas (Joseph and Raj, 2010).

According to the traditional usage of *Aloe vera*, it is beneficial for the control of the female reproductive system and the complications linked with it, such as PCOS (Nadkarni, 1976; Risvan et al., 2017). Numerous studies have demonstrated the effectiveness of *Aloe vera* gel in modifying metabolic status by showing hypoglycemic, anti-dyslipidemic, and antioxidant, effects (Desai et al., 2012b; Misawa et al., 2012; Tanaka et al., 2006). The abundance of phytochemicals in *Aloe vera* gel accounts for its diverse pharmacological activities. The polysaccharides, glycosides, flavonoids, tannins, chromones, alkaloids, anthraquinones, organic substances, pyrones, phytosterols, anthrones, fatty acids, sterols, terpenoids, hormones, vitamins, proteins, and mineral components are present in *Aloe vera*. Even though traditional usage is described, the scientific investigation remains unclear.

In this line, findings from our laboratory demonstrated that the *Aloe vera* gel (10mg dry weight daily for 60 days) could improve ovarian structure-function and reduce co-morbidities like dyslipidemia and hyperglycemia in the PCOS rat model (Maharjan et al., 2010a; Radha et al., 2014a; Desai et al., 2012b; Radha & Laxmipriya, 2015). Additionally, it was shown by solvent-based extraction of *Aloe vera* gel that oral administration of non-polar petroleum ether extract (NPE) (25 μg/kg body weight for 60 days) in the Letrozole-induced PCOS rat model may significantly boost the metabolic and reproductive problems associated with PCOS. The presence of fatty acids, phytosterols, and terpenoids in the NPE was associated with the observed efficacy (Radha & Laxmipriya, 2016a).

From the previously discussed section and lab studies, it is interesting to note the insufficient scientific data regarding the treatment of indigenous plants when used for the improvement of embryo-endometrium function and/or pre-conceptive therapy in PCOS pathology. With this background, the present study is directed toward understanding the molecular signatures

involved in the embryo-endometrial microenvironment in PCOS phenotype and investigating the efficacy of the phytocompounds present in petroleum ether extract of *Aloe vera* gel in the correction of the embryo-endometrium milieu of PCOS, this could be due to the direct or indirect effect. Thus, the probable molecular interactions of bio-actives present in the petroleum ether extract of *Aloe vera* gel with the key targets of early pregnancy were also examined in this study.

To summarize, PCOS is associated with clinical pregnancy complications, an increased rate of spontaneous abortion/early pregnancy loss, and preterm delivery. However, molecular alterations in PCOS pregnancy that originates from the mother, embryo, or both are still not clear. Thereby, there exists a need for a model system wherein molecular events of early pregnancy could be studied with ease and dissection of pathways. Hence, the first part of the present study emphasizes the probable regulatory mechanism for the organization of early pregnancy events in the letrozole-induced PCOS mouse model. Further, targeting them for therapeutic interventions could help us in the management of early pregnancy complications linked to PCOS pathology. In this direction, the second part of the present study was an attempt to elucidate the therapeutic potential of phytocompounds present in petroleum ether extract of *Aloe barbadensis* when given as a pre-conceptive agent in the improvement of embryo-uterine transmission for the establishment of pregnancy in letrozole induced PCOS mouse model. Also, preliminary prediction of the interaction of molecular targets with important key regulators of pregnancy has been studied to delineate the effects being direct or indirect. ***Thus, the overall aim of the study is to Bio-prospect the petroleum ether extract of Aloe vera gel as a pre-conceptive agent in the management of PCOS pregnancy.***

1.10 Aim of the study

The aim of the current study was to decode the molecular cascade of embryo-uterine modulators in early pregnancy loss of letrozole-induced PCOS mouse model and to elucidate the therapeutic potential of petroleum ether extract of *Aloe barbadensis* when given as a pre-conceptive agent in the improvement of embryo-uterine transmission of PCOS mouse model.

1.11 Specific objectives:

The major objectives of the present study are:

I. To assess the key regulators of early pregnancy in the letrozole-induced PCOS mouse model.
II. To evaluate and validate the role of the non-polar extract of *Aloe vera* gel on early pregnancy in the PCOS mouse model.
III. To identify the targets of the phytocomponents of non-polar extract of *Aloe vera* gel towards modulation of early pregnancy using the *"in-silico"* approach.