

Final degree project

Grau en Bioquímica i Biologia Molecular

ROLE OF GUT MICROBIOTA IN THE PATHOGENESIS AND PROGRESSION OF POLYCYSTIC OVARY SYNDROME (PCOS) IN WOMEN.

A SYSTEMATIC REVIEW.

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is the most prevalent endocrine-metabolic pathology in women on reproductive age, which poses a significant public health issue. It is a multifactorial and heterogeneous disorder with four recognized clinical phenotypes, each with different implications. It is mainly characterised by increased ovarian androgen biosynthesis and is related to metabolic complications, like insulin resistance. But to be considered PCOS (according to the currently more accepted diagnosis) at least two of these signs must be present: hyperandrogenism, ovulatory dysfunction, and/or polycystic ovarian morphology. The resulting alterations negatively impact patients' quality of life, and these long-term effects transcend well beyond life.

However, despite significant progress in understanding the pathophysiology over last years, it remains underdiagnosed, invisible, and misunderstood. Recently, a new perspective of the syndrome has appeared: the hypothesis that gut microbiota dysbiosis is involved in the pathogenesis and development of PCOS has been postulated. However, the specific mechanisms by which gut microbes play a role in PCOS remain elusive. The aim of this bibliographic study is deep into the connection of molecular pathways, like insulin resistance; sex and gastrointestinal hormones; bile acids; interleukin-22; and microbial metabolites, with the altered intestinal bacteria composition that end up forming a typical profile in PCOS patients.

In a future perspective, novel treatments with gut microbiota as a therapeutic target could be proposed and tested in patients. Even so, more research is still needed to make progress.

KEYWORDS: PCOS, pathology, women, microbiota, dysbiosis, novel treatment.

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1. GLOSSARY

AE: androgen excess

AMH: anti-Müllerian hormone

ESHRE/ASRM: European Society of Human Reproduction and Embryology / American Society for Reproductive Medicine

FAH: functional adrenal hyperandrogenism

FMT: faecal microbiota transplantation

FOH: functional ovarian hyperandrogenism

FSH: follicle stimulating hormone

HDACs: histone deacetylases

IL-22: interleukine-22

IR: insulin resistance

LH: luteinizing hormone

LPS: lipopolysaccharides

NIH: National Institutes of Health

PCOM: polycystic ovarian morphology

PCOS: polycystic ovary syndrome

PCOS-A: functionally atypical PCOS

PCOS-T: functionally typical PCOS

PYY: peptide YY

SCFAs: short chain fatty acids

2. INTRODUCTION

2.1. Polycystic ovary syndrome (PCOS)

PCOS is the most common endocrinopathy that affects more than 116 million women (3,4%) worldwide, according to the World Health Organization (WHO) estimation (1). Other sources cite 6-10% of reproductive-aged women as being affected by PCOS globally (2). Thus, being a major reason for female infertility around the world and one of the most prevalent nowadays. It is no longer considered simply as a disease of ovary (3). The pathologic state of PCOS is a life-long condition, which begins to manifest at puberty. This syndrome is also linked to metabolic disorders such as insulin resistance (IR) hyperinsulinism¹, type 2 diabetes, and obesity (4). It is defined as a multifactorial pathology, determined by the association of many factors: genetics, endocrine, intrauterine environment, and lifestyle, have been considered as contributors in the development of PCOS (5). Accordingly, warrants reconsideration in light of the substantial data that emerging from last studies. (6)

2.1.1. Evolutionary perspective and diagnosis of PCOS

PCOS evolved as a means of preserving anabolism and reproductive capacity in times of nutritional deprivation. In ancient eras of scarce nutrition, the higher androgen² levels of such women would have been advantageous to reproduction as well as survival, though in current times of plenty this phenotype is disadvantageous. The diversity of apparently unrelated gene variations that are associated with PCOS suggests that multiple mechanisms support this common phenotype. (7)

Due to the heterogeneity within PCOS, there are no unified diagnostic criteria for this pathology, and it has progressed considerably since the last years. Currently,

¹ IR and compensatory hyperinsulinemia: IR is the inability of exo/endogenous insulin to increase glucose uptake and utilisation. That can develop IR hyperinsulinism because peripheral tissues required higher concentrations of insulin to maintain the normoglycaemia. (5)

² Androgens: steroid hormones that regulates the development and maintenance of male characteristics. They are synthesized in testes, ovaries, and adrenal glands. Androgens are the precursors to oestrogens. (26)

there are three widely accepted diagnostic criteria: the National Institutes of Health (NIH) criteria, the Rotterdam Criteria, and the Androgen Excess (AE)-PCOS Society Criteria (8). In 1992, the NIH defined PCOS by two features: (i) chronic anovulation, with (ii) clinical and/or biochemical hyperandrogenism, in absence of other disorders. In 2003, a meeting of experts in Rotterdam revised the criteria, and according to the European Society of Human Reproduction and Embryology / American Society for Reproductive Medicine (ESHRE/ASRM), PCOS diagnosis was based on the presence of at least two of the following features (always excluding another etiologist): (8) (9)

- Ovulatory dysfunction (oligo - and/or anovulation): anovulation means a lack of ovulation or absent ovulation; oligo-ovulation is called when ovulation is irregular but not completely absent.
- Clinical and/or biochemical signs of hyperandrogenism: the evidence of hyperandrogenism can be rated as free testosterone or free androgen index, or the calculated bioavailable testosterone level.
- Polycystic ovarian morphology (PCOM): the presence of 12 or more follicles measuring 2-9 mm throughout the entire ovary (it is an accumulation of smaller follicles than normal ones), or an ovarian volume = 10 cm³ defines the PCOM.

Then, the overall prevalence of PCOS varied from 6% when applying the NIH criteria to 10% according to Rotterdam or AE-PCOS Society Criteria. This last criteria is still the most widely used worldwide for both individual diagnosis and research.

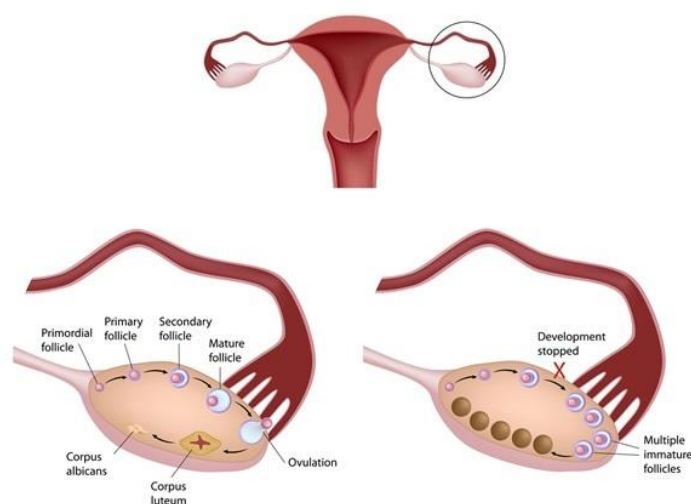


Figure 1. **Normal ovarian cycle (left) and polycystic ovary cycle (right).** From (10)

2.1.2. PCOS phenotypes

The PCOS pathogenesis is explained through several signalling pathways but is mainly focused on an imbalance in the hypothalamic-pituitary-ovarian axis. That ties in with Functional Ovarian Hyperandrogenism (FOH), which is characterised by generalized ovarian steroid hormone hyper-responsiveness to Luteinizing Hormone (LH)³ (6). From that, different phenotypes of PCOS can be distinguished:

Clinical phenotypes

PCOS phenotypes vary with ethnicity, region, age, and weight. The Rotterdam criteria generate four clinical phenotypes, each characterised by combinations of at least two typical symptoms of PCOS. They fall on a spectrum of decreasing specificity in parallel with a decreasing clinical severity (7), as listed in *Table 1*.

Table 1. Clinical phenotypes of PCOS according to Rotterdam criteria.
Adapted from (7).

PHENOTYPE 1 (“Classic PCOS”)
Clinical and/or biochemical evidence of hyperandrogenism
Evidence of ovulatory dysfunction
Ultrasonographic evidence of a polycystic ovary (the PCOM)
PHENOTYPE 2 (“Essential NIH Criteria”)
Clinical and/or biochemical evidence of hyperandrogenism
Evidence of ovulatory dysfunction
PHENOTYPE 3 (“Ovulatory PCOS”)
Clinical and/or biochemical evidence of hyperandrogenism
Ultrasonographic evidence of a polycystic ovary
PHENOTYPE 4 (“Non-hyperandrogenic PCOS”)
Evidence of ovulatory dysfunction
Ultrasonographic evidence of a polycystic ovary

³ Luteinizing Hormone (LH) is a gonadotropic hormone that stimulates androgen hormone secretion and female ovulation and luteinization. (12)

Biochemical Phenotypes

For determine and categorize it, is necessary to involve testing ovarian and adrenal androgenic function status. Below, it is showed the functional classification of PCOS according to source of androgen excess: (7) (11) (12)

- FUNCTIONALLY TYPICAL PCOS (PCOS-T). The typical pattern of FOH indicates dysregulated steroidogenesis, now particularly at the level of CYP17, the rate-limiting enzyme in androgen and oestrogen biosynthesis which is not appropriately regulated in PCOS. Then, the resultant inappropriately elevated serum testosterone levels are from ovarian origin. It represents a prevalence among PCOS of 67-69% (the most common form of PCOS).
- FUNCTIONALLY ATYPICAL PCOS (PCOS-A). In this case, FOH may indicates an atypical and heterogeneous pathogenetic mechanism because now the characteristic CYP17 abnormality is lacking. Normally, it coexists with Functional Adrenal Hyperandrogenism (FAH), which is due to a disturbance of adrenal steroidogenesis like that affects the PCOS ovary. The prevalence of FOH with FAH among PCOS is 20-30%. A small number of cases (5%) are due to isolated FAH. Then, most of testosterone levels are from suprarenal origin.

2.1.3. Molecular and clinical symptomatology

The molecular characteristics of PCOS women can be ovulatory dysfunction, polycystic ovarian morphological changes, and as mentioned above, increasing androgen biosynthesis (also referred to as FOH or AE).

This last pathophysiologic abnormality is demonstrable in nearly 90% of PCOS and can account for all the clinical features of the syndrome (11). In addition, in the majority of FOH patients has been found insulin resistance and compensatory hyperinsulinemia. It is estimated that between 44% and 70% of women with PCOS are prone to have an impairment of insulin action (13). The resultant hyperinsulinemia leads to an increase in testosterone production in the ovarian

Theca cells⁴ and in the adrenal glands. Then, increased circulating androgens levels stimulate adipogenesis (especially the visceral), which in turn increases IR, and other PCOS-related disorders (5). Accordingly, the hyperinsulinemia is an extra-ovarian modulator that aggravates the ovarian hyperandrogenism in different ways, creating a vicious cycle. These disorders also affect apoptosis, proliferation, and differentiation of follicular cells, because ovulation is hindered and follicular growth in early phases is speeded. Simultaneously, this is accompanied by hormone secretion abnormalities, like LH and Anti-Müllerian Hormone (AMH)⁵ excess, while Follicle Stimulating Hormone (FSH)⁶ tend to be lower (2) (8) (11). Therefore, it has been shown that the gonadotropin-releasing hormone, which is secreted by the hypothalamus and stimulates the release of the gonadotropins LH and FSH, in the case of PCOS only promotes LH synthesis, resulting in an increased LH/FSH ratio in women with PCOS (1).

The typical clinical manifestations that the majority of PCOS women present include excessive hair growth (hirsutism), acne, irregular or no menstrual periods, pelvic pain, infertility, weight changes, pregnancy complications, and psychological comorbidities (anxiety, depression, eating disorders and poor body image and bad quality of life). It arises the risk for associated comorbidities like endometrial cancer, or type II diabetes (1). These features can evolve during the woman's life cycle (12). With increasing age, the syndrome evolves from a reproductive disease to a more metabolic disorder (14).

2.1.4. Aetiology

The factors contributing to PCOS are the result of complex host intrinsic factors, like epigenetics and the gut microbiota; and extrinsic factors such as lifestyle, diet, and exercise, which may alleviate PCOS symptoms. (8)

⁴ Theca cells are a group of endocrine cells in the ovary made up of connective tissue surrounding the follicle. Is the predominant site of androgen production and maturation of the ovarian follicle. (11)

⁵ Anti-Müllerian Hormone (AMH) is a biological marker of ovarian reserve (number of growing follicles) and acts inhibiting the excessive follicular recruitment by FSH and the CYP17. (12)

⁶ Follicle Stimulating Hormone (FSH) is a gonadotropic hormone that stimulates the production of oocytes and oestrogen hormones. (12)

There is some evidence of a genetic component. Some women have a higher risk of developing PCOS due to predominant genes. Several data revealed specific loci and alleles that play a major role in PCOS phenotype identification (1). A hypothesis says that PCOS consist of a congenital predisposing factor (first hit) that becomes manifest upon exposure to a postnatal provocative factor (second hit). The congenital factor causes abnormal androgenic function and may have heritable or environmentally acquired causes. In fact, the hyper exposure to testosterone in the utero can have a risk influence later in life. The postnatal condition is insulin-resistant hyperinsulinism, which may also either have a congenital or acquired basis and seems to predispose to the development of obesity and PCOS (11) (14). So, there are many hypotheses attempting to explain the primary defect, which the most commonly accepted is IR generating a hormonal and metabolic imbalance (13).

Although, there are different risks factors for PCOS, the pathogenesis is complicated, and the origin of the syndrome is still unknown for certain. But another one possible aetiology factors, which is gaining more and more interest, is the gut microbiota and its diversity. Altered gut microbial community has been reported in PCOS patients. (5)

2.1.5. Treatments

PCOS is a challenging condition to manage, and it is difficult to treat. However, early diagnosis and management strategies are crucial. To date, the optimal therapeutic approach is still unknown, but the main goal of PCOS treatment is to obtain a metabolic and hormonal balance to improve the clinical signs and symptoms (1).

Currently, the most effective and the first line of PCOS treatment is to change lifestyle; personalised dietary interventions; and, where necessary, lose weight (15). There are additional interventions to treat the clinical symptoms (*Figure 2*), so, the use of (a) oral contraceptives pills (containing artificial versions of female hormones) have been shown effective and is one of the most common in today's population. Other options are (b) the addition of antiandrogens, which decreases the androgen secretion by androgen receptor inhibition; (c) upregulation of

adiponectin, an anti-inflammatory adipocytokine that is diminished in PCOS women and controls ovarian steroidogenesis and folliculogenesis, fatty acid and glucose metabolism. Also, the (d) administration of different drugs, like insulin-sensitizing drugs (metformin) or statins (1). What is more, natural substances as a strategy to reduce the PCOS features are in increasing attentiveness to affected patients (15). Some of these are specific food supplements considering energy restriction as well as dietary macro- and micronutrient composition, such as: (e) inositol, which seems to have also efficacy due to its numerous valuable health effects; (f) vitamin D supplementation, which is involved in sex hormone synthesis and insulin metabolic pathway (14); (g) vitamins B (B9/folate and B12/cobalamin) supplementation, because it can also reduce IR; (h) polyphenols from plant-based foods, which improve acute insulin secretion and sensitivity; (i) supplementation with omega-3 fatty acids because has shown anti-inflammatory and antioxidant effects and insulin sensitivity improvement; etc. (12) (14) (16)

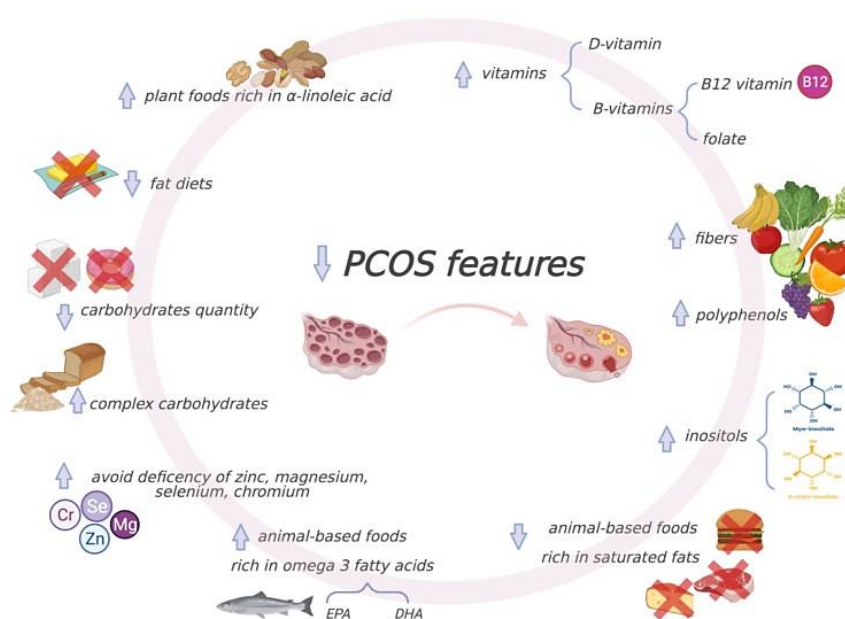


Figure 2. **Nutritional options with beneficiary effects in PCOS features.** From (15)

However, each case is treated differently adapted to the severity of the pathology and to the patient concerned. It also should be symptom-oriented, dynamic, and appropriated to the changing circumstances and personal needs. So, research is still underway to improve and tailor the PCOS treatments. (15)

2.2. Gut microbiota

Human microbiota is the set of microorganisms and their taxonomic diversity of a given environment. It is specific to each individual and it changes throughout stages of life. Its mass is about 200-1500 g, so it is considered “the forgotten human organ”. Human body is made up of about 10^{13} to 10^{14} microbial cells and there are more microbial genes (microbiome) than human genes (genome) (17).

Human microbiota varies according to the location in the body where it is found. The gastrointestinal tract microbiota, and specifically that of the colon, is the most abundant and it contains wide diversity of microbial species, as shown in *Figure 3*. This gut microbiota is an element of the bacterial ecosystem in all mammalian organisms. However, in the past, the microbiota was largely ignored and mostly inaccessible to investigation. Until recently, researchers realize that it has co-evolved with humans hosts and consistently provide a set of benefits to them. These symbiotic microorganisms play an important part in the strengthening the immune system, pathogen prevention, aiding the digestive system, and supporting intestinal development and function, from the moment of birth (5) (18). The main function of gut bacteria is to help the host digest nutritional and/or non-nutritional substrates, for the purpose of getting an effective uptake, absorption and utilization of metabolites (8) (9).

The study of the gastrointestinal microbiota is carried out using faecal samples from which DNA is extracted to find out which microorganisms exist and what function they have in the organism. It is based on bacterial 16s rRNA sequences analysis, as they are highly conserved. The most represented microorganisms are bacteria, especially anaerobes, but the intestinal microbiota also includes viruses, protozoa, archaea, and fungi. The most typical bacterial phyla are Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria. (9)

The presence of diverse but balanced intestinal microbiota is defined as eubiosis, which is characterised by a predominance of beneficial microbiota species, mainly belonging to two phyla, Bacteroidetes and Firmicutes. Although pathogenic species may be present, they are in the minority. But the composition of this ecosystems can be altered by pathology, diet, long-lasting stress, dietary habits, antibiotics ingestion, exposure to environmental factors, age, etc. For

example, a human population-based study demonstrated that the gut microbiota phylum predominating in aging individuals is Bacteroidetes, while the more common phylum in younger adults is Firmicutes. (18)

This disturbance on the composition and functioning of the gut microbial community is known as dysbiosis and influences the maintenance of host health. Its role in many pathological states by influencing physiology, metabolism, nutrition, and immune function is taken into consideration and lately is gaining a lot of relevance. (17)

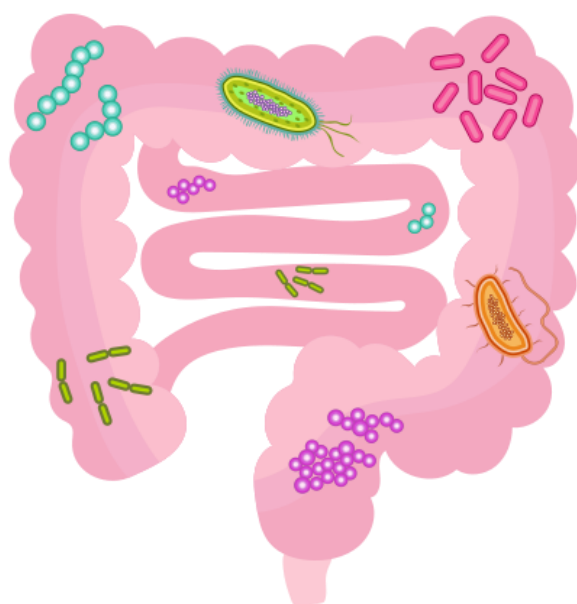


Figure 3. **Schematic illustration of gut microbiota diversity.** *From Database Center for Life Science*

3. HYPOTESIS AND AIMS

The main hypothesis is that gut microbiota is involved in the pathogenesis and development of polycystic ovary syndrome, affecting several critical points. This assumption is supported by other pathological cases, where the gut microbiota has been shown to play a determinant role.

The aim of this bibliographic research is to summarize and review the available literature data about the relationship between biochemical ovarian function in women with PCOS and the structural and functional profiles of the gut microbiota. Also, trying to assess the different associated mechanisms of gut microbiota dysbiosis on the developmental progress of PCOS. Finally, with all the findings, try to rationalize different suggestions for innovative treatments that could be effective in improving the pathology.

The motivation to make women's diseases more visible, to know more about them and to give the gut microbiota the importance it deserves, will be essential to achieve the proposed aims.

4. METHODOLOGY

This study consists of an overview of the current literature about all possible gut microbiota risk factors and their potential relationship with PCOS, that contribute to the developing and severity of the pathology. In order to write it, various information search strategies have been consulted.

Firstly, general literature search on the MEDLINE database (accessed through PubMed) has performed to introduce and situate the topic, with 'polycystic ovary syndrome OR PCOS' and 'gut microbiota' as key words. In addition, articles have filtered by year of publication to further refine the search to compact and better organise the information. The selected ones have been trending articles, most published in the years 2020-2021, as the literature is quite a lot of current.

Also, some Medical Subject Headings (MeSH) terms have been used to screen and identify studies: 'polycystic ovary syndrome' [Majr] with 'diagnosis', 'ethology', 'history', 'metabolism', etc. as subheadings; AND 'gastrointestinal microbiome' [Mesh]. This database provides a list of terms or keywords useful for indexing articles for PubMed. It is also possible selecting the option Major Topic (Majr) to give relevance to the word so that it is the main topic.

In those searches, articles published as conference papers or abstract only have been skim-read to get some general idea or have been excluded directly. Instead, it has been included full-text scientific papers, reviews or studies concerning the alterations of gut microbiota under PCOS conditions and reporting the mechanisms by which intestinal microbiota influenced the pathogenesis and progression of PCOS, which have carefully read and studied. Therefore, other keywords have been later added, such as 'molecular mechanisms', 'insulin resistance', 'hyperandrogenism', 'dysbiosis', 'phenotypes', 'diagnosis', etc. to go into each sub-section in more detail. When appropriate, further articles retrieved from their references have been also reviewed with the aim to include other critical studies that could provide new information. Finally, a useful list of bibliographical references related to the topic and that meet the inclusion criteria of interest has been obtained.

5. RESULTS AND DISCUSSION

In this section, different discoveries about the project are exposed and reviewed.

5.1. Possible links and mechanisms between gut microbiota and PCOS

All possible contributors that relate the two fundamental subjects of this project are explained in this section.

5.1.1. Alterations of gut microbiota composition in PCOS patients

The growing interest in the literature for the correlation between the microbiota composition and the development of numerous diseases have led to think that the gut microbiota also plays a major role in the pathogenesis of PCOS. (9)

Microbiota composition and the delicate balance between it and the host can determine organism's health status. Conversely, it is suggested that the dysbiosis of microbiota, either for its species diversity richness or its species abundance, could underlie negative effects when this disruption of homeostasis occurs. This supposes an instability of the ecosystem and compromises the microbiota, turning it into less flexible to changes and with weakened resilience. (9)

Gut microbiota composition differs between PCOS patients (adolescents and adults) and healthy controls, as demonstrated in many clinical studies and animal models (4). It has been found that the intestinal microbiota may have bidirectional interactions with PCOS: gut bacteria play a role in the pathogenesis of PCOS, and PCOS conditions lead to composition changes in the gut bacteria as well. Although changes in lower genital tract microbiota have also been noted in few studies, these are less noticeable and their impact in PCOS is still unknown (8) (9).

Below are detailed this composition alteration of some gut bacterial levels (classified by each category) in women with PCOS, which can produce diverse effects. (2) (4) (8) (9)

- Imbalance of Bacteroidetes phylum: increase in *Bacteroides* genus (specific increase in *Bacteroides vulgatus* and *Bacteroides fragilis*) and in *Prevotella* genus.
- Imbalance of Firmicutes phylum: decrease in *Lactobacillus*, *Clostridium* and *Ruminococcus* genus, but increase in *Streptococcus* genus.

These alterations involve an altered production of short-chain fatty acids; gut barrier integrity; immune system activation and inflammatory response; body weight gain; etc.

- Imbalance of Proteobacteria phylum: specific increase in *Escherichia* and *Shigella* genus.

They produce anti-inflammatory metabolites, so there will be an adverse or negative inflammatory effect depending on its proportion in PCOS patients.

- Imbalance of Actinobacteria phylum: decrease in *Bifidobacterium* genus.

Those are beneficial bacteria who enhance immunity, mucosal barrier function, and nutrient absorption. In PCOS cases, are significantly reduced.

- Imbalance of Verrucomicrobia phylum: decrease in *Akkermansia* genus.

This bacterium has both regulatory and anti-inflammatory properties. Is also decreased in obesity. It shows an opposite relationship with sex-hormone, brain-gut peptides, body weight, and peripheral inflammation in adipose tissue. Its decreased prevalence is also associated with lipopolysaccharides (LPS)⁷ bacterial metabolite production, and an impaired mucosal maintenance. (8) (19)

⁷ Lipopolysaccharide or endotoxin are a major component of the outer membrane of Gram-negative bacteria. It is a stimulant of the immune system with a powerful toxic effect, among other functions. (26)

Table 2 summarizes the relationship between changes in major gut microorganisms and its effects in PCOS patients. In Figure 4 are demonstrated with a bar chart.

Table 2. The effects of imbalance in major gut microorganisms in PCOS patients. Adapted from (9).

MICROORGANISMS IMBALANCE IN PCOS		EFFECTS
Phylum	Genus	
Bacteroidetes	↑ <i>Bacteroides</i>	Altered production of SCFAs
	↑ <i>Prevotella</i>	Altered gut barrier integrity
Firmicutes	↓ <i>Lactobacillus</i>	Altered immune system activation and inflammatory response
	↓ <i>Clostridium</i>	
	↓ <i>Ruminococcus</i>	Body weight gain
	↑ <i>Streptococcus</i>	
Proteobacteria	↑ <i>Escherichia</i>	Adverse or negative inflammatory effect
	↑ <i>Shigella</i>	
Actinobacteria	↓ <i>Bifidobacterium</i>	Reduced immunity Altered mucosal barrier function Altered nutrient absorption
Verrucomicrobia	↓ <i>Akkermansia</i>	Body weight gain Peripheral inflammation in adipose tissue Altered secretion of sex hormones and brain-gut peptides LPS bacterial metabolite production Impaired mucosal maintenance

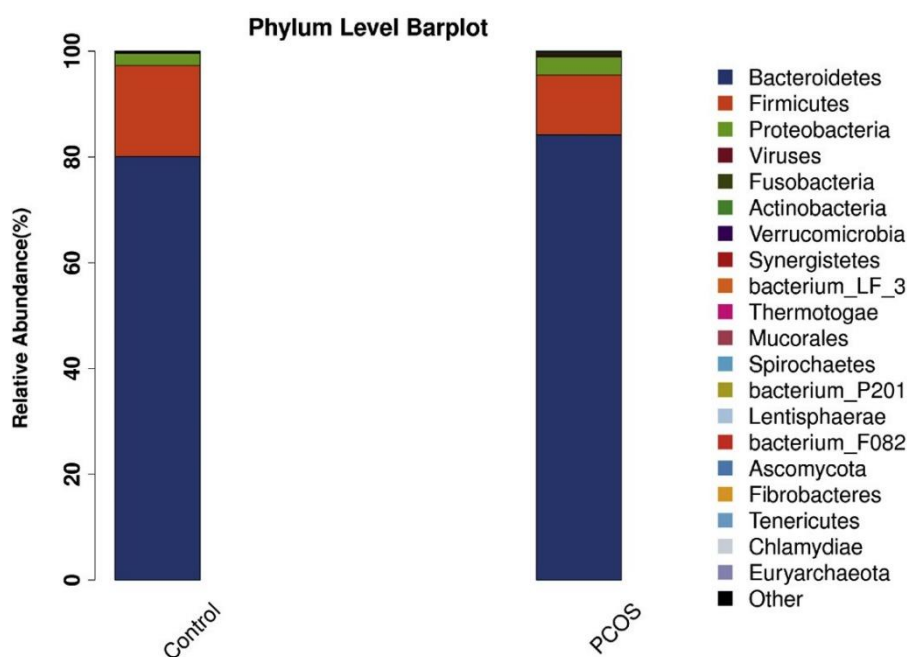


Figure 4. Relative abundance of gut microbes in control and PCOS groups at phylum level. Adapted from (2).

The gut microbes enriched in the control group are critical to stable and beneficial gut microbiota constitution. By contrast, the microbes that correlated positively in PCOS patients may function together under specific pathologic conditions and make the host more susceptible to disorders and inflammation, contributing to the PCOS pathology (2). Also, decreased bacterial diversity alters the normal function of the gut and leads to PCOS phenotypes (8). But these general modifications of the gut microbiota in PCOS are different, sometimes controversial, and not yet fully understood. However, attempts have been made to explore several pathways that connects intestinal bacteria involvement in the developmental progress of PCOS.

5.1.2. Insulin-resistance (IR)

Insulin resistance is closely related to the gut microbiome, as it plays a role in the development of the polycystic ovary syndrome. Additionally, as the microbiota field has come to the fore, it has been shown that mechanisms leading to IR factors may originate in the gut. (20)

There are diverse molecular pathways significantly changed in the gut microbial communities of IR-PCOS forms compared to PCOS patients and healthy controls. Also, patients with IR-PCOS display a much more marked difference in the abundance of some gut microbial strains. Indeed, this modification of gut microbiota structure increases the intestinal permeability and consequently, the LPS or endotoxins escape from gut lumen into the bloodstream. It induces a systemic-low grade inflammation by activating the immune system and it has been shown to disrupt various organ functions. So, the production of pro-inflammatory cytokines⁸ interferes with insulin receptor function. It seems to selectively affect the metabolic pathway of insulin action (the PI3-K pathway), distinguished by a pattern of abnormal phosphorylation. Whereby, the increased insulin and blood glucose levels boost testosterone and finally lead to PCOS phenotypes. The consequent hyperandrogenaemia typical of PCOS can stimulate decomposition of visceral adipose tissue, leading to an increase in free fatty acids. Higher levels of circulating free fatty acids damage multiple cell types, including oocytes, by increasing reactive oxygen species. All this further aggravate IR, creating a vicious cycle that promoting the occurrence and development of PCOS. (5) (9)

One experiment demonstrates that bacterial LPS produced by some bacteria belonging to the genus *Bacteroides*, *Streptococcus* and *Escherichia/Shigella*, whose are significantly increased in the gut of PCOS women, can induce chronic inflammation and metabolic disorders, like obesity and IR, in LPS-infused mice. Hence, the dysbiosis of gut microbiota, as increasing of harmful bacteria and decreasing protective bacteria, may correlate with the development of metabolic disorders in PCOS, leading to aggravating the PCOS syndrome. (19)

However, there is still no consistent conclusion regarding features of the gut microbial composition in the case of IR-PCOS patients. The gut microbiota profile in that patients has been only investigated in *Zeng's et al.* study (5). For example, regarding the *Bacteroidetes* phylum, IR-PCOS patients have the highest level of bacterial species in *Bacteroides* genus and a greater decrease of bacterial

⁸ Proinflammatory cytokines: signalling molecules secreted by immune cells that promote inflammation and can lead to disease progression. (11)

species in *Prevotella* genus. The effect this has involve gastrointestinal hormones, like Ghrelin and Peptide YY (PYY), which have a significantly negative correlation with testosterone. It has been reported a lower level of both hormones production in women with PCOS and gut microbiota may cause an altered secretion of these hormones, all leading to IR too. (19)

Figure 5 summarizes and illustrates the triggering characteristics of this profile.

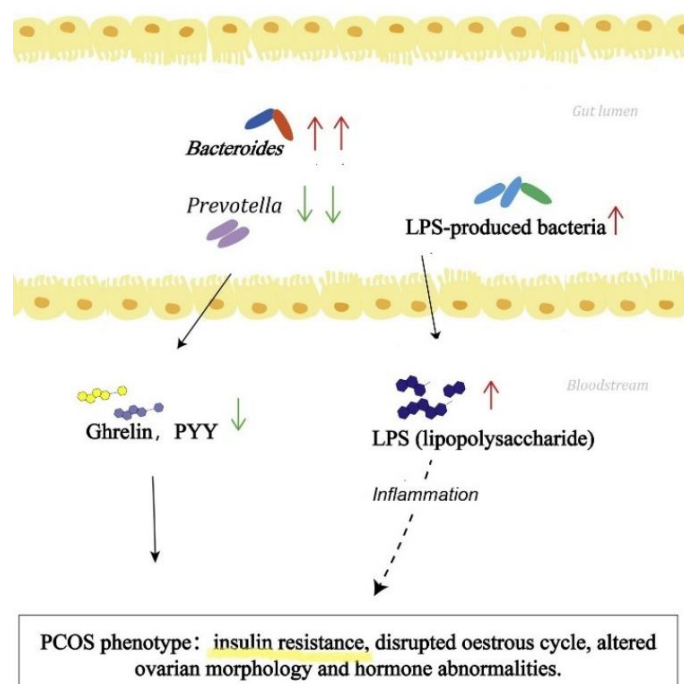


Figure 5. **IR pathway via which gut microbiota influences PCOS.** The mechanisms represented by dashed lines refer to that have not yet been verified. Red arrows mean increasing levels and green arrows, decreasing. *Adapted from* (8).

5.1.3. Sex hormones

Colonisation of the microbiota begins in the placenta. After that, the microbiota of the newborn will vary according to the type of labour (natural, caesarean, or preterm) and the breastfeeding. Later, the gut microbiota matures, and its composition differs between women and men sex (17). These differences are caused by sex hormones; being the testosterone the primary male sex hormone, and the oestrogen (like oestradiol) and progesterone the main ones for female

sex. Thus, sex hormones can determine the profile and some alterations of gut microbiota, and the gut microbiota also has effects on the production of sex hormones (9).

Indeed, the microbiota of women is characterised by greater bacterial diversity but a relatively smaller representation of species in *Bacteroides* genus, compared to that of men. *Lactobacillus* is positively correlated with oestradiol levels, so is more abundant in women. Otherwise, *Prevotella* genus has a strong positive correlation with testosterone and negative associations with oestradiol concentrations, so is more abundant in men than women. In change, men had a lower level of *Clostridium*. Other differences in the microbiota of the two sexes are highlighted. (21)

Aside from the mechanism discussed above regarding IR, where gut microbiota eubiosis was disrupted, there is another one, now due to sex hormones. This relationship perhaps is causal or consequential, is still unclear. But evidence says that in PCOS patients, there is highlighted a reduction in the number and biodiversity of microbiota species (9). This special situation has an inverse correlation between hyperandrogenism. In other words, lower microbial diversity is strongly associated with higher testosterone concentrations (4). Besides, some gut bacterial species enriched in PCOS patients are positively correlated with serum levels of reproductive hormones, such as testosterone, AMH and LH. For example, the increased abundance of *Prevotella* under PCOS conditions upregulates androgen levels, but decreased abundance of *Lactobacillus* downregulates oestradiol levels, contributing to androgen excess (2). The bidirectional interaction between gut microbes and changes in sex hormones, therefore, influences on the pathogenesis of PCOS (8). It can be observed in *Figure 6*.

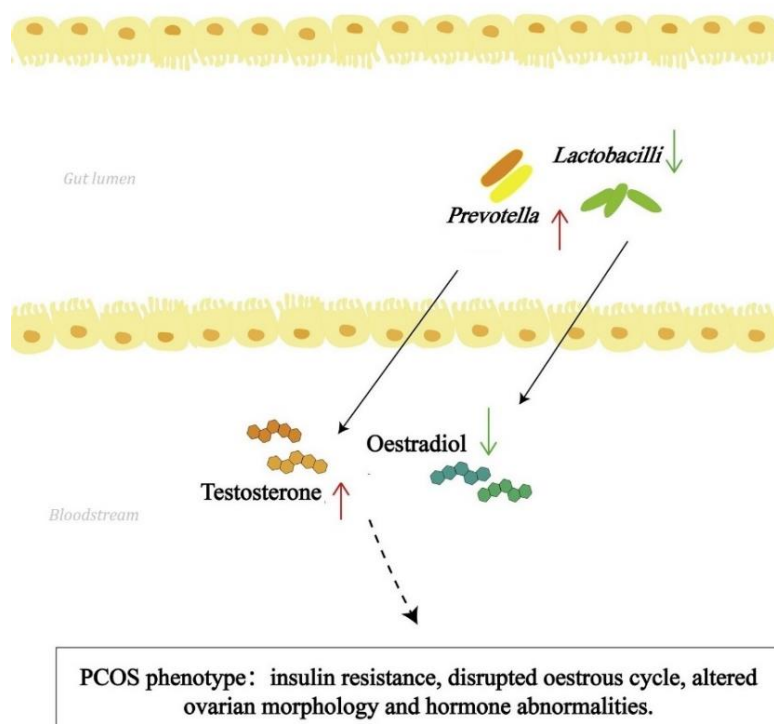


Figure 6. **Sex hormones pathway via which gut microbiota influences PCOS.** The mechanisms represented by dashed lines refer to that have not yet been verified. Red arrows mean increasing levels and green arrows, decreasing. Adapted from (8).

5.1.4. Bile acids, IL-22 and *B. vulgatus*

One of the suggested mechanisms through which gut microbes are associated with PCOS is related to bile acids, Interleukine-22 (IL-22) and *Bacteroides vulgatus* (*B. vulgatus*) specie belonging to the *Bacteroides* genus, as all shown in Figure 7.

This bacterium, as mentioned above, is significantly increased in PCOS patients compared with healthy controls and plays an important role in the pathogenesis of PCOS. Its increased presence is accompanied by a remarkably increase in the abundance of bile salt hydrolases⁹ and, consequently, by a reduced

⁹ Bile salt hydrolase enzyme catalyses the hydrolysis of the amide bond in glycol- and tauro-conjugated bile acids to de-conjugation them and release free bile acids. Is produced by intestinal bacteria. (8)

concentrations of bile acids because *B.vulgatus* is known to deconjugate conjugated bile acids synthesized in the liver (22). It negatively influences the production of cytokines by intestinal immune cells that modulate immune response and influence bile acid metabolism, like IL-22. This protein is normally very effective in ameliorating PCOS features, as some of its mechanisms are the resolution of inflammation targeting ovarian cells; upregulation the expression of brown-fat related gens (like Ucp1 or Cox8b) which helped improve many symptoms of PCOS; regulation the disrupted oestrous cycles; improve the infertility and the IR... But, IL-22 in both intestinal mRNA and serum levels are substantially reduced in PCOS patients, which is not able to function well to reverse the PCOS phenotypes. (8) (9) (22)

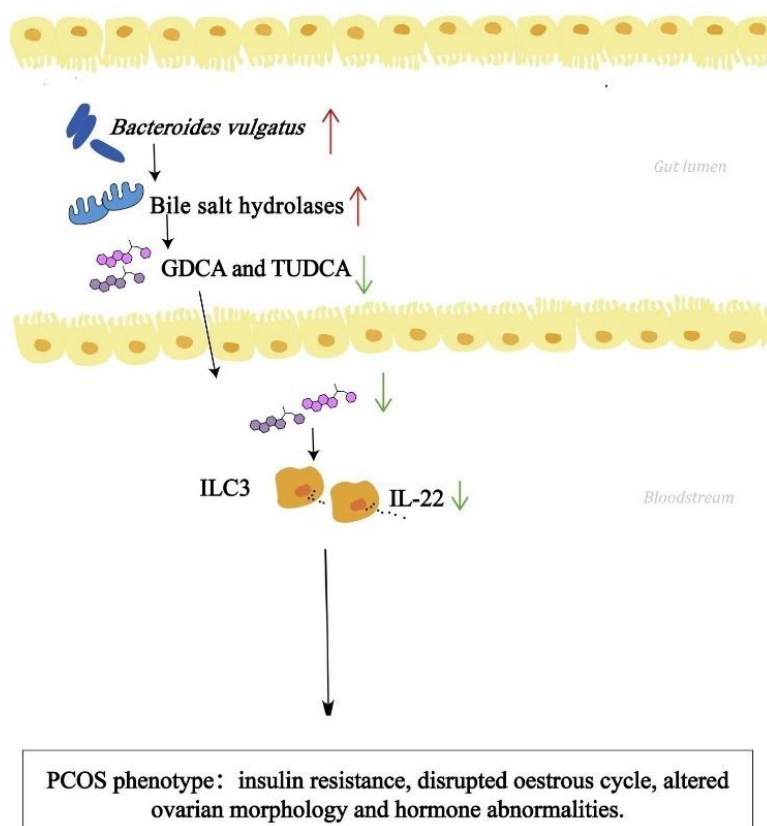


Figure 7. **Bile acids, IL-22 and *B. vulgatus* pathway via which gut microbiota influences PCOS.** Red arrows mean increasing levels and green arrows, decreasing. (GDCA, glycodeoxycholic bile acid; TUDCA, tauroursodeoxycholic bile acid; ILC3, type 3 innate lymphoid cells). *Adapted from* (8).

5.1.5. Short Chain Fatty Acids (SCFAs) and gut-brain mediators

Another mechanism of gut microbiota to effect on PCOS is specifically based on SCFA-producing gut bacteria, like *Bifidobacterium* and *Akkermansia* (8). Analysis of this beneficial microbial metabolites, the SCFAs, in terms of their effects on molecular regulatory mechanisms improve the understanding of the etiology of many common diseases, such PCOS. (18)

The secretion of some mediators of the gut-brain axis is regulated by gut microbiota (19). In particular, SCFAs (mainly butyrate, propionate, and acetate) bind to their receptors located at enteroendocrine cell membranes and its activation initiates the signal transmission cascade inside the cell. It directly stimulates the release of gut-brain mediators, such as gastrointestinal hormones ghrelin and PYY (19), whose presence can influence the proper secretion of sex hormones by the hypophysis and hypothalamus through the gut-brain axis. The result is an improvement of the PCOS signs and symptoms.

But the opposite case happens in PCOS pathology (*Figure 8*). It is worth noting that in the faecal samples of PCOS patients there are low concentrations of SCFAs. In the same way, they show lower levels of ghrelin and PYY; as well as higher concentrations of sex hormones due to their inverse correlation with the gut-brain mediators and by the fact that decreased levels of those gut-brain mediators are related to an aggravated secretion of sex hormones (8). Then, sex hormones deregulation can determine alterations of gut microbiota by activating the receptors presents in the gastrointestinal tract, by altering beta-glucuronidase activity¹⁰, or by modulating systemic or intestinal immunity. In addition, SCFAs deficiency cannot inhibit histone deacetylases (HDACs) activity and it leads to an increased expression of this molecules in the intestine, which are involved in promoting pathogenesis (18). And also, low levels of SCFAs cannot cope with gut barrier function and cause an increased translocation of endotoxins across the gut wall, resulting in more inflammation. (9) (23)

¹⁰ Beta-glucuronidases are members of the glycosidase family of enzymes that catalyse breakdown of complex carbohydrates. (14)

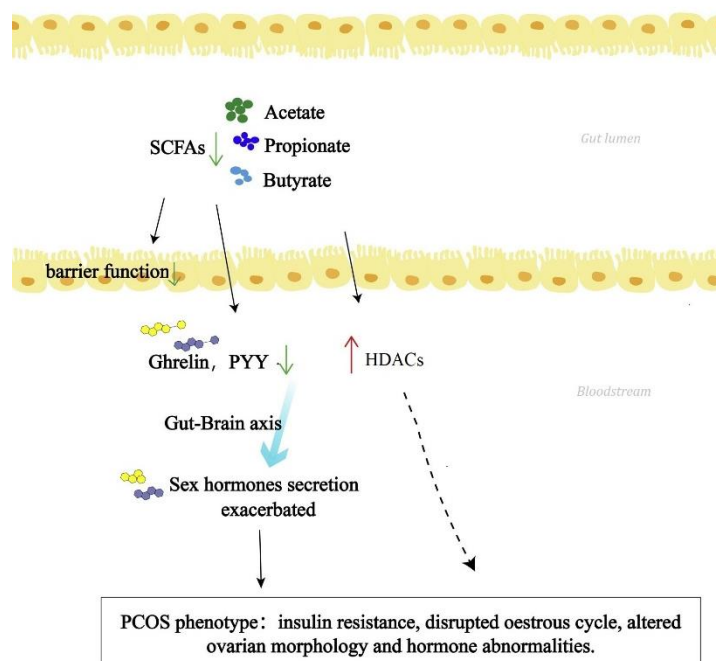


Figure 8. **SCFAs and gut-brain mediators' pathway via which gut microbiota influences PCOS.** The mechanisms represented by dashed lines refer to that have not yet been verified. Red arrows mean increasing levels and green arrows, decreasing. *Adapted from (8).*

Thereby, the potential interrelation with SCFAs, gut-brain mediators release and sex hormones secretion may contribute to the pathogenesis of PCOS. This can be reversed with the administration of gut probiotics¹¹, which promoted the growth of SCFA-producing bacteria, therefore increasing intestinal SCFAs and achieving ameliorated PCOS symptoms. (8)

¹¹ Probiotics are “live microorganisms that, when administrated in adequate amounts, confer a health benefit on the host”, according to the definition of the *Food and Agriculture Organization (FAO)* and *World Health Organization (WHO)*. (21)

6. THERAPEUTIC OPPORTUNITIES FOR PCOS

Current research has revealed close correlation between the pathological process of PCOS and the intestinal microbiota. The observed gut dysbiosis profile in PCOS patients raises a health alert from which emerges the need to innovate with new treatments and future drugs development, or to improve the present ones (such as a standardization) for the management of this syndrome, which is so common and current in women. More emphasis should also be given to the opportunity of reducing the possible healthcare costs by the new treatment options.

Most of them are focused on the gut microbiota because is closely associated with therapeutic targets of many diseases. It holds a considerable potential for improving the pharmacological practice. Nevertheless, most recent studies have been conducted in PCOS rat models; more studies on PCOS patients would be needed for further exploration in the therapeutic field. (8)

First, microbiota can exert an impact on drug and therapy response. So, accurate modulation of the microbiota may be adopted as an adjuvant therapy and monitoring intestinal microbiota as a pathologic indicator. Second, microbiota itself is a source of new therapeutics (8). So, Faecal Microbiota Transplantation (FMT) is a strategy that consists of the administration of a faecal matter solution from a healthy donor into the intestinal tract of a recipient, in order to directly change their gut microbiota, normalize the composition and confer a health benefit (20). This kind of treatment results to be associated with a normalization of ovarian function and metabolic improvements. However, it is not a therapeutic strategy with a large-scale applicability due to the presence of critical limitations and many conditions are required for it to be appropriate (20).

Other promising methodologies to manipulate and reconstitute gut microbiota communities include nanotechnology, genetically engineered microorganisms to express specific genes, regulation of microbial metabolites levels, probiotics, prebiotic¹² and symbiotic¹³ agents (8),... Many studies have been demonstrated

¹² Prebiotics: substrates selectively used by microorganisms that stimulate the growth and/or activity of beneficial bacteria and confers benefits upon host well-being and health. (21)

¹³ Symbiotics: mixture containing pre- and probiotics together. (21)

that probiotics supplementation therapy for a period of 12 weeks has shown a positive effect on the pathological signs, like a reduction of inflammatory indices, and on the metabolic profile in women with PCOS, like a decrease in plasma glucose and serum insulin levels with an additional improvement of lipid profile (9) (21). Furthermore, factors such as lifestyle and diet, whose change normally are recommended in order to achieve a health improvement, at the same time, they take into account the microbiota's health and this ends up converging in the microbiota state (24). For example, only one relevant study has described the implications of diet with gut microbiota in PCOS condition. It has been demonstrated that a 21-day treatment with inulin¹⁴ and metformin in mice could cause a decrease of ovarian damage and attenuation of PCOS, through the increase of *Lactobacillus* and *Bifidobacterium* levels and the decrease of inflammatory indicators from plasma and ovary (25). Another example demonstrates that diets rich in fibre and low carbohydrate have been shown to interact directly with gut microbes, they increase SCFAs and impact gut microbiota composition, diversity, and richness (15). Given those associations, there may be a significant therapeutic utility for PCOS in regulating microbial composition through diet. It would be interesting to continue research on the possible mechanisms of action of certain products in diet, to those which beneficial effects have already been attributed. Such as, analyse the feasibility of a synergism between a bioactive compound¹⁵ and a drug to delay PCOS progression.

Apart from that, other options are valuable for the treatment of PCOS. The therapeutic role of IL-22 has been studied, as low levels of this intestinal immune factor have been reported in PCOS individuals, but it is still not so clear. (9)

¹⁴ Inulin is a non-digestible polysaccharide based on fructose with probiotic function. It belongs to a class of dietary fibres known as fructans. (26)

¹⁵ Bioactive compound could be defined as phytochemicals, which can be extracted from foods or foods by-products, and able to regulate metabolic functions leading to beneficial effects. (26)

7. CONCLUSIONS

This study showed a broad view of the intimate association between PCOS pathogenesis and progression with gut microbiota in women.

PCOS patients have reduced microbial diversity and altered microbiota composition. In addition, the compositional dysbiosis contribute to the syndrome through the possible mechanisms discussed above: upregulation or downregulation of hormone secretion, gut-brain mediators, cytokines, bile acids, and microbial metabolites production.

Limitations also exist in the current research about this topic. Further studies are required to elucidate the specific role of each microbiota's genus, and to explore more the gut microbiota potential mechanisms (or if there are newly involved) for provide a useful theoretical basis on how exactly gut microbiota can impact PCOS progress.

While may seem rather intuitive that the gut microbiota and host metabolism pathways would be interrelated, disentangling cause and effect remains a challenge. I.e., alterations in the gut microbiota may influence the PCOS, or the contrary, they may be the reflective of PCOS. The direction of this existing relationship is yet to be confirmed.

After all this knowledge, a wide range of possibilities opens to develop new treatments options for the pathology. These could be directed towards the microbiota and its different mechanisms of action. In that way, an enhanced lifestyle, both physically and mentally, could be achieved for these many PCOS affected patients.

Finally, to conclude this project I would like to recall the quotation of a Russian anarchist philosopher and sociologist who said "Uniformity is the death, diversity is the life" (*Mijaíl A. Bakunin*). It becomes clear that diversity is rich and beneficial in every way. This fact can be extrapolated to humanity, as well as to what occurs inside us. As has been demonstrated in the case of PCOS women, a poor gut microbiota composition can trigger a host of health problems. All in all, diversity could be a requisite for survival.

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