Effect of Oral Administration of Dehydroepiandrosterone on PCOS-Like Phenotype of Female C57BL/6 Mice

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ABSTRACT

We aim to evaluate the efficacy and optimal dose of orally administered DHEA in the PCOS mice model by assessing their ovarian morphology and serum FSH, LH, and testosterone levels. Female C57BL/6 mice were divided into a control group (n=5, received daily injections of 0.2 ml sesame oil) and an experimental group, which was further classified into 1) D-45 group (n=5), 2) D-60 (n=5), and 3) D-90 group (n=5) that were treated with 45, 60, and 90 mg/Kg body weight of oral DHEA. After modelling, mice in the control group had a regular estrous cycle, while mice in all experimental groups had a disturbed estrous cycle. Ovarian histology showed several growing follicles and some corpora lutea (CL) in the control, D-60, and D-90 groups. However, some large antral follicles and decreased CL were observed in the D-45 group. Serum FSH was significantly lower in the D-45 group than in the control group (3.73 ± 0.12 vs. 5.28 ± 0.31 mIU/mL, P<0.01), but D-60 and D-90 groups had a similar FSH level to the control group (P>0.05). The serum level of LH and testosterone were significantly elevated in the D-45 group than in the control group (2.52 ± 0.43 vs. 1.30 ± 0.33 mIU/mL, P<0.01 and 1.80 ± 0.32 vs. 1.24 ± 0.23 ng/mL, P<0.01, respectively). Still, the level of LH and testosterone in the D-60 and the D-90 groups was comparable to the control group (P>0.05). Our study suggests that oral administration of DHEA is efficacious in establishing PCOS-like phenotype in mice with the suggested optimal dosage of 45 mg/Kg body weight.

Keywords: DHEA, PCOS-mice model, polycystic ovary syndrome, animal modelling

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a hormonal disturbance characterised by anovulation, hyperandrogenism, and polycystic ovaries among women at their reproductive age (Deswal et al., 2020). PCOS is the first cause of female infertility, as the absence of ovulated oocytes leads to difficulty in natural conception (Azziz, 2006). A growing body of evidence also suggested that PCOS is associated with metabolic syndrome (MetS). MetS is a cluster of metabolic dysregulation that directly increases the risk of cardiovascular disease and type 2 diabetes mellitus (Fahed et al., 2022; Fu et al., 2023). It was reported that the risk of MetS among women with PCOS is 11-fold higher than age-matched control, thus illuminating the underlying mechanism behind PCOS is essential to improve the quality of life of the sufferers and to reduce PCOS-associated comorbidities (Karee et al., 2020; Lorincz et al., 2024). However, ethical issues often limit obtaining human materials for PCOS research. Consequently, the use of animal models for this purpose is undeniable (Ren et al., 2022).

Various animal models to mimic PCOS phenotype have been explored in the past few decades. Mice are the most widely used animal to study PCOS, and their advantages are their stable genetic background, smaller size, short reproductive lifespan, high reproduction index, and easy handling and maintenance (Shi & Vine, 2009; Walters et al., 2012). Currently, several methods to induce PCOS in mice have been developed, including subcutaneous injection of androgens, such as testosterone propionate (TP), dihydrotestosterone (DHT), and dehydroepiandrosterone (DHEA); treatment with other reagents, such as estrogens, anti-Müllerian hormone (AMH), and letrozole; exposure to constant light; and transgenic mice model (Shi & Vine, 2009; Wu et al., 2023). Among these induction strategies, the DHEA-induced PCOS mice model is the most common model used in the research of PCOS (Wu et al., 2023).

DHEA is a steroid hormone synthesized in the adrenal cortex, converted to testosterone (T) and DHT in the ovary. Reports suggested that the DHEA-induced mice model exhibited characteristics of human PCOS, including hyperandrogenism, acyclicity anovulation, and polycystic ovaries (Shi & Vine, 2009). Wu et al. (2023) demonstrated that daily subcutaneous injection of 6 mg per 100 g body weight for 20 consecutive days resulted in a disturbed estrous cycle, ovarian cysts formation, decreased number of corpus luteum, and increased serum T level in the induced mice model. Studies also reported similar results (Sander et al., 2006; Palmerini et al., 2023). However, despite the well-established protocol for the DHEA-induced mice model, little is known about the effect of different administration routes on the efficacy of DHEA for PCOS animal modelling.

This study aims to evaluate the efficacy and optimal dosing of orally administered DHEA in a PCOS-induced mouse model by evaluating FSH, LH, and testosterone serum levels and ovarian histology of treated animals. To the best of our knowledge, this is the first study to investigate the effect of oral administration of DHEA on a PCOS-like phenotype in mice and scrutinize the optimal dosage.

MATERIALS AND METHODS

Animal and PCOS Modelling

Female C57BL/6 mice at the age of 21 days were purchased from iRatco laboratory (Bogor, Indonesia). PCOS induction was performed as described previously (Wu et al., 2023; Palmerini et al., 2023). Briefly, all animals were maintained at $22°C \pm 2°C$ under 12 hours light/dark cycle with free access to food and water. The mice weighing 21-25 grams were divided into a control group (n=5) and an experimental group on postnatal day 25. Mice in the experimental group were further stratified into 1) Experimental 1 group (n=5), 2) Experimental 2 group (n=5), and 3) Experimental 3 group (n=5). Mice in the control group were fed normal chow and subcutaneously injected daily with 0.2 ml sesame oil per 100g body weight (Sigma-Aldrich, St. Louis, CO, USA). The mice in the PCOS-induced group were fed with normal chow and subcutaneously injected daily with 45 mg per Kg body weight DHEA for 21 consecutive days, 60 mg per Kg body weight DHEA for 21 consecutive days, and 90 mg per Kg body weight DHEA for 21 consecutive days (Sigma-Aldrich et al., USA) from experimental 1 to experimental 3 group, respectively, dissolved in 0.1 ml sesame oil Sigma-Aldrich, St. Louis, CO, USA). The control group received the same maintenance as the experimental group without DHEA treatment for 21 days. Experimental 1 group, Experimental 2 group, and Experimental 3 group were further designed as D-45 group, D-60 group, and D-90 group.

Estrous Cycle Determination

The estrous cycle was determined by daily vaginal smear starting from the seventh day of DHEA or sesame oil injection. A vaginal smear was performed by vaginal lavage by which vaginal cells were flushed with saline solution, dried on a glass slide, and then examined under a light microscope under 10× magnification after methylene blue staining (Sigma-Aldrich, St. Louis, CO, USA). The predominance of nucleated epithelial cells indicated the proestrus stage, the estrus stage was characterized by the predominance of cornified squamous epithelial cells, the metestrus stage was identified by the predominance of cornified squamous epithelial cells and leukocytes, and the diestrus stage was displayed by the predominance of leukocytes.

Tissue Collection and Histology Analysis

After the modelling process was ended, mice were anaesthetized, and blood samples were collected. Ovaries were dissected, fixed overnight in 4% paraformaldehyde at 4°C, and embedded in paraffin. The fixed ovaries were then cut into serial sections with microtomes in 6-μm thickness. Sections were stained with hematoxylin and eosin and observed by a light microscope under 100x magnification.

Hormone Serum Measurement

The serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were measured by the enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (ELK Biotechnology, CO, USA). The sensitivity limits of LH, FSH, and testosterone were 0.28 mIU/mL, 0.67 mIU/mL, and 17.7 pg/mL, respectively.

Statistical Analysis

Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) version 20 (Chicago, USA) and visualized using GraphPad Prism 9.0 (Boston, USA). Data are presented as mean±SEM. The differences among the examined group were

analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test.

RESULTS AND DISCUSSION

Estrous Cycle and Ovarian Morphology

The estrous cycle of the animal in the control group was regular, and the proestrous, estrous, metaestrous, and diestrous stages occurred at regular periods. As a typical feature of PCOS, the estrous cyclicity of animals in all the experimental groups was disturbed. An irregular estrous cycle was observed in the D-45 group, D-60 group, and D-90 group. Ovarian histology analysis of the control group showed several follicles at different developmental stages and several corpora lutea (CL) (Figure 1A). In the D-45 group, a sizeable antral follicle and a decrease in CL indicated abnormal ovulation (Figure 1B). Meanwhile, ovarian histology from the D-60 and D-90 groups showed a similar morphology with the control group in which follicles at different developmental stages were observed with a lack of large antral follicles (Figure 1C-D).
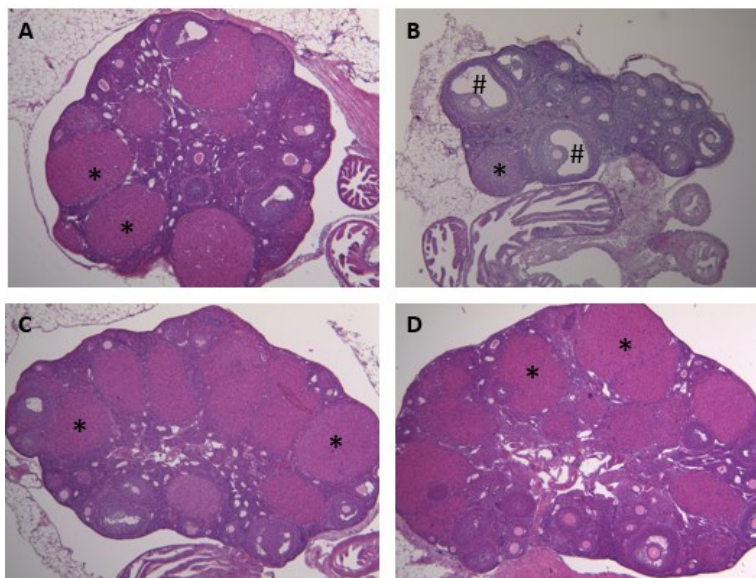

Figure 1. Representative ovarian histology of A) Control group, B) D-45 group, C) D-60 group, and D) D-90 group. (*) indicates CL; (#) indicates large antral follicle.

Hormone Serum Level

Regarding blood serum level, immunoassay analysis showed that serum FSH was significantly lower in the D-45 group compared to the control group (3.73 ± 0.12 vs. 5.28 ± 0.31 mIU/mL, $P<0.01$). Serum FSH levels in the D-60 and D-90 groups were comparable to the control group (5.37 ± 0.59 mIU/mL vs. 5.28 ± 0.31 mIU/mL; $P>0.05$ and 5.22 ± 0.40 mIU/mL vs. 5.28 ± 0.31 mIU/mL; $P>0.05$, respectively) (Figure 1B). The serum level of LH was significantly elevated in the D-45 group than in the control group (2.52 ± 0.43 vs. 1.30 ± 0.33 mIU/mL, $P<0.01$) (Figure 2B). The level of LH in the D-60 and the D-90 group was comparable to the control group (1.74 ± 0.17 mIU/mL vs. 1.30 ± 0.33 mIU/mL, $P>0.05$ and 1.70 ± 0.26 mIU/mL vs. 1.30 ± 0.33 mIU/mL, $P>0.05$, respectively) (Figure 2B). The D-45 group also had a raised testosterone level than the control group (1.80 ± 0.32 vs. 1.24 ± 0.23 ng/mL, $P<0.01$), but D-60 and D-90 had a comparable testosterone level to the control group (1.67 ± 0.19 ng/mL vs. 1.24 ± 0.23 ng/mL, $P>0.05$) and D-90 group (1.37 ± 0.26 ng/mL vs. 1.24 ± 0.23 ng/mL, $P>0.05$) (Figure 2C).

Various animal models have been developed and explored to study PCOS in humans. The primary method used to establish mice models for PCOS was subcutaneous injection of DHEA pre- or postnatally. However, very little is known about the effect of different administration routes on the PCOS phenotype of the mice model. Our study demonstrated that oral DHEA administration effectively established PCOS phenotype in mice.

Our study showed that mice in all experimental groups (D-45, D-60, and D-90) had disturbed estrous cycles and altered serum hormone levels compared to the control. Moreover, mice in the D-45 group showed the formation of large antral follicles and a decreased number of corpus luteum.
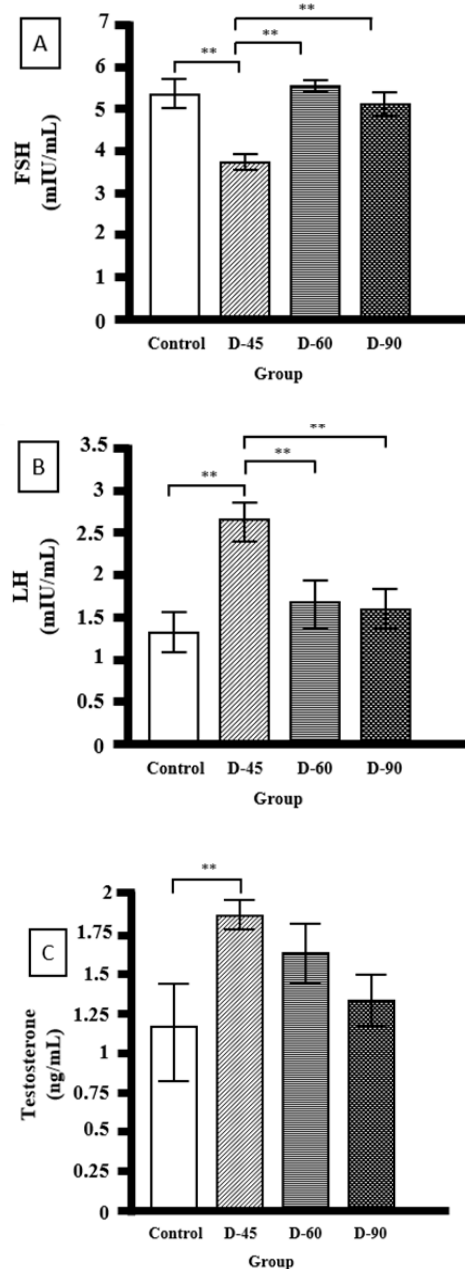

Figure 2. Serum level of A) FSH, B) LH, and C) testosterone. Data presented as mean ± standard deviation (SD) analyzed by one-way ANOVA followed by Bonferroni post hoc test. (**) indicates P<0.01.

This finding was consistent with a previous report by which Wang et al. (2022) reported that subcutaneous injection of DHEA 6 mg/100 g body weight was able to induce pathophysiological defects of PCOS in mice which showed by irregular estrous cycle, elevated testosterone level, formation of the antral follicle, and decreasing corpus luteum number. Wu et al. (2023) similarly found that a female mice model treated with 6 mg/100 g body weight DHEA injection for 20 consecutive days exhibited a disturbed estrous cycle, remarkable ovarian cysts, decreased corpus luteum number, and high testosterone level.

DHEA is a precursor of sex steroid hormones primarily produced in the cortex of the adrenal gland. Women with PCOS exhibited high levels of DHEA due to excessive adrenal precursor androgen production; thus, DHEA is often utilized to generate PCOS models (Osuka et al., 2018). Reports have shown that DHEA induction in rats stimulates inflammation and oxidative stress, resulting in large ovary cysts (Li et al. 2019). Zhang et al. (2024) further revealed that oxidative stress can promote excessive testosterone production by up-regulating 17α-hydroxylase enzyme synthesis. Moreover, DHEA is a pre-substance of testosterone by which DHEA is converted to testosterone in ovarian tissue. We assumed that DHEA induction in the experimental group triggers inflammation and oxidative stress and increases the bioavailability of circulating DHEA for testosterone conversion, which promotes sizeable antral follicle formation and testosterone level surge.

Compared to the D-60 and D-90 groups, our study found that the D-45 group is the ideal PCOS model, indicated by disturbed estrous cycles, significant fall of FSH serum level, and significant rise of LH and testosterone serum level compared to the control. Moreover, a visible large antral follicle was only observed in the D-45 and not in the other groups. This finding implied that 45 mg/Kg body weight is the optimal dosage to establish a PCOS-like phenotype for oral administration in mice. A recent study on humans showed that DHEA could restore ovarian function in CTX-induced POI rats by promoting ovarian vascular remodelling (Zhao et al., 2024). Tsui et al. (2015) further reported that DHEA supplementation could rescue atretic follicles, promote preantral follicle growth, and suppress apoptosis, improving ovarian reserve and oocyte yields in women with poor ovarian response. As such, we assumed that higher DHEA dosage benefits ovarian function, as depicted by improved

ovarian morphology in the D-60 and D-90 groups in the present study.

CONCLUSION

In conclusion, our study demonstrated that oral administration is efficacious in establishing a PCOS-like phenotype in mice with the suggested optimal dosage of 45 mg/Kg body weight. Further research with more dosage and duration variation is required to establish a typical protocol for generating PCOS mice models through oral administration.

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