Short Communication

Effect of Delta (δ)-Tocotrienol Supplementation on the Blood Follicle-Stimulating Hormone (FSH) and Luteinising Hormone (LH) Levels in Female Mice: A Preliminary Study

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Abstract

One of the factors causing female infertility is the deficiency in blood follicle-stimulating hormone (FSH) and luteinising hormone (LH) levels. Both hormones are important for the maturation and growth of follicular cells and ovulation. Vitamin E first discovered in 1992 is a potent lipid-soluble antioxidant and has been reported to exert beneficial effects on the reproductive system. However, its effects particularly on the FSH and LH levels have not been widely reported. Hence, this study was conducted to determine the effect of vitamin E, particularly delta (*δ*)-tocotrienol supplementation on the blood FSH and LH levels in female mice. Thirty (30) female mice were divided into five groups, with 4 treatment groups supplemented with 0.1ml corn oil (G2), 10 (G3), 20 (G4) and 30 (G5) mg/kg/day of *δ*-tocotrienol while one group without any treatments (G1 = control) for 7 days. On the 8th day, blood samples were collected and analysed using ELISA analysis. Based on the results obtained, mice treated with 10 mg/kg/day of δ-tocotrienol (G3) showed the highest blood FSH and LH levels compared to the other treatment groups. Thus, δ-tocotrienol is suggested to exert improved hormonal regulation in females, and further studies are needed to confirm the effects.

Keywords: Delta-tocotrienol, vitamin E, Follicle-Stimulating Hormone, Luteinising Hormone, infertility, antioxidant

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1.0 Introduction

Luteinising hormone (LH) produced by the anterior pituitary plays a major role in regulating the function of the gonads. LH stimulates the production and secretion of testosterone from the testes via Leydig cells in males, whereas in females, it stimulates the production of oestrogens and progesterone from the ovary. During ovulation, the concentration of LH is high (1,2). Follicle-stimulating hormone (FSH) is also produced by the anterior pituitary gland and it acts on the Sertoli cells in males while, in females, it acts on the ovarian granulosa cells (3). FSH functions to enhance the maturation of the germ cells in both testes and ovaries and stimulates follicular development and oestradiol synthesis in females (4).

Vitamin E was first discovered in 1922 at the University of California, United States of America, in the laboratory of Herbert M. Evan (5). This lipid-soluble antioxidant consists of eight subtypes, which are α-, β-, γ-, δ- tocopherol (TOC) and α-, β-, γ-, δ-tocotrienol (TCT) (6). The difference between TOC and TCT is in their aliphatic chain, where TOC has a phytyl side chain and is saturated while TCT contains farnesyl with an unsaturated side chain (7). Both compounds have the same basic chemical structure, consisting of a long isoprenoid side chain attached at position 2 of 6-chromanol ring (6–8).

Vitamin E plays a vital role against lipid peroxidation by protecting the cell membrane from being attacked by free radicals. Free radicals are unstable atoms that may bind to other atoms to gain electrons and complete its outer shell. This process will lead to oxidative stress and cause damage to cells in the body. Oxidative stress may also disrupt the functions of the female reproductive system by causing structural changes in both the ovaries (9) and embryos (10).

Despite the widely reported protective effects of vitamin E, including on the reproductive system (11), reports on the effect of vitamin E on reproductive hormones mainly FSH and LH, are still lacking. Hence, this study was conducted to determine if vitamin E, particularly the δ-TCT subtype, can improve blood FSH and LH levels.

2.0 Materials and Methods

2.1 Animal treatments

An approval by the university's Committee on Animal Research and Ethics (CARE) was obtained prior to beginning this study (Approval No. 197/2017). Thirty (30) female mice were divided into five groups (G1 – G5) with six mice each. G1 (control) received no treatment, G2 was given 0.1 ml of tocopherol-stripped corn oil, G3 was treated with 10 mg/kg/day of *δ*-TCT, G4 was given 20 mg/kg/day of *δ*-TCT and G5 received 30 mg/kg/day of *δ*-TCT. The treatments were given once daily for 7 consecutive days through oral gavage.

2.2 Blood sample collection and assay procedure

Following the completion of the treatments, an amount of 0.2 – 0.5ml of blood samples were collected for FSH and LH level analysis using the ELISA kit (Elabscience® ELISA Kit). The samples were processed following the manufacturer's instructions. Briefly, an amount of 100 µL of the standard solution and samples were added into the wells on the 96-well plate and incubated for 90 minutes at 37°C. After 90 minutes, the solution was removed and 100µL of biotinylated detection Ab working solution was immediately added to each well before being incubated again for 1 hour at 37°C. Following the incubation, the solution from each well was removed and 350µL of wash buffer was added. After the washing step, 100µL of HRP conjugate working solution was added to each well and incubated for 30 minutes at 37°C. After the incubation, the solution from each well was removed and

the washing step was repeated. Next, 90µL of substrate reagent was added to each well, incubated again for about 15 minutes at 37°C and finally added with 50µL of stop solution. The results were measured using a microplate reader set to 450nm, with the standard curves generated from mouse FSH and LH standards ranging from 0 to 100 and from 0 to 30 ng/mL, respectively.

2.3 Data Analysis

The obtained data were calculated and analysed using ANOVA (SPSS24) followed by post-hoc Tukey's test.

3.0 Results

3.1 Blood FSH level in female mice of each treatment group

Results from the ELISA assay showed that G2 (corn oil) had the highest amount of FSH (56.64 ng/ml), followed by G3 (10 mg/kg/day of δ-TCT). The concentration values for blood FSH level in G1 (no treatment), G4 (20 mg/kg/day of δ-TCT) and G5 (30 mg/kg/day of δ-TCT) were 15.22, 16.68 and 15.94 ng/ml respectively. The differences in the results are shown in Figure 1.

3.2 Blood LH level in female mice of each treatment group

The obtained results showed that G3 (10 mg/kg/day of δ-TCT) had the highest value of LH which was 29.61ng/ml, followed by G2 (corn oil) with 27.81ng/ml, G5 (30 mg/kg/day of δ-TCT) with 26.52ng/ml, G1 (no treatment) with 24.23ng/ml and G4 (20 mg/kg/day of δ-TCT) with 24.73 ng/ml. The differences in the results are shown in Figure 2.

4.0 Discussion

Findings from the present study showed that vitamin E, specifically *δ*-TCT supplementation, could improve hormonal regulation by increasing blood FSH and LH levels in female. Mice with the treatment of 10 mg/kg/day of δ-TCT (G3) shows higher concentration (non-significant) of FSH after 7 days of treatment compared to mice given 20 and 30 mg/kg/day of δ-TCT (G4 & G5). The results of both treatments with 20 and 30 mg/kg/day of δ-TCT were also non-significantly higher compared to mice in G1 (no treatments). Meanwhile, in the analysis of blood LH level, the 10 mg/kg/day supplementation of δ-TCT also had the highest concentration among the five groups of treatment, with 20 mg/kg/day of δ-TCT having the lowest concentration of blood LH level. In comparison to G1, the 20 and 30 mg/kg/day of δ-TCT supplementation showed a non-significant increase in blood LH level in female mice.

Vitamin E has been long reported to possess oestrogenic, androgenic and progesterone-like properties and it acts synergistically with ovarian hormones and testosterone (12). This was also observed in this study, where vitamin E, specifically δ-TCT supplementation, may have an effect on improving the hormonal regulation by increasing blood FSH and LH levels in female mice. Previous studies on the effects of δ-TCT on the regulation of FSH and LH in mice are not available; hence, the similar comparisons to the present results could not be discussed. However, there are earlier studies reported on the role of vitamin E in the hypothalamic control of luteinizing hormone-releasing hormone (LHRH) release in rats (13). Results from another *in vitro* study also indicated the possibility of vitamin E stimulating the expression of gonadotropin hormones in the pituitary of turbot (14). A recent systematic review by Tefagh et al. (2022) (15) also reported that supplementary regimens containing vitamin E can positively affect the hormonal parameters in polycystic ovary syndrome (PCOS) patients (15). In comparison with the previous studies, the findings of the present study are in line with and supported by those reports.

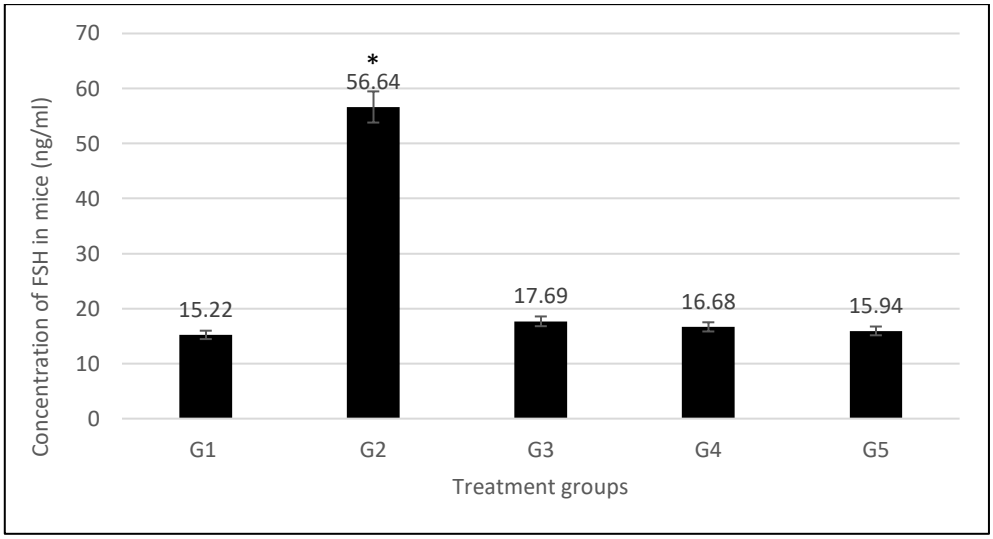

Figure 1 Blood FSH level in female mice of each treatment group (*p = 0.03: indicates significant against G1)

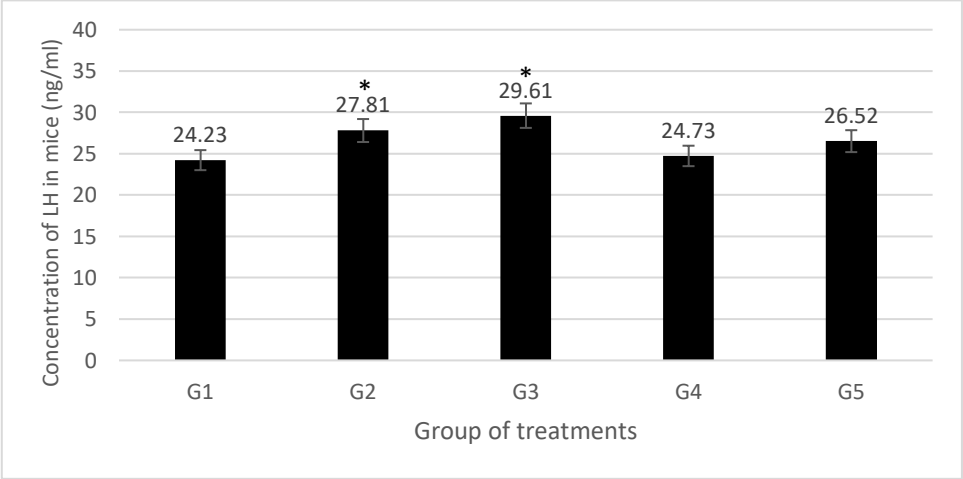

Figure 2 Blood LH level in female mice for each treatment group (*p<0.05: indicates significant against G1)

5.0 Conclusion

In the experimental mice, varying vitamin E doses led to variations in blood FSH and LH levels. From this study, the results suggest that δ-TCT could increase blood FSH and LH levels. The optimal dose of δ-TCT supplementation that increased blood FSH and LH levels was 10 mg/kg/day. The results were obtained following a short period of treatment duration, and hence further studies are needed to study more about these effects.

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Conflict of Interest

The authors declare that there are is no conflict of interest.

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