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ORIGINAL ARTICLE

# The effects of low-level laser therapy on polycystic ovarian syndrome in rats: three different dosages

**Bülent Polat[1,2] · Damla Tuğçe Okur[1] · Armağan Çolak[1] · Kader Yilmaz[3] · Mustafa Özkaraca[4] · Selim Çomakli[5]**

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## Abstract

The main objective of this in vivo study was to investigate the effect of different low-level laser therapy (LLLT) doses on polycystic ovary syndrome (PCOS). In the present experimental study, a single dosage of estradiol valerate (EV) was administered to induce PCOS in female rats. After administration of the EV for induction of PCOS, rats were divided into 5 groups ($n = 8$/group): C group (animals that were not exposed to any form of procedure), PC group (no treatment following EV induction), $L_1$ group (1 J/cm$^2$ LLLT treatment following EV induction), $L_2$ group (2 J/cm$^2$ LLLT treatment following EV induction), $L_3$ group (6 J/cm$^2$ LLLT treatment following EV induction). The results indicated that no significant difference was found in the serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone (P4) between the C and $L_2$ groups ($p < 0.05$). Although the serum levels of testosterone (T) were significantly higher in the C group compared with other groups ($p < 0.05$), the $L_2$ group was determined to be the closest to the C group. Additionally, the LH, FSH, and T receptor level of the $L_2$ group was closest to the C group. In conclusion, a 2 J/cm$^2$ dosage of LLLT ($L_2$ group) can be considered the most potentially effective treatment of PCOS in the rat. However, more studies are needed to determine the optimal dose of LLLT for the treatment of PCOS.

**Keywords** Low-level laser therapy · Polycystic ovary syndrome · Rat · Estradiol valerate

## Introduction

Folliculogenesis is a process which results in oocyte reproduction [1]. Disruptions in this process may result in ovarian diseases such as polycystic ovary syndrome (PCOS) in women and female of domestic animals [2]. PCOS, an endocrine metabolic disorder that Stein and Leventhal initially identified in 1935, affects 10% of women worldwide who are of reproductive age [3, 4]. Although PCOS is a widespread endocrine disorder, its etiopathogenesis is unknown [5]. Previous studies have suggested that elevated luteinizing/follicle-stimulating hormone (LH/FSH) ratios caused by abnormal Gonadotropin-releasing hormone (GnRH) secretion, as well as increased serum LH and decreased FSH production, may play a significant role in the etiology of PCOS [6, 7]. PCOS induces follicular development, anovulation, polycystic formation, and ovarian enlargement by causing hyperandrogenism, aberrant gonadotropin production, and an elevated LH/FSH ratio [7, 8]. Therefore, PCOS is a disorder that affects women and female of domestic animals during their lives and is characterized by hyperandrogenism, chronic anovulation, and polycystic ovaries [9, 10].

Although the treatment methods used for PCOS effectively reduce symptoms, it has been reported that they commonly produce serious side effect [11, 12]. For instance, regularly prescribed metformin and antiandrogens resulted in cardiovascular disease and fatal/nonfatal lactic acidosis, respectively [13, 14]. In addition, treatment methods such as gonadotropin and surgical intervention are both costly

✉ Damla Tuğçe Okur
damla.tugce.okur@gmail.com

1 Department of Obstetrics and Gynecology, Faculty Veterinary Medicine, Atatürk University, Erzurum, Turkey

2 BIL-TEC, TEKNOKENT, TR-25240 Erzurum, Turkey

3 Celal Oruç Animal Production School, Ağrı İbrahim Çeçen University, Ağrı, Turkey

4 Department of Pathology, Faculty Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey

5 Department of Pathology, Faculty Veterinary Medicine, Atatürk University, Erzurum, Turkey

and time-consuming [12]. Because the etiology of PCOS is unknown, treatment strategies are mostly focused on improving symptoms [15]. Thus, several researchers investigate cost-effective treatment approaches with low side effects [16]. Additionally, low-level laser therapy (LLLT) has been reported to be a non-pharmacological, safe, and effective alternative treatment option [17].

LLLT is based on the interaction of low-energy-density light with cells and tissue without a thermal effect produced [18]. Previous studies have reported that LLLT could improve connective tissue cells, accelerate connective tissue healing, and function as an anti-inflammatory agent [19, 20]. LLLT is a type of photomodulation in which photons are utilized to influence biological activity, and its biochemical effects on living tissues at the cellular level are not harmful [21]. In contrast to other medical laser techniques, LLLT employs a photochemical effect, which implies that light is absorbed and results in a chemical change, rather than an ablative or thermal mechanism [22]. The reason why the technique is termed low level is that the optimum levels of energy density delivered are low, and it is not comparable to other forms of laser therapy as practiced for ablation, cutting, and thermal tissue coagulation [23]. Additionally, LLLT can reduce cell apoptosis and induce cell proliferation and migration [24].

A previous study conducted in rats with PCOS induced by estradiol valerate (EV) reported that LLLT (1 J/point, for 18 s of irradiation time on each ovarian) decreases the number of follicular cysts while increasing the number of ovarian follicles and the corpus luteum [25]. However, to the author's knowledge, there has been no study comparing different dosages of LLLT on a rat with PCOS induced by EV. For this reason, the goal of the in vivo study was to determine three different dosages of LLLT on rats with PCOS induced by EV. We hypothesized that dosage does affect the efficiency of LLLT in rats with PCOS induced by EV.

## Materials and methods

### Animals

Forty adult female Wistar rats (average weight of 250 g) were obtained from the Medical Experimental Application and Research Center of Atatürk University for the current study. The animals were housed four per standard cage and were acclimated for 1 week prior to the beginning of the study. The rats were maintained under the humanity ranged from 40 to 60% and a uniform temperature of 22 ± 2 °C with a 12:12h light: dark cycle. They were fed a commercially available diet and water ad libitum. Atatürk University Local Board of Ethics Committee for Animal Experiments approved the experimental protocol (No: 2019/111). The regularity of the estrous cycle was evaluated [26], and only rats with at least four consecutive regular 4-day estrous cycles were included in this study.

### Induction of polycystic ovary

In the current study, the rats were injected with intramuscular (IM) 2 mg/kg (0.2 ml) EV (Sigma-Aldrich, MO, USA) with dissolved in mineral oil to induce the formation of follicular cyst in order to mimic the PCOS [27, 28].

### Experimental groups

Rats were randomly assigned to one of five groups, with eight animals each: C group (animals that were not exposed to any form of procedure), PC group (no treatment following EV induction), $L_1$ group (1 J/cm$^2$ LLLT treatment following EV induction), $L_2$ group (2 J/cm$^2$ LLLT treatment following EV induction), $L_3$ group (6 J/cm$^2$ LLLT treatment following EV induction). All treatment procedures were initiated 60 days after EV administration to the rats.

### Laser irradiation

A low-energy gallium arsenide (GaAs) laser device (Lasermed 2200, Eme Physio, Italy) at the wavelength (λ) of 904 nm (10.000 Hz, 25 mW, 1 cm$^2$ beam area, and continuously mode), with a peak power 25 mW, in a dose of 1 J/cm$^2$, 2 J/cm$^2$, and 6 J/cm$^2$ for 60, 120, and 240 s of irradiation time were performed on each ovary 3 times a week for a total of 4 weeks. A monodiode laser probe (MLA 1/25) was used to the direct contact with the skin with slight pressure at the central part of the dorso-abdominal. No anesthetic drugs were administered during LLLT procedures, and the same person performed all LLLT treatment protocols at the same time between 8 and 12 a.m.

### Hormone assay

Following 4 weeks of treatment procedures, rats were euthanized and approximately 5 ml of blood was obtained from each animal's heart. Collected blood samples were centrifuged at 1200 G for 20 min at 4 °C, serum samples were taken into 0.5 ml godets and stored at −20 °C until hormonal analyses. Measurement of serum LH, FSH, progesterone (P4), and testosterone (T) levels was performed using rat specific ELISA assay kit (Rat ELISA Kit, Sunred YL Biotech Co., Ltd). BIOTEK brand ELISA device was used for this analysis [29].

### Western blot analysis

The right and left ovaries of the rats were collected for western blot analysis before euthanasia. Western blotting was performed on normal and PCOS-created ovary tissues to assess the expressions of rat LH, FSH, progesterone, and androgen receptors. Before homogenization, frozen tissue samples were put into liquid nitrogen for 30 s. Then, transferred into a steel homogenizer chamber that contains liquid nitrogen and homogenized at 30/s frequency for 30/s frequency by using Tissue Lyser II. Finally, 0.1 g of the homogenized sample was mixed with 1 ml 5% SDS prepared in RIPA buffer (pH 7.4) and homogenized again by using Tissue Lyser Lt at 30/s frequency for 1 min. For western blotting, 0.5 ml of obtained liquid samples were mixed with 4.5 ml of electrophoresis sample buffer (125 mmol/L Tris, 10% [v/v] glycerol, 1% sodium dodecylsulfate [SDS], 2.5% β-mercaptoethanol, and 0.012% bromophenol blue) and boiled for 1 min. A fraction of each sample (20 µg protein) was separated by using 12% SDS-polyacrylamide gel; the proteins were then transferred onto PVDF membranes (1704272, Bio-Rad, Hercules, CA, USA). Nonspecific binding sites were blocked with 4% (w/v) bovine albumin fraction V (8076.2, Carl Roth GmBH, Karlsruhe, Germany) prepared in TBS-T (0.1% Tween 20 in, 0.05 mol Tris, 0.9% sodium chloride; pH 7.5) and probed with rabbit polyclonal antibody to rat and (bs-5192R), FSH (bs-20658R), LH (bs-6431R), and Pro (bs-23376R) receptors (Bioss, Woburn, MA, USA, 1 µg IgG/µL, 1:300 dilution in BSA TBS-T) and monoclonal antibody to rat β-tubulin (66240-1-Ig, Proteintech, St. Leon-Rot, Germany) (1427 µg/ml IgG, 1:20000 dilution in BSA TBS-T). After overnight incubation at +4 °C, primer antigens were labeled with horseradish peroxidase-labeled goat seconder antibodies against rabbit and mouse (ab205718, ab6789, Abcam, Cambridge, UK) and detected by using enhanced chemiluminescence substrate (Super Signal West Femto, 34094, Thermo Scientific, Rockford, IL, USA) [30].

### Statistical analysis

All data were analyzed by using the SPSS Version 20.0 (IBM Company, SPSS Inc., IL, USA). Prior to analysis, the normality of the results was assessed by the Shapiro-Wilk test. The Kruskal-Wallis test was employed to evaluate statistical differences between groups. followed by the Mann-Whitney *U* test. Data expressed as mean ± SEM (standard error of the mean), and a $p$-value of $< 0.05$ was considered statistically significant.

## Results

All procedures were successfully completed without any complications during the experimental study. Following 4-week treatment periods, LH levels were significantly lower in the $L_1$ and $L_2$ groups compared to the $L_3$ groups ($p = 0.030$). Additionally, there was no significant difference between C (14.8 ± 0.5 ng/mL) and $L_2$ (15.3 ± 0.1 ng/mL) group ($p = 0.44$), and no significant difference was found between PC (16.9 ± 0.4 ng/mL) and $L_3$ (16.4 ± 0.2 ng/mL) groups in serum LH level ($p = 0.89$, Fig. 1, Table 1). The assessment of LH at the receptor levels also showed statistical differences between groups. The LH receptor level was statistically higher in the PC (154 ± 1.9 ng/mL) group, while was lower in the $L_2$ (113 ± 1.5 ng/mL) group ($p = 0.021$, Fig. 2, Table 2).

The rat serum FSH levels in PC (18.7 ± 0.3 ng/mL) groups were significantly higher compared to the C, $L_2$, and $L_3$ groups at the end of the study ($p = 0.027$). There were no significant differences among C, $L_1$, $L_2$, and $L_3$ groups ($p = 0.62$, Fig. 1, Table 1). The FSH receptor level was statistically higher in the PC (137 ± 2 ng/mL) group, while was lower in the $L_2$ (106 ± 1.5 ng/mL) group ($p = 0.014$, Figure 2, Table 2).

The rat serum P4 levels were significantly lower in the C and $L_2$ groups compared to the PC, $L_1$, and $L_3$ groups ($p = 0.041$). No significant difference was observed between C (64 ± 1.9 ng/mL) and $L_2$ (96 ± 1.8 ng/mL) groups ($p = 0.79$, Fig. 1, Table 1). There were significant differences among all groups according to the P4 receptor level ($p = 0.011$); the highest level was measured in the C (100 ± 1.2 ng/mL) group, while the lowest level was measured in the PC (68 ± 1.4 ng/mL) group ($p = 0.032$, Fig. 2, Table 2).

There was a significant difference in serum T levels between C and PC, $L_1$, $L_2$, and $L_3$ groups ($p = 0.001$). No significant difference was observed between L1 (104 ± 1.1 ng/mL) and $L_3$ (107 ± 1.5 ng/mL) groups ($p = 0.65$). Also, there was no significant difference between PC (112 ± 2.2 ng/mL) and $L_3$ (107 ± 1.5 ng/mL) groups ($p = 0.71$, Fig. 1, Table 1). There were significant differences among all groups according to the P4 receptor level ($p = 0.016$); the highest level was measured in the PC (139 ± 2.1 ng/mL) group, while the lowest level was measured in the C (100 ± 1.3 ng/mL) group ($p = 0.003$, Fig. 2, Table 2).

## Discussion

The current study evaluated the effect of three different dosages of LLLT on experimental PCOS in rats. Our results confirmed that $L_2$ groups significantly decreased

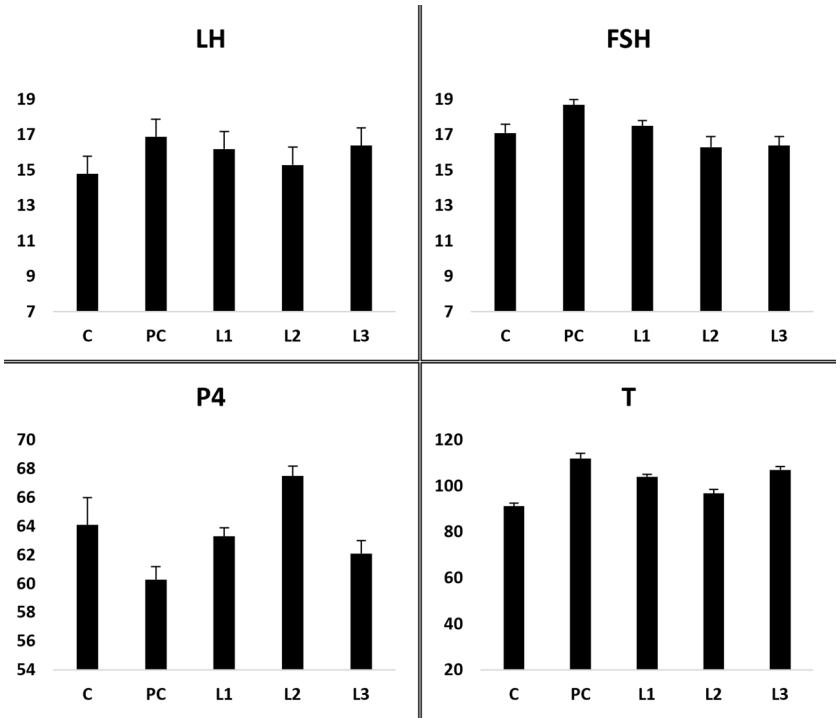


**Fig. 1** Serum LH, FSH, P4, and T levels of C group (animals that were not exposed to any form of procedure), PC group (no treatment following estradiol valerate induction), $L_1$ group (1 J/cm$^2$ LLLT treatment following EV induction), $L_2$ group (2 J/cm$^2$ LLLT treatment following EV induction), $L_3$ group (6 J/cm$^2$ LLLT treatment following EV induction). LH luteinizing hormone, FSH follicle-stimulating hormone, P4 progesterone, T testosterone

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| Group | *n* | Serum level of hormones | | | |
|---|---|---|---|---|---|
| | | LH | FSH | P4 | T |
| C | 8 | $14.8 \pm 0.5^{c}$ | $17.1 \pm 0.5^{b}$ | $64.1 \pm 1.9^{a}$ | $91.2 \pm 1.4^{d}$ |
| PC | 8 | $16.9 \pm 0.4^{a}$ | $18.7 \pm 0.3^{a}$ | $60.3 \pm 0.9^{b}$ | $112 \pm 2.2^{a}$ |
| $L_1$ | 8 | $16.2 \pm 0.3^{ab}$ | $17.5 \pm 0.3^{ab}$ | $63.3 \pm 0.6^{b}$ | $104 \pm 1.1^{b}$ |
| $L_2$ | 8 | $15.3 \pm 0.2^{bc}$ | $16.3 \pm 0.6^{b}$ | $67.5 \pm 0.7^{a}$ | $96.8 \pm 1.8^{c}$ |
| $L_3$ | 8 | $16.4 \pm 0.2^{a}$ | $16.4 \pm 0.5^{b}$ | $62.1 \pm 0.9^{b}$ | $107 \pm 1.5^{ab}$ |

*LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *P4* progesterone, *T* testosterone, different letters in the same column indicated a significant difference

the serum level of LH, FSH, and T and increased the serum level of P4 compared with PC groups. Additionally, $L_2$ groups significantly decreased the receptor level of LH and FSH compared with PC groups.

Several methods have been described to produce an experimental PCOS model in rats, including androgens, hcG, EV, or continuous light exposure [25]. Long-acting EV leads to dysregulated release and storage of LH, resulting in hypothalamic-pituitary dysregulation of GnRH [31–34] and hence results in an enhance in LH and the LH/FSH ratio, anovulation, immature follicles, multiple cysts, and increased ovarian volume [7, 8]. An increased number of polycystic ovaries was observed in rats exposed to EV following 8 weeks with a single dose of EV administration as observed in our study [35, 36]. Similarly, the current experimental study indicated that EV-induced rats exhibited anovulation and polycystic ovarian characteristics similar to those of PCOS [37].

Previous studies stated that LLLT can be performed on different tissues with direct skin contact or without skin contact methods [38, 39]. To avoid causing damage to the ovarian vascular system, intraovarian hemorrhage, and ovarian dysfunction, an experimental study conducted on women recommended that LLLT should be performed at a location distant from the ovary instead of intraovarian application [40]. Therefore, LLLT was performed on direct contact with the skin with slight pressure at the central part of the dorsoabdominal in our study. Similarly, Alves et al. performed LLLT with dorsa-abdominal region approach to modulation of experimental PCOS model in rats [25].

A sudden increase in LH levels (LH surge) is a necessary process for ovulation; however, in PCOS, a continuous increase in LH levels causes the development of antral follicles without ovulation, leading to the formation of cystic follicles [41]. As the ratio of LH to FSH increases in PCOS patients, androgen production in the ovaries enhances T levels [42]. In addition, the overproduction of LH in patients with PCOS results in anovulation and low P4 levels [43]. In the current study, LH, FSH, and T levels increased while P4

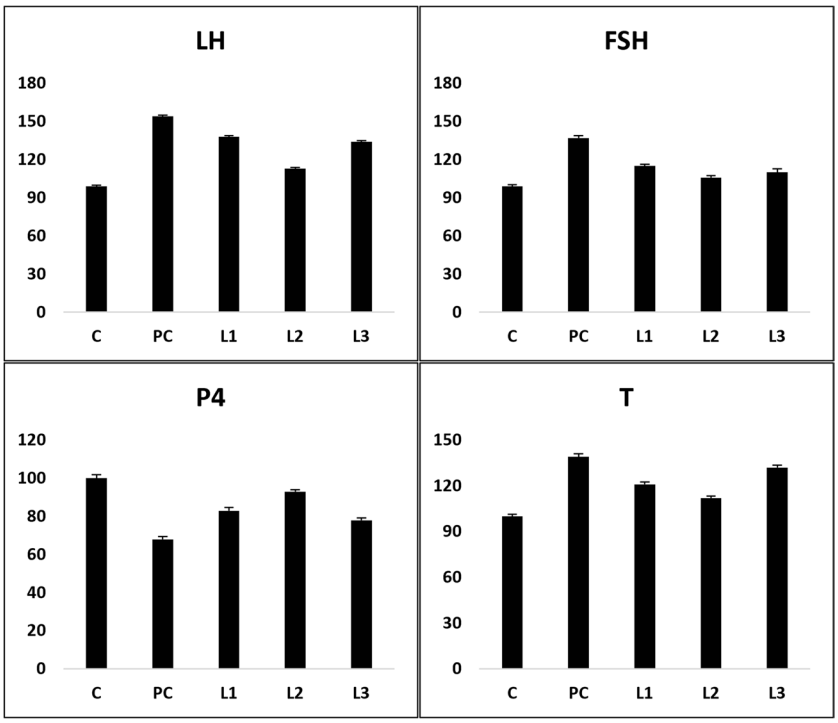


**Fig. 2** The LH, FSH, P4, and T receptor levels of C group (animals that were not exposed to any form of procedure), PC group (no treatment following estradiol valerate induction), $L_1$ group (1 J/cm$^2$ LLLT treatment following EV induction), $L_2$ group (2 J/cm$^2$ LLLT treatment following EV induction), $L_3$ group (6 J/cm$^2$ LLLT treatment following EV induction). LH luteinizing hormone, FSH follicle-stimulating hormone, P4 progesterone, T testosterone

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| Group | *n* | Receptor level of hormones | | | |
|---|---|---|---|---|---|
| | | LH | FSH | P4 | T |
| C | 8 | $99 \pm 1.3^{d}$ | $99 \pm 1.4^{d}$ | $100 \pm 1.9^{a}$ | $100 \pm 1.3^{e}$ |
| PC | 8 | $154 \pm 1.9^{a}$ | $137 \pm 2^{a}$ | $68 \pm 1.4^{e}$ | $139 \pm 2.1^{a}$ |
| $L_1$ | 8 | $138 \pm 2.3^{b}$ | $115 \pm 1.5^{b}$ | $83 \pm 1.8^{c}$ | $121 \pm 1.7^{c}$ |
| $L_2$ | 8 | $113 \pm 1.5^{c}$ | $106 \pm 1.5^{c}$ | $93 \pm 1.1^{b}$ | $112 \pm 1.4^{d}$ |
| $L_3$ | 8 | $134 \pm 1.7^{b}$ | $110 \pm 2.8^{bc}$ | $78 \pm 1.3^{d}$ | $132 \pm 1.6^{b}$ |

*LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *P4* progesterone, *T* testosterone, different letters in the same column indicated a significant difference

levels decreased in the EV-induced PC groups compared to the C groups, as reported in previous studies [41].

The current study demonstrated that LLLT may modulate different levels of hormones. The results of this study are consistent with the previous studies reporting that LLLT stimulates folliculogenesis in rats [25]. In a previous study evaluating the number of blood vessels in the ovary of rats with PCOS, it was reported that the number of blood vessels reduced, which seriously impacted folliculogenesis and disrupted the control of effective hormones during the cycle. Moreover, an increase in the number of blood vessels causes an enhancement in the follicular production activity of the ovarian tissue [44]. Similarly, a study by Abramovich et al. reported that an increase in the number of blood vessels causes an increase in the activity of ovarian tissue to produce various follicles and mature oocytes in rats. According to the same study reported, LLLT regulates ovarian function by increasing angiogenesis in the ovaries [45]. Another previous study hypothesized that LLLT stimulates cells to increase ATP synthesis and blood vessels in the ovary, thereby enhancing the activity of the ovaries [46].

Mitochondria are the principal location of steroidogenesis in ovarian organelles. By acting on mitochondrial membranes, LLLT may have affected steroid synthesis activities by changing the actions of the cytochrome-P450 complex steroidogenic enzymes [25]. LLLT has been demonstrated to cause ligand-dependent nuclear redistribution and transcriptional activation of steroid hormone receptors [25, 47, 48]. LLLT can modify the mitochondrial steroidogenic enzyme activities of theca and granulosa cells, which are related to follicular development and ovarian cyst formation [49].

In this study, three different LLLT dosages (1 J/cm$^2$ [$L_1$], 2 J/cm$^2$ [$L_2$], and 6 J/cm$^2$ [$L_3$]) were performed on rats with PCOS. Although LLLT groups with different doses have effects on hormone levels, the most effective results were obtained in the $L_2$ group compared with $L_1$ and $L_3$ groups. These results could be related to the high dosage of LLLT ($L_3$) employed, which causes a reduction of cell viability and ATP activity, and inhibition of cell proliferation, as reported

by a previous study [50]. Another possible explanation for the low dosage of LLLT ($L_1$) is that the combination of a given energy density or fluency ($J/cm^2$) and a number of exposures is insufficient for LLLT stimulant effects for the ovaries.

## Conclusion

In conclusion, our results support that LLLT can modulate the ovarian hormonal mechanism in a dose-dependent manner in rats with PCOS. But still, further molecular and genetic research is required to explain the ovarian pathways altered by LLLT. Further research is required to establish the appropriate dosage of LLLT for PCOS modulation. Additionally, EV-induced PCOS was also used as a study model for infertility. But this model might not accurately reflect what occurred in actual diseases, and this might be a limitation of the current work.

**Author contribution** **Bülent Polat, Damla Tuğçe Okur**: literature search, study design, data collection and interpretation, and manuscript preparation. **Damla Tuğçe Okur, Armağan Çolak, Kader Yilmaz**: statistical analysis, data collection, interpretation, and manuscript preparation. **Selim Çomaklı, Mustafa Özkaraca**: histopathological analysis and evaluation.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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