DOES SUPPLEMENTATION WITH ALPHA TOCOPHEROL (A-TOC) AFFECTS BLOOD FOLLICLE-STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) LEVELS IN NORMAL FEMALES? A PRELIMINARY STUDY ON MOUSE MODEL

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ABSTRACT: *Alpha-tocopherol (α-TOC) is a subtype of tocopherol, which is one of the components of vitamin E. It was commonly referred to as 'vitamin E' during the initial years of its discovery, due to its ability to act as an antioxidant and anticancer in human. Following its discovery as the vitamin for reproduction in 1922, α-TOC has been continuously reported to exert good effects on reproductive health. These were mainly based on the studies conducted on the use of vitamin E on diseased animal or cell models, however, our focus here was on what will be the results if vitamin E is given to the normal or healthy animals. Thus, this study aimed to determine the effects of supplementation with α-TOC (this subtype was chosen as it is known as the most effective among the subtypes) on the blood follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in normal female mice. Twenty-four female mice divided into four treatment groups (G1-G4) with 6 mice each. Treatment with 10 mg/kg/day, 20 mg/kg/day and 30 mg/kg/day of α-TOC were given for 7 days. On Day 8, blood samples were collected and analyzed using the ELISA method. Present preliminary results showed that the differences in the blood FSH and LH levels following the given doses were significant ($p<0.05$) compared to control, suggesting that short-term supplementation with α-TOC affected the blood hormonal levels in normal females. Further studies need to be done with different dosages and treatment durations to determine the changes under different experimental settings.*

Keywords: Alpha-tocopherol (α-TOC), vitamin E, follicle-stimulating hormone (FSH), luteinizing hormone (LH), reproductive health

1. INTRODUCTION

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are among the important female reproductive hormones, besides estradiol, progesterone, prolactin, and human chorionic gonadotropin (hCG). FSH functions to stimulate the follicular development, estradiol synthesis, and germ cell maturation in the ovaries, whereas LH functions to stimulate the ovary to secrete estrogen and progesterone [1]. The combination of both hormones in stimulating steroidogenesis and the growth of ovarian follicles is summarized in figure (1) [1].

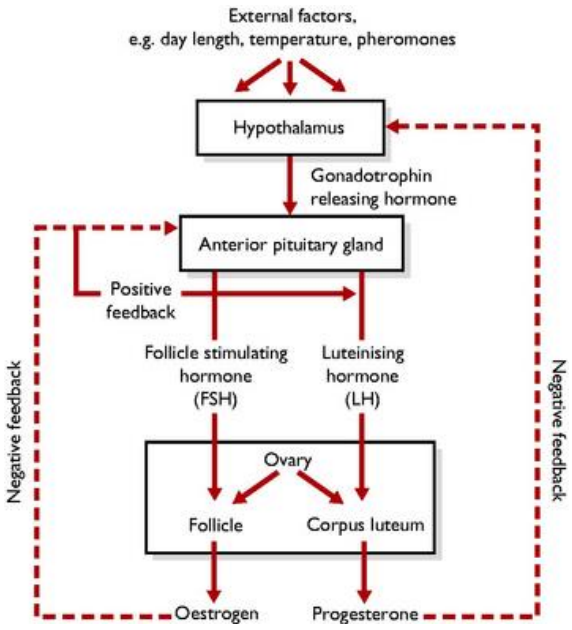

Fig (1) Hormonal effects on the female reproductive system [1].

Vitamin E is an essential lipid-soluble vitamin that acts mainly as an antioxidant in the human body [2-3]. It was first discovered in green leafy vegetables by the researchers from the University of California, USA, Herbert Evans and Katherine Bishop in 1922 [4]. Evans and Bishop also described vitamin E as a "substance X" which is important for fertility and reproduction in rats.

Vitamin E must be consumed from the diet as it is not produced in the human body. There are two substances present in vitamin E which are tocopherols (TOCs) and tocotrienols (TCTs). Both are present in eight different subtypes, namely α-TOC, β-TOC, γ-TOC, δ-TOC, α-TCT, β-TCT, γ-TCT, and δ-TCT [5]. From these subtypes, α-TOC has been widely reported to be used to treat diseases such as cataracts [reviewed in (4)] and cancers [6–8].

Vitamin E also has been reported to have beneficial effects against reproductive disorders due to its antioxidant properties, for instance, it was reported to improve the thin endometrium by increasing the endometrial thickness and improve the endometrial response in women with unexplained infertility [9]. Other effects of vitamin E on the female reproductive system from studies conducted using *in vitro / in vivo* models were also reviewed in [10].

In this review [10], it was reported that palm tocotrienol-rich fraction (TRF) improved the pregnancy outcomes and embryonic development in nicotine treated mice as well as in the actin intensity of the early stage embryos. Besides, the percentages of oocytes' DNA damages and numbers of fragmented oocytes were also reduced. Palm γ-TCT was also reported to ameliorate the damages induced by nicotine on the oocytes' ultrastructures [reviewed in (10)].

Studies on vitamin E and reproductive disorders have been actively reported since the last decade [reviewed in (10-11)],

however, most of them were conducted on the effects on diseased animal or cell models. Thus, in the present study, the focus was to determine the effects of α-TOC supplementation in normal females (using mouse model), particularly on the blood follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels.

2. EXPERIMENTAL DETAILS

Ethics Approval

Ethics approval was obtained prior to commencing this project. All procedures were carried out in accordance with the approved guidelines.

Animal treatments

Twenty-four female mice (*Mus musculus*) (4-6 weeks old, 20-30g) were obtained from Laboratory Animal Facility and Management (LAFAM) UiTM Cawangan Selangor, KampusPuncakAlam, Malaysia. All animals were acclimatized for a week at a 12-h light/dark cycle. Animals were fed with vitamin E-free pellets (Gold Coin Holdings, Kuala Lumpur, Malaysia), and water was given ad libitum. A sample of α-TOC was provided by American River Nutrition Inc. (ARN), Hadley, MA, United States of America (USA).

The animals were randomly divided into 4 groups (G1-G4) with 6 mice each (n=6). G1 served as a control group and was not given any treatment. G2, G3, and G4 were treated with 10, 20, and 30 mg/kg/day of α-TOC respectively through oral administration (oral gavage). Treatments were given for 7 days. On Day 8, the blood samples were collected and processed for ELISA analysis(Elabscience® ELISA Kit).

ELISA analysis

i. Sample collection

Serum collection: Blood samples were allowed to clot for 2 hours at room temperature before centrifugation for 15 minutes at 1000×g at 2~8^{o}C. Then, the supernatant (serum) was collected and kept at -20 ^{o}C.

ii. Reagent preparation

1. All reagents were brought to room temperature (18~25 ^{o}C) before use. The microplate reader was set-up and pre-heated for 15 min before use.
2. 30 mL of Concentrated Wash Buffer was diluted with 720 mL distilled water to prepare 750 mL Wash Buffer.
3. Standard Working Solution: The standard was centrifuge at 10,000×g for 1 min. 1 mL of Reference Standard & Sample Diluent was added and left to stand for 10 min and inverted gently several times. After it has fully dissolved, it was mixed thoroughly with a pipette. This reconstitution produced 100 ng/mL (FSH) or 30 ng/mL (LH) of working solution. The serial dilutions then conducted following the recommended dilution gradient for FSH (100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0 ng/mL) and LH (30, 15, 7.5, 3.75, 1.88, 0.94, 0.47 and 0 ng/mL).
4. Biotinylated Detection Ab working solution: The stock tube was centrifuged prior to use. The 100× Concentrated Biotinylated Detection Ab was diluted to 1× working solution with Concentrated HRP Conjugate Diluent.
5. Concentrated HRP Conjugate working solution: The 100× Concentrated HRP Conjugate was diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

Assay procedure

100 µL of standard working solution and 100 µL of samples were added according to the plate design as suggested by the manufacturer. Then, the plate was incubated for 90 minutes at 37^{o}C. After 90 minutes, the liquid from each well was removed and 100 µL of biotinylated detection Ab working solution was added immediately. Again, the plate was sealed and incubated for 1 hour at 37^{o}C. After 1 hour, the solution in each well was aspirated and the washing process was done 3 times repeatedly using 350 µL of wash buffer.

Following that, 100 µL of HRP conjugate working solution was added to each well. The plate was sealed and incubated for 30 minutes at 37^{o}C. After 30 minutes, the plate was removed from the incubator, washed five times repeatedly, and added with 90 µL of substrate reagent. The plate was again incubated for 15 minutes at 37^{o}C. After 15 minutes, 50 µL of stop solution was added into each well. The plate was ready for determination of the optical density (OD) value using a microplate reader set to 450 nm. Lastly, the result was calculated, and data analysed using the student's t-test (SPSS24.0).

3. RESULTS

Blood FSH Level

The present finding showed a significant increase (p=0.03) in FSH levels in G2 (10 mg/kg/day) compared to the control group. Treatment with higher dosages resulted in a non-significant increase in G3 (20 mg/kg/day) and a non-significant decrease in G4 (30 mg/kg/day). The differences in the FSH levels are as shown in figure (2).

Blood LH Level

In contrast to the results of blood FSH levels, present results showed significant increases in LH levels in G3 (20 mg/kg/day) and G4 (30 mg/kg/day), and a non-significant increase in G2 (10 mg/kg/day). Figure (3) shows the differences in the LH levels between the groups.

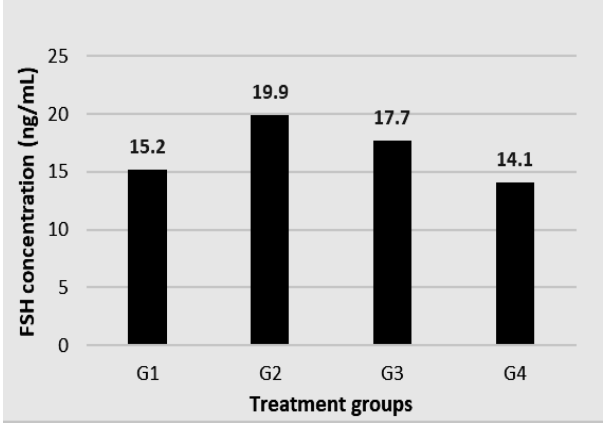

Fig (2) Blood FSH levels following supplementation with α-TOC in normal female mice

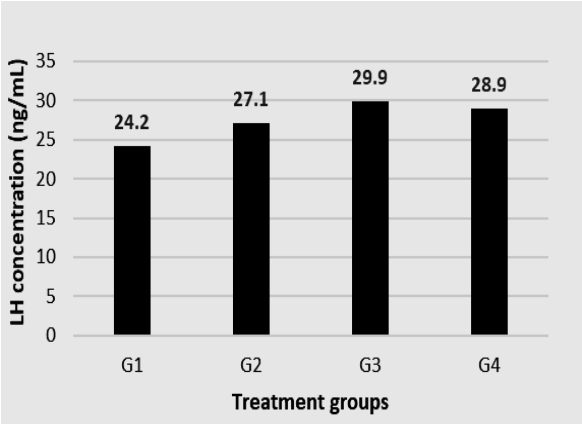

Fig (3) Blood LH levels following supplementation with α-TOC in normal female mice

4. DISCUSSION

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are among the main essential reproductive hormones produced by the human body [1]. Studies on the effects of vitamin E (or its subtypes) on reproductive health have been reported previously, however reports on the blood hormone levels are still lacking. An earlier study on the effects of vitamin E deficiency on the pituitary-gonadal function in rats revealed that the vitamin E deficiency gives a suppressive effect directly on the gonadal function to decrease the hormone synthesis [12], and in rats supplemented with vitamin E, the FSH and LH content in pituitary tissue was significantly higher than the controls, although there was no significant rise in basal FSH and LH level in plasma [13].

Another study on the effects of administration of vitamin E on fertilization capacity in male rats exposed to noise stress reported that the levels of FSH, LH, and testosterone were significantly decreased, and administration of vitamin E significantly increased the level of hormones [14]. In addition, Kenani *et al*. [15] and Rajabzadeh *et al*. [16] also reported on the effects of honey and vitamin E on the levels of sex hormones and male fertilization capacity of noise-exposed rats. Both studies reported that honey and vitamin E improved the regulations of the hormones and enhanced fertility rate by increasing the rate of healthy alive fetuses.

A study on the effects of vitamin E on reproductive hormones and testis structure was reported in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The results showed that TCDD decreased the levels of brain gonadotropin-releasing hormone (GnRH), testis luteinizing hormone (LH), and follicle-stimulating hormone (FSH), serum testosterone and testis spermatozoa number, and damaged testis structure. Treatment with vitamin E at 20 mg/kg was reported to alleviate the decrease of GnRH. In addition, the study also reported that vitamin E at 20 mg/kg, 100 mg/kg, and 500 mg/kg antagonized the decline of LH and FSH, vitamin E at 20 mg/kg and 100 mg/kg reversed the decrease of testosterone and spermatozoa number and vitamin E at 100 mg/kg decreased the damage of the testis structure caused by TCDD [17].

Our previous study on the effect of α-TOC supplementation at 60 mg/kg/day on the histological structures of murine ovaries and uteruses in nicotine-treated females also showed that α-TOC increased the average number of ovarian follicles and the thickness of the endometrium layer [18], suggesting these resulted from the improvement in the hormonal regulations.

In relation to the present study, the available study reports on vitamin E and blood hormones as discussed above were mainly of the stressed or chemically exposed animals. As reported in those studies, vitamin E did improve the diseased conditions, however, the effects of vitamin E (particularly α-TOC) supplementation in normal animals was not much discussed. Hence, our present findings indicated an interesting result in which α-TOC supplementation in general increased the blood levels of FSH and LH in normal female mice (except a slight decrease in FSH level in G4 (Fig. 1)). This suggests that even a short-term (7 days) supplementation with α-TOC improves the blood hormonal levels in normal females.

5. CONCLUSIONS

Short-term (7 days) supplementation with α-TOC increased the blood FSH and LH hormonal levels in normal female mice. Therefore, it is possible to consider α-TOC as a recommended supplementation to help improve the hormonal regulation and the overall reproductive health in females. However, further studies need to be done with different dosages and treatment durations to determine the changes in different experimental settings.

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