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The Effects of Selenium Supplementation on Gene Expression Related to Insulin and Lipid in Infertile Polycystic Ovary Syndrome Women Candidate for In Vitro Fertilization: a Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract This study was conducted to evaluate the effects of selenium supplementation on gene expression related to insulin and lipid in infertile women with polycystic ovary syndrome (PCOS) candidate for in vitro fertilization (IVF). This randomized double-blind, placebo-controlled trial was conducted among 40 infertile women with PCOS candidate for IVF. Subjects were randomly allocated into two groups to intake either 200-μg selenium (n = 20) or placebo (n = 20) per day for 8 weeks. Gene expression levels related to insulin and lipid were quantified in lymphocytes of women with PCOS candidate for IVF with RT-PCR method. Results of RT-PCR demonstrated that after the 8-week intervention, compared with the placebo, selenium supplementation upregulated gene expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) (1.06 ± 0.15-fold increase vs. 0.94 ± 0.18-fold reduction, P = 0.02) and glucose transporter 1 (GLUT-1) (1.07 ± 0.20-fold increase vs. 0.87 ± 0.18-fold reduction, P = 0.003) in lymphocytes of women with PCOS candidate for IVF. In addition, compared with the placebo, selenium supplementation downregulated gene expression of low-density lipoprotein receptor (LDLR) (0.88 ± 0.17-fold reduction vs. 1.05 ± 0.22-fold increase, P = 0.01) in lymphocytes of women with PCOS candidate for IVF. We did not observe any significant effect of selenium supplementation on gene expression levels of lipoprotein(a) [LP(a)] in lymphocytes of women with PCOS candidate for IVF. Overall, selenium supplementation for 8 weeks in lymphocytes of women with infertile PCOS candidate for IVF significantly increased gene expression levels of PPAR-γ and GLUT-1 and significantly decreased gene expression levels of LDLR, but did not affect LP(a).

Clinical trial registration number: http://www.irct.ir: IRCT201704245623N113.

Keywords Selenium supplementation · Gene expression · Insulin · Lipid · Polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent heterogeneous endocrine disorder affecting 6–10% of the women in reproductive age depending on the diagnostic criteria used [1]. In the in vitro fertilization (IVF) setting, the multifolliculogenesis as a response to exogenous gonadotropin stimulation is more frequent in insulin-resistant subjects who are, therefore, more prone to progress ovarian hyperstimulation syndrome [2, 3]. The peroxisome proliferator-activated receptor (PPAR-γ) is involved in several metabolic pathways, such as insulin and lipid metabolism and cellular differentiation [4]. In addition, PPAR-γ may regulate the steroidogenesis, which in turn contributes to the regulation of ovarian function [5]. Oxidized low-density lipoprotein (ox-

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LDL) concentrations were also statistically significantly higher in untreated PCOS women than controls [6], which are considered as an independent risk factor in cardiovascular diseases (CVD) [7].

Previous studies have documented that trace elements are required for normal body function and a deficiency or overabundance may result in a diverse range of diseases, including diabetes and obesity [8–11]. Selenium is an essential micronutrient, which plays an important role in redox reactions, such as glutathione peroxidase (GPx) and thioredoxin reductase [12]. More recently, evidence has also been presented that selenium could affect carbohydrate and fat metabolism. Selenium, apart from its anti-inflammatory, anticarcinogenic, and antioxidative effects, influences the way of PPAR-γ action [13]. In a study by Gao et al. [14], it was observed that selenium deficiency inhibited PPAR-γ activity and promoted nuclear factor-κB (NF-κB) pathway activation. In addition, selenium supplementation has been demonstrated to increase the synthesis of a PPAR-γ activator in macrophages [15]. Indeed, increased gene expression of PPAR-γ or transcriptional activity has anti-inflammatory effects [15]. Another study conducted by Hussein et al. [16] has seen that selenium supplementation for 3 months among kidney transplant recipients decreased the enhanced susceptibility to lipid peroxidation and ox-LDL.

Since insulin resistance and dyslipidemia in PCOS women contribute to both reproductive and metabolic disorders [17], it is important to identify methods to decrease complications related to insulin resistance and dyslipidemia through lifestyle changes and pharmacological interventions. This evidence might support the importance of selenium supplementation in women with PCOS candidate for IVF. According to the best of our knowledge, data on the effects of selenium supplementation on gene expression related to insulin and lipid in women with PCOS candidate for IVF are limited. The aim of current study was to evaluate the effects of selenium supplementation on gene expression related to insulin and lipid in women with PCOS candidate for IVF.

Methods

Trial Design and Participants

This randomized double-blind, placebo-controlled trial, registered in the Iranian website for registration of clinical trials (http://www.irct.ir: IRCT201704245623N113), was conducted among 40 infertile women aged 18–40 years old with PCOS diagnosis according to the Rotterdam criteria [18], who were IVF candidate from the Research and Clinical Center for Infertility and the Mahdieh Clinic, Tehran, Iran, between April 2017 and July 2017. Diagnosis of PCOS was done according to the Rotterdam criteria [18]. Those with two of the following criteria were considered as having PCOS: (1) oligo- and/or anovulation (defined as delayed menses > 35 days or < 8 spontaneous hemorrhagic episodes/year), (2) clinical (hirsutism using the modified Ferriman-Gallwey score of ≥ 8) [18] and/or biochemical signs of hyperandrogenism, and (3) polycystic ovaries (12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume > 10 ml^3) [18]. Clinical hyperandrogenism was assessed as the self-reported degree of hirsutism using the modified Ferriman-Gallwey scoring method based on a chart displaying degree of hair growth in nine regions [19] or acne. We excluded pregnant women and participants with elevated levels of prolactin, thyroid disorder, and endocrine diseases. This study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from all participants. The research was approved by the ethics committee of the Shahid Beheshti University of Medical Sciences.

Study Design

Firstly, all participants were matched for age (< 30 and ≥ 30 years) and BMI (< 25 and ≥ 25 kg/m^2). Participants were then randomly divided into two groups to receive either 200-μg selenium as selenium yeast-free other supplements such as zinc and copper (n = 20) or placebo (n = 20) per day for 8 weeks. Selenium supplement and placebo (cellulose) tablets were identical in color, shape, size, and packaging and were manufactured by Nature Made (California, USA) and Barij Essence (Kashan, Iran), respectively. To assess the compliance, participants were asked to bring the medication container. In addition, participants received a daily reminder message on their cell phones to take their supplements regularly. Randomization assignment was conducted using computer-generated random numbers as blindness by a trained midwife at the clinic. Participants were requested not to alter their routine physical activity and not to take any nutritional supplements during the trial or the previous 3 months (wash-out). All patients completed 3-day food records and three physical activity records at the baseline, 3rd and 6th weeks, and at the end of trial. Daily macro- and micronutrient intakes were analyzed by nutritionist IV software (First Databank, San Bruno, CA).

Assessment of Anthropometric Measures

Weight and height of participants were determined in an overnight fasting status using a standard scale (Seca, Hamburg, Germany) at the baseline and the end of the intervention. BMI was calculated as weight in kilograms divided by height in meters squared.

Assessment of Outcomes

Gene expression of PPAR-γ and glucose transporter 1 (GLUT-1) were considered as the primary outcomes, and gene expression levels of low-density lipoprotein receptor (LDLR) and lipoprotein(a) [LP(a)] were considered as the secondary outcomes.

Isolation of Lymphocyte, RNA Extraction, and cDNA Synthesis

Twenty milliliters of blood samples was collected in the anticoagulant EDTA tubes. Lymphocytes were isolated using 50% percoll solution (Sigma-Aldrich, Dorset, UK) gradient by centrifugation for 20 min and 3000 rpm at 4 °C [20]. Total RNA was extracted based on acid guanidinium-phenol-chloroform procedure using RNX™-plus reagent (Cinnacolon, Tehran, Iran) according to the manufacturer's instructions. RNAs was treated with DNAase I (Fermentas, Lithuania) for the elimination of any genomic DNA contamination. Concentration, integration, and purity of RNA samples were determined by spectrometry and gel electrophoresis. Three micrograms of total RNA was used for complementary DNA (cDNA) synthesis with random hexamer and oligo (dT) 18 primers through RevertAid™ reverse transcriptase (Fermantase, Canada) in the total 20-μl reaction mixture [20].

Real-Time PCR Analysis

Appropriate primers for PPAR-γ, GLUT-1, LDLR, and LP(a) and glyceraldehyde-3 phosphate dehydrogenase—as an internal control—were designed (Table 1). Quantitative real-time PCR was performed by the LightCycler® 96-sequence detection systems (Roche Diagnostics, Rotkreuz, Switzerland) using 4 μl of 5× EVA GREEN I master mix (Salise Biodyne, Japan), 10-ng cDNA, and 200 nM of each forward and reverse primers in the final volume of 20 μl. The PCR was performed through the following instruction: an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 54–62.1 °C for 15 s, and extension at 72 °C for 30 s. The specificity of PCR products was evaluated by 1.5% agarose gel electrophoresis and melting curve analysis. All experiments were performed at least in triplicate.

Statistical Methods

To evaluate whether the study variables had been normally distributed, we used the Kolmogrov-Smirnov test. To detect differences in anthropometric measures as well as in macro- and micro-nutrient intakes between the two groups, we applied independent samples *T* test. To determine the effects of selenium supplementation on gene expression related to insulin and lipid, we used independent samples *T* test. Adjustment for changes in baseline values of gene expression levels related to insulin and lipid, age, and baseline BMI was conducted by analysis of covariance. The *P* value of < 0.05 was considered statistically significant. All statistical analyses used the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL, USA).

Results

At the baseline, we invited 45 PCOS women of IVF candidate; however, 5 women were excluded from the study because of not meeting inclusion criteria. Therefore, 40 women with PCOS candidate for IVF [placebo ($n = 20$) and selenium ($n = 20$)] completed the trial (Fig. 1). On average, the rate of compliance in our study was high, such that higher than 90% of supplements were taken throughout the study in both groups. No side effects were reported following the intake of selenium in women with PCOS candidate for IVF throughout the study.

Mean age, height and weight, and BMI at baseline and at the end of trial were not statistically different between the two groups (Table 2).

Based on the 3-day dietary records obtained at baseline, at the end of trial, and throughout the study, we found no significant difference in mean dietary macro- and micro-nutrient intakes between the two groups (data not shown).

Results of RT-PCR demonstrated that after the 8-week intervention, compared with the placebo, selenium supplementation upregulated gene expressions of PPAR-γ (1.06 ± 0.15-fold increase vs. 0.94 ± 0.18-fold reduction, $P = 0.02$) and GLUT-1 (1.07 ± 0.20-fold increase vs. 0.87 ± 0.18-fold reduction, $P = 0.003$) in lymphocytes of subjects with PCOS candidate for IVF (Figs. 2 and 3). Adjustments for baseline values of PPAR-γ and GLUT-1, age, and baseline BMI did not influence our findings (data not shown).

Compared with the placebo, selenium supplementation downregulated gene expression of LDLR (0.88 ± 0.17-fold reduction vs. 1.05 ± 0.22-fold increase, $P = 0.01$) in lymphocytes of subjects with PCOS candidate for IVF (Fig. 4). Adjustments for baseline values of LDL, age, and baseline BMI did not influence our findings (data not shown).

We did not observe any significant effect of selenium supplementation on gene expression of LP(a) in lymphocytes of subjects with PCOS candidate for IVF (Fig. 5).

Discussion

We found that selenium supplementation for 8 weeks in lymphocytes of PCOS women of candidate for IVF significantly increased gene expressions of PPAR-γ and GLUT-1,

Table 1 Specific primers used for real-time quantitative PCR

Gene	Primer	Product size (bp)	Annealing temperature (°C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG R: TCTTCCTCTTGTGCTCTTGCTGG	126	61.3
PPAR-γ	F: ATGACAGACCTCAGACAGATTG R: AATGTTGGCAGTGGCTCAG	210	54
GLUT-1	F: TATCTGAGCATCGTGGCCAT R: AAGACGTAGGGACCACACAG	238	62.1
LDLR	F: ACTTACGGACAGACAGACAG R: GGCCACACATCCCATGATTC	223	57
Lp(a)	F: GACACAGCACGTTCATTCCA R: ACACCCCCCTACAATGCTTC	200	55

GAPDH glyceraldehyde-3-phosphate dehydrogenase, *GLUT-1* glucose transporter 1, *Lp(a)* lipoprotein(a), *LDLR* low-density lipoprotein receptor, *PPAR-γ* peroxisome proliferator-activated receptor gamma

significantly decreased gene expression of LDLR, but did not influence LP(a). To our knowledge, this study is the first report of the effects of selenium supplementation on gene expression related to insulin and lipid among women with PCOS candidate for IVF. It must be kept in mind that in the current study, no side effects were reported following supplementation with selenium in PCOS women candidate for IVF throughout the study. Mean dietary plus supplemental selenium intake of study participants was lower than upper limits (400 μg). However, data on the effects of selenium supplementation on health status even in subjects with high dietary selenium intake are conflicting. For instance, Burk [21] observed that intake of moderate (200 μg/day) to large (600 μg/day) selenium supplements as selenomethionine was safe among volunteers ages ≥ 18 years for 16 weeks. However, Aldosary et al. [22] reported hair loss and dermatitis as the adverse effects of selenium supplementation.

Subjects with PCOS are susceptible to several metabolic disorders, such as insulin resistance and dyslipidemia [23, 24]. The current study demonstrated that selenium supplementation for 8 weeks in lymphocytes of PCOS women candidate for IVF upregulated gene expression of PPAR-γ and GLUT-1 expressions. Decreased PPAR-γ expression was reported in PCOS rats compared with the control group [25]. Similarly, decreased concentrations of PPAR-γ in obese children were reported compared with normal controls [26]. In a study by Mueller et al. [27], it was observed that supranutritional selenate in type 2 diabetic animals significantly increased gene

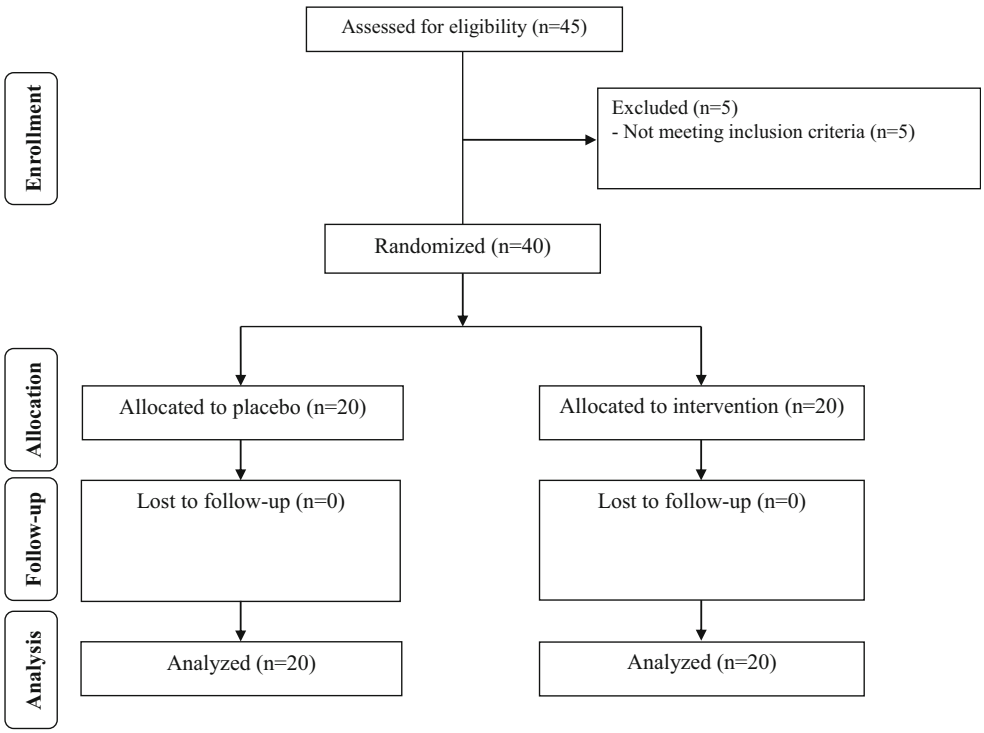

Fig. 1 Summary of patient flow diagram

Table 2 General characteristics of study participants

	Placebo group (n = 20)	Selenium group (n = 20)	P^1
Age (year)	31.4 ± 3.6	31.1 ± 4.7	0.82
Height (cm)	161.0 ± 3.4	162.4 ± 4.5	0.26
Weight at study baseline (kg)	70.7 ± 7.1	69.8 ± 10.7	0.74
Weight at the end of trial (kg)	70.8 ± 7.0	70.0 ± 10.4	0.77
Weight change (kg)	0.1 ± 0.9	0.2 ± 0.8	0.71
BMI at study baseline (kg/m^2)	27.3 ± 2.6	26.5 ± 4.1	0.46
BMI at the end of trial (kg/m^2)	27.3 ± 2.6	26.6 ± 4.0	0.48
BMI change (kg/m^2)	0.04 ± 0.3	0.1 ± 0.3	0.69

Data are means ± SDs

[1] Obtained from independent T test

expression of PPAR-γ and significantly reduced the activity of liver cytosolic protein tyrosine phosphatases, which in turn can be assumed as being responsible for the changes in intermediary metabolism, such as gluconeogenesis and lipid metabolism. In another study, selenium supplementation significantly increased the production of 15-deoxy-delta-12,14-prostaglanin J2 which is an activator of PPAR-γ [15]. Moreover, combined insulin and selenium could significantly revive normoglycemia and restored the disturbances in insulin receptor substrate 1, phosphatidylinositide 3-kinases, and GLUT-4 levels in cardiac muscle of diabetic rats [28]. However, sodium selenite supplementation in mice fed with a high-fat diet downregulated the mRNA expression of PPAR-γ and sterol regulatory element-binding protein-1 [29]. PPAR-γ is a member of the nuclear receptor superfamily and regulates carbohydrate and lipid metabolisms, the immune system, and inflammatory responses [30]. Downregulation PPAR-γ plays a key role in the pathology of numerous diseases including diabetes, CVD, and cancer [31]. Insulin resistance is frequently found in PCOS women, and this trait has cause-consequence relationships with increased risk of CVD [32], gestational diabetes mellitus, and type 2 diabetes mellitus [33] as well as dyslipidemia [34]. A previous meta-analysis has documented that selenium levels were inversely associated with coronary heart disease risk in observational studies [35]. Other randomized trials which have examined the effect of selenium supplements in combination with other vitamins or minerals on CVD end points have yielded inconclusive findings [36, 37]. Selenium, aside from its anti-inflammatory, anticarcinogenic and antioxidative effects, affects also the way of PPAR-γ action. It has been reported that insulin resistance can be attenuated by the inhibition of NF-κβ and mitogen-activated protein kinase (MAPK) signaling pathways and the activation of PPAR-γ [38]. Selenium intake may increase PPAR-γ expression through reducing gene expression of pro-inflammatory genes, inhibiting NF-κβ and p38MAPK activation [39] and the activation of PPAR-γ [40].

Our study supported that selenium supplementation for 8 weeks in lymphocytes of PCOS women candidate for IVF upregulated gene expression of LDLR, but did not influence Lp(a) expression. In a study by Ghaffari et al. [41], it was observed that selenium plus vitamin E supplementation in diabetic rats resulted in significant decreases in ox-LDL and malondialdehyde levels. In addition, antioxidant supplementation (selenium, zinc, copper, manganese, *N*-acetylcysteine, vitamins E and C, and glutamine) for 4 weeks in irradiated hypercholesterolemic rabbits significantly decreased the cholesterol-induced, radiation-enhanced circulating and tissue ox-LDL concentrations [42]. Few studies have reported the association between selenium and Lp(a) levels. For instance, in a study by Alissa et al. [43], it was observed that the serum Lp(a), a coronary risk factor, was strongly related to selenium status in healthy adults. It has been generally documented that increased ox-LDL levels contribute to atherogenesis [44]. Ox-LDL can also promote inflammatory reactions in monocyte-derived macrophages, which in turn can result in endothelial dysfunction [45]. In addition, it has been reported that gene expression levels of matrix metalloprotease 2 (MMP2) increase in atherosclerotic lesions [46]. On the other hand, ox-LDL stimulatory

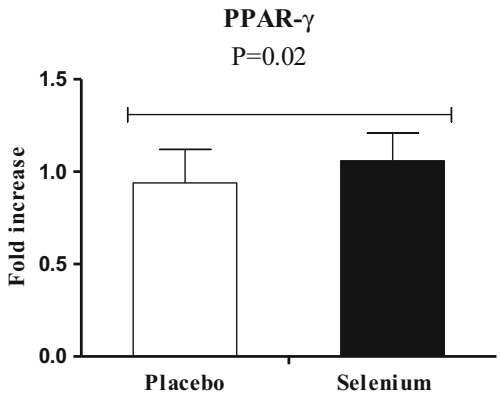

Fig. 2 Effect of 8-week supplementation with selenium or placebo on expression ratio of PPAR-γ gene in lymphocytes of PCOS women of IVF candidate

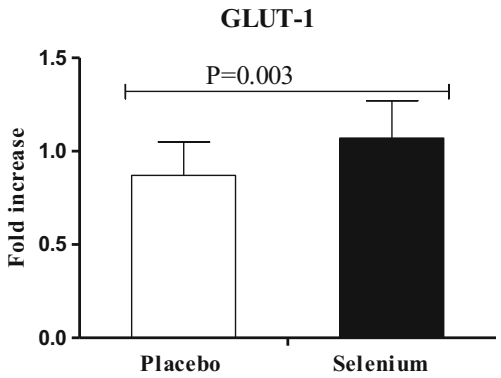

Fig. 3 Effect of 8-week supplementation with selenium or placebo on expression ratio of GLUT-1 gene in lymphocytes of PCOS women of IVF candidate

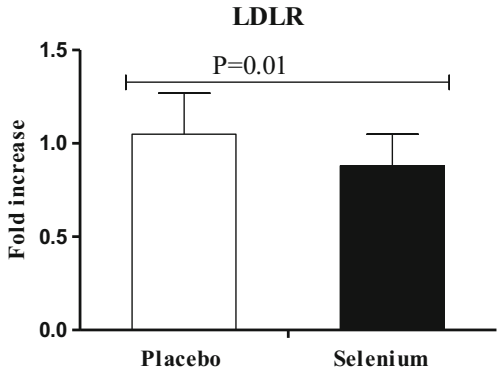

Fig. 4 Effect of 8-week supplementation with selenium or placebo on expression ratio of LDLR gene in lymphocytes of PCOS women of IVF candidate

effect on gene expression of MMP2 in smooth muscle cells has also been documented experimentally [47]. ox-LDL as product of lipid peroxidation is generated under high levels of unscavenged free radicals and can result in the diabetes-associated pancreatic damage [48]. Selenium is an essential component of the erythrocyte glutathione system [49], which functions as part of an antioxidant defense to protect polyunsaturated fatty acids and proteins from the damaging effects of free radicals, peroxides, and lipid hydroperoxides [50]. Decreased ox-LDL levels following the supplementation of selenium in the current study may have indirectly resulted from decreased ROS, free radicals, and lipid peroxidation.

The current study had few limitations. Due to funding limitations, we did not evaluate selenium or GPx levels at baseline and at the end of trial. In addition, we could not assess gene expression levels related to inflammation and oxidative stress.

Overall, selenium supplementation for 8 weeks in lymphocytes of infertile PCOS women candidate for IVF significantly increased gene expressions of PPAR-γ and GLUT-1 and significantly decreased gene expression of LDLR, but did not affect LP(a).

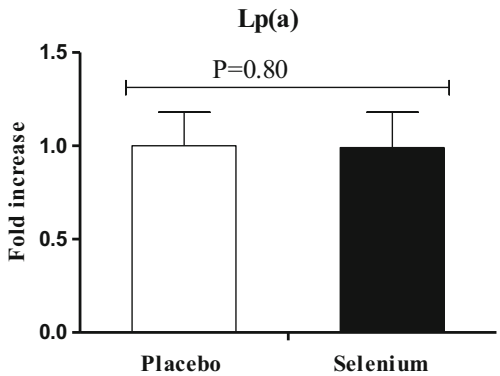

Fig. 5 Effect of 8-week supplementation with selenium or placebo on expression ratio of Lp(a) gene in lymphocytes of PCOS women of IVF candidate. GLUT-1, glucose transporter 1; Lp(a), lipoprotein(a); LDLR, low-density lipoprotein receptor; PCOS, polycystic ovary syndrome; PPAR-γ, peroxisome proliferator-activated receptor gamma

Authors' Contributions ZA contributed in conception, design, statistical analysis, and drafting of the manuscript. SZ, ZH, FF, ZR, and EA contributed in data collection and manuscript drafting. All authors approved the final version for submission. ZA supervised the study.Funding InformationThis study was founded by a grant from the vice-chancellor for research, Shahid Beheshti, University of Medical Sciences, Tehran, Iran.**Compliance with Ethical Standards** This study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all participants. The research was approved by the ethics committee of Shahid Beheshti University of Medical Sciences.

Conflict of Interest The authors declare that they have no conflict of interest.

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