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The Effect of FSH and Estradiol Benzoate on Hormonal Levels and Growth in Rats Treated with Tamoxifen Citrate

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ABSTRACT

Tamoxifen disturbs spermatogenesis and administration of FSH and estradiol benzoate (EB) may improve spermatogenesis. Spermatogenesis and hormonal levels have direct relation. Therefore, this study was conducted to investigate the effect of FSH and EB on hormonal levels and growth in male rats treated with Tamoxifen citrate (TC). Rats were assigned to 4 groups (n=5) including rats treated with TC 1) without hormone therapy, 2) daily dose of 7.5 IU FSH for 10 consecutive days, 3) daily dose of 12.5 µg EB per rat for 10 consecutive days and 4) daily dose of 7.5 IU FSH per rat and 12.5 µg EB per rat for 10 consecutive days. The blood samples were collected to measure of FSH, LH and testosterone. Results showed that oral gavage of TC and hormones had no significant effect on growth performance of rats. Oral gavage of TC significantly decreased the serum concentrations of testosterone, FSH and LH. Administration of FSH and FSH+EB also increased the serum concentrations of testosterone, FSH and LH. However, administration of EB did not improve hormonal levels. It can be suggested the use of FSH for treatment of infertility in men with spermatogenesis deficiency.

Keywords: Estradiol, FSH, Testosterone, Tamoxifen, Wistar rat.

INTRODUCTION

Tamoxifen (TAM), a triphenylethylene derivative, is known to have estrogen agonist or antagonistic responses and also bond to estrogen receptors [1]. TAM is broadly used for treatment of estrogen-dependent of patients with breast cancer at different stages

[2,3]. TAM is accepted as appropriate way for treatment of breast cancer; however, it has side effects such as sexual dysfunction [4]. On the other hand, some studies have been shown side effects of TAM on male reproductive system in human such as sexual dysfunction and sex hormone in male rat [5,6]. Study has been shown that administration of 0.25 mg/kg daily of TAM for 14 weeks could induce histological alterations in testes and epididymis and also decrease serum testosterone, FSH, LH concentration, sperm count and sperm motility [1]. Estradiol in men is known to have essential role in spermatogenesis. It has been shown estrogen role as influencing factor on spermatogenesis in men with nonobstructive azoospermia [7]. It is reported that estradiol not only exists in the reproductive system of the adult male, but it is also found brain as well [8]. It is stated significant concentrations of estradiol in the male reproductive tract and semen compared with serum [9]. Studies have shown estradiol production by Leydig and Sertoli cells; however, studies are not still reported estradiol synthesis by germ cells within the seminiferous tubules [10]. However, antifertility effects of estradiol in adult male rats have been also reported [6]. FSH is necessary hormone for the pubertal development of full complement of Sertoli cells, the lack of FSH during pubertal development influences spermatogenesis; resulting in reduced sperm output in the adult. It is reported that FSH hormone acts through specific transmembrane receptors [11]. FSH has been reported as essential factor for maintaining fertility in men [12]. Studies have shown that transgenic hypogonadal mice which express FSH can maintain spermatogenesis by the completion of meiosis [13]. Studies have reported that lack of FSH prevents spermatogonial proliferation and their transition to spermatocytes [14]. Spermatogenesis destroy can disturb hormonal levels. It was hypothesized that TAM destroys spermatogenesis and administration of FSH and estradiol improves spermatogenesis. Therefore, this study was conducted to investigate the effect of FSH and estradiol benzoate (EB) on hormonal levels and growth in rats treated with Tamoxifen citrate (TC).

MATERIALS AND METHODS

Animals

This research was conducted in Animal Reproduction Laboratory of Tabriz University of Medical Science, Iran. All the used procedures approved by ethical principles were in agreement with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and approved by the Tabriz University of Medical Science Animal care and Ethics Committee (No. 1509, 2016). Twenty adult male Wistar rats prepared from Pastor Institute were used. The animals were 6 weeks of age and had weight mean of 220 gr. Animals were kept at a temperature of 22-23°C, humidity 50% and lighting cycle of 12 h L/12 h D. Animals had *ad libitum* to commercial rat pellets and water. The rats were weighed at start and end of experiment for weight changes. Food consumption was measured by measuring the amount of the daily consumed and refused feed. Feed conversion ratio (feed intake/body weight gain) was also calculated for each group. TC (Iran Hormone, Tehran, Iran), FSH (Gonaser®, Laboratorios Girona, Spain) and EB (each ml of Vetastrol contains 2 mg estradiol benzoate, CinnaGen Biopharma Co, Tehran, Iran) were purchased. This experiment was lasted for 40 days.

Experimental treatments

All rats received oral gavage of 600 µg/kg of TC for 30 consecutive days and received different levels of FSH and EB for 10 days. Thus, rats were allocated to 4 groups (n=5), as follows; 1) rats treated with TC and without hormone, 2) rats treated with TC and daily dose of 7.5 IU FSH for 10 consecutive days, 3) rats treated with TC and daily dose of 12.5 µg EB per rat for 10 consecutive days and 4) rats treated with TC and daily dose of 7.5 IU FSH per rat and 12.5 µg EB per rat for 10 consecutive days.

Blood collection and handling

On d 40, all animals were weighed and anaesthetized by administration of xylazine (0.64 mg) and ketamine (20 µg). The blood samples were collected from heart and placed into non-heparinized tubes to reaching serum for hormone analysis. The samples were kept at room temperature for 1 h and then centrifuged at 3000 rpm for 20 minutes. The sera samples were stored at -20 C until analysis. Commercial kits (Mono bind Inc. Lake Forest, CA 92630, USA) were used for measurement of FSH, LH and testosterone.

Statistical analysis

The data are shown as mean ± standard deviation (mean ± SD). Significance of the differences was examined by analysis of variance (ANOVA) test. The levels of significance were done at ($P<0.05$). Figures were illustrated by Graph pad prism.

RESULTS

Performance analysis

Effects of TC and administration of EB, FSH and EB+FSH did not change ($P>0.05$) body weight change (Figure 1), feed intake (Figure 2) and feed conversion ratio (Figure 3).

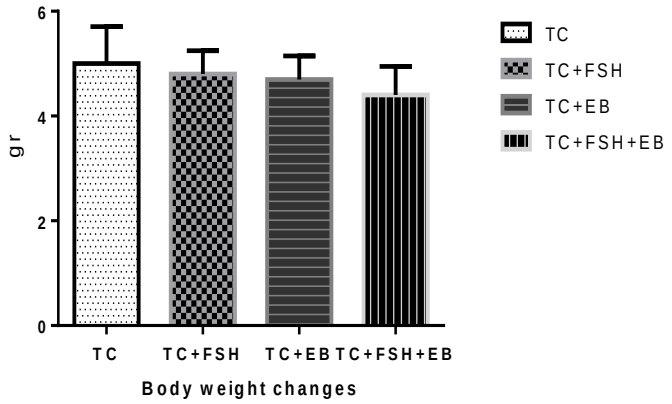

Figure 1: Effects of experimental treatments on body weight changes (g)

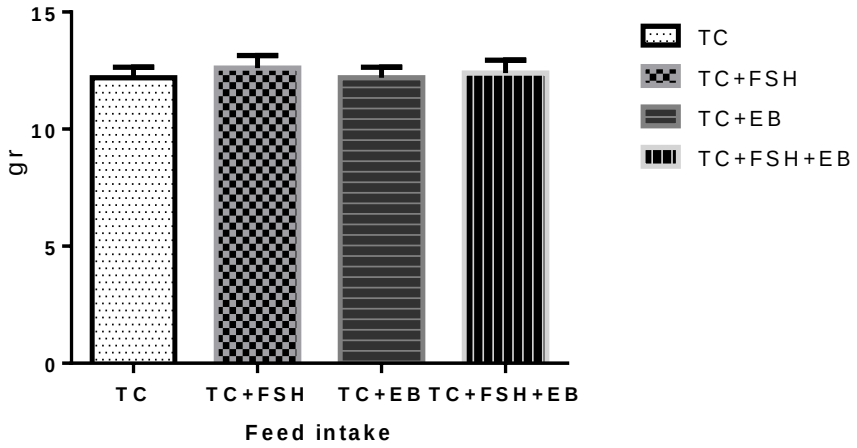

Figure 2: Effects of experimental treatments on feed intake (g)

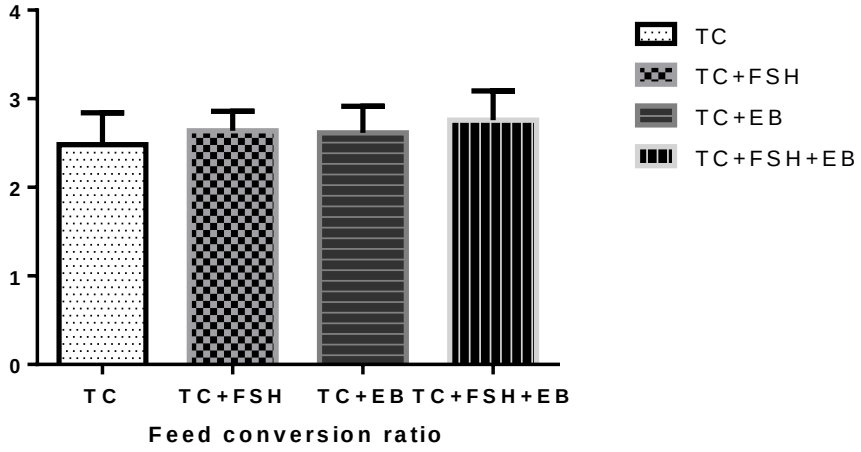

Figure 3: Effects of experimental treatments on feed conversion ratio

Hormonal levels

Oral gavage of TC significantly (P<0.05) decreased the serum concentrations of testosterone (Figure 4), FSH (Figure 5) and LH (Figure 6). Administration of FSH and EB+FSH significantly (P<0.05) increased the serum concentrations of testosterone, FSH and LH. Administration of EB alone did not change compared to TC group (P>0.05).

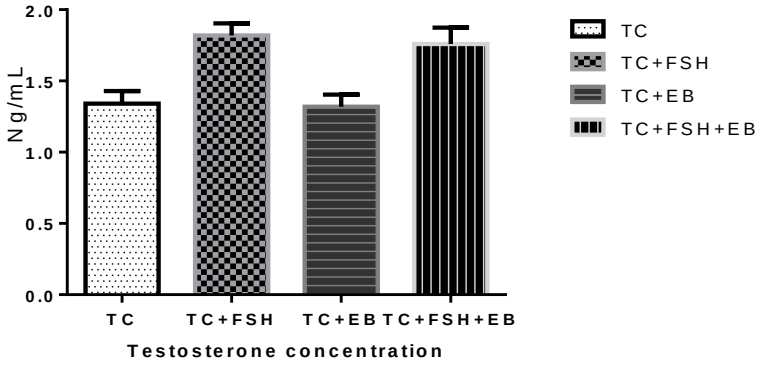

Figure 4: Effects of experimental treatments on testosterone concentration (ng/mL)

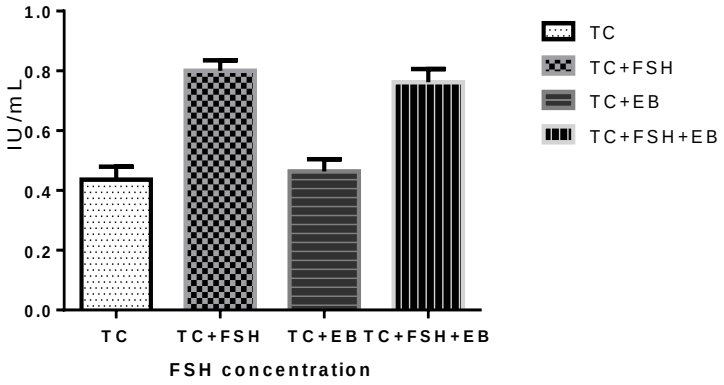

Figure 5: Effects of experimental treatments on FSH concentration (IU/mL)

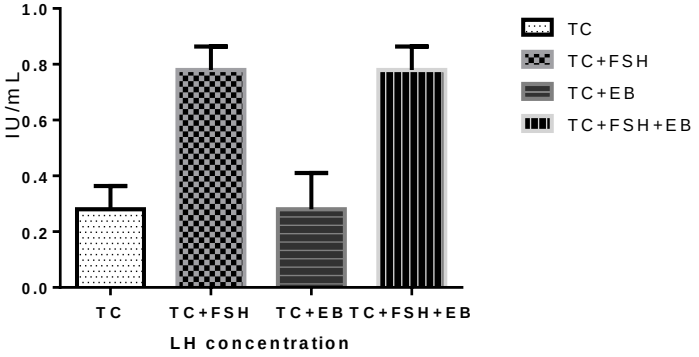

Figure 6: Effects of experimental treatments on LH concentration (IU/mL)

DISCUSSION

As results indicated, oral gavage of TC and hormone therapy had no significant effect on the growth performance. There is not any study showing the effects of TC, EB and FSH on body weight of rats. Body weight is severely influenced by food consumption and on the other hand, feed conversion ratio is function of weight gain and feed intake. In the research, feed intake was not influenced by TC and hormone therapy; resulting in body weight and feed conversion ratio were not also affected. It can be stated that TC and decreased hormonal levels have not relations together. Oral gavage of TC lowered the serum concentrations of testosterone, LH and FSH. Studies have reported that rats treated with TC show lower testosterone, LH and FSH [1]. Other studies have shown that TC administration significantly decreased the levels of LH and testosterone in mice and male rats

[15,16]. Studies have related the decreased testosterone levels of testosterone with defaulted responsiveness of Leydig cells to LH in rats treated, since LH acts particularly on Leydig cells in the testis and is the initial regulator of testosterone secretion [1,17]. However, other studies have been suggested that TC has direct effect on Leydig cells and prevents testosterone synthesis [18]. *In vitro* studies have shown that TC prevents testosterone production, under incubation of Leydig [19]. It is reported that TC plays role as estrogen agonist at the hypothalamus pituitary axis by preventing the secretion of LH through the pituitary, since estrogens acts as negative feedback on the synthesis and secretion of gonadotropins by receptors present in the pituitary and hypothalamus [20,21].

Administration of EB had no effect on hormonal levels of rats compared with TC group. However, administration of FSH and EB increased hormonal levels which can be attributed to administration of FSH and synergism interaction effect between estradiol and FSH. It has been reported that combined of FSH and estradiol is essential to the mRNA transcription of N cadherin that are protein responsible for cell-to-cell adhesion [22,23]. Other studies have also shown that testosterone resulting from Leydig cells and FSH from the anterior pituitary are essential to for Sertoli cells to transduce signals and produce essential factors for nurture germ cells [24,25]. It is shown that estrogen receptors convert testosterone to estrogen and are enough found in brain, penis, and testis, organs important for sexual function [7]. Some studies have used testosterone therapy not only for optimization of physiologic levels of testosterone and also optimization its metabolites such as estradiol, which shows relation estradiol and testosterone [26]. Estradiol is found not only in the reproductive tract of the adult male, but also in the brain [8]. It was expect that estradiol improves hormone levels. However, it had not significant effect on hormonal levels. The most studies have reported reducing effect of estradiol on hormone levels. It is reported that estrogen prevents the hypothalamus-pituitary axis and then FSH and LH that subsequently reduces circulating testosterone [27]. Study has been shown that administration of estradiol reduces the serum concentrations of FSH, LH and testosterone [6]. It is reported that administration of estradiol prevents LH effect on Leydig cells and high estrogen exposure decreases serum testosterone levels by this action [28]. However, all the mentioned studies implicate on decreasing effect of estradiol on hormones, but such results did not achieve in the current study. It seems that TC decreases hormonal levels and hormonal levels cannot be more decreased. This study also indicated that FSH alone and in combination with EB increased hormonal levels. Studies have also indicated that GnRH-deficient hypogonadal (hpg) mouse, hypophysectomized rat or GnRH neutralized rat treated with FSH have increased germ cell numbers by several fold by increasing the complement of spermatogonia and spermatocytes [29-34]. Administration of gonadotropins increases signals which enhance hormone testosterone produced from Leydig cells in response to LH signaling [11]. Being increase spermatogenesis by completion of meiosis has been reported in transgenic hpg mice that express FSH [13].

CONCLUSION

It can be concluded that oral gavage of TC and hormones had no significant effect on growth performance of rats. TC significantly decreased the serum concentrations of testosterone, FSH and LH. Administration of FSH and FSH+EB increased the serum concentrations of testosterone, FSH and LH. However, administration of EB had did not improve hormonal levels. It was not found any relation between growth and hormonal levels. It can be suggested the use of FSH for treatment of infertility in patients with spermatogenesis deficiency.

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