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Animal Models of PCOS: A Comprehensive Review on Current Advancement, Diet-Microbiota Interactions and Future Trend on Developing PCOS Induced Rodent Models

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Keywords: Rodent model; Gut microbiota; Genetic remodeling; Polycystic ovary syndrome.

Abstract

Polycystic Ovary Syndrome (PCOS) accounts for a major form of dysovulatory infertility, often observed in ~15% women belonging to reproductive age. Being the etiology undecipherable and the management requiring a diversified approach, the call for a suitable animal model is warranted that will be able to mimic both reproductive as well as metabolic facets of the syndrome. Even though numerous animal models are widely used, none of them could essentially fulfil both of the above criteria and thereby lack vivid clarity in the selection of the predicted methods. Thus, this review concentrates on putting forward some of the reliable methodologies for rodent model/s of PCOS along with the meticulous understanding of the physiological, hormonal, and metabolic changes involved in the process. Furthermore, we detail the perception of gut microbiota-induced PCOS phenotype, which has not been yet translated entirely in laboratory research along with a precise food-metabolite-gene loop working behind the manifestation of the syndrome. The benefits and restrictions of different hormonal treatments or genetic manipulation of animal models for the heterogeneous endocrinopathy are also highlighted along with the current development and future trend.

Abbreviations: A: Androstenedione; AR: Androgen Receptor; BMD: Bone Mineral Density; DHEAS: Dehydroepiandrosterone Sulfate; DHT: Dihydrotestosterone; ER: Estrogen Receptor; EV: Estradiol valerate; FSH: Follicle Stimulating Hormone; GCs: Granulosa Cells; GM: Gut Microbiota; GnRH: Gonadotropin Releasing Hormone; hCG: Human Chorionic Gonadotropin; HFHS: High Fat High Sugar; HPO: Hypothalamus Pituitary Ovary; IGF-1: Insulin like Growth Factor 1; IR: Insulin Resistance; IR/LepRPOMC: Knockout- Mice Lacking Insulin and Leptin Receptors in Pro-Opiomelanocortin Neurons of the Hypothalamus; IRS-1: Insulin Receptor Substrate 1; KO: Knock Out; LH: Luteinizing Hormone; NGF: Nerve Growth Factor; PAI-1: Plasminogen Activator Inhibitor-1; PCOM: Polycystic Ovary Morphology; PCOS: Polycystic Ovary Syndrome; PCOD: Polycystic Ovary Disease; POMC: Pro-Opiomelanocortin; SCFAs: Short Chain Fatty Acids; T: Testosterone; IU: International Unit

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Introduction

Polycystic Ovary Syndrome (PCOS) is probably one of the most frequently observed ovulatory disorders that arrests approximately 10-15% woman of reproductive age [1]. Extensive investigation during recent years have equivocally designated PCOS as a multifactorial disease, however, etiology and the mechanism of the disease not yet fully elucidated. This perhaps stands as a major hurdle in fixing the targeted therapeutic strategy for the disease [2].

Considering unavoidable limitations on undertaking comprehensive studies on humans, an array of PCOS induced animal models have been developed mimicking the syndrome to some extent. However, the diverse etiology with wide phenotypes of the disease brings down the potential of a single model to display the pathogenesis of the syndrome in all dimensions. While some models do represent the characteristic morphological features of the disease, they lack to reproduce the metabolic aspects and vice versa [3]. Moreover, familial and heritable aspects of PCOS demand attention towards the contribution of genetic, epigenetic and developmental conditions behind the etiopathogenesis of the disease [4]. Eventually, PCOS has been described where genetic influences for energy conservation may negatively impact reproductive potential under particular environmental circumstances. However, progress towards early detection of PCOS is held back due to undeciphered pre-PCOS diagnostic biomarkers, thus advocating interdisciplinary research and developments in this direction [5].

Clinical Picture of PCOS: The Androgen Circle

The clinically prominent hallmarks of PCOS include hyperandrogenism, menstrual dysfunction and polycystic ovarian morphology [5]. In accordance to Rotterdam criteria, four types of PCOS phenotypes had been put across, namely, type A, B, C and D. (Table 1).

Table 1: Four PCOS phenotypes [6].

Type of PCOS phenotype	Hyperandrogenism	Ovulatory dysfunction	Polycystic ovary morphology	Prevalence worldwide
Type A	√	√	√	40-45%
Type B	√	√		
Type C	√		√	~35%
Type D		√	√	~20%

The ultrasound finding of Polycystic Ovarian Morphology (PCOM) characterized by the presence of more than 12 follicles with increased ovarian volume is often observed in females of different ages, which may be triggered by many factors including certain pharmacological treatments [7]. Thus, diagnosis of PCOS on basis of PCOM stands isolated and needs to be backed up by the presence of other hallmarks, which include ovulatory dysfunction and hyperandrogenism. Clinically, hyperandrogenism is evaluated particularly through skin conditions including hirsutism, acne, acanthosis, skin tags and irregular menstrual cycle [8]. The pathogenesis of PCOS is indeed a genetically mediated ovarian disorder where elevated androgen synthesis in early life may transpire into a hormonal imbalance leading to PCOS in adulthood. Thus, experimental hyperandrogenism forms the basis of developing PCOS induced animal models. Rodent/s expressing PCOS phenotype/s display endocrine dysfunctions [9] with minimum metabolic disturbances in single or in combination like Insulin Resistance (IR), glucose tolerance, hyperlipidemia, obesity, type 2 diabetes and even cardiovascular disease which are the most prominent comorbidities associated with human PCOS [10,11]. IR and hyperandrogenemia stimulate each other in a reciprocal fashion. The enhanced bioavailability of androgen in conjunction with IR thwart the cyclic rhythm of follicular recruitment [12]. Recruitment and development of ovarian follicle during reproductive cycle is related to P13K-Akt signaling pathway. This pathway is initiated by the binding of insulin receptor substrate 1 (IRS-1) with its ligand insulin or IGF-1, followed by phosphorylation of IRS-1. Phosphorylation of IRS-1 induces a downstream signaling cascade which results in upregulated FOXO1 expression in cystic follicles inducing oxidative stress mediated apoptosis in the granulosa cells [13,14,15].

Obesity regulates PCOS in a positive feedback loop; hence, a bold prospect of adipose tissue intervention in the pathogenesis is evident. Leptin release by white adipose tissue regulates *Kiss1* neuron present in hypothalamus, which in turn activates GnRH neuron/s finally leading to secretion of LH from the pituitary gland. Perturbation of HPO axis with a "danger alarm" in the positive feedback loop of leptin-*Kiss1* reduces the negative feedback of estrogen to hypothalamus leading to increased pulse frequency of LH. Hence, this culminates into vicious cycle of hyperandrogenism producing several clinical manifestations of PCOS [3]. It is worthy to note that the possible contribution of visceral lipolysis towards metabolic malfunctioning in PCOS can also be triggered by androgens. However, influence of hyperandrogenism on lipolysis and adipocyte functioning in PCOS patient remains largely incoherent. Manifestation of physiological hallmarks of PCOS is summarized in Figure 1.

Relevance and purpose

Laboratory animal models like rodent/s exhibit mammalian similarities on reproductive tract development and hormone actions with that of humans, although there exists a perceptible variance in reproductive physiology with respect to ethological behavior, ovulation number, duration of breeding cycle, ovulation pattern and hormonal sensitivity etc. [16]. In addition, animal models often exhibit a single trait of the Rotterdam criterion for PCOS like hyperandrogenism, which is considered as PCOS like trait, but is not adequately PCOS in totality [12]. For comprehensive perception of PCOS induced rodent model, it is thus imperative to decipher overall reproductive physiology along with the updated molecular pathophysiology which will further assist in contributing the understanding of the disease within humans. A combined perception of physiological, hormonal and metabolic changes is therefore essential for planning and executing experiments with this model/s. The purpose of the review is to deliver an overview of hormone-induced and genetically modelled PCOS paradigm/s highlighting prominent and influential food omics approach towards manifestation of PCOS with easy interpretation.

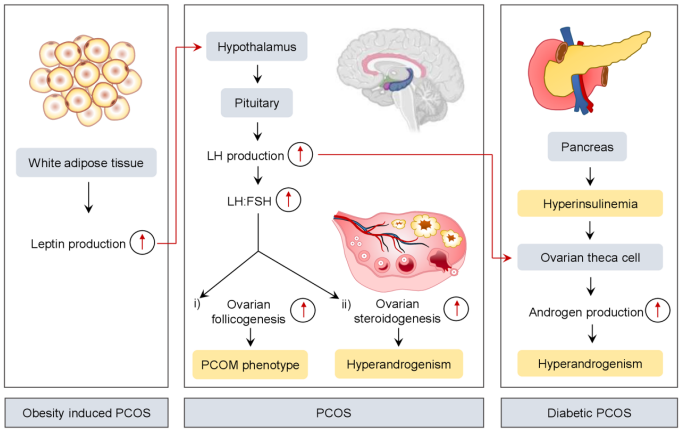

Figure 1: Manifestation of physiological hallmarks of PCOS.

Reproductive Physiology: Understanding common bridges between species

The parts of brain consisting of hippocampus, hypothalamus, thalamus, amygdala, and pineal gland contribute in formation of the limbic system [17]. In female rats, GnRH is released from the neural elements in close proximity to medial preoptic nucleus of hypothalamus like that of the humans. GnRH neurons receiving estrogen signalling is crucial for coordinating the preovulatory surge of LH that brings about the follicular development and maturation, followed by ovulation [18,19]. The short duration of estrous cycle of 4-5 days in rodents makes them alluring model/s for exploring all the key physiological changes associated with the reproductive stages.

The recurring pattern of cellular growth and differentiation of ovarian tissues is coordinated by ovarian factors along with the production of stimulatory hormones from anterior pituitary and hypothalamus. However, mechanism/s for the blockade of GnRH surge in rats showing uninterrupted estrous cycle is inadequately deciphered. The major characteristic feature of PCO is arrested follicular maturation leading to an abnormal ovarian endocrine environment. Presence of vaginal cornified cells in the smear taken from rodent vagina for a minimum period of 10 days is referred as the sign of persistent vaginal cornification. This is considered as a signal for cystic follicle [20]. Moreover, timing of the opening of vagina in response to ovarian steroids is taken as one of the prime indication of onset of puberty in rodents. However, the vaginal opening generally depends on the body mass, rather than chronological age. The event may be a consequence of a combination of endocrine changes that occur early in postnatal life. The association of earlier sexual maturation with obesity is also well described. Girls with early sexual maturation were twice as likely to be obese than other girls [21]. The causal nature of this relationship remains blurred, but it is possible that increased androgens may play a role in this phenomenon. It is to be remembered that during PCOS, the H-P-O axis regulated estrous cycle is glitched by the elevated androgens and insulin resistance, which conclusively culminates to anovulation and delayed estrous cycle [2].

Barker's hypothesis and fetal programming in PCOS: Significance of androgen receptor

The developmental origin of Barker's hypothesis speaks off the permanent alteration of the fetal development, morphology and physiology due to critical exposure(s) during gestation, and thereby increases the susceptibility to reproductive track associated disease and also likely influences the phenotypic expression and trans-generational transmission of PCOS [22]. This backdrop forms the basis of understanding of female reproductive system in rodent/s and its association with the maternal androgen level/s. The sexual dimorphism in mouse is brought about by the essential actions of androgens [23] which is even more understood by the difference in positioning of the Mullerian ducts and the urogenital junction in male and female mice [24]. It is to be noted that PCOS women have daughters displaying two strong markers associated with in-utero androgen exposure, an extended anogenital distance and elevated sebum production in face [25]. Surprisingly, increased urogenital distance after postnatal androgenisation is also observed in rodent/s [26]. These clinical observations back up Barker's hypothesis, which underlines the influence of excess fetal androgen stimulating the developmental programming of PCOS.

As hyperandrogenism is a pivotal feature in PCOS, it is important to stroke the mechanistic chord of androgen action in ovary via the Androgen Receptor (AR). AR expression is present throughout most stages of follicular development with distinct patterns among mammalian species. Interestingly, during most of follicular developmental stages, Granulosa Cells (GCs) predominantly express AR with substantial increased amount of small preantral and antral follicles [27]. This cues to a change in ovarian picture resulting PCOM. Wang and his co-workers in 2015 showed that GCs with alternative splice variants of AR cause impaired function of transcription factor/s, which ended into increased androgen production and follicle growth, thereby manifesting to PCOS phenotypes [28]. These circulating androgens brought forth the altered expression of genes associated with folliculogenesis (*DHCR24* and *DHRS13*), along with *C1GALT*, *PRSS23*, *IGFBP7*, *PGK1*, *IGFBP5*, *TMSB10* and *HSPG2*, *FBN1*, *SPARC*, *PLOD2* which regulates ovarian steroidogenesis and ovulation respectively. Recently, Aflatounian et al. documented similar findings in AR-associated PCOS phenotype [29]. Caldwell and his colleagues in 2017 demonstrated that specific loss on AR signalling in the brain of a hyperandrogenized PCOS mice protects it from developing metabolic PCOS traits [30]. Moreover, silencing of AR signalling on theca cells of ovarian follicles of PCOS induced mice had shown restoration of cyclicity and ovulation [31]. With the advancements on CRISPR/Cas9 technology, cell specific AR knockout rodent models are holding big promise on revealing mechanistic investigations on androgen - AR associated PCOS etiology.

Animal models of PCOS: Advantages of androgenized rodent

Hyperandrogenism in PCOS is primarily of theca origin [32]. Presence of androgen/s is observed in peripheral fluid/s in the form of Androstenedione (A), Testosterone (T), Dehydroepiandrosterone Sulfate (DHEAS) to name a few [33].

The foetus of rhesus monkeys and sheep when exposed to high levels of androgens resulted in manifestation of PCOS phenotypes during their adolescence. A research team lead by Teresa in 2007 showed that exposure of monkey to testosterone during 30 to 90 days of fetal life resulted in offspring with low birth weight, hypergonadotropism, multifollicular ovaries, and early cessation of cyclicity [34]. Same workers reported that time-mated pregnant ewes treated with testosterone propionate twice weekly from day 30 to 90 of gestation led to an increased fetal weight ratio with respect to that of control. After fetal exposure to high levels of androgens, adolescent rhesus monkeys and sheep show many features of PCOS. However, use of these models to study the etiology of PCOS is exorbitantly costly.

Neonatal/early postnatal androgenization in rats was reported to express the basic doctrine of human PCOS [35]. Among the different induction protocols, female rats when treated with Testosterone (T) or 5β-Dihydrotestosterone (5β -DHT) during early 5 days postpartum showed persistent anovulatory estrous syndrome during adulthood of all the rats; 74% injected with 5β -DHT displaying anovulation ~120 days of age [36]. Furthermore, administration of androstenedione [37] or DHEA [38] to neonatal or adult female rats induced PCO. Lately, Walter et al. in 2010 documented sub-fertility with perturbed follicular development and ovulation in AR knockout mouse model [39].

In a rodent model, daily administration of testosterone for 7-35 days displayed PCO morphology along with apoptotic ovarian follicles. Moreover, the rats also exhibited disturbed glucose and insulin levels, resonating with the fact that high levels of androgens may result to IR [40]. A single subcutaneous injection of Estradiol Valerate (EV) to young adult female rats induced persistent vaginal cornification and anovulatory polycystic ovaries but fail to induce the prime metabolic disturbances associated human PCOS [41]. Earlier researchers have also tried with the antiprogestin treatment [10] or administration of aromatase inhibitor, letrozole [42] to mimic the condition of PCO in 6-week-old female rats. These treatments resulted in acyclicity, endocrine disturbances and PCO morphology, but the majorities lack the prime metabolic disturbances related to human PCOS. Manneras et.al in 2007 reported a DHT model, which featured both ovarian and metabolic attributes of PCOS [43]. Development of an animal model mimicking both reproductive as well as metabolic aspects of the syndrome is highly perplexing.

The foregoing table (Table 2) refers to a number of laboratory models of PCOD, induced by different hormonal agent/s and/or drug/s reflecting heterogeneity of the syndrome, albeit only a few have focussed on the metabolic dysfunction that represents a vital attribute of human PCOS. In nearer time, use of knockout and newly developed rodent model/s possibly will accelerate the multidimensional understanding on PCOS pathogenesis.

Any of the described methods depicted in Table 2 cannot entirely reiterate the human PCOS in its entire dimension. As for instances, obesity accompanied PCOS is not portraited in most of the prevalent PCOS rodent models, although reports speak off obesity causing aberrations in female reproduction system [12]. Likewise, testosterone treated rodent models display only one of the PCOS phenotypes. However, DHEA treated rat models develop two of the PCOS traits. The potential limitations in chemical induced rodent models can be worn down by coupling the chemical treatment with genetic remodeling in animal models. Treating genetically modified PCOS mice/rats models with appropriate PCOS inducing hormones might open up a ground-breaking avenue in understanding the different dimensions of the syndrome.

Table 2: Different methods of hormone and chemical induced PCOS mouse model.

Inducing agents	Treatment	Ovarian and Reproductive abnormalities	Hormonal irregularities and other metabolic abnormalities	Reference
Dihydrotestosterone (DHT)	Prenatal treatment: Female mice treated with DHT (250 µg) on 16-18 days of gestation bring about reproductive abnormalities in the female offsprings	Increased estrous cycle length Hyperandrogenism More number of small antral follicles in ovary Follicles contain thin granulosa cell layer and thick theca cell layer	Increased serum LH and testosterone Increased size of visceral adipocytes Elevated fasting glucose level Decreased glucose tolerance	[38,44, 45]
Estrogen	20 µg estradiol 17β is subcutaneously injected in neonatal mice of 5-7 days of age	Follicular cysts appear Anovulation is observed Corpora luteum is lacking	Endocrine profiling and metabolic functioning of the treated mice need to be studied in detail	[44,46]
Testosterone	Female mice are treated with 100 mg of testosterone propionate during 1-5 days of age. Not frequently administered to mice	Polyfollicular ovaries are observed Anovulation is observed Corpora luteum is lacking	Endocrine profiling and metabolic functioning of the treated mice need to be studied in detail.	[40,47]
Letrozole	Mice were treated with 9-36 µg/day letrozole for 5 weeks	Acyclicity is observed in estrous cycle Polycystic ovaries are observed	Elevated testosterone Obesity Increased fat mass Elevated basal glucose levels Impaired glucose tolerance	[42,48]
Antiprogesterone (RU486)	Adult rat treated with 2-4 mg/100 g body weight for 2 weeks	Enlarged ovaries with arrested follicular development Hyperthecosis Large atretic follicular cysts Hypertrophy of granulosa cells Luteinized cysts Acyclicity in estrous cycle	Elevated serum LH, Testosterone, and Prolactin Decreased FSH level enlarged pituitary Elevated serum insulin-like growth factor- I (IGF-I) Increased serum insulin	[10,48]
Estradiol valerate (EV)	One dose of 2 mg/day in adult rat	Ovaries reduce in size Large cystic follicles Hyperthecosis Presence of degenerative secondary follicles Ovaries lack postovulatory corpora lutea Acyclic estrous cycle Does not develop hyperandrogenemia	Low serum LH concentrations Decreased plasma Testosterone, FSH Develop hypertension	[10,11, 48]
Dehydroepiandrosterone (DHEA)	Rats treated with 6 mg/100 g body weight, for 4 weeks.	Anovulation is observed Multiple follicular cysts develop Weight of ovary increase	Serum Testosterone, FSH, LH, Prolactin increase Elevated fasting serum glucose and insulin Increase in resistin mRNA level of white adipose Aberrations in glucose metabolism is observed	[10,38, 48]
Insulin + Human chorionic gonadotropin (hCG)	Rats were treated with insulin of 0.5 IU/day and the dose gradually increased to 6.0 IU/day and continued to 3 weeks along with hCG of 3.0 IU/day.	Increase in uterine weight Acyclic estrous cycle	Increase in LH, testosterone Increase in free androgen index Increase in fasting glucose level	[49,50]

Table 3: Genetically engineered mouse models showing PCOS-like symptoms.

Genetic mouse model	Rationality behind the model	Ovarian phenotypes	Reproductive abnormalities	Metabolic alterations	Reference
1. hCG-/LH- over-expressing transgenic female mice	Increased levels of hCG/LH are found in women suffering from PCOS.	i) Arrest of ovarian folliculogenesis at the antral follicle stage. ii) Develop cystic ovaries with thickening in theca cell layer and luteinization of stroma cells iii) Display elevated levels of serum estradiol and testosterone	i) Reduced fertility	i) Abdominal fat accumulation and increase in body weight ii) Develop hyperinsulinemia, hypertriglyceridemia and dyslipidemia, as well as glucose intolerance and insulin resistance iii) Increase in BMD iv) Develop hyperprolactinemia, mammary tumors and pituitary adenomas v) Develop degenerating kidneys	[12,33,56,57]
2. IR/LepR^{POMC} knockout female mice	Leptin and insulin action in the brain is essential for coordinated reproduction due to their ability to indirectly modulate GnRH release.	i) Reduced ovulation or anovulation ii) Occasional formation of cyst-like follicles iii) Display elevated serum LH and testosterone levels iv) Increased ovarian androgen production	i) Reduced fertility	i) Increased body fat mass and adipocyte hypertrophy ii) Develop hyperinsulinemia, glucose intolerance and insulin resistance iii) Presence of inflammation in perigonadal adipose tissue, liver and ovary iv) Exhibit gestational hyperglycemia	[12,33,58,59]
3. PAI-1 overexpressing transgenic female mice	The main physiological inhibitor of plasminogen activation PAI-1 is enhanced in women with PCOS	i) Develop polycystic ovaries with thickened theca cell layer and rare corpora lutea ii) Hyperandrogenism with higher testosterone plasma levels	i) Reduced fertility	i) Develop hyperinsulinemia	[61,3]
4. ESR1 knockout (ESR1 KO) female mice	ESR1 mediates estrogen action in regulating at all levels of the hypothalamus-pituitary-ovary axis	i) Ovaries have fewer corpora lutea but more antral follicles ii) Display irregular estrous cycles iii) Display enhanced levels of serum testosterone and FSH iv) Display lower or undetectable LH levels	i) Loss of fertility prematurely	i) Develop adipocyte hyperplasia and hypertrophy ii) Develop insulin resistance and glucose intolerance	[62,63]
5. Aromatase knockout (Ar KO) female mice	Granulosa cells synthesize Estrogens by the conversion of androgens, with the involvement of the enzyme P450 aromatase	i) Develop haemorrhagic ovarian cysts ii) Display elevated serum LH, FSH and testosterone levels iii) Display lower or undetectable estrogen levels iv) Disrupted folliculogenesis v) Anovulation	i) Infertile	i) Increased fat accumulation and body weight ii) Impaired lipid metabolism	[11,18,64,65]

hCG: Human Chorionic Gonadotropin; LH: Luteinizing Hormone; BMD: Bone Mineral Density; IR/LepR^{POMC}: Knockout- Mice Lacking Insulin and Leptin Receptors in Pro-Opiomelanocortin (POMC) Neurons of the Hypothalamus; GnRH: Gonadotropin-releasing hormone; PAI-1: Plasminogen Activator Inhibitor-1; ESR1: Estrogen Receptor-α; FSH: Follicle-Stimulating Hormone.

Genetic remodeling for development of PCOS mouse model

Customized genetically manipulated animal model/s has been developed for understanding the molecular basis of etiopathogenesis of PCOS. Many studies have successfully conceived the idea of familial clustering in PCOS and clearly specified that susceptibility to this disorder has a genetic foundation [12]. A considerable degree of penetrance of the symptoms is observed between first degree relatives of women with PCOS and is now widely accepted that the disorder is oligogenic. This is possibly based on a few key genes namely, CYP11a, CYP17, CYP19, insulin gene variable number tandem repeat, follistatin locus on chromosome 5 etc to name a few [51] which perhaps becomes the basis of targeting few particular genetic components in animal models with distinctive genetic mutations [52].

Since, human PCOS is frequently linked with metabolic disturbances, care should be taken while selection of appropriate transgenic or knockout (KO) models based solely on their ovarian phenotype. ERα and/or ERβ knockout mice model/s has been studied for evaluation of role of ERα in the regulation of theca cell function. Similarly, female mice over-expressing LH/hCG or lacking plasminogen activator inhibitor-1 (PAI-1) exhibited theca cell hyperplasia, hyperandrogenemia and hyperinsulinemia as observed in human PCOS [3].

Dissen et al. in 2009 demonstrated that the transgenic overexpression of nerve growth factor (NGF), a neurotrophin and a marker of sympathetic hyperactivity, perturbs 17 α-hydroxylase/ C17–20 lyase promoter (17NF mice) and resulted in ovarian anomalies similar to that observed in the ovaries of women with PCOS [53]. At normal levels, NGF not only plays a pivotal role in functioning of the peripheral nervous system but also aids in the processes of follicular development and ovulation. However, an excess of intraovarian NGF in the 17NF mice leads to arrest of antral follicle growth at an intermediate stage accompanied by elevated apoptosis of granulosa cells and increased synthesis of androgens in response to stimulation by FSH. On the other hand, there was no formation of follicular cysts, which developed only in the presence of a sustained elevated plasma LH level. These mice also displayed delayed puberty, irregular estrous cycles, reduced ovulatory response and decreased fertility. Besides causing reproductive abnormalities,

excessive ovarian NGF production in 17NF mice resulted in several metabolic alterations, including hyperinsulinemia, glucose intolerance and IR; increase in body fat, visceral fat and lean body mass leading to increased body weight, enhanced bone mineral content and bone mineral density, systemic sympathetic hyperactivity, all of which are generally seen in PCOS women [54]. In a recent Swedish study, Manti and co-workers in 2020 demonstrated that excessive ovarian NGF impaired embryonic development of the female foetuses, which subsequently displayed irregular estrous cycles, altered ovarian expression of steroidogenic markers and an increased systemic sympathetic outflow in the adulthood [55]. Moreover, adult 17NF mice develop glucose intolerance, increased fat mass, decreased energy expenditure, and liver steatosis55. Thus, the above studies suggest that ovarian overexpression of NGF in the mouse model 17NF causes embryonic defects as well as reproductive and metabolic abnormalities in the adult mice that are characteristic of PCOS in women, and propose that ovarian sympathetic hyperactivity contributes to the development and/or progression of the disorder.

Other important genetically engineered mouse models have been documented in Table 3. Transgenic mice that display anomalies in glucose and/or lipid metabolism, such as, the New Zealand obese mice, IR/LepRPOMC knockout mice, Mito-ob mice, mice over-expressing the human insulin-like growth factor-I (IGF-I), protein kinase B-β (PKBβ/Akt2) KO mice, Ptenfl/fl–Cyp17iCre (tPtenMT) mice, usually develop ovarian cysts and metabolic abnormalities [3]. Moreover, transgenic mice in which specific endocrine system-related genes have been deleted or over-expressed, like aromatase KO (Ar KO) mice, hCG-/LH- over-expressing mice, inhibin-subunit-over-expressing mice, display PCOS-like characteristics [3]. Though genetically modified mouse models might not always represent the full spectrum of human PCOS, they exhibit most of the reproductive and metabolic abnormalities associated with the disorder, which may help to unravel the mystery behind the development of PCOS in women.

Diet - Gut microbiota remodeling for PCOS development

Human gut houses more than 100 trillion microbes referred to as the Gut Microbiota (GM) [60]. In the past, the commensal microbiota residing in gut lumen was largely neglected and was mostly inaccessible to investigation. Recently, studies have revealed that GM takes an imperative role in digestion, immune modulation, maintaining healthy gut and other metabolic activities [66]. GM being remarkably metabolically active releases certain array of metabolites, which are playing an unavoidable part in maintaining host homoeostasis and health. Interestingly, recent reports are of this opinion that the composition of commensal microbes of male and female animals diverge at the time of puberty, which implies that the sex hormone levels probably exert specific influences on the composition of the microbiota [67,68]. Therefore, learning of gut microbiome composition and its metabolites are inevitable in deciphering host-microbiome interactions, which is basically executed by the faecal metabolic profiling. After all, the faecal metabolome provides a functional readout of the microbial activity within the host body [69].

GM has been also lined to sex steroids, neurotransmitters, obesity, hypertension, cardiovascular disease, diabetes, metabolic dysregulation, cancer, and depression [70,71,72]. Many of these features are often unavoidable in women with PCOS [73]. Precisely, few recent studies from China and Europe have reported that GM is altered in PCOS women and rodent models [74,75]. It is worthy to note that there are reports documenting the association between dysgenesis of GM with sex hormones and obesity in women with PCOS [76]. Thus, there is a hint of the existence of a close link between PCOS and obesity backed up by epidemiological data [77]. Moreover, gradual change in GM from birth indicates the significance of diet in colonization of the microbiota profile. Echoing this concept, Daniel and his team in 2014 reported altered gut microbiota in mice fed with High Fat High Sugar (HFHS) diet for 12 weeks [78]. The probable unidirectional concept of diet-GM-PCOS in human as depicted in Figure 2 starts with the intake of food with high saturated fat and refined sugar content [79]. A hypothesis proposed by Tremellen et al. in 2012 named DOGMA (dysbiosis of gut microbiota), elucidates a probable series of events behind the pathophysiology of PCOS [80]. The events are as follows: 1) Prolonged HFHS diet and/or low dietary fibre diet end up in imbalance on GM; 2) this imbalance brings about loosening of tight junction between intestinal epithelial cells, thereby increasing the permeability of the gut mucosal, often referred to as leaky gut; 3) Leaky gut might facilitate in leakage of Lipopolysaccharide (LPS) from gram negative bacteria residing within gut lumen into systemic circulation which stimulates secondary activation of immune system; 4) this immune activation may probably intervene the functioning of insulin receptor, thereby causing Insulin Resistance (IR); 5) IR/Hyperinsulinemia is prone to promote elevated production and secretion of testosterone, hence interfering with ovarian follicles maturation and development.

Actually, consumption of food with less dietary fibre culminates into reduction in population of good gut bacteria like *Bifidobacteria* and *Lactobacilli* etc. [81]. These beneficial bacteria ferment dietary fibre and produce Short Chain Fatty Acids (SCFAs) along with lactic acids which aid in lowering colonic pH and thereby inhibiting the growth of bad gut bacteria like *Prevotella* etc. Consequently, the SCFAs in form of acetate, propionate and butyrate induce enhanced production of MUC-2 mucin from colonic mucosal cells which prevent trans-mucosal passage of bacteria / bacterial endotoxins from the gut lumen [82,83]. Moreover, SCFAs help in maintaining tight junction of the colonic epithelial cells, hence decreasing permeability and thereby preventing leakage of Lipopolysaccharides (LPS) into systemic circulation. From these facts, it can be deduced that the lowering of good gut bacteria facilitates in growth of certain gram-negative bacteria, which imparts harmful influences causing manifestation of the PCOS phenotypes. LPS from these gram-negative bacteria migrate to the systemic circulation and bring about strong immune stimulation. This chronic activation of innate immunity in hepatic and muscles cells cause impairment of insulin receptor functioning and thus causing insulin resistance, one of the driving factors of PCOS [81]. This conception is backed up by the studies conducted by Volk et al. (2017) and Roberts et al. (2017) where prolong feeding of rodent with High Fat High Sugar (HFHS) diet resulted in shooting up of serum testosterone and insulin level along with alteration in gene expression involved in folliculogenesis and steroidogenesis [84,85]. The unspoken influence of GM on pathophysiology of PCOS resonated with the study conducted by Torres and co-researchers in 2019 where faecal microbiota transplantation technique was set forth. The study had displayed that the transplantation of faeces from PCOS patients into mice by oral lavage resulted in development of PCOS phenotypes including IR, ovarian cysts, increased level of testosterone and luteinizing hormones, followed by decreased fertility [86].

Furthermore, the concept of GM remodelling for PCOS phe-

notype was also conceived by a recent study from China which have analysed faecal bile acid metabolites along with microbiome in women with PCOS. They found that transplantation of faecal microbiota from PCOS patients or mice receiving *B. vulgatus* colonies lead to increased distortion of ovarian functions, IR, altered bile acid metabolism, and infertility [87]. Apart from bile acid metabolites, the GM mediated dietary fibre fermentation in large intestine releases SCFA, which stimulate release of other gut hormones like Peptide YY (PYY), Glucagon Like Peptide 1 (GLP1), ghrelin and leptin, whose individual role/s are pivotal to PCOS pathophysiology [88].

The composition of the gut microbial communities impacts estrogen levels through secretion of β-glucuronidase enzyme which deconjugates estrogen to its active form and interacts with its receptor [89]. The hyperandrogenic microenvironment encountered in PCOS women may lead to gut dysbiosis and alteration in both estrogen and androgen metabolism. Up-regulation of estrogen metabolism gene i.e. *Crb1* and *Ste2* gene is documented which are involved in both folliculogenesis and steroidogenesis [90]. Other studies have also reported the association of gut dysbiosis with menstrual cycle regularity and infertility [91,92]. In point of fact, gut microbiome and metabolome is rapidly becoming new avenue for personalized medicine. As a sub-continental group of author/s, impact of the gut microbiome is important given the vast diversity of geographic, dietary habits (most importantly a predominantly vegetarian diet) followed in India and increased prevalence of PCOS in adolescents when the GM is still being shaped and dietary intervention may prove most beneficial. In summary, given that PCOS has far-reaching reproductive and metabolic concerns, understanding microbial profile specific to PCOS may enable to understand another significant facet to PCOS pathophysiology.

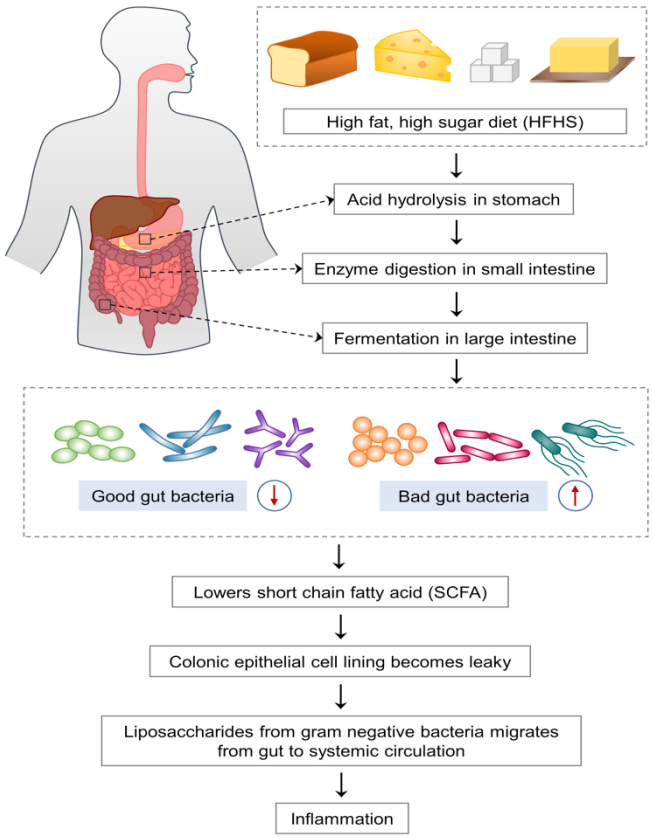

Figure 2: Influence of High Fat High Sugar diet on GM.

Conclusion and future scope

PCOS being a common endocrine disorder in women and imposing prominent financial burden, still falls short of early diagnosis and curative therapy based on its molecular pathogenesis. The etiology of PCOS is enveloped by several postulates, which claim the need of studying the undeciphered aspects on different animal models mimicking the PCOS phenotypes. Various methods of inducing PCOS in rodent/s have been documented; however, selection of the predicted methods is not univocal. Invasive research work needs to be concentrated in putting forward the most reliable methodology for inducing PCOS phenotype with meticulous details of the physiological, hormonal and metabolic changes involved in the process.

One of the potential methods, gut microbiota induced PCOS phenotype has not been yet translated entirely in laboratory research, thereby advocating attention of the researchers in this direction. A precise food-metabolite-gene loop works behind the manifestation of the disease, which is yet unexplored. Thus, it is essential to examine metagenome-hyperbole-diet interaction in susceptibility of the disease. Probing the different facets of PCOS is imperative to pull out an exclusive pre-PCOS biomarkers, which will not only facilitate in early diagnosis but also can effectively, prevent in manifestation of the disease. Furthermore, women in this speeding life era are habitually under the clutches of lifestyle diseases like diabetes, obesity etc. which make them more vulnerable to the adverse effects of the disease. Thus, a very vivid, critical and multidimensional research and development is of prime importance in understanding the closely knitted network between these diseases and thereby, curbing down the overall impact of these diseases.

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