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Polycystic Ovary Disease (PCOD)- An Insight into Rodent Models, Diagnosis and Treatments

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1. Abstract

PCOD could be an advanced heterogenous metabolic disorder affecting 10% of women at their fertile age. A well effective treatment for a complete cure for PCOD is still a challenge and evaluation of new strategies to treat this disease is of great priority. This review focuses on two aspects of PCOD, such as types of rodent screening models and diagnosis of major symptoms. A fully convincing animal model to study PCOD is still in challenge as they are multi-ovular species. But in many ways the rodent models are similar to human PCOD. This study explores on the parallels and problems associated with the use of different rodent models. Oligo- or anovulation, clinical or biochemical hyperandrogenism and polycystic ovaries represent the key clinical options on that the diagnosing ought to be primarily based. Determination of diagnosis parameters such as estrus cyclicity, insulin sensitivity, lipid profile, anti-oxidant status, weights of reproductive system and histopathological features of ovary were discussed along with the currently available treatment methods for PCOD.

2. Introduction

Polycystic Ovary Syndrome (PCOS) or Polycystic Ovary Disease (PCOD) is that the most typical disorder, moving 5–10% of women about in fruitful age1. PCOD posses heterogeneous assortment of signs and symptoms with severe disturbance of fruitful, endocrine and metabolic functions 2. Recently European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) united a refined definition of PCOD: particularly the presence of 2 out of the subsequent 3 criteria: (i) oligo- and/or anovulation; (ii) hyperandrogenism (clinical and/or biochemical); (iii) polycystic ovaries, with the exclusion of different aetiologies3. Clinically PCOD women diagnosed with associate inflated sterility risk like dysfunctional haemorrhage, obesity, carcinoma, sort a pair of polygenic disorder, dyslipidemia, cardiovascular disease, and presumably disorder [4, 5]. PCOD might manifest at any age, ranging from childhood (premature puberty), adolescent years (hirsutism and discharge abnormalities), early adulthood and middle life (infertility and aldohexose intolerance) to later life (diabetes mellitus and CV diseases) [1]. Menstrual irregularities are associated with Oligo-and anovulation. Less than 8 ovulations in a year is called Oligo ovulation. In case anovulation is gift no expelling hemorrhage happens and therefore the intercycle interval forever exceeds half-dozen months or 183 days [6]. Anovulation is that the key issue of inflicting expelling irregularities and it are reported that forty nine you look after ladies with PCOD suffer expelling irregularities seven. Prevalence of PCOD is according as 52% and is found highest in South Asian immigrants in UK, of whom 49.1% had expelling irregularity [7]. Excessive amounts of androgenic hormones indicates hyperandrogenism, resulting in acne and hirsutism. In PCOD the excess androgen production alter the gonadotropins induced estrogen and progesterone synthesis in the ovaries. In the ovaries normally testosterone and androstenedione are converted by the

enzyme aromatase P450 to estrogens and hyperandrogenism may be due to the decreased activity of enzyme in PCOD condition [5].

In PCOD insulin resistance and compensatory hyperinsulinemia are prominent features. Insulin resistance leads to reproductive abnormalities in women with PCOD. Insulin resistance is seen upto 50% of women with PCOD in both obese and non-obese patients [6]. Insulin resistance in cellular and molecular mechanisms of PCOD are well investigated and there could also be a genetic regulation abnormalities of hypoglycemic agent receptor phosphorylation, leading to hyperbolic hypoglycemic agent-independent amino acid phosphorylation and shrivelled insulin-dependent amino acid phosphorylation which ends up decrease insulin responsiveness [8]. PCOD shows the presence more than 12 follicles measuring 2–9 mm in diameter in each ovary, and/or an increased ovarian volume (N10 mL). Ovarian pathophysiology in PCOD shows folliculogenesis and alterations in theca and granulosa cell function. Ovaries in PCOD shows polycystic morphology with antral follicles more than the normal [9, 10].

As PCOD is the most complex, heterogenous female endocrine disorder, development of new treatment methods with fewer side effects are of higher priority. A fully convincing animal screening model to study PCOD is still in challenge. Assessing disruption of fertility by rodent's models is clearly valuable. But the major concern is that they are multiovular species. They have atleast six ovulations in any traditional polyoestrous cycle and various giant follicles are present at an instance within the rat and mouse ovaries. But in many ways the rodent models are similar to human PCOD.

2.1. Clinical Signs and Symptoms

- Hyperandrogenism (Acne, hirsutism and alopecia)
- Anovulation (Oligomenorrhea and amenorrhea)
- Infertility
- Obesity
- Recurrent abortion spontaneous
- Depression/anxiety
- Dyslipidemias (Increased total cholesterol, increased LDL, Decreased HDL and increased triglycerides)
- Coronary atherosclerosis
- Hypertension
- Diabetes Mellitus-2
- Coronary atherosclerosis
- Cancer in uterus
- Cardiovascular diseases

2.2. Causes of Pcos

2.2.1. Genetics

The genetic component appears to be an inherited fashion in PCOS. From a PCOS parent every kid has probability of inheritable the predisposing genetic variant is 50% [11]. Familial clustering shows genetic evidence, that there is a two fold increase in consistency of PCOS in identical than the non identical twins [12-14].

2.2.2. Prenatal Exposure to Androgens

The reported research works support that exposure of foetus to maternal contribute to inducing the PCOS phenotype in offspring/children. In PCOS higher testosterone levels equivalent to male levels, have been found in the umbilical vein in female infants [15, 16].

2.2.3. Insulin Resistance

In obese and non-obese women PCOS patients, insulin resistance is seen in up to 50% [5]. Insulin resistance is the usual driving force in overweight women with PCOS. The excess fat tissue is often causes a person to become resistant to the action of insulin. This impaired insulin function causes high risk of developing into compensatory hyperinsulinemia and which may lead to hyperandrogenism and gonadotropin aberrations.

In PCOD the cellular and molecular mechanisms of hormone resistance shows that genetic abnormalities in regulation of hormone receptor phosphorylation, reduced hormone responsiveness through accumulated insulin-independent aminoalkanoic acid phosphorylation and weakened insulin-dependent tyrosine phosphorylation [17, 18].

2.2.4. Insulin and Ovarian Function

Theca cells and granulosa cells are lined in ovary follicles. Theca cells helps to convert cholesterol into androstenedione, a weak male hormone. Theca cells pass the androstenedione and converted to oestrone, a weak oestrogen or female hormone in adjacent granulosa cells and then into oestradiol (strong oestrogen or female hormone) [6]. In females with insulin resistance stimulate Cytochrome P450c 17-α in each the ovaries and therefore the adrenal glands to supply increased amounts of male hormones. The surplus of male hormones comes from each the ovaries and therefore the adrenal glands in polycystic ovary syndrome. Increased amounts of LH secretion by pituitary gland stimulated by high level of insulin in the blood stream [13, 19].

2.2.5. Obesity

Obesity features a wide result on the manifestation of PCOS. Family studies have shown that in susceptible population, weight gain might promote composition of PCOS. Many women with PCOS (between 38% and 88%) are overweight or obese [20]. Symptoms of hyperandrogenism and ovulatory function in women with PCOS decrease with modest weight loss of 5%. Hence avoirdupois plays a major in development and maintenance of PCOS and extremely influences the severity of clinical and endocrine options [21, 22].

2.2.6. Hypothalamus - Ovarian Axis Dysfunction

The physiology of huge proportion PCOS ladies show associate degree increased ICSH (LH) secretion to gonadotrophic hormone quantitative relation, associate degree increased pulse frequency of the hypothalamic Gonadotropin-Releasing Hormone (GnRH) might favor the over production of LH over Follice Stimulating Hormone [23]. This elevated level of LH, increases the production of androgens in ovarian theca cells. Pituitary gland is stimulated by high level of insulin in blood stream to produce increased amounts of LH. The developing vesicle and egg don't respond to stimulation by luteinizing hormone as follicles theca cell layer thickens. However high levels of endocrine, however, cause the developing sac and egg to retort to stimulation by ICSH at an earlier stage of development, at 4mm diameter rather than at 9.5 mm. As no extra development of the sac is possible once ICSH stimulation, the growth of the sac is thus stopped at a diameter of eight mm and conjointly the sac is left too immature to eject [3,9]. PCOD shows the presence of twelve or additional follicles in every ovary mensuration 2–9 millimeter in diameter, associate degreed/or an exaggerated sex gland volume. PCOD ovaries contain further antral follicles than ancient therefore forming the polycystic morphology.

2.2.7. Hormonal Imbalance

Ovulation and progestin production square measure inseparably coupled functions; biological process (release of the egg from the ovary) dependably triggers the ovary to provide the endocrine progestin for the next twelve to fifteen days. Women with PCOS release sometimes or not the least bit, they manufacture progestin sometimes or not the least bit cause the irregular or absent discharge periods.

If biological process doesn't occur, the vesicle continues to provide steroid hormone for a few time, inflicting the liner of the female internal reproductive organ to grow thicker than usual nine. True is created worse as a result of the ovary doesn't turn out Lipo-Lutin if biological process has not occurred [9]. The liner of the female internal reproductive organ then breaks away in associate erratic fashion. This causes the emission harm to be long, usually with giant quantities of blood and tissue, inflicting catamenia which will be serious, painful and prolonged.

Persistent (rather than cyclic) estrogen secretion is seen with PCOS women. Periodic drops in sex hormone production are necessary to trigger the secretion changes that result in egg development and biological process [24]. Taken along, the abnormal endocrine atmosphere in PCOS girls, with the secretion of luteinizing hormone, elevated androgens, elevated insulin, and follicle-stimulating hormone deficiency altogether impair the event of the maturing follicles [9, 25].

2.2.8. SHBG (Sex Hormone Binding Globulin) Production

SHBG is formed in the liver. Females with PCOS have belittled levels of SHBG that is caused by repressive effects of agent on the SHBG production. Additionally, overweight/obesity decreases SHBG production even a lot of badly.

Decreased SHBG levels lead to enlarged levels of androgens, as commonly concerning 80% of androgen and 8% of androstenedione is usually certain to SHBG [26-28].

2.2.9. Adrenal Androgen Production

The secreter synthesizes all the three major androgens; Dehydroepiandrosterone (DHEA), androstenedione and sex hormone, and this are the alternative major location of female hormone production, besides the ovaries.

Among 60-80% of PCOS girls have elevated concentrations of current androgenic hormone [26, 29]. The mechanisms of the adrenal sex hormone excess in PCOS continues to be unclear, though it's been projected that it should result from exaggerated metabolism of corticosteroid, that may results in feedback on adrenocorticotropin (Adreno cortico tropic hormone) secretion [30].

2.3. Consequences of Pcos

2.3.1. Infertility

Clinically, diagnosed PCOD ladies were found with an magnified risk for infertility and that is usually because of oligo/anovulation and metabolic alterations. Miscarriage rates believed to be higher in PCOS ladies than in ancient ladies [30]. A recent-analysis verified the results of many alternative studies and showed that an enlarged prevalence of physiological state polygenic disorder, physiological state cardiovascular disease, pre toxemia of pregnancy and premature births area unit ascertained in PCOD ladies. Additionally, the infants of PCOS ladies were additional typically admitted to a babe medical aid unit and therefore the prenatal mortality was higher, severally of multiple pregnancies [31].

2.3.2. Cancer

Endometrial, female internal reproductive organ and breast carcinoma are having a potential association with PCOS.

- **Endometrial Cancer**

Carcinoma in PCOS is also because of the stimulation by excess estrogens on the mucous membrane, which may cause mucous membrane dysplasia with multiplied risk of atypia and eventually results in carcinoma [32]. Research work showed virtually thrice higher risk of developing endometrial carcinoma for PCOS ladies compared to ladies while not PCOS [33].

- **Ovarian Cancer**

The risk of ovarian cancer is more in case of women with anovulation and polycystic ovaries with early menarche and late menopause. The extensive use of drugs like clomiphene for induction of ovulation in PCOS women are also a possible reason for ovarian cancer [34]. PCOD women posses a double risk in developing into ovarian cancer than normal women.

- **Breast Cancer**

Regarding carcinoma, the mentioned reason behind an association with PCOS, is that of fatness, hyperandrogenism, long time-exposure to steroid and of sterility [35]. Previous analysis works supports the positive association between PCOS and also the presence of case history of carcinoma. During a study of 217 girls the proportion woemn with positive case history of carcinoma was considerably higher in women with PCOS compared with controls [36].

2.3.3. Type 2 Diabetes mMellitus (T2DM)

It was reported that PCOS patients suffer impaired insulin secretion and that carries an associated risk of progression into diabetic mellitus-2. The main mechanism responsible for T2DM is insulin resistance and approximately 50% to 70% of women with PCOS have insulin resistance [5]. Insulin resistance is strongly associated with PCOS and causes compensatory hyperinsulinema. Insulin resistance combined with abdominal blubber could account for the upper prevalence of sort two diabetes in PCOS. However the chance of developing sort two diabetes is additionally increased in non-obese girls with PCOS. Women under 45 suffering from type two diabetes also are diagnosed with PCOS.

A substantial proportion of PCOS ladies have disordered and insufficient beta-cell response inflicting hexose intolerance, they are at a high risk of developing T2DM. Research works support that women with physiological condition polygenic disorder are found with high prevalence of PCOS once gestation [13].

2.3.4. Obesity/Abdominal Obesity

Several studies show that PCOS ladies of fertile age have accumulated abdominal avoirdupois with accumulated visceral/abdominal fat compared with traditional controls. Abdominal obesity has been shown to be associated with insulin resistance, hypertension, dyslipidemia and cardiovascular diseases. The abdominal obesity is measured as Waist to Hip Ratio (WHR), and it have been associated with a strong risk factor for myocardial infraction. A WHR >0.85 or a waist circumference >88 cm area unit each correlative to hyperandrogenism, enhanced aldohexose intolerance, dyslipidemias and important enhanced risk of developing CVD. Obese women doesn't change into hyperandrogenemia and don't have PCOS. Associate degree enhanced sex hormone production has been reported in women with upper-body obesity [37-39].

2.3.5. Hyperlipidemias

According to the National Cholesterol Education Program (NCEP), ~70% of PCOS ladies have abnormal humor macromolecule levels and dyslipidemia is taken into consideration put together of the foremost common metabolic abnormality in PCOS. The dyslipidemia in PCOS includes elevated levels of compound protein and triglycerides and attenuate levels of alpha-lipoprotein [13]. As PCOS women tend to own augmented abdominal fat, they're a lot of susceptible to dyslipidemia, because the centrally placed adipocytes manufacture associate in nursing adverse result on blood supermolecule level. The parameters like hypoglycemic agent resistance and hyperandrogenemia, end in increased catecholamine-induced lipolysis and additionally the unhitch of free fatty acids into the circulation. Elevated free fatty acids flux to the liver and stimulate the assembly and secretion of very beta-lipoprotein producing hypertriglyceridemia [13, 40, 41].

2.3.6. PCOS and Blood Pressure

Women with PCOS also have hypertension and the possible mechanism of developing hypertension in PCOS is due to the excess androgen levels in PCOS which causes an increase in oxidative stress [4]. Hypertension is that the fourth strongest modifiable risk issue for heart muscle offence and one in all the strongest risk factors for stroke [39]. Redoubled pulsation pressure level has been noted in patients with PCOS even once adjustment for body mass index.

Cardiovascular Diseases (CVD) risk factors

Increased levels of androgens in PCOS women pose a risk of cardiovascular disease in young age as well as after menopause. PCOS women with avoirdupois, cigaret smoking, dyslipidemia, high blood pressure, impaired aldohexose tolerance and tube-shaped structure unwellness square measure in danger, whereas those with metabolic syndrome and/or kind II polygenic disorder square measure at high risk for CVD [39]. Research works reveals that coronary artery calcification and aortic calcification were observed in PCOS women in comparison with control [42]. Evidence strongly support the following concepts that

1. The prevalence of both impaired glucose tolerance and T2DM substantially increases the condition of cardiovascular risk, in patients with PCOS.

2. Imaging studies in ladies with PCOS have uniformly known a better prevalence of anatomic and useful abnormalities indicative of disorder or disfunction 43.

2.3.7. PCOS and (BMD) Bone Mineral Density

PCOS ladies with amenorrhoea have a lower BMD than often cycling PCOS ladies. Therefore amenorrhic ladies are susceptible to have an enlarged risk of pathology and fractures [44]. Several factors area unit acknowledge owning effects on bone mineral density, most notably age and biological time standing. Several of the common morphological and endocrine connected symptoms of PCOS, like blubber and hyperandrogenism, might have semi-permanent implications on bone density [45]. Blubber and also the high waist-to-hip quantitative relation body composition in several PCOS ladies (commonly called “apple shaped”) may end up in focused physical bone stress, which might result in inflated bone density [46, 47].

2.3.8. Effect on Thyroid Glands

Some studies support the higher existence of increased TSH (Thyroid Stimulating Hormones) levels 48 and associations of hypo-

thyroidism in PCOS. It is speculated hypothyroidism are common in PCOS women, as gland disease is related to irregular periods, exaggerated BMI (Body Mass Index) and endocrine resistance in non-PCOS women [49]. Studies relating to gland disease in biological time PCOS women are lacking.

3. Types of Rodent Models

3.1. Letrozole Induced Rat Model

Letrozole is an oral non-steroidal aromatase P450 inhibitor showed many histological and biochemical findings similar with human PCOD. Aromatase P450 is an enzyme responsible for the conversion of testosterone and androstenedione into estradiol and estrone respectively [50]. Decrease in aromatase activity results in elevated ovarian androgen production and a lesser estrogens levels that may leads to the development of PCOD.

Six-week-old female rats (mean body weight, 180 g) with 4-day regular estrus cycle are selected and estrus cycles are monitored by daily examination of vaginal smears. In this experiment the symptoms of PCOD is achieved by administration of letrozole at a concentrations of one mg/kg p.o. dissolved in 1% CMC (2 mL/kg) once daily for twenty one days. throughout this era, channel smears are collected daily and evaluated microscopically for heat cycle determination. Twenty four hours when the last dose of letrozole, all the animals are sacrificed by decapitation. Trunk blood is collected and sera are unbroken during a Deepfreeze for later internal secretion determination (follicle-stimulating internal secretion [FSH], gonadotrophic hormone [LH], estradiol, progestin, and testosterone). Uteri and ovaries ar excised and weighed to judge the result of the take a look at compounds. Ovaries are cut through at longest longitudinal dimension and perform histopathology to determine the formation of cyst [51].

This methodology showed high androgenic hormone levels that mirrored the buildup of sex hormones as a result of conversion of androgen substrates into estrogens is briefly blocked. The levels of sex hormone and gonadotrophic hormone are elevated. Slashed progestin production, which can have mirrored anovulation, in conjunction with slashed levels of sex hormone was another biochemical finding of this model, in keeping with human PCOD. The histopathology of ovaries show the presence of various subcapsular cysts lined with a thin layer of granulosa cells and abnormalcy of theca interna cells. These general anatomy findings indicate the presence of biologically active levels of hormone, enlarged gonadotrophic hormone, and lack of interaction between granulosa and theca cells, which could otherwise cause process.

Merits- Histology of ovaries taken from letrozole-treated animals exhibited terribly placing similarity to human PCOD. It showed elevated androgen and elevated LH which were considered the most consistent bio-chemical feature of PCOD [50]. The method could achieve reversibility of reproductive function after withdrawal from letrozole.

Demerits - The method showed an increased FSH concentrations which is not typical with human PCOD, appeared to be the main drawback of this model [51, 52].

3.2. Dehydroepiandrosterone Induced Model

Dehydroepiandrosterone (DHEA) is found to be one of the most abundant circulating androgens in women with PCOD. Roy et al developed an animal model for the study of PCOD by injecting DHEA. Two methods of DHEA models are available which include female mice and female rats [53].

In the 1st animal model the immature feminine mice's are injected with DHEA (6 mg/kg b w, 0.75 mg DHEA per mouse) for twenty consecutive days. This dose of DHEA ensures a hyperandrogenized standing admire that found in women with PCOD [54, 55]. Histological examination of immature ovaries of DHEA treated mice reveals a rise in fat and stromal tissue and increased white blood corpuscle infiltration. The sex gland cortex shows a rise within the variety of atretic follicles and also the formation of quite 2 cysts in every ovary. The cysts show a compacted formation of granulosa cells with the absence of a vascularized theca interna. A decrease in insulin sensitivity, increased prolactin level and an altered lipid metabolism [55] is reported with this method. The method shows an enhanced oxidative stress and which is typical with PCOD women along with immune disturbances and impaired ovarian function.

The second method is performed in both adult and immature female rats. The immature rats are administered with DHEA from around 22 days of age for 7–21 days [56, 57]. High levels of androstenedione and testosterone are achieved after injection with DHEA when compared with normal levels [58]. The adult female rats exhibit anovulation. This method shows an increase in FSH followed by a reduced LH levels in blood. Histopathology of ovaries shows the appearance of cystic and atretic follicles. The ovaries show associate absence of corpus lutea indicating an absence of biological process with or while not the presence of cystic follicles.

Merits - DHEA iatrogenic PCOD model exhibits several of the salient options of human PCOD like hyperandrogenism, hypoglycaemic agent resistance, altered steroidogenesis, abnormal maturation of sex gland follicles, aerophilic stress and biological process [53-58].

Demerits- The gonadotropin and prolactin profiles are not equivalent with human PCOS.

3.3. Estradiol Induced Rat Model

Female wistar rats of body weight of 190-230 gm with a regular fourday estrous cycle are selected for the study. Animals are injected with four mg of estrogen valerate in 0.2ml vegetable (sesame) oil for 33 days [59]. Once the induction of experimental PCOD intracardiac blood sample is taken underneath intraperitoneal (xylazine 6 mg/kg and Ketamine 30 mg/kg) anesthesia and blood

glucose level, followed by antioxidative standing are determined [60]. Histopathological studies of sex gland tissue samples show polycystic sex gland tissue formation. The variety of follicles ar hyperbolic, and the cystic wall is thickened characterised by a thickened theca cell layer and a diminished granulosa cell layer. A significant decrease in plasma levels of gonadotrophin, gonadotrophic hormone and androgen was seen suggesting that a protracted term result of oestrogen at the pituitary/hypothalamus, in all probability decreases these secretion levels.

In another study 8week old virgin female rats are administered with a single i.m. injection of 4 mg of EV in 0.2 ml of oil for 30 and 60 days [61]. Complete cystic ovaries are developed at 60 days of drug administration. This method evidenced by higher levels of tyrosine hydroxylase enzyme in the ovaries and higher sympathetic activity.

Merits- A well developed polycystic ovary is observed with characteristic features of thick theca and thin granulose cell layers

Demerits- This method could not achieve a significant elevation in blood sugar and oxidative stress marker levels [59-61].

3.4. Dihydrotestosterone Induced Rat Model

The Dihydrotestosterone (DHT) evoked rat technique could be a distinctive model that exhibits several characteristic options of PCOD women and it's helpful to check the mechanisms accountable for PCOD-mediated cardiovascular disease [62].

Sprague-Dawley feminine rats of 4-6 weeks elderly are elite for the study. Rats ar deep-rooted subcutaneously on the rear of the neck with continuous unleash of DHT pellets (7.5 mg/90 days; daily dose of 83µg). During and after 14 weeks of treatment the body weight, blood pressure and estrous cycle were determined regularly. Blood samples are withdrawn from anaesthetized animals and various biochemical parameters such as plasma insulin, leptin, cholesterol, glucose, oral glucose tolerance test, inflammation, oxidative stress and serum sex hormone levels are determined.

DHT induced animal model achieved an increased bodyweight and blood pressure [22]. Along with the increase in body weight, this method shows an increase in blood glucose levels. The abnormal oral glucose tolerance test indicates insulin resistance. It have been reported that approximately 50% to 70% of women with PCOD have insulin resistance [3]. This model exhibited a complete absence of estrus cycle followed by increases in leptin, oxidative stress and cholesterol levels. The plasma estradiol level was unchanged and testosterone levels were below the levels of detection whereas the most potent hormone dihydrotestosterone level is elevated. In another method DHT is administered for rats from prepubertal 21 days to 56 days or 90 days [63]. This method also shows irregular estrus cycle and anovulation. Hisopathology of ovaries shows the presence of atretic and many cystic follicles with thickened theca and decreased granulose cells. The levels of FSH remains normal with lowered LH and increased prolactin levels.

Merits - The method showed insulin resistance, oxidative stress, high blood pressure, elevated dihydrotestosterone, increased levels of cholesterol and leptin [62, 63].

Demerits- the unchanged FSH level with lower LH is not the consistent feature of PCOD

3.5. Testosterone Induced Rat Model

Testosterone induced young female rat model of PCOD is performed to visualize the implications of sex hormone on cancellate bone and marrow adipocytes [64]. Sprague-Dawley rats are selected for the study. All the rats are injected subcutaneously with testosterone propionate (0.1 mg in 0.004 mL olive oil) [65] at ninth day after birth. After 16th week blood samples from the heart is collected and serum biochemical parameters are determined. Body weights are measured before sacrificing them and after sacrifice, the ovaries are removed from each rat and histopathological studies are performed.

Female rats those received androgen for nine days after birth develop polycystic ovaries, high bone volume, low bone turnover, and lower fat content at intervals the bone marrow. All the rats show prolonged and irregular estrus cycles. Many atretic follicles with degenerating oozytes are observed. Multiple follicles cysts are present within the ovaries with luteinized theca and stroma within the absence of endocrine. This impact is related to traditional gonadotropin, low traditional luteinizing hormone, however multiplied gonadotropin levels in plasma [64].

Merits- The consequences of PCOD in bone density can be studied. Administration of testosterone in fetal or early in neonatal life of rats interferes with the effect of estradiol and causes anovulation.

Demerits- The method shows low levels of LH and which is not typical with PCOD [64, 65].

4. Laboratory Scale Diagnostic Parameters

4.1. Estrous Cyclicity

Prolonged monoestrous cycle is one amongst the most important symptoms of PCOD and monoestrous periodicity is monitored by feminine rat cytosmear examination. The presence or absence of cell varieties and its relative cell kind determines the stage of the oestrus cycle [66]. Duct smears are collected daily by a fragile insertion of a measuring device or a cotton bud dipped in ancient saline at intervals the duct gap of the female rats and a swab is obtained. The cotton bud is rolled on a clean grease free slide to make a smear and allowed to air dry. Few drops of methyl alcohol is additional to repair the cells within the smear. The slide was air dried. Giemsa stain is additional to the slide to hide the smear. The slide is unbroken lined in petridish for five minutes. When five minutes H2O is additional to the giemsa and gently rocked. An inexperienced scum appeared on high of the slide. The

slide is stained for ten minutes in dilute giemsa. The stained slide is drained and then washed in mild stream of faucet water. The washed slide is air dried and observes underneath the magnifier in 40x objective [67]. Traditional monoestrous cycle of rat lasts for 4-5 days showing four phases in serial order like metaestrous, diestrous, proestrous and monoestrous (Figure-1). Estrus phase is characterised by non-nucleated, cornified animal tissue spherical cells. Metestrus has generally a coffee cell variety, typically with loads of cell scrap. Anestrum contains largely lymphocytes. Protestrus is outlined by the presence of additional little cell organ animal tissue cells largely in spherical. PCOD induced animal's exhibit irregular or prolonged estrus cycles [66].

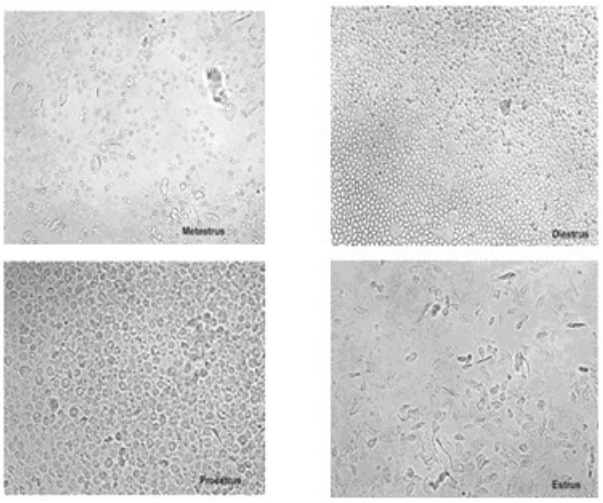

Figure 1: Stages of normal estrous cycle.

4.2. Insulin Sensitivity

Dunaif et al. reported that PCOD patients suffer from impaired hypoglycemic agent secretion, that carries an associated risk of progression to type-2 diabetes [5]. Hypoglycemic agent resistance in girls with PCOD is seen in up to five hundredth of patients in each weighty and non-obese women [3]. The hyperinsulinemic-euglycemic clamp technique is that the most scientifically sound technique for mensuration hypoglycemic agent sensitivity. Various methods involve Glucose Tolerance Test (OGTT), Insulin Tolerance Test (ITT), Insulin Sensitivity Test (IST), and Continuous Infusion of Glucose with Model Assessment (CIGMA). Treatment with Malaysian herb Labisia pumila increased insulin sensitivity of 36% in PCOD rats [68].

4.3. Hyperinsulinemic-Euglycemic Clamp Technique

The rats are anaesthetised with thiobarbital sodium (130 mg/kg i.p). Catheters are inserted into the left arteria and also the right venous blood vessel. A surgical process is performed to facilitate respiration. Body temp is maintained at 37°C with a heater. Insulin (100 IU/ml) along with 0.2 millilitre of simple protein (albumin) and 10 millilitre of physiological saline is infused at 24, 16, and 12 mU/min/kg for 1, 2, and 3min, severally, followed by 8mU/min/kg for the remainder of the clamp. At the same time, 20% glucose in physiological saline is administered to maintain blood sugar levels at a euglycemic level (6.0 mM). The glucose infusion rate is radio-controlled by measurement of glucose concentration each five min with a B-glucose instrument. The mean glucose infusion rate, normalized to weight, is calculated at a gentle state (after roughly 50–70 min) as associate index of endocrine sensitivity. Blood samples are taken at the top of the clamp to see endocrine concentrations [68].

4.4. Oral Glucose Tolerance Test

All the animals are kept 12 h of fasting. Blood samples are collected in anticoagulant vials. After blood collection all the animals are fed with glucose 300mg/kg body weight and blood samples are withdrawn at time intervals of 30, 60, 90,120 minutes. Blood samples are centrifuged and the plasma obtained is used to determine the blood glucose levels [69].

4.5. Preparation of Blood Serum

After decapitation of rodents under anaesthetized the blood samples of every animal square measure collected and allowed to clot for forty five min at temperature. Serum is separated by centrifugation for 15min with 3000 revolutions per minute and analyzed for numerous biochemical parameters [68].

4.6. Hormonal Balance

PCOD is one in all the main feminine endocrine disorders that have an effect on the degree of major hormones like LH, FSH, androgenic hormone, Lipo-Lutin and estrogens. In women with hyperandrogenemia 72.1% were thought of to possess PCOD3. The clinical indicator for hyperandrogenism is hairiness and skin disorder in women. Assessment of the free androgenic hormone (T) or free T (Free Androgen Index (FAI)) is that the additional sensitive strategies of deciding hypernadrogenaemia. Strategies for the assessment of free T include: equilibrium qualitative analysis, calculation of free T from the measurement of Sex Hormone Binding Globulin (SHBG) and total T; and ammonia sulphate precipitation [70]. An elevated LH was monitored on 60% of women with PCOD. Both the elevated testosterone and LH is considered as the most consistent biochemical feature of PCOS. An elevated serum LH concentration has been related to a reduced probability of conception and an accumulated risk of miscarriage 3. All the hormones can be analyzed by Radioimmunoassay (RIA) using a commercially available RIA kits.

4.7. Lipid Profile

Body weight encompasses a vital impact on traditional sex gland perform. PCOD is related to blubber and probably ends up in CV risks [60]. The lipid profiles of animals during and after the experiments can be monitored by commercially available kits. The effect of test drugs on plasma Total Cholesterol [TC], Low-Density Lipoprotein Cholesterol [LDL-C], High-Density Lipoprotein Cholesterol [HDL-C], and Triglycerides [TG] are determined [68]. PCOD model such as letrozole induced [51] and DHT induced [53]

is found to increase the lipid profiles in induced animals. Studies proved the lipid lowering effects of Gymnema sylvestre [71] and Linum usitatissimum [72] in PCOD induced animals.

4.8. Uterus Weight

Rat or mice uteri is excised and weighed to guage the result of the check compound on endocrine balance of animals. Test drugs which show an increase in uterus weight indicates estrogenic effect [68] It have been reported that a Malaysian herb Labisia pumila, Cocus nucifera [73] and Bambusa bambos [74] have shown an increase in uterus weight of PCOD induced rats.

4.9. Ovary Weight

Rat or mice ovaries are collected and weighed separately by using electronic balances to evaluate the development of poly cystic follicles. In PCOD induced animals the weights of ovaries increases than control indicates the developed cyst [75]. The test drug which shows a decrease in ovary weight of PCOD induced animals indicates the curative effect from cystic ovaries.

4.10. Anti-Oxidant Status

Women with PCOD shows a rise within the current aerophilous stress markers compared with healthy ladies that is answerable for the multiplied impact on CVdiseases and different major symptoms. Free radicals that originated a sequence of organic chemistry reactions cause the formation of the many extremely reactive intermediates and which can play a task within the method of fertilization and implantation in female internal reproductive organ. Previous works support that PCOD women posses a high oxidant status and an insufficient antioxidant status [76]. In PCOD elicited rodents the antioxidant levels are evaluated by determination of corpuscle reduced glutathione (GSH) concentration, catalase, SOD (SOD) activities and Total Antioxidant Status (TAS) in female internal reproductive organ tissue material.

4.11. Preparation of Uterus Homogenate

Isolated and ice chilled uterus tissues were finely minced and homogenized in five volumes of 0.15 M Tris-HCl buffers (pH 7.4). The fraction was assayed for the marker enzymes like SOD (SOD) and reduced glutathione (GSH).

Determination of erythrocyte reduced glutathione (GSH) concentration

GSH estimation is predicated on a reaction of reduced GSH with 5-5' dithiobis-2- nitrobenzoic acid. The tissue material (in 0.1 M phosphate buffer hydrogen ion concentration 7.4) is taken and more with equal volume of 20% acetic acid (TCA) containing one mM EDTA to precipitate the tissue proteins. The mixture is allowed to square for five min before action. The supernatant liquid is then transferred to a brand new set of test tubes and more with 1.8 ml of the Ellman's chemical agent (5,50-dithiobis-2- nitrobenzoic acid (0.1 mM) ready in 0.3 M phosphate buffer with 1 % of sodium citrate solution). Then make the volume to 2ml in all the test tubes. On completion of the full reaction, solutions square measure measured at 412 nm against blank [78].

4.12. Superoxide Dismutase (SOD) Activities

It involves the inhibition of superoxide-dependant reduction of tetrazolium dye alkyl thiazolyl tetrazolium to its formazan. Tissue stuff (0.5 millilitre) is diluted with one mL of water. To this mixture, 2.5 millilitre of ethyl alcohol and 1.5 millilitre of chloroform is added and jolted for one min at 4ºC then centrifuged. The supernatant liquid is employed for assay. The assay mixture contained 1.2 millilitre of sodium pyrophosphatebuffer (0.025 M, pH 8.3), 0.1 millilitre of l M potassium metabisulfite (PMS), 0.3 millilitre of l M nitroblue tetrazolium (NBT), 0.2 millilitre of l M NADH, befittingly diluted protein preparation and water during a total volume of three millilitre. Reaction is started by the addition of NADH. when incubation at 30°C for ninety min the reaction is stopped by the addition of one millilitre glacial acetic acid. The reaction mixture are stirred vigorously and jolted with four millilitre of n-butanol. The absorbance of butyl alcohol layer is measured at 560 nm against butyl alcohol blank [79].

4.13. Histopathological Analysis of Ovaries

The histological examinations of ovaries are performed by standard histological methods. The PCOS induced ovaries (Figure-2) shows the consistent histological features 1-3 of

- Whole ovarian hypertrophy,
- Elevated number of sub capsular follicle cysts,
- Deficient of corporea lutea or albicantia,
- Hyperplasia and fibrosis of the ovarian stroma
- Thin granulosa cell layer
- Premature luteinization of theca cells

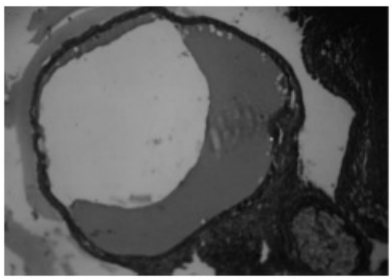

Figure 2: Histopathology of Letrozole induced PCOS ovary showing large cystic follicle

5. Current Status of Treatments

5.1. Lifestyle Modification

Obese with PCOD would benefit of a regime of diet and exercise. Diet ought to be low in sugar and fat and high in fruit, pulses, recent vegetables and dish. Lightweight exercise like walking, sport or swimming for a minimum of associate hour would be useful to cut back the load [80].

5.2. Oral Contraceptives

Oral Contraceptives (OCPs) are the foremost economical means that of steroid suppression created by the ovary. It ends up in lower and static LH levels while not surges. OCPs typically offer sure and consistent withdrawal hemorrhage, removing a very important supply of frustration. They scale back gonad male internal secretion, sometimes up skin disease and excess hair, though they donot reduce endocrine male internal secretion. Above all, they supply reliable family planning and up to date study report that OCPs create hypoglycaemic agent resistance worse and additionally increase the tendency for clots in PCOS [81].

5.3. Anti Androgens

Drugs like cyproterone acetate and spironolactone is a competitive inhibitor for the androgen binding receptor. Most anti-androgens act by block the action of androgen at the follicle. Corticoid (spironolactone) is used on its own or whiles not a prophylactic device and this will be helpful in smokers or weighty. This treatment can prevent unwanted hair growth and menstrual irregularities along with some side effects like weight gain, lethargy and headache [82].

5.4. Metformin

Metformin (dimethyl-biguanide) is associate oral medicament that decreases gluconeogenesis and will increase peripheral glucose uptake by muscles. It decreases enteral aldohexose absorption, decreases fatty acid oxidization, and will increase the utilization of aldohexose [83]. Metformin is effective in reducing androgen levels and in creating menstrual cycle more regular. It acts by creating the body a lot of sensitive to insulin secretion. Metformin in addition to clomiphene showed an increase in ovulation and pregnancy rates. Metformin requires a prolonged period of usually 3–6 months or longer. As a result, studies support the occurrence of high noncompliance rates and side effects include abdominal discomfort, nausea, diarrhea, anorexia, and lactic acidosis [82-84].

5.5. Clomiphene Citrate

Most recently developed conventional treatment for PCOD is ovulation induction by clomiphene citrate. It is used for PCOD women those who wish to become pregnant. The overall physiological condition rate in women treated with Clomid is over 50%. Clomid is an anti-estrogen that is employed to treat physiological state in women with PCOD by inducing ovulation. As ovulation induction aims restoration of the maturation of a dominant follicle and unharness of one gametocyte, this method is usually troublesome to manage, leading to multiple vesicle development, multiple pregnancy, and sex gland hyperstimulation. Metformin in combination with clomiphene citrate has been the most extensively used drug therapy for PCOD [83-85].

5.6. Supportive Treatment

Removal of unwanted hair and acne by electrolysis or by newly introduced laser therapy may improve the physical appearance of PCOD patients [82].

5.7. Hormone Fertility Treatments

The endocrine follicle-stimulating hormone and gonadotropic hormone will be given by daily injection for ovulation induction. Low doses are used at the start of a cycle so accumulated betting on the results of watching with endocrine tests, or a lot of typically with an ovarian ultrasound. The foremost aspect impact of this sort of treatment is ovarian hyperstimulation syndrome and which ends up multiple pregnancies [9, 84].

5.8. Laparoscopic Ovarian Drilling

It is a surgery which will trigger ovulation in women who have PCOD and who haven't responded to weight loss and fertility drugs. Electrocautery or a optical device is employed to destroy parts of the ovaries. Studies of ladies with PCOD have reportable that ovarian drilling ends up in associate 80% ovulation rate and a 50% gestation rate, however different studies have shown less success. Young women and people with a body mass index within the traditional vary are presumably to learn from laparoscopic female internal reproductive organ drilling [86].

5.9. Surgery

Surgical treatment has been only moderately effective. The goal of ovarian surgery is to get rid of or destroy a little of the female internal reproductive organ tissue. This ends up in a lowering of female internal reproductive organ male secretion and estrogen production, thereby making secretion surroundings a lot of contributive to egg-development and biological process [87].

5.10. In vitro fertilization

IVF may be the only option 87 for PCOS women who actively wanted to become pregnant where all other treatments have failed.

6. Herbal Medicines for PCOD

Herbal medicines are plant substances with stripped-down or no industrial processing that has been accustomed treat varied diseases. Ancient herbal medicines are becoming important attention in international health. When put next to traditional medical care the phytotherapy is safe with lesser aspect impacts and presence of multiple active compounds in healthful herbs altogether provides a potentiating effect. it's become necessary to point out that phytotherapy will match alternative fields of medication within the painstakingness of its scientific work and its sensible use [88]. An herbal approach to PCOD may helps to initiate ovulation, improve fertility, decrease insulin resistance, treat cyst and improve hirsutism. Along with reduction of major symptoms, natural plants are well known to maintain the oxidative status of body.

A large number of medicinal plants have been reported for various pharmacological activities using rodent models and it was limited in case of PCOD. Previous reported works shows plants such as Panax ginseng [89-91], Aloe barbadensis [68,92], Matricar-

ia Chamomilla [93,94], Labisia pumila [68], Tephrosia purpurea [95], Paeonia lactiflora [96], Tribulus terrestris [97], Gymnema sylvestre[71], Vitex agnus-castus [98], Glycyrrhiza glabra [99], Linum usitatissimu [72], Cinnamomum zeylanicum [100], Silybum marianum [101] ,Cocus nucifera [73], Bambusa bambos [74] have shown favorable results in decreasing the major symptoms. (Table No.1).

Table 1: Reported medicinal plants for treatments of various symptoms of PCOD

S. No	Plants	Estrous cycle	Insulin sensitivity	Hormonal balance	Uterus weight	Anti-oxidant status	Ideal Lipid profile	Normal histology of ovary
1	*Panax ginseng*	NR	+	NR	NR	+	+	+
2	*Aloe barbadensis*	+	+	+	NR	+	+	+
3	*Matricaria Chamomilla*	+	+	+	NR	+	NR	+
4	*Labisia pumila*	+	+	+	+	+	NA	NR
5	*Tephrosia purpurea*	+	+	NR	NR	+	+	NR
6	*Bambusa bambos*	+	+	+	+	+	+	+
7	*Cocus nucifera*	+	+	+	+	+	+	+
8	Paeonia lactiflora	+	NR	+	NR	+	NR	NR
9	Gymnema sylvestre	NR	+	NR	NR	+	+	NR
10	Tribulus terrestris	NR	NR	+	NR	+	NR	NR
11	Vitex agnus-castus	+	+	+	NR	+	NR	NR
12	*Glycyrrhiza glabra*	+	+	+	NR	+	+	NR
13	*Linum usitatissimum*	+	+	+	NR		+	NR
14	*Cinnamomum zeylanicum*	NR	+	NR	NR	+	+	NR
15	*Silybum marianum*	+	+	+	NR	+	+	NR

7. Conclusion

PCOD is associated with multivariate symptoms and a single biochemical diagnostic criterion is not sufficient for clinical diagnosis. A complete convincing animal model which characterizes all the symptoms of PCOD similar to human PCOD was not well established. This review clearly discusses the merits and demerits of various rodent screening models and also the methods to determine the major symptoms of PCOD. In many ways these rodent models are similar to human PCOD and to develop a new treatment for the complete cure is of great importance. This review support researchers who work in development of new treatment strategies for PCOD.

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