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Original Research Article

Effect of Probiotic Supplementation on FSH, LH Levels and Folliculogenesis

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Abstract

Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder that often occurs in women of reproductive age. The main therapy currently used to treat PCOS patients is insulin sensitizer. In PCOS, there is an imbalance in the intestinal flora which causes activation of the immune system and an inflammatory response that leads to insulin resistance. The effect of probiotic supplementation on insulin resistance can have an impact on changes in reproductive hormone levels in PCOS women.

Objective: Analyze the effect of probiotic supplementation on levels of FSH, LH, and folliculogenesis in a study of PCOS Wistar rats.

Methods: Experimental research with a post-test only controlled group design. The research sample was 35 Wistar rats which were divided into 5 groups, (K-) were healthy rats, (K+) were PCOS rats without treatment, (P1) were PCOS rats received Metformin, (P2) were PCOS rats received Probiotics, and (P3) were PCOS rats that received Metformin + Probiotics. The intervention was carried out for 14 days. The dependent variables were levels of FSH, LH and folliculogenesis. Research data were analyzed using one-way ANOVA test, Fisher Exact test. Differences between groups and controls were tested with Dunnett's post hoc test. Significant $p<0.05$

Results: Metformin + probiotic supplementation resulted in increased FSH levels, decreased LH levels and increased folliculogenesis when compared to the PCOS rats without treatment (K+).

Conclusion: Metformin + probiotic supplementation causes levels of FSH, LH and folliculogenesis activity in PCOS rats to resemble healthy rats.

Keywords: *PCOS; metformin; probiotic; LH; FSH; folliculogenesis*
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INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder that often occurs in women of reproductive age. PCOS is often associated with obesity and reproductive health disorders.[1] PCOS occurs in around 116 million women in the world, with a percentage of 3.4% of the global population. PCOS accounts for 70% of anovulatory infertility.[2] Hyperandrogenism can be detected by increasing serum testosterone, serum androstenedione, or dehydroepiandrosterone. As a result of hyperandorgenism, PCOS can also have clinical manifestations in the form of hirsutism, acne, and/or alopecia.[3]

Many therapeutic methods have been applied to treat PCOS patients, including insulin sensitizers, lifestyle changes, and dietary interventions. The main therapy given as a treatment for PCOS patients currently is insulin sensitizer.[4]

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Table 1. Differences in FSH levels according to the treatment group

Variable	Group					p
	K -	K +	P1	P2	P3	
FSH Levels, IU/L	133.16 ± 12.79; 132.22 (119.29 – 159.73)	53.33 ± 11.75; 52.07 (34.03 – 68.95)	200.08 ± 26.76; 189.41 (173.48 – 235.20)	185.96 ± 31.93; 192.85 (117.18 – 210.41)	130.49 ± 18.79; 131.49 (106.89 – 162.92)	<0.001

ANOVA; significant p<0,05

Table 2. Post-Hoc test results for different FSH levels between groups

Group (I)	Group (J)	Mean Difference (I-J)	p
P3	K-	-2.67	1.000
	K+	77.16	0.000
	P1	-69.58	0.000
	P2	-55.47	0.000

Bonferroni Post-Hoc; significant p<0,05

Table 3. Differences in LH levels according to the treatment group

Variable	Group					p
	K -	K +	P1	P2	P3	
LH Levels, IU/L	22.71 ± 9.71; 21.75 (11.55 – 41.75)	75.09 ± 7.66; 75.37 (61.53 – 85.42)	37.58 ± 12.60; 40.57 (11.55 – 51.79)	42.55 ± 4.89; 42.94 (36.07 – 51.79)	26.81 ± 3.62; 26.06 (20.95 – 31.75)	<0.001

ANOVA; significant p<0,05

Table 4. Post-Hoc test results for different LH levels between groups

Group (I)	Group (J)	Mean Difference (I-J)	p
P3	K-	-9.43	1.000
	K+	-61.80	0.000
	P1	-24.29	0.223
	P2	-29.27	0.014

Bonferroni Post-Hoc; significant p<0,05

Table 5. Folliculogenesis Rates Between Groups

K+ (I)	Group (J)			
	K-	P1	P2	P3
Primary Follicle	0.42 + 1.13	1.57 + 1.27	1.42 + 1.39	1.28 + 1.70
Secondary Follicle	0.57 + 1.27	0.57 + 0.78	0.85 + 1.77	0.71 + 1.49
Tertiary Follicle	1.57 + 1.51	0.29 + 2.13	-0.57 + 0.97	0.86 + 1.34
Graff's Follicle	0.57 + 1.98	-1.14 + 1.46	1.00 + 1.41	0.42 + 1.27
Corpus Luteum	3.71 + 2.81	0.71 + 0.95	1.71 + 1.79	3.57 + 2.22

Table 6. Differences in Ovarian Histopathology between groups

K-	Group			
	K+	P1	P2	P3
Primary Follicle	0.474	0.608	0.371	0.608
Secondary Follicle	0.292	1.000	0.062	0.619
Tertiary Follicle	0.138	0.523	0.068	0,776
Graff's Follicle	0.608	0.042	0.510	1.000
Corpus Luteum	0.295	0.211	0.538	0.899

Fischer Exact; significant p<0,05

Dysbiosis of gut microbiota (DOGMA) proposes the hypothesis that after an imbalance in the intestinal flora, there is an increase in intestinal permeability which can cause lipopolysaccharide leakage into the systemic circulation, which causes activation immune system and inflammatory responses that lead to insulin resistance.[5]

The use of probiotics and microorganisms in the digestive tract as a therapeutic method can have an impact on metabolic, inflammatory, and oxidative markers. Recent studies have shown data on a decrease in insulin concentration and insulin resistance in subjects given probiotics. The possible mechanism by which probiotic supplementation provides a beneficial effect is from the balance of the host's energy metabolism.[4]

The effect of probiotic supplementation on insulin resistance can also have an impact on changes in reproductive hormone levels in PCOS women. Several studies have also identified changes in hormone levels related to reproductive function in subjects receiving probiotic supplementation.

The purpose of this study was to prove the effect of probiotic supplementation on levels of FSH, LH, and folliculogenesis in a study of PCOS Wistar rats.

MATERIALS AND METHODS

Experimental research with a post-test only controlled group design. The study was conducted on the PCOS rat model induced by testosterone proprionate through subcutaneous injection at the Faculty of

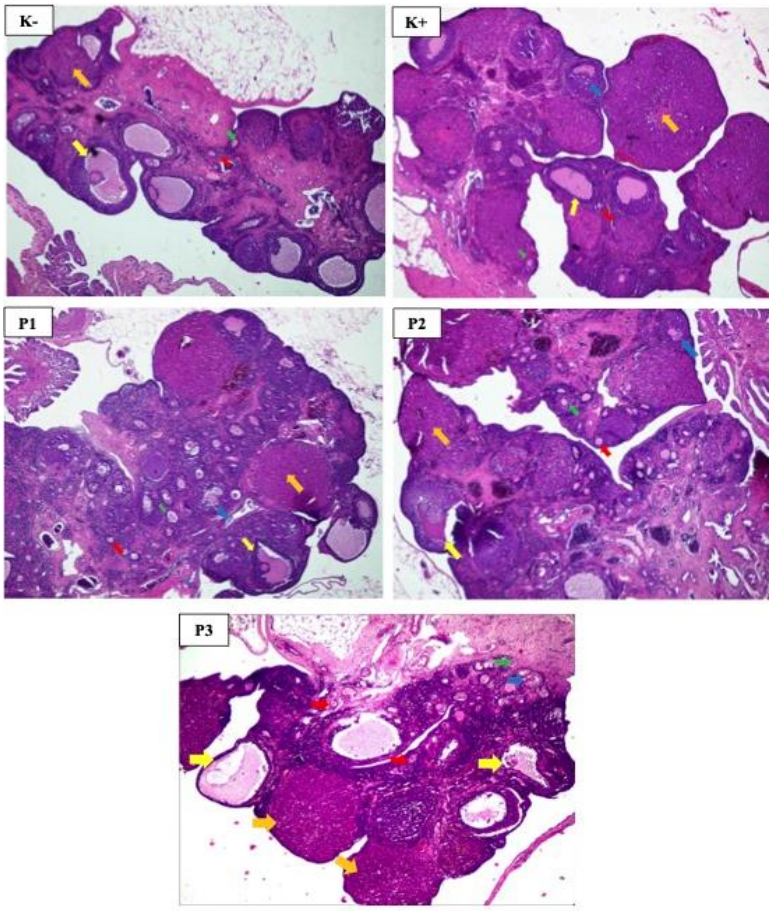

Figure 1. Features of folliculogenesis (K-, K+, P1, P2, P3). Red arrow = primary follicle; green arrow = secondary follicle; blue arrow = tertiary follicle; yellow arrow = Graff follicle; orange arrow = corpus luteum. HE. 100x

Veterinary Medicine, Universitas Airlangga, Surabaya. The research sample was 35 wistar rats which were divided into 5 groups, (K-) were healthy rats, (K+) were PCOS rats without treatment, (P1) were PCOS rats received Metformin, (P2) were PCOS rats received Probiotics, and (P3) were PCOS rats that received Metformin + Probiotics. The intervention was carried out for 14 days. Probiotic supplementation was carried out using acidophilus liquid probiotics with a preparation of 45,000 bacteria per 1 mL, given at a dose of 6.15 mL/KgBB/rat/day, for 14 days, where rats are estimated to have experienced 3 cycles of ovarian hormone secretion. The dependent variables were levels of FSH, LH and folliculogenesis. The ELISA method was used to assess FSH and LH levels using blood samples obtained from the retroorbital vein as much as 3cc. Ovarian histopathological examination with hematoxylin eosin staining was used to assess folliculogenesis using ovarian samples obtained from laparotomy with left and right ovarian samples. Calculation of the number of ovarian follicles was carried out by Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga Research data were analyzed using one-way ANOVA test, and Fisher Exact test. Differences between groups and controls were tested with Dunnett's post hoc test. The data is said to be significant if the p value <0.05

Research by intervening in animal models had been carried out based on approval and ethical eligibility from the Ethics Commission of the Faculty of Medicine, Universitas Diponegoro with no.130/EC/H/FK-UNDIP/XI/2022 All costs related to research became the responsibility of the researcher.

RESULTS

Based on the assessment conducted on 35 rats which were divided into 5 research groups namely K- (non-SOPK), K+ (SOPK), P1 (SOPK + Metformin), P2 (PCOS + Probiotics), and P3 (SOPK + Metformin + Probiotics), the following results were obtained. Table 1 explains the differences in FSH levels between study groups. In the PCOS (K+) group, low FSH levels were found. In the intervention group (P1, P2, and P3) there was an increase in FSH levels when compared to the PCOS group (K+). Based on table 2, FSH levels in the P3 group have the most similar values to FSH levels in the healthy group (K-).

Table 3 explains the differences in LH levels between study groups. In the PCOS (K+) group, high LH levels were found. In the intervention group (P1, P2, and P3) there was a decrease in LH levels when compared to the PCOS group (K+). Based on table 4, LH levels in the P3 group have the most similar values to LH levels in the healthy group (K-).

Figure 1 and table 5 explains the differences in folliculogenesis rates based on each follicular development between groups. When compared to the PCOS group (K+), there was an increase in the degree of

folliculogenesis in the intervention group (P1, P2 and P3) at almost all stages of follicular development. However, the increase in folliculogenesis in the P3 group resembled that of the healthy (K-) group. Based on table 6, it was found that, when compared with the healthy group (K-), there was no significant difference in the level of folliculogenesis between the P3 groups at all stages of follicular development.

DISCUSSION

PCOS rats without treatment (K+) had much lower FSH levels than healthy samples (K-). Intervention in all groups (P1, P2 and P3) resulted in an increase in FSH levels.

Khashchenko E, et al in his study on the assessment of hormone levels in PCOS patients and healthy patients found that PCOS patients had higher FSH levels than healthy patients, although there was no significant difference between the two groups ($p=0.285$).[6] This result was also supported by the study of Jindal P, et al found that the average patients FSH levels before and after treatment were 10.55 ± 6.22 mIU/ml and 9.69 ± 4.07 mIU/ml, respectively ($p<0.137$).[7] The results in this study were different from the results obtained by Khashchenko. E and Jindal P are suspected because both studies used human subjects, while this study used rat samples that received testosterone proprionate injection. The results in this study were supported by Dardmeh F, et al who obtained similar results that probiotic supplementation increased testosterone, LH and FSH levels in research subjects.[8]

Karimi et al.[9] and Heshmati et al.[4] stated that PCOS patients generally have insulin resistance and increased serum insulin and abnormal lipoprotein metabolism. Rice S, et al in a study on the effect of using metformin on FSH levels in PCOS patients found that metformin significantly reduced FSH levels but not expression and aromatase activity stimulated by forskolin. This effect arises through inhibition of ligand- and basal-induced upregulation of FSH receptor expression. Metformin also reduces FSH-induced CREB phosphorylation and hence CRE activity, potentially interfering with the CRE-binding CREB-CREB2 protein coactivator complex at promoter II of the aromatase gene. This condition is mediated in a manner that is independent of AMP-activated protein kinases and does not involve changes in cAMP levels.[10]

PCOS is directly related to insulin disorders. Insulin resistance will cause hyperinsulinemia, which directly affects the role of ovarian receptors, inhibits insulin-binding protein and sex hormone-binding protein, while releasing testosterone and increasing ovarian androgens. Therefore, metformin is used to regulate insulin secretion and achieve the aim of effectively improving PCOS. Most approved weight management drugs are contraindicated in women of reproductive age, but metformin has fewer side effects, safer, and recommended for use in the treatment of PCOS.[11]

PCOS can also cause systemic metabolic disorders (such as hyperinsulinemia and insulin resistance, obesity, increased risk of type II diabetes, cardiovascular disease) have played a fundamental role. The overwhelming evidence on the correlation between the gut microbiome and the occurrence of metabolic disorders has led to the hypothesis that changes in the microbiome also involved in the pathogenesis of PCOS.[12] Dysbiosis of gut microbiota (DOGMA) demonstrated that, along with an imbalance in the gut flora, increased intestinal permeability can lead to leakage of lipopolysaccharide (LPS) into the systemic circulation. The net effect is activation of the immune system and an inflammatory response leading to insulin resistance.[5]

The condition that shows the role of the gut microbiome is the presence of lower concentrations of short chain fatty acids (SCFA) in stool samples of PCOS patients.[13] The growth of Faecalibacterium prausnitzii, Bifidobacterium and Akkermansia was driven by probiotic supplementation, which are SCFA-producing bacteria, and led to an increase in intestinal SCFA. This SCFA binds to its receptor on the enteroendocrine cell membrane and directly stimulates the release of gut-brain mediators such as ghrelin and PYY, the increase of which can affect the secretion of sex hormones by the pituitary and hypothalamus through the gut-brain axis, thereby improving PCOS symptoms.[14] Increased SCFA production also contribute to intestinal barrier function and reduce endotoxin translocation across the intestinal wall, thereby reducing inflammation and insulin resistance. Ultimately, there is a potential interaction between sex hormones and the gut microbiota, and this interaction may contribute to the pathogenesis of PCOS.[15]

Arab A, et al who conducted a study on the effect of probiotic supplementation on hormone levels and clinical outcomes of PCOS patients found that after 12 weeks of administration there was a decrease in FSH levels, but there was no significant difference ($p=0.188$).[16] Consumption of probiotics balances intestinal pH and microbial flora, improves the absorption and digestion of nutrients, prevents the production of inflammatory cytokines, and improves lipid and carbohydrate metabolism in the gut.[17] Probiotics also reduce blood glucose, insulin resistance, and de novo cholesterol formation, which in turn reduces the production of androgens including SHBG, DHEA, FAI, and testosterone.[18]

In this study, different results were obtained, namely a decrease in FSH levels in PCOS patients and an increase in FSH levels after the intervention of metformin and/or probiotics. Dardmeh F, et al found that after supplementation with Lactobacillus rhamnosus there was an increase in FSH, LH and testosterone levels between before and after administration ($p <0.05$).[8] Szydlowska I, et al got similar results, namely an increase in FSH from the baseline value after administration of probiotics.[19]

PCOS samples that were not given treatment (K+) had much higher LH levels than healthy samples (K-). Giving interventions to all groups (P1, P2 and P3) caused a decrease in LH levels. There was a significant difference in LH levels between the K- and P1 and P2 groups.

Khashchenko E, et al also assessed LH levels, found that PCOS patients had higher LH levels than healthy patients, with a significant difference between the two groups ($p<0.001$).[6] Jindal P, et al found similar results where the average LH level was before and after treatment respectively 22.34 ± 11.49 mIU/ml and 15.65 ± 6.09 mIU/ml ($p < 0.0001$).[7]

Obese women with PCOS exhibit higher LH levels to stimulate androgen secretion, leading to excessive insulin and androgen resistance.[20] Based on existing guidelines, it is currently recommended that overweight women with PCOS use metformin for weight control and for endocrine and metabolic disorders. 106 High levels of LH and androgens are considered to be the reason for the increasing number of oligomenorrheal patients. Metformin treatment reduces hyperinsulinemia. This is thought to be the cause of changes in pituitary sensitivity to gonadotropin-releasing hormone leading to excessive LH secretion. Administration of metformin causes a decrease in LH response to GnRH.[16]

The type of intervention that gave the most similar level of ovarian folliculogenesis to healthy samples (K-) was the P3 group, namely administration of metformin and probiotic supplementation.

Khashchenko E, et al found that the number of ovarian follicles in the PCOS group was higher than the control group with a significant difference between the two groups ($p<0.001$).[6]

Primordial follicular growth is largely independent of gonadotropins and is primarily influenced by paracrine/endocrine factors, including several proteins of the TGFβ superfamily (i.e., TGFβ, anti-mullerian hormone [AMH], inhibin, activin, bone morphogenetic protein-15 [BMP15] and growth differentiation factor 9 [GDF9]).[21] Several of these TGF-β-related proteins are dysregulated in PCOS follicles. Oocytes in PCOS patients experience a decrease in GDF9 mRNA levels, which interferes with growth from the primordial follicle to the development of small antral follicles, and is accompanied by impaired follicle growth.

Insulin sensitivity in PCOS patients is intrinsically impaired from abnormal post-receptor signal transduction, reducing insulin-mediated glucose uptake, but not ovarian steroidogenesis. Consequently, the hyperinsulinemia of insulin resistance in PCOS is independent of and related to obesity, with the combination of PCOS and obesity severely impairing glucose-insulin homeostasis, while enhancing ovarian steroidogenesis. Consequently, hyperinsulinemia due to insulin resistance in PCOS contributes to hyperandrogenism. This condition also promotes premature luteinization of granulosa cells in small antral PCOS follicles, as evidenced by LH receptor overexpression and P4 hypersecretion, leading to arrest of cell proliferation and follicular growth.

In this study, administration of metformin and probiotics to samples with PCOS resulted in a histopathological appearance of the ovaries resembling that of healthy samples (K-). It is suspected that the mechanism underlying this condition is through improvement in LH and FSH levels that resemble those of healthy samples (K-).

CONCLUSION

Metformin + probiotic supplementation causes levels of FSH, LH and folliculogenesis activity in PCOS rats to resemble healthy rats.

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