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Perspectives of PCOS Pathophysiology: Exploring the Interplay between PCOS and the Gut Microbiota

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Abstract

PCOS is a gynecological and metabolic concern for numerous women of reproductive age. Its pathophysiology broadly entails hyperandrogenism, insulin resistance, and neuroendocrine dysfunction, and is heavily influenced by genetic and epigenetic factors. However, its precise aetiology remains unclear. The gut microbiome is a major endocrine organ and plays a key role in host metabolism through its metabolites which regulate diverse host physiology like metabolism, immunity, etc. Numerous studies have described associations of altered microbiota in the progress and development of major human diseases. The studies conducted on animals and humans have suggested that the gut microbiota and its metabolites are involved in the pathogenesis of PCOS and its associated attributes such as insulin resistance, obesity and inflammation. Moreover, supplementation of probiotics/prebiotics has been reported to relieve the adverse metabolic and hormonal parameters effectively. Knowledge of this link between gut dysbiosis and PCOS has also spurred research interest in exploring novel management of PCOS. In this review, we have discussed the role of gut microbiota dysbiosis and its metabolite in the progression and treatment of PCOS.

Keywords: Gut Microbiota, Gut Metabolites, Hyperandrogenism, Insulin-Resistance, Obesity, PCOS

1. Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder observed in women of reproductive age, with a global prevalence ranging from 2.2-48 %[1]. It is characterized by skewed LH:FSH ratio, Hyperandrogenism (HA), Polycystic Ovarian Morphology (PCOM) and Anovulation/Oligomenorrhoea (OA). About 50% of women with PCOS are obese, and 60-70 % are insulin resistant[2]. Women with PCOS are at higher risk of developing Type 2 Diabetes mellitus (T2D), dyslipidemia, metabolic syndrome and cardiovascular disease[3]. Sets of criteria are laid for diagnosing PCOS, with the National Institute of Health (NIH), 1990, being the first to establish the condition on the presence of clinical and/or biochemical hyperandrogenism and oligo/amenorrhea anovulation. The Rotterdam consensus criteria, 2003, requires the presence of any two of three features: anovulation or oligo-ovulation, hyperandrogenism and polycystic ovarian morphology seen on ultrasound for the diagnosis of PCOS. Later, the Androgen Excess Society defined PCOS as the presence of hyperandrogenism and either polycystic morphology or/and anovulation/oligomenorrhea[2]. As this is a complex heterogeneous disorder with various characteristic features, a meeting by the National Institute of Health (NIH) in 2012 proposed characterizing the disease based on a phenotype observed using the Rotterdam criteria. The four different phenotypes observed are Phenotype A (HA + OA + PCOM), phenotype B (HA + OA), phenotype C (HA + PCOM) and Phenotype D (PCOM + OA) (Evidence-based Methodology Workshop (EbMW) program, 2012).

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2. Pathophysiology of PCOS

The pathophysiology of PCOS is multifactorial and includes neuroendocrine dysfunction, hyperandrogenism and insulin resistance. The GnRH pulse frequency is high in women with PCOS, leading to increased LH secretion and skewed LH: FSH ratio. This impacts the normal folliculogenesis process leading to follicular growth arrest at the preantral stage, which fails to select the dominant follicle leading to anovulation. Excessive secretion of LH and insulin leads to increased androgen levels, leading to follicular arrest. Hyperinsulinemia is a compensatory mechanism for insulin resistance often seen in women with PCOS and is suggested to be a result of a defect in post-insulin receptor binding.

Further, hyperinsulinemia is known to induce androgen synthesis both directly and indirectly. Insulin is reported to stimulate the activity of enzymes involved in the steroidogenesis pathway; it increases 17-a-hydroxylase and 17-20 lyase activity of enzyme P450c17, which is the rate-limiting enzyme in the production of androgens in both luteinized theca cells and adrenal glands. Insulin also decreases the levels of Sex Hormone Binding Globulin (SHBG), increasing the levels of free testosterone[4]. A state of chronic low-grade inflammation and oxidative stress[5] also characterizes the syndrome. An increase in Reactive Oxygen Species (ROS) production leading to oxidative stress has been known to play a role in the aetiology of PCOS[6]. ROS is known to induce insulin resistance through mitochondrial dysfunction leading to the production of TNF-α. ROS also induces hyperandrogenism and affects oocyte quality[6].

PCOS has a strong genetic component confirmed by studies reporting the prevalence of the disease up to 55-60 % in a first-degree relative of PCOS women. In comparison, twin studies have reported 72% genetic variance in risk of PCOS[7]. Several candidate gene studies and GWAS have been conducted to identify genetic predisposition profiles in PCOS in different ethnic populations. These studies have reported an association of PCOS and its related phenotypes with various polymorphisms in genes involved in steroidogenesis, hypothalamic-pituitary axis function, and metabolic and inflammatory pathways[8]. Although it is clear that PCOS is a multigenic syndrome, no genetic biomarker has been established yet. In addition to genetic association, epigenetic changes in adipose tissue and granulosa cells have also been reported to play a role in the pathology of the disease[9].

The gut is a major endocrine organ harbouring trillions of microorganisms, mainly bacteria, viruses, and fungi. The gut microbiota is involved in the digestion of indigestible carbohydrates, producing metabolites beneficial to the host, metabolism of proteins, nutrients, and immune functions. The gut microbiome encodes enzymes that convert non-digestible dietary fibers to gases and Short-Chain Fatty Acids (SCFA). Gut microbiota metabolizes proteins into peptides, branched fatty acids and gases like ammonia, H_2, CO_2, and H_2S due to its wide range of proteolytic enzyme activity. Gut microbiota is also known to synthesize vitamins like vitamin K and vitamin B complex, including biotin, cobalamin, folates, nicotinic acid, etc., and maintain the diversity of the bile acid pool[10]. Gut microbiota is also known to modulate the regulation and development of innate and adaptive immune components. A mechanical and immune barrier mechanism exists to prevent the interaction of gut microbiota and with the host so as not to cause immune system activation[11]. Gut microbiota dysbiosis may be defined as the loss of beneficial microbes resulting in atypical changes in its role in metabolism and energy homeostasis leading to inflammation. Gut microbiota dysbiosis is associated with various autoimmune conditions, neurodegenerative diseases, metabolic diseases like obesity, insulin resistance, and cardiovascular diseases[12].

Despite rigorous efforts, the pathophysiology of PCOS remains largely elusive to date. In 2012, an interesting theory was hypothesized by Tremellen *et al.*, known as the Dysbiosis of Gut Microbiota (DOGMA), to elucidate the aetiology of PCOS. The authors proposed that the imbalance in gut microbiota due to a high-fat diet leads to disruption of intestinal epithelial cells, thereby increasing gut mucosa's permeability and leading to Lipopolysaccharide (LPS) leakage in the gut, resulting in chronic inflammation and insulin resistance[13]. Studies have drawn attention to a relationship between gut microbiota dysbiosis with obesity, insulin resistance and chronic inflammation, which are often observed in women with PCOS. Evidence on gut microbiota dysbiosis in women with PCOS has emerged recently, which has shown an association with markers of hyperandrogenism, insulin resistance, inflammation and metabolic syndrome

in them. Further, treatments directed towards improving gut health, such as supplementation of probiotics and fecal microbiota transplantation to manage PCOS, have also been explored[14].

3. Composition of Gut Microbiota

Gut microbiome mapping projects such as the NIH Human Microbiome Project (HMP), the integrative HMP (iHMP), and the Metagenomics of the Human Intestinal Tract (MetaHIT) have enhanced our understanding of host-microbiome interaction[15].

The gut comprises the stomach and small and large intestines, each with different physiological environments that can harbour different microorganisms. Acid-resistant bacterial strains like *Streptococcus, Neisseria* and *Lactobacillus* mainly inhabit the acidic conditions of the stomach. The duodenum being aerobic, consists of bacteria belonging to phyla Firmicutes and Actinobacteria. In contrast, the jejunum consists of both aerobic Gram-positive and facultative anaerobic bacteria like *Lactobacilli, Enterococci* and *Streptococci*. The distal end of the small intestine mostly consists of anaerobes and Gram-negative organisms[15]. The large intestine has the highest density of anaerobes, with *Bacteroides, Bifidobacterium, Streptococcus*, *Enterobacteriaceae, Enterococcus, Clostridium, Lactobacillus* and *Ruminococcus* being the predominant microbes[15].

The microbiota composition varies at different stages of development and depends on diet, gender, ethnicity and geographical origin. The most commonly found taxa in the gut belong to the phyla Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia and Cyanobacteria[16]. The Bacteroidetes and Firmicutes constitute more than 90% of the total population. Firmicutes have a higher capacity to harvest energy from food[17], and Bacteroides are often termed as friendly commensals, although they could also be pathogenic. The Firmicutes to Bacteroides (F/B) ratio is often determined to assess host health status and is influenced by sex, age and diet. An altered ratio is reported to be associated with obesity, T2D, inflammatory disease and certain cancers[18,19].

The genome of the gut microbiota is called the microbiome and is about 150 times greater than that of the host[20]. The microbiome, together with the host genome, is known as the hologenome. Studies to determine the composition of gut microbiome use two approaches, 16s rRNA amplicon sequencing and whole genome sequencing. A commonly used approach for bacterial identification is 16s rRNA sequencing, which involves sequencing 16S rRNA gene hypervariable regions to assign bacterial taxa. Metagenomics or whole genome sequencing also provides information on the functional genes in the sample. With advancements in next-generation sequencing, many studies involving varied conditions have reported alterations in microbiota composition, popularly defined by diversity indices such as alpha and beta diversity. Alpha diversity is defined as the estimation of richness (number of taxonomic groups observed) and evenness (abundance of the group) in an ecosystem or within a sample. Beta diversity estimates the difference in richness and evenness between two ecosystems or two samples[21.] It has become an area of interest to examine the causative links behind these changes and their possible link to PCOS pathophysiology.

4. Gut Microbiota Dysbiosis in PCOS Animal Model

Gut microbiota is complex and plays a key role in host metabolism. Studies have shown that Fecal Microbiota Transplantation (FMT) from *ob/ob* mice to germ-free mice resulted in increased body weight in them[17]. In another study, FMT from lean donors into individuals with metabolic syndrome improved insulin sensitivity[22]. Alteration in gut microbial composition is associated with various conditions like obesity, insulin resistance and inflammation, all of which are characteristic features of PCOS as well.

Several animal models to study PCOS have been developed, which include non-human primates, sheep and rodents, developed by prenatal, prepubertal or adult exposure to Testosterone (T) or Dihydrotestosterone (DHT), which display characteristic reproductive and metabolic features of PCOS[23]. Reports of gut microbiota dysbiosis playing a role in the aetiology of PCOS have emerged mainly using rodent models.

In a first-ever study, Kelley *et al.* observed diet-independent dysregulation of gut microbiota in a letrozole-induced PCOS mouse model with a decrease in alpha diversity which was inversely correlated with testosterone levels[24]. In another study, control and DHT-induced PCOS rats fed with varying concentrations

of protein, carbohydrate and fat diets showed altered beta diversity[25]. This suggests the importance of diet modulation in controlling gut microbiota dysbiosis and PCOS. Further, hyperandrogenemia and high-fat diet synergistic actions have been reported to decrease gut microbial diversity and increase plasma inflammatory markers in DHT rats[26]. Torres *et al.* indicated that co-housing of letrozole-induced PCOS mice with placebo mice ameliorated PCOS metabolic and reproductive phenotypes[27]. This is one of the early studies which supports the therapeutic approach of FMT in the management of this syndrome. Thus, studies in rodents indicate a strong correlation between gut microbiota and PCOS pathophysiology.

5. Gut Microbiota Dysbiosis in Women with PCOS

Several studies report lower alpha diversity and beta diversity in women with PCOS compared to healthy control, respectively[14]. In a pilot study by Lindheim *et al.*, the alpha diversity in PCOS women was 15% lower with a significantly lower abundance of members of phylum Tenericutes[28]. The gut of women with PCOS is depleted of beneficial bacteria and is enriched with opportunistic or pro-inflammatory pathogens like *Escherichia spp., Shigella spp., Enterobacteria phage SfV, and Parabacteroides merdae*[29,30]. Gut dysbiosis could be detected from early age, as seen in another study in adolescent girls with PCOS which showed lower alpha diversity, accompanied by a lower abundance of *Bacteroidaceae* compared to non-PCOS adolescent girls[31,32]. Torres *et al.* reported a low abundance of SCFA-producing bacteria in PCOS women[33], which could partially explain increased serum levels of zonulin and calprotectin, markers of gut permeability and inflammation, respectively, observed in them[34].

The close relationship among obesity, PCOS and gut microbiota has also been investigated, and gut microbiota composition, in terms of bacteria and fungi, was found to be different between lean and obese women with PCOS and their respective control counterparts[35]. Liu *et al.* found that the gut of obese PCOS women is enriched with Gram-negative LPS-producing bacteria and a decrease in spore-forming bacteria with an increased ratio of *Escherichia/Shigella,* similar to the microbial composition of obese non-PCOS women[36]. Obese women with PCOS are also stated to have an increased abundance of the *Catenibacterium* and *Kandleria* genera, previously linked to infectious and autoimmune disorders[37]. The enrichment

Table 1. Summary of studies of gut microbiota in women with PCOS

Reference	Ethnicity	Study Population	Outcomes in the PCOS group
Lindheim *et al.*, 2017[28]	Austrian	PCOS (n = 24); Control (n = 19)	Reduced alpha diversity and altered beta diversity
Liu *et al.*, 2017[36]	Chinese	Obese PCOS (n = 21); Non-obese PCOS (n = 12); Obese controls (n = 6); non-obese (n = 9)	Reduced alpha diversity
Insenser *et al.*, 2018[52]	Spanish	Obese PCOS (n = 8); Non-obese PCOS (n = 7); Obese control (n = 8); Non obese control (n = 8)	Reduced beta diversity in obese PCOS women compared to their lean counterpart
Torres *et al.*, 2018[48]	Polish	PCOS (n = 73); Control (n = 48); women with PCOM (n = 42)	Reduced alpha diversity, altered beta diversity
Zeng *et al.*, 2019[42]	Chinese	IR-PCOS (n = 9); Non-IR PCOS (n = 8); Controls (n = 8)	Alteration in gut microbial composition
Qi *et al.*, 2019[43]	Chinese	PCOS (n = 50); Control (n = 43)	Reduced beta diversity
Lull *et al.*, 2020[44]	Finnish	PCOS(n = 102); Control(n = 210)	No difference

Mammadova *et al.*, 2020[39]	Turkish	PCOS (n = 24); Control (n = 22)	No difference
Eyupoglu *et al.*, 2020[45]	Turkish	PCOS (n = 17); Control (n = 15)	No difference
Chu *et al.*, 2020[30]	Chinese	Obese PCOS (n = 7); Non-obese PCOS (n = 7)); Obese Control (n = 7); Non-obese control (n = 7)	Observed microbial species difference
Liang *et al.*, 2020[46]	Chinese	Obese PCOS (n = 8); Non obese (n = 10); Control (n = 9)	Reduced alpha diversity, altered beta diversity
Zhou *et al.*, 2020[35]	Chinese	Obese PCOS (n = 30); Non-obese (n = 30); Obese controls (n = 11); Non-obese (n = 30)	Gut microbial composition alteration
Jobira *et al.*, 2020	American	PCOS (n = 37); Control (n = 21)	Reduced alpha diversity, altered beta diversity
Haudum *et al.*, 2020[47]	Austria	PCOS (n = 24); Control (n = 20)	Reduced alpha diversity
Zhou *et al.*, 2020[48]	Chinese	PCOS (n = 18 obese); Control (n = 15 non obese)	Reduced alpha diversity
Liang *et al.*, 2021[49]	Chinese	Lean PCOS (n = 10); Overweight PCOS (n = 10) 10); Lean control (n = 10); Overweight control (n = 10)	Gut microbial composition alteration
Beltran *et al.*, 2021[31]	Spanish	PCOS (n = 23); Control (n = 31)	Reduced alpha diversity
He and Li, 2021[50]	Chinese	IR-PCOS (n = 14); Non-IR PCOS (n = 12); Control (n = 10)	Gut microbial composition different between IR PCOS, non-IR PCOS and control
Dong *et al.*, 2021[51]	Chinese	PCOS (n = 45); Control (n = 37)	Reduced alpha diversity [a]
Chen *et al.*, 2021[52]	Chinese	PCOS (n = 98); Control (n = 38)	Reduced alpha diversity, altered beta diversity
Zhu *et al*, 2021[53]	Chinese	PCOS (n = 54); Control (n = 33)	Reduced alpha diversity
Yang *et al.*, 2021[54]	Chinese	PCOS (n = 56); Control (n = 31)	Increased alpha diversity, altered beta diversity
Yang *et al.*, 2022[29]	Chinese	PCOS(n = 32); Control (n = 18)	Reduced alpha diversity, altered beta diversity
Hassan *et al.*, 2022[41]	Indian	PCOS (n = 19); Control (n = 20)	Increased alpha diversity
Yu *et al.*, 2022[55]	Chinese	PCOS (n = 20); Control (n = 20)	Reduced alpha diversity

[a] Not significant when sub-grouped; IR-Insulin resistance; non-IR- non-insulin resistance

of *Lactobacillus, Prevotella,* and *Megamonas* was observed in obese women with PCOS, whereas *Lactococcus, Paraprevotella, Alloprevotella*, and *Clostridium cluster XIVb* were enriched in the non-obese PCOS women[35,36,38-40].

Table 1 summarizes the studies on the association of gut microbiota in women with PCOS. Most of the available studies have been carried out in European and Chinese populations and have reported contrasting results regarding significant changes in alpha and beta diversity in women with PCOS. A single Indian study in Kashmiri women has been reported thus far, indicating increased microbial diversity in women with PCOS[41].

Although studies carried out so far have not conclusively pinpointed the abundance of any particular microbe influencing PCOS, the compositional alteration of gut microbiota is evident in women with PCOS. The differences in the results may be attributed to phenotypes of PCOS, age of the participants enrolled and other confounding factors like ethnicity, dietary habits, and lastly, the relatively small sample sizes used so far. Further use of different methodologies for the collection and processing of stool samples and analytical approaches for interpreting the metagenomic data may also influence these observations.

6. Association of Sex Hormones with Gut Microbiota in PCOS

The potential relationship of sex hormone levels with the gut microbiota has been termed as "microgenderome". The relationship between host and microbiota is bidirectional and is gender-dependent[20]. Women's gut microbiota composition markedly differs from men's, indicating an influence of sex hormones on microbiota composition and these changes often occur after puberty under the influence of sex steroids[56,21]. As mentioned earlier, women with PCOS present with androgen excess along with altered levels of estrogen and progesterone, and this hormonal dysregulation could further alter the composition of the microbiota and its associated metabolites.

Estrobolome is a group of gut microbes known to regulate estrogen levels in the host. This group of bacteria secretes the β-glucuronidase enzyme that deconjugates estrogen, thereby preventing its excretion and allowing its reabsorption in the gut. Lowered enzyme activity due to a decrease in the diversity of gut microbiota was found to decrease estrogen levels in the host leading to conditions like metabolic syndrome, cardiovascular diseases etc[57]. On the other hand, an increased enzyme activity leads to the release of high levels of hormone into enterohepatic circulation leading to condition like endometrial cancer etc[58]. β- glucuronidase activity was found to be significantly high in fecal samples of women with PCOS[59], which could partially account for the dysregulated levels of estrogen observed in them. PCOS rats receiving FMT from healthy rats showed elevated levels of estradiol and estrone, indicating that alterations in gut microbiota can result in fluctuations in hormone levels. In another study, ovariectomized female mice showed an increased abundance of LPS-producing *Escherichia/Shigella* compared to control[60].

Studies have shown that exposure to androgens *in utero*, during the prenatal or neonatal period, results in the development of PCOS phenotype at the adult stage. This also results in gut microbiota dysbiosis characterized by decreased alpha diversity and metabolic complications such as glucose homeostasis, obesity, hypertension, etc[61–63]. Further, the abundance of certain bacteria was also found to correlate with the level of testosterone. The genus *Prevotella*, family Bifidobacteriaceae, showed a negative correlation, while phylum Proteobacteria, Enterobacteriaceae, and the genus *Bacteroides* showed a positive correlation with testosterone levels. Hyperandrogenism influences gut microbiota composition in PCOS[33]. Importantly, it was seen that deconjugation of DHT and T does not occur in the absence of gut microbiota, indicating that gut microbiota participates actively in androgen metabolism[64]. All, in all, these findings indicate that gut microbiota alterations can result in fluctuation of hormone levels in women with PCOS[65].

7. Association of Gut Microbiota with Insulin Resistance in PCOS

Hyperandrogenemia and hyperinsulinemia are the principal corner stones of PCOS pathophysiology. He *et al.*, 2023, reported a decrease of Bacteroides, Bifidobacterium, and Lactobacilli, with an increase in Enterococci abundance and decrease in SCFA concentration in T2D[66]. SCFAs are known to indirectly

regulate glucose levels by regulating gluconeogenesis, increasing insulin secretion and controlling appetite[67].

In a pilot study by Zeng *et al.,* the gut microbiota showed notable difference between Insulin Resistant (IR) and non-IR women with PCOS. It was demonstrated that Firmicutes/Bacteroides ratio was significantly increased in non-IR PCOS, while IR-PCOS women showed a significant decrease[42]. Along similar lines, He and Li also observed that the gut microbial composition of IR-PCOS differs from that of non-IR- PCOS and healthy women. The gut microbiota of IR-PCOS women was enriched with a relative abundance of *Rothia, Ruminococcus*, *Lachnospira,* and *Enterococcus,* while non-IR PCOS was enriched with *Lactobacillus* and *Akkermansia.* Interestingly, both these studies reported significantly decreased abundance of *Prevotellaceae* and *Prevotella,* known producers of SCFA, in the IR-PCOS group compared to the healthy control[50]. Thus, the difference in gut microbiota between IR-PCOS and non-IR-PCOS should be considered while designing new therapeutic approaches.

8. Crosstalk between Gut Microbiota, Inflammation and Metabolism

The metabolites derived from gut microbiota are important for several host processes, and any alterations in microbiota composition change the levels of these metabolites. Indigestible carbohydrates are metabolized by a diverse group of bacteria in the gut microbiota to SCFA, mainly into acetate, propionate, and butyrate. The SCFAs serve as the energy source for intestinal epithelial cells and are important for mediating intestinal gluconeogenesis, and inhibiting the accumulation of triglycerides and lipolysis in adipose tissue. Obese PCOS women showed a lower abundance of several SCFA-producing bacteria, including *Lachnospiraceae_UCG-010*, *Subdoligranulum*, *Anaerococcus spp., Odoribacter spp., Roseburia spp.,* and *R. bromi* compared to the healthy controls[33,38]. On administration of sodium acetate (a known salt component of SCFA) to PCOS-induced rat model, improvement in endocrine profile, ovarian histomorphology, glucose, and lipid profile was observed[68], indicating a potential role of SCFA as a therapeutic treatment for PCOS.

Leaky gut refers to the entry of LPS through the intestinal barrier, causing endotoxemia and inflammation[13]. LPS binds to TLR4 receptors leading to secretion of various pro-inflammatory cytokines like TNF-α, IL-6, and INF-γ, which increases gut permeability by disrupting the tight junction proteins of the intestinal epithelium. SCFAs are also involved in the maintenance of gut permeability by induction of genes encoding tight-junction proteins like zonulin and occludin[69]. The increase in gut permeability in PCOS women is evident with elevated concentrations of zonulin in serum[34]. Butyrate is an important SCFA involved in inhibiting translocation of LPS, thereby preventing immune cell activation leading to inflammation. This was supported by a study in a human ovarian granulosa tumour, called KGN cell line, which were stimulated with LPS to mimic the inflammatory condition in PCOS and were later treated with butyric acid. It was observed that butyric acid improved glucose metabolism, and mitochondrial membrane potential and inhibited inflammation through a complex mechanism[70]. This could indicate the role of gut microbiota metabolites in ovarian functions.

Branched-Chain Amino Acids (BCAA) are essential amino acids obtained through plant-based diet and synthesized by the gut microbiota[71]. Decreasing BCAA consumption was found to improve glucose and lipid homeostasis in obese rats[72]. Elevation in BCAA levels has been linked to increased insulin resistance in obese non-PCOS women via altered levels of signalling of mTOR, JNK, and IRS1[73]. Recent serum metabolomic studies have shown that women with PCOS had significantly higher BCAA levels than controls, which was further influenced by obesity[74]. Another study reported that BCAA levels are elevated in the serum of PCOS women and are found to be associated with insulin-resistance along with increased abundance of the species *Prevotella copri* and *Bacteroides vulgatus*. This indicates that gut microbiota could contribute to dysregulated amino acid metabolism in women with PCOS[75].

Gut microbiota also plays an essential role in Bile Acid (BA) metabolism and in maintaining the diversity of the BA pool, which regulates lipogenesis, gluconeogenesis, and intestinal inflammation via BA receptors. Bile acids are conjugated in the liver, via N-acyl amidation with glycine or taurine; sulfation; ester glucuronidation; ethereal conjugation and N-acetylglucosamination to be then secreted in the intestine[76]. Conjugated primary bile acids (PBA) are first deconjugated and then acted upon by the intestinal flora to be converted into Secondary Bile Acids (SBA) like Lithocholic Acid (LCA) and Deoxycholic Acid (DCA). The deconjugation process for SBA production

provides energy in the form of glycine and taurine residues. This deconjugation is carried out by microbial enzymes Bile Salt Hydrolases (BSHs), widely encoded by several Gram-positive and negative bacteria across various phyla[77]. Women with PCOS have been reported to have an increased abundance of *B. vulgatus,* with low levels of bile acid, Glycodeoxycholic Acid (GDCA) and Tauroursodeoxycholic (TUDCA) acid levels[43]. These acids induce the lymphoid cells of the small intestine to secrete IL-22, which improves insulin sensitivity. IL-22 is anti-inflammatory and functions to maintain the gut epithelial lining, along with inducing expression of genes involved in proliferation, wound healing and regulation of tight junction proteins[78]. Administration of IL-22 or GDCA acid to PCOS mouse model improves insulin resistance and estrous cyclicity.

Further, increased abundance of *B. vulgatus* modulates bile acid profiles and reduces IL-22 levels in the DHEA-induced PCOS mouse model. It was also observed that administration of IL-22 on the DHEA-induced PCOS mouse model improved insulin sensitivity, estrous cycle, and abnormal ovary morphology[43]. Recently Gao *et al.* showed that administration of Troxerutin, a naturally occurring flavonoid, to DHT rats enhanced secondary bile acid profiles, which positively correlated with serum IL-22 level[79]. These results indicate a role of IL-22 as a therapeutic approach in the management of PCOS that could be explored.

Choline, mainly derived from food, is metabolized by gut microbiota to Trimethylamine (TMA) and converted to Trimethylamine-N-oxide (TMAO) in liver[80]. TMAO levels are reported to be associated with an abundance of *Prevotella* and *Akkermansia mucinophilia.* Dysregulation in the concentration of TMAO has been reported to have a potential role in the aetiology of various diseases like CVD, atherosclerosis, chronic kidney disease, metabolic syndrome and cancers[81]. In a PCOS rat model, treatment with TMAO inhibitor 3,3-dimethyl-1-butanol (DMB) alleviated metabolic symptoms by improving the PI3K/Akt-related signalling pathway. This pathway is activated by the binding of insulin to its receptor leading to increased glucose uptake, thereby decreasing glucose level in serum[82]. Women with PCOS also showed elevated serum levels of TMAO and its precursor, which correlated with testosterone and could also contribute to elevated CVD risk in them[45,81,83].

Overall, alterations in gut metabolite profiles could serve as important diagnostic markers and therapeutic targets for understanding PCOS pathophysiology and management.

9. Association of Brain-Gut Axis with Gut Microbiota in PCOS

Gut bacteria and gut hormones are known to communicate with the brain via the gut-brain axis through the Enteric Nervous System (ENS), the Hypothalamic Pituitary Adrenal (HPA) axis and the Autonomic Nervous System (ANS)[84]. Glucagon-Like Peptide-1 (GLP-1) is an anorexigenic gut hormone whose secretion is stimulated by SCFA[85]. GLP-1 stimulates insulin release, in response to food intake and regulates gluconeogenesis. It inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes[86]. Members of gut microbiota are reported to degrade GLP-1 and induce GLP-1 resistance, indicating the role of gut dysbiosis in influencing glucose homeostasis via GLP-1[87,88]. GLP-1 agonists are widely used in treating PCOS women for improving insulin sensitivity, weight loss, etc[89,113,114]. Peptide YY (PYY) is another anorexigenic hormone that promotes energy absorption in the intestinal tract and is influenced by SCFA produced by gut microbiota[85]. Reduced level of PYY is reported in women with PCOS along with a negative correlation with insulin resistance and BMI[90].

The gut microbiota metabolites communicate with the brain via vagus nerve[91]. Gut microbiota is reported to produce neurotransmitters like GABA, dopamine, and serotonin[92]. GABA can stimulate the arcuate nucleus leading to increased LH secretion[93]. Liang *et al.* reported an increase abundance of GABA-producing bacteria *Parabacteroides distasonis* and a positive relationship between *Bacteroides fragilis* and *Escherichia coli* with serum LH levels and LH: FSH ratios in PCOS women[49]. This finding could support the previously reported discovery of high levels of GABA in the cerebrospinal fluid of women with PCOS by Kawwass *et al*[94]. Women with PCOS also showed a significant decrease in serotonin, which is involved in overall behavioral and neuropsychological processes, and this decrease in levels of serotonin may explain the increased odds of depression

and anxiety in these women leading to lower quality of life[95].

The dysbiosis in gut microbiota leads to alteration in gut hormone secretion, which may impede the gut-brain axis and could influence signs and symptoms of PCOS[96].

10. Therapeutics

PCOS is a multifactorial disorder where both genetic and environmental factors play a role in its pathogenesis. With growing evidence linking gut microbiota dysbiosis with the etiology of PCOS, treatments focusing on shifting the gut microbiota dysbiosis towards eubiosis are being studied. The use of prebiotics, probiotics, synbiotics, and Fecal Microbiota Transplants (FMTs) in the management of PCOS is currently being widely studied.

10.1 Probiotics, Prebiotics and Synbiotics

The FAO/WHO defines (2002) probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host**".** Bacterial genera commonly used as probiotics include *Lactobacillus, Bacillus, Bifidobacterium, Streptococcus*, and *Enterococcus*[97].

In a randomized, double-blind, placebo-controlled trial of 60 women with PCOS, including both phenotype A and D, the probiotic (*Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum)* receiving group (n = 30) showed significant weight reduction, BMI, FPG, serum insulin concentrations and triglycerides after 12 weeks of treatment in comparison to placebo group (n = 30)[98]. Similarly, Rashad *et al.* observed that probiotic supplementation showed favourable effects on hormonal and inflammatory profiles, particularly

Table 2. Meta-analysis studies to evaluate the effect of probiotics, prebiotics, and synbiotics in treating women with PCOS

Reference	Supplements evaluated	Participants	Conclusion
Miao *et al.*, 2021[107]	Probiotic and Synbiotic	PCOS = 486	Significant reduction in HOMA-IR and serum insulin levels
Li *et al.*, 2021[108]	Probiotics, Prebiotics, and Synbiotics	PCOS = 1049	Decreased HOMA-IR, FPG, FINS and increased QUICKI, decreased TG, TC, LDL-C, and VLDL-C Pro-, pre-, and synbiotics consumption has a beneficial effect
Cozzolino *et al.*, 2020[109]	Probiotic and Synbiotic	PCOS = 294; Control = 293	Reduced testosterone FPG and systemic inflammation
Heshmati *et al.*, 2019[110]	Probiotics or Synbiotics supplementation	PCOS = 236; Control = 235	Improved QUICKI and decreased TG in the PCOS group receiving probiotics (or synbiotics) as compared to controls [a]
Shemasbi *et al.*, 2019[110]	Probiotics, Prebiotics, and Synbiotics	PCOS = 438; Control = 417	Improved hormonal (FAI, SHBG) and inflammatory (NO, MDA) parameters
Liao *et al.*, 2018[111]	Probiotic	PCOS = 406	Improved QUICKI, TG and VLDL-C Daily probiotic consumption has beneficial effects

[a] Not significant; FAI; Free Androgen Index; FINS: Fasting plasma insulin level; FPG: Fasting plasma glucose; HOMA IR: Homeostatic Model Assessment for Insulin Resistance; LDL- C: Low density lipoprotein cholesterol; MDA: malondialdehyde; NO: nitric oxide; QUICKI: Quantitative insulin sensitivity check index; SHBG: sex hormone binding globulin; TC: Total Cholesterol; TG: Triglyceride; and VLDL-C: very low-density lipoprotein cholesterol

concerning macrophage migration inhibitory factor, in women with PCOS; however, they found no significant effects on fasting serum insulin and HbA1c[99]. In another randomized, double-blind, placebo-controlled trial study, probiotic supplementation resulted in significantly increased serum levels of Sex Hormone-Binding Globulin (SHBG), reduced total serum testosterone levels, reduced hirsutism, and improved chronic inflammatory states as indicated by decreased levels of serum high-sensitivity C-Reactive Protein (hs-CRP) and Malondialdehyde (MDA) concentrations in women with PCOS[100]. Another probiotic supplementation intervention for 12 weeks led to a decrease in weight, insulin resistance, triglycerides and VLDL-cholesterol concentrations in PCOS women[98]. An 8-week trial with multispecies probiotics supplementation positively affected pancreatic β-cell function[101]. Lactobacillus supplementation along with Cyproterone acetate was found to reduce the inflammation through reduced IL-6, hs-CRP and increase in IL-10 along with weight loss in PCOS women[102]. Co-supplementation of Vitamin D and probiotics was associated with improved depression and anxiety scores in women with PCOS and reduced levels of testosterone and CRP and increased total antioxidant capacity[103]. Similar results were obtained with the administration of selenium and probiotic[104]. Intake of probiotic *Bifidobacterium lactis* V9 has been shown to increase SCFA levels in PCOS group[105].

Similar results are also reported with the use of prebiotics, which are non-digestible saccharides that can only be digested by the gut flora. Prebiotic supplementation is reported to improve secretion of GLP-1, which in turn can reduce insulin resistance[106]. Intervention with isoflavone supplementation in PCOS women increases gut microbiota alpha diversity and improves fasting glucose and insulin sensitivity. Table 2 summarises the meta-analyses performed to evaluate the effect of probiotics, prebiotics, and synbiotics in treating women with PCOS.

10.2 Fecal Microbiota Transplantation (FMT)

FMT is the process of transplanting a fecal sample from a healthy person into a recipient via a nasogastric tube or a nasointestinal tube to normalize the gut microbiota composition. The procedure is widely used to treat *Clostridium difficile* infections with an effectiveness of 90%. European Consensus for FMT in clinical practices provides guidelines for safe and accurate FMT practices to promote pathogen-free transplantation of fecal matter from a donor to recipient[112]. Vrieze *et al*. showed FMT from lean donors to recipients with metabolic syndrome resulted in improved insulin sensitivity[113]. A systematic review with meta-analysis evaluated the role of FMT in the treatment of obesity and metabolic syndrome and found that the treatment is safe and may be encouraged as "adjuvant therapy"[114]. FMT may thus also aid in managing PCOS and its related traits by altering microbial profiles to better metabolic, inflammatory, and hormonal anomalies. Evidence suggestive of the use of FMT to manage PCOS mainly comes from the PCOS mouse model. Transplantation of fecal suspension from control rats into letrozole-induced PCOS rats showed improved oestrous cycles and a significant increase in estradiol and estrone levels compared with those in non-treated PCOS rats[65]. In another study, fecal microbiota from women with PCOS was transplanted into pseudo-sterile rats for a period of 21 days, which then displayed features of PCOS like increased cystic follicle numbers and elevated levels of T, LH and LH/FSH. Further, when letrozole-induced PCOS rats received fecal microbiota from a healthy donor, they showed improved estrous cycle, ovarian pathophysiology, and OMA IR index[29]. Although no human study has been reported so far for FMT in women with PCOS, the results observed in PCOS mouse models, and use of FMT in treatment of other disease conditions, advocates the usefulness of the treatment as a possible therapeutic approach for PCOS.

11. Conclusion

Gut microbiota dysbiosis is reported in a variety of disease conditions like T2D, obesity, and even neurological disorders like Alzheimer, and Parkinson's Autism Spectrum Disorder. The decrease in diversity of microbes in PCOS is evident in different studies in human and animal models. Although many studies point out the abundance of certain bacterial phyla/genera in the diseased condition, an abundance of a single bacterium has not yet been clearly defined in the condition. The effect of dietary habits across the different populations may heavily impact the gut microbiota, and the complex nature of gut microbiota may contribute to variation in

the results of different studies where the heterogeneous nature of PCOS may further add to this burden.

Thus, it may be concluded that a panel of microbiota and their metabolites could be explored for their potential for developing a prognostic marker for PCOS and offer new therapeutic options in treatment.

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13. References

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