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ORIGINAL ARTICLE

The effect of myoinositol on ovarian blood flows in women with polycystic ovary syndrome

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ABSTRACT

To evaluate whether 4 gram myoinositol and 400 mcg folic acid(MYO) therapy has any effects on ovarian stromal blood flow by using pulsed and color Doppler at 3 months follow-up period in polycystic ovary syndrome (PCOS). One-hundred eighty patients were designed into six groups; Group 1: PCOS patients that received OCP containing 30 mcg ethinyl estradiol (EE) plus 3 mg drospirenone (DRP); Group 2: PCOS patients that received MYO; Group 3: PCOS patients that received no medication. Group 4: Healthy patients that received OCP; Group 5: Healthy patients that received MYO; Group 6: Healthy patients that received no medication. Resistance index (RI) and pulsatility index (PI) of both ovaries were assessed. There was a significant increase in RI and PI of both ovarian stromal blood flow women with PCOS who received OCP (Group 1, $p < .001$) and MYO (Group 2, $p < .001$). The rate of increment in both RI and PI values were similar for OCP users (Group 1) and MYO users(Group2) in PCOS patients. MYO therapy reduced ovarian vascularization in both PCOS and healthy users after 3 months and this decrease is especially noticeable in women with PCOS compared to healthy women. OCP therapy also reduced ovarian vascularization just like MYO therapy.

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Myoinositol; polycystic ovary syndrome; ovarian doppler indices

Introduction

In 1935, polycystic ovary syndrome (PCOS) was first investigated by Stein-Leventhal, however, its etiopathogenesis is still obscure [1]. The prevalence of PCOS varies depending on the criteria used to make the diagnosis. According to the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM), the prevalence is as high as %15–22 in reproductive-aged women [2,3]. PCOS is defined as a heterogeneous disorder characterized by biochemical and clinical signs of hyperandrogenism, chronic anovulation, and polycystic ovaries. It is frequently associated with insulin resistance [4,5]. Clinical importance of the polycystic appearance of the ovaries still remains uncertain and the appearance alone is not enough for the diagnosis. Evidence suggests that patterns of blood flow is directly related with the morphology and function of the relevant organ. 3-dimensional power Doppler ultrasound is a beneficial noninvasive method to assess the vascularization of the ovarian stroma. Although the Doppler analysis of ovarian circulation is not included in the Rotterdam Diagnosis criteria, detection of changes in stromal ovarian blood flow patterns make us think that ovarian stromal Doppler evaluation might be helpful in understanding the pathophysiology of this syndrome [6,7]. Some studies [8–10] have shown that PCOS cases have significantly higher ovarian blood flow and other ones [6,11,12] have not shown these relations. Elevated luteinizing hormone levels are accused of increased stromal vascularization by inducing neoangiogenesis [8].

Combined oral contraceptives (OCPs) are considered as the first line treatment for women with PCOS who do not desire fertility. They suppress LH secretion and androgen production. There are several studies which investigate the Doppler indices of ovary during the OCP treatment for PCOS, but the data are limited and the results are conflicting [10,13,14].

Inositol (hexahydroxycyclohexane) belongs to the vitamin B complex group; it is a 6-carbon ring compound, having a hydroxyl group linked to each carbon of the ring, with nine possible stereoisomeric forms depending on the epimerization of the six-hydroxyl groups [15]. Among them, myoinositol and folic acid (MYO) is the most represented isoform with very relevant functions. MYO plays a key role in cell morphogenesis and cytogenesis, lipid synthesis, the structure of cell membranes and cell growth. MYO administration improves hormonal profile, reduces LH, androgen production and insulin resistance [16,17]. MYO may affect ovarian stromal blood flow, by reducing LH secretion and by increasing insulin sensitivity. Yet there is no data showing the MYO effect on ovarian blood flow.

The primary outcome of this study was to observe and to compare the ovarian blood flow of PCOS patients and healthy women. Secondly, we aimed to evaluate whether MYO therapy or OCP treatment have any effects on ovarian stromal blood flow by using pulsed and color Doppler at the of 3 months follow up period in women with PCOS. In addition, our goal was to investigate the relation of biochemical parameters with doppler findings to understand the pathophysiology of the polycystic ovary syndrome.

Material/method

The present prospective study was conducted in the Division of Reproductive Endocrinology, Department of Obstetrics and

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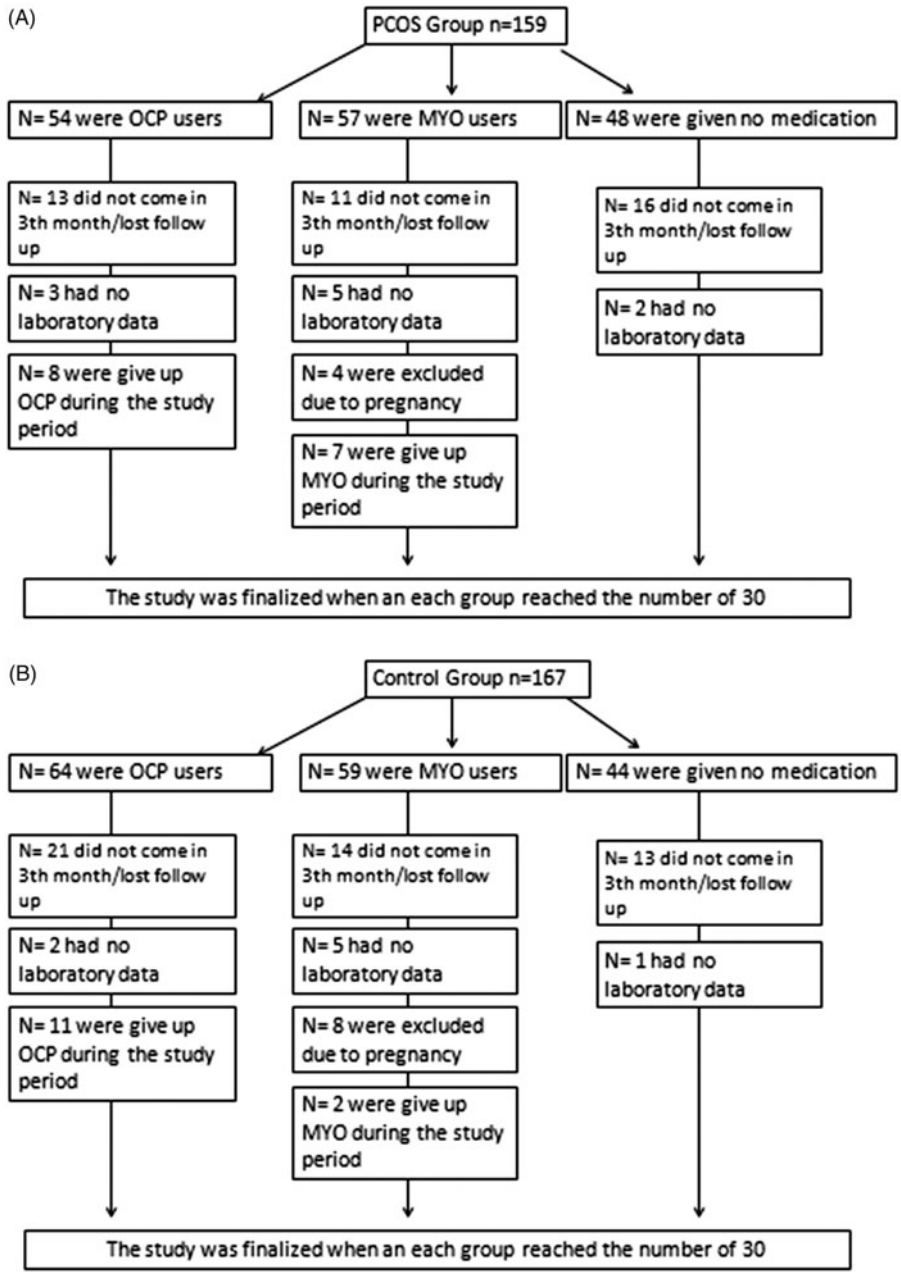

Figure 1. Flow chart of the study. (A) PCOS group. (B) Healthy women.

Gynecology at Dokuz Eylul University, Izmir, Turkey, between April 2012 and May 2015. The study protocol was approved by our Institutional Review Board and informed consent was obtained from all patients.

The study started with 326 patients, but 180 patients' results were analyzed. The patients were designed into six groups as follows; Group 1: PCOS patients that received OCP containing 30 mcg ethinyl estradiol (EE) plus 3 mg drospirenone (DRP) (Yasmin; Schering AG, Berlin, Germany) for 3 months ($n = 30$); Group 2: PCOS patients that received 4 gram myoinositol and 400 mcg folic acid ($n = 30$) for 3 months; Group 3: PCOS patients that received no medication ($n = 30$). Group 4: Healthy patients that received OCP (EE + CA) for 3 months ($n = 30$); Group 5: Healthy patients that received MYO ($n = 30$) for 3 months.; Group 6: Healthy patients that received no medication ($n = 30$). (Figure 1(A,B))

Twenty-six participants per each group were required with power of 80%, based on two-sided significance level alpha of 0.05 and medium effect size 0.25 [18]. Therefore, the study was continued until reaching the number 30. (Figure 1(A,B)).

A detailed medical and gynecologic history was taken for all the women in the study. Body mass index (BMI), waist-hip- ratio (WHR) and Ferriman-Gallwey score (FGS) were calculated. All women in group 4, 5 and 6 (healthy women) have regular cycles and normal initial baseline transvaginal ultrasound imaging without any gynecological symptoms and fertility problems. In group 1, 2 and 3 (PCOS group), women with fertility problems were also excluded. The diagnosis of PCOS was made in accordance with criteria from Rotterdam Consensus [3]. Hirsutism was defined as FGS ≥ 8. Exclusion criteria were as follows: age of <18 or >35 years, BMI $\geq 30\,kg/m^{2,}$ current pregnancy, smokers, the presence dyslipidemia and or/insulin resistance, hyperprolactinemia, thyroid disease, congenital adrenal hyperplasia, personal history of endometriosis or endometriosis-related symptoms, a history of ovarian surgery or tubal sterilization, any other systemic disease or drugs that could influence hypothalamic pituitary

ovarian axis, androgen production, insulin and or/glycemic metabolism, patients who received any hormone therapy including contraceptives up to 6 months before study. The presence of any ovarian lesion, ovarian cyst or follicle ≥10 mm diameter in both ovaries were also considered within exclusion criteria.

Initial physical examination included weight, height, waist and hip circumferences, to calculate waist/hip ratio (WHR) and body mass index (BMI). BMI was calculated as kg/m^2, was used as a measure of overall obesity. The WHR was measured at the midpoint of the lowest margin of 12th rib and the lateral iliac crest during the normal expiration. The hip circumference was measured at the maximum distance between major trochanters. All anthropometric measurements were made by the same operator.

Serum levels of fasting plasma glucose and insulin, follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), thyroid stimulating hormone (TSH), total and free testosterone, dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG) and progesterone were measured. Normal insulin sensitivity was defined by fasting serum glucose and insulin levels with homeostatic model of insulin resistance (HOMA-IR) HOMA-IR was calculated by the formula: HOMA-IR = fasting blood glucose (mg/dL)×fasting insulin (μIU/mL)/405. HOMA-IR ≥2.5 was accepted as insulin resistance. All peripheral blood samples were taken after an overnight fasting within first 5 days of the menstrual cycle. In amenorrheic cases, after pregnancy has been ruled out, a dosage of 5 mg/day medroxyprogesterone acetate (TARLUSAL; Deva Holding A.Ş. Istanbul, Turkey) for 5 days was administered in order to induce vaginal bleeding.

All Doppler ultrasound examinations were performed between 8.00 am and 11.00 am to exclude the effects of the circadian rhythmicity on ovarian blood flows by the same gynecologist (A.C.O) using Voluson 730Expert (GE Medical Systems, Solingen, Germany) ultrasound machine equipped with a 7–9 MHz transvaginal transducer. The examiner was blinded to clinical data and hormonal status of all the women in order to avoid biases. Patients rested in a waiting room for at least 15 min before being scanned and completely emptied their bladders in order to minimize the external effects on blood flow. For ovarian stromal blood vessel measurements, color signals were sought in the ovarian stroma at a maximum distance from the surface of the ovary. Blood vessels located close to the wall of a follicle were not measured. For each variable, at least three consecutive measurements were taken and the average value was obtained. Resistance index (RI) and pulsatility index (PI) were electronically calculated using the following formula: PI = (S–D)/mean, RI = (S–D)/S, where S is the peak shifted doppler frequency, D is the minimum doppler shifted frequency over the cardiac cycle.

Treatment of OCP (Group 1 and 4) or MYO (Group 2 and 5) was started on the 3rd day of menstruation. For OCP treatment, we prescribed a monophasic OCP containing 30 mcg ethinyl estradiol (EE) plus 3 mg drospirenone (DRP) (Yasmin; Schering AG, Berlin, Germany) once a day for 3 months. For MYO treatment, the patients were given 2 packs of 1 gram myoinositol plus 100 μg folic acid (Inofolic; Lo.Li. Pharma, Rome, Italy) twice a day in the morning and in the evening. In total 4 gram myoinositol plus 400 μg folic acid was given for 3 months. Rest of the cases (Group 3 and 6) did not receive any medication. No changes in lifestyle were implemented throughout the study. Follow-up examinations were made 3 months later in all participants.

Data are shown as mean ± standard deviation unless otherwise stated and are analyzed using Statistical Program for Social Sciences (SPSS) version 16. (SPSS, Chicago, IL). The level of significance was accepted when $p < .05$.

Results

The mean values of BMI, WHR, and Ferriman gallery score were significantly higher in PCOS group compared to healthy subjects initially (Table 1).

Initially, LH/FSH ratio, LH and free testosterone levels were significantly higher in PCOS compared to healthy ones. Table 2 outlines the comparison of the hormonal parameters of women in each group before and after OCP therapy. Table 3 shows the comparison of the hormonal parameters of women in each group before and after MYO therapy. After the treatment with MYO and OCP in patients with PCOS, there was a significant decrease in LH/FSH ratio and testosterone levels and an increase in SHBG. (Tables 2 and 3).

Healthy women had higher levels of RI and PI values compared to women with PCOS in the baseline measurement. (Table 4). There was a significant increase in RI and PI of both ovarian stromal blood flow in women with PCOS who received OCP (Group 1, $p < .001$) and MYO (Group 2, $p < .001$) (Table 5). The rate of increment in both RI and PI values were similar for OCP users (Group 1) and MYO users (Group2) in PCOS patients. Whereas RI and PI values of both ovaries remained unchanged in all untreated women with or without PCOS (Group 3 and 6).

Table 1. Comparison of characteristics of PCOS and healthy controls.

	PCOS ($n = 90$)	Healthy controls ($n = 90$)	p value*
Age (years)	22.67 ± 2.86	21.54 ± 3.54	.116
BMI(kg/m2)	23.6 ± 1.33	21.5 ± 2.19	.002
Waist hip ratio	0.81 ± 0.06	0.75 ± 0.03	<.001
Ferriman Gallwey score	12.3 ± 2.1	6.3 ± 1.9	<.001
Blood pressure(mmHg)	115.4 ± 7.5	116.2 ± 6.3	.451
HOMA-IR	1.74 ± 0.61	1.63 ± 0.72	.573

BMI: body mass index; HOMA-IR: homeostasis model assessment-insulin resistance.
*$p < .05$ is statistically significant. Independent samples t test.

Table 2. Hormonal assessment of PCOS and healthy controls after 3 months OCP treatment.

	Study groups		Months		p values 0 vs. 3 mo*
	Cases	OCP	0	3	
LH (IU/L)	PCOS	+	5.91 ± 1.21	4.28 ± 1.44	<.001
		–	5.99 ± 1.11	5.53 ± 1.08	.53
	Healthy	+	3.21 ± 1.76	2.91 ± 1.65	.61
		–	3.76 ± 1.43	3.71 ± 1.99	.60
LH/FSH ratio	PCOS	+	1.98 ± 0.21	1.43 ± 0.29	<.001
		–	1.84 ± 0.38	1.82 ± 0.24	.06
	Healthy	+	1.15 ± 0.35	0.93 ± 0.20	.19
		–	0.89 ± 0.11	0.91 ± 0.58	.42
Free testosterone (pg/ml)	PCOS	+	2.86 ± 0.67	1.83 ± 0.34	.004
		–	2.79 ± 0.89	2.61 ± 0.21	.21
	Healthy	+	1.45 ± 0.22	1.28 ± 0.13	.32
		–	1.54 ± 0.32	1.76 ± 0.31	.95
SHBG (nmol/L)	PCOS	+	36.76 ± 0.13	93.76 ± 0.24	.003
		–	38.71 ± 0.21	36.66 ± 0.15	.86
	Healthy	+	78.36 ± 0.16	95.43 ± 0.25	.44
		–	86.79 ± 0.22	93.26 ± 0.34	.56
E2(pg/mL)	PCOS	+	45.12 ± 15.2	35.12 ± 16.8	.73
		–	44.15 ± 11.3	40.34 ± 14.3	.78
	Healthy	+	39.11 ± 11.9	41.33 ± 19.2	.62
		–	40.25 ± 12.8	44.91 ± 22.1	.91

FSH: Follicle stimulating hormone; LH: Luteinizing Hormone; E2: Estradiol; SHBG: sex hormone binding globulin.
*$p < .05$ is statistically significant. Paired samples t test.

Table 3. Hormonal assessment of PCOS and healthy controls after 3 months MYO treatment.

	Study groups		Months		*p* values 0 vs. 3 mo*
	Cases	MYO	0	3	
LH (IU/L)	PCOS	+	5.78 ± 1.28	3.51 ± 1.91	.002
		−	5.83 ± 1.99	5.91 ± 1.83	.77
	Healthy	+	4.91 ± 1.02	3.88 ± 1.09	.81
		−	4.34 ± 1.22	4.98 ± 0.87	.86
LH/FSH ratio	PCOS	+	2.15 ± 0.27	1.54 ± 0.15	<.001
		−	1.95 ± 0.45	2.21 ± 0.33	.28
	Healthy	+	1.08 ± 0.21	0.96 ± 0.71	.67
		−	0.97 ± 0.28	0.93 ± 0.42	.91
Free testosterone (pg/mL)	PCOS	+	2.73 ± 0.24	1.66 ± 0.23	<.001
		−	2.89 ± 0.37	2.31 ± 0.67	.69
	Healthy	+	1.36 ± 0.18	1.10 ± 0.85	.67
		−	1.55 ± 0.27	1.75 ± 0.17	.59
SHBG (nmol/L)	PCOS	+	36.76 ± 0.13	99.08 ± 0.54	.002
		−	31.04 ± 0.22	27.56 ± 0.11	.54
	Healthy	+	101.54 ± 0.18	109.36 ± 0.19	.76
		−	95.45 ± 0.28	89.34 ± 0.23	.98
E2(pg/mL)	PCOS	+	56.33 ± 11.7	44.81 ± 21.9	.91
		−	34.12 ± 18.8	42.12 ± 28.5	.55
	Healthy	+	40.21 ± 10.6	46.11 ± 11.4	.98
		−	42.98 ± 11.9	43.21 ± 18.3	.59

FSH: Follicle stimulating hormone; LH: Luteinizing Hormone; E2: Estradiol; SHBG: sex hormone binding globulin.
*$p < .05$ is statistically significant. Paired samples *t* test.

Table 4. Comparison of initial doppler measurements of PCOS and control patients.

	PCOS $N = 90$	Controls $N = 90$	*p* value*
Right PI	1.56 ± 0.33	2.35 ± 0.27	<.001
Right RI	0.68 ± 0.29	1.06 ± 0.14	<.001
Left PI	1.48 ± 0.27	2.28 ± 0.35	.02
Left RI	0.70 ± 0.18	1.15 ± 0.21	<.001

RI: resistance index; PI: pulsatility index.
*$p < .05$ is statistically significant. Independent samples *t* test.

Table 5. Changes in Doppler parameters after 3 months OCP and MYO treatment in PCOS patients.

	Right ovary		Left ovary		*p* value*
	Before OCP	After OCP	Before OCP	After OCP	
PI	1.67 ± 1.01	2.33 ± 0.98	1.35 ± 0.87	2.02 ± 1.26	<.001
RI	0.76 ± 0.35	1.31 ± 0.37	0.82 ± 0.11	1.27 ± 1.32	<.001
	Before MYO	After MYO	Before MYO	After MYO	
PI	1.56 ± 0.89	2.18 ± 0.27	1.45 ± 0.76	2.31 ± 0.95	<.001
RI	0.66 ± 0.35	0.97 ± 0.44	0.76 ± 0.12	1.04 ± 0.20	<.001

RI: resistance index; PI: pulsatility index.
*$p < .05$ is statistically significant. Paired samples *t* test.

Discussion

Our study data demonstrated a significantly lower PI and RI values of baseline ovarian blood flow in women with PCOS compared to healthy women. These results were supported by many other reports which show the increased stromal vascularization in the polycystic ovaries [19–21]. However, there are conflicting results which did not find any significant changes of vascular indices in ovaries of PCOS patients compared to controls [6,11,12]. These different results may be due to the different phenotypic features of PCOS patients [8].

In 2015, Ozdemir et al. [22]. found that elevation of LH and LH/FSH ratios were related with the hyperplasia of ovarian theca/stromal cells which may lead to stromal vascularization by affecting neoangiogenesis. This data is supported by our study results. Our PCOS patients had significantly higher LH and LH/FSH ratios initially compared to controls. With the treatment with OCP and MYO, we found a reduction in LH and LH/FSH ratios. This may be related with the reduced RI and PI values after treatment with MYO and OCP. Several publications have proposed that serum LH levels might play a critical role in adjusting ovarian blood flow by several different mechanisms [19,21,23]. Elevated LH levels induce androgen production by theca cells in PCOS which results in androgen overproduction. In addition, Amin et al. [24] found that the increase in LH levels was correlated with vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1). VEGF and IGF-1 play a role in neoangiogenesis which lead to increased stromal vascularization in PCOS.

OCPs are used as the first choice treatment for women with PCOS who have menstrual irregularity, hirsutism complaint and who do not desire fertility. OCPs suppress LH secretion and androgen production. There are several studies which investigate the Doppler indices of ovary during the OCP treatment for PCOS, but the data is limited and the results are conflicting [10,13,14]. Our study data supports that OCPs reduce LH/FSH ratio and reduce androgen production and this is correlated with the blood flow of the ovary. After the treatment with OCPs, we found increased levels of RI and PI values. This effect on ovarian blood flow is most likely based on decreased levels of androgen levels, suppressed LH levels and decreased LH/FSH ratio. There are some studies which claim that OCPs are associated with impaired insulin sensitivity [25]. Insulin may act together with LH to induce the synthesis of androgens and may decrease RI and PI values of the stromal blood flow of the ovaries. Our study population did not have insulin resistance and after OCPs treatment there was no significant change in HOMA-IR levels. Therefore, our opinion is that the change in ovarian blood flow may be due to the decreased androgen levels and suppressed LH/FSH ratio rather than the insulin resistance.

MYO is a precursor of inositol triphosphate, acting as an intracellular second messenger and regulating reproductive system hormones and insulin [26,27]. MYO administration improves hormonal profile, reduces LH, androgen production and insulin resistance [16,17]. Our study population were normal weight and did not have insulin resistance. When MYO is administered to normal weight PCOS patients, it may modulate insulin secretion and peripheral tissue sensitivity. Genazzani et al's. [28] study about MYO effect in normal weight patients support our data. Also, Genazzani showed that PCOS patients are characterized by an exaggerated LH response to the GnRH stimulation test [28]. MYO may affect ovarian stromal blood flow, by reducing LH secretion and by increasing insulin sensitivity [29]. Yet, there is no data showing the MYO effect on ovarian blood flow. To the best of our knowledge, this is the first prospective case-controlled study which evaluated MYO effect on ovarian doppler indices. Our data shows that MYO increases RI and PI values, therefore the stromal vascularization decreases. MYO may show its effect on vascularization of the ovary by down regulation of IGF-1 and suppression of LH. Our results support that MYO treatment decreases androgen production. Further studies are needed to prove the consequences.

Conclusion

MYO therapy reduced ovarian vascularization in both PCOS and healthy users after 3 months and this decrease is especially noticeable in women with PCOS compared to healthy women. OCP therapy also reduced ovarian vascularization just like MYO therapy. The effect of MYO and OCP therapy were similar in

PCOS patients. MYO or OCP treatment is not superior to each other in terms of ovarian blood flow indices. There are limitations to this study such as this study was conducted with only one type of combined oral contraceptive pill and it may not be concluded as a direct effect of all OCPs on ovarian vascularity. Study groups are relatively small and there were dropouts during the study. The results presented in this study should be assessed with further data.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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