Journal of Advanced Veterinary and Animal Research
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2016.c180
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

December 2016
Vol 3 No 4, Pages 420-424.

Short Communication

Effects of estradiol benzoate injection to intact and castrated male rabbits on LH, FSH, testeosterone and prostate tissues

Tamour Elkhier, Shams-Eldein Hassaballa, Shadia Abdo-Elatti Omer and Abdelnasir Adam

• **Received:** Oct 29, 2016 • **Revised:** Dec 7, 2016 • **Accepted:** Dec 8, 2016 • **Published Online:** Dec 13, 2016

AFFILIATIONS

- Tamour Elkhier

Department of Physiology and Biochemistry, Faculty of Veterinary Science, University of Nyala. Nyala-Sudan.

- Shash-Eldine Hasaballaha
- Shadia Abdo-Elatti Omer

Department of Basic Science (DBS), Collage of Veterinary Medicine and Animal Production- Sudan University of Science and Technology, Khartoum North, Kuku-Sudan.

- Abdelnasir Adam

Department of Pathology, Faculty of Veterinary Science, University of Nyala. Nyala-Sudan.

CORRESPONDENCE

Tamour Elkhier
Department of Physiology and Biochemistry, Faculty of Veterinary Science, University of Nyala. Nyala- Sudan.
E-mail: elkhiertmur@gmail.com

ABSTRACT

Objective: This study was conducted to investigate the effects of estradiol benzoate injection to intact and castrated male rabbits on LH, FSH, testosterone and prostate tissue.

Materials and methods: A total of 72 mature male rabbits were used in this study. The rabbits were randomly divided into two groups (36 intact and 36 castrated) and each group was further divided into four sub-groups. The rabbits of each subgroup were injected estradiol benzoate dosed at 0, 40, 80 and 120 µgm/rabbit, through intramuscular (IM) route, on each alternative day over a period of 30 days. The levels of LH, FSH, and testosterone were measured in serum samples. Prostate tissue samples were taken from each sub-groups, histological examination was done.

Results: The mean serum levels of LH and FSH were not affected by injection of estradiol benzoate in all intact and castrated rabbits sub-groups ($P>0.05$). However, the results of testosterone levels were showed insignificant increase in all intact and castrated male rabbits sub-groups, except the intact male rabbit sub-group that received estradiol benzoate at 120 µg/rabbit ($P<0.05$). Furthermore, the effects of estradiol benzoate in prostate tissues were ranged from hyperplasia with dysplasia or dysplasia only in intact male rabbits; hyperplasia was represented by papillary projection in castrated male rabbits.

Conclusion: The present study revealed no difference in the serum levels of LH, FSH in intact or castrated male rabbits, however, testosterone hormone did not show any change except in sub-group of intact male rabbits that was injected dosed at 120 µg estradiol benzoate. The effect of estradiol benzoate on prostate tissue was found to be induce hyperplasia in both intact and castrated males rabbits.

KEYWORDS

Castrated, LH, FSH, Male rabbit, Prostate tissues, Testosterone

How to cite: Elkhier T, Hassaballa SE, Omer SAE, Adam A (2016). Effects of estradiol benzoate injection to intact and castrated male rabbits on LH, FSH, testeosterone and prostate tissues. Journal of Advanced Veterinary and Animal Research, 3(4): 420-424.

INTRODUCTION

Estrogens are steroid hormones include estrone, estradiol, and estriol that regulating reproductive development and function of female. Estrogen was historically believed to be a female hormone, but there is growing evidence of a biological role of this steroid in the male reproduction (Tohidi, 2010). The source of estrogen in males may be germ cells (Carreau et al., 2003), or liver, adrenal glands, adipose tissue, and testes (Tohidi, 2010). There is a differences in action between the natural and synthetic steroids due to difference in affinity for binding to globulin , biological activity in plasma, metabolism, and half-life (Anna-Maria and Niels, 1999).

Estrogen play a physiological role in regulation the reproductive functions of mammalian including the regulation of gonadotropin feedback (Carreau et al., 2003) and steroid genesis (O'Donnell et al., 2001). Endogenous estradiol-17β exerts negative feedback effects on the secretion of gonadotropins FSH and LH, affecting the hypothalamo-pituitary system (Bellido et al., 1990; Sharpe and Skakkebaek, 1993; Sharpe et al., 1998).

The hormones are play an important roles in prostate function, disease and as well as in the treatment of specific disorders with hormone therapy. Prostate growth, differentiation and function are primarily controlled by androgens but estrogens modulate these effects in several ways. the direct effect may be due to elicited of external hormones or by estradiol produced by local aromatization of testosterone (Harkonen and Makela, 2004), and the indirect could be due to interference with androgen production by repression of the hypothalamic-pituitarygonadal axis (Wellerson et al., 2008). There is however, strong experimental evidence shows that excessive or untimely exposure to estrogens can facilitate development of prostatic changes, disorders and even malignancies (Ho, 2004) as in adult rodents was found to be cause squamous metaplasia of the anterior prostate lobe (Risbridger et al., 2001) hyperplasia and severe dysplasia in ERKO mice by (Prins et al., 2001) aberrations in prostate epithelial growth in dogs, monkeys, and humans (Coffey and Walsh, 1990; Prins and Korach, 2008).

The objective of this study was to investigate the effects of estradiol benzoate injection via intramuscular route at a different doses to intact and castrated male rabbits for 30 days on serum concentration of LH, FSH, testosterone hormones and prostate tissue.

MATERİALS AND METHODS

Animals: Seventy two adult male rabbits weighing 2.1±0.1 kg at the beginning of the experiments housed under individual cage 50 x 50 cm were used in this study. They had free access to standard rabbit chow and tap water. The necessary ethical approval for the animal experimentation was taken from the Nyala University's Animal Welfare Committee.

Treatments: Two groups of rabbits (36 per group). One group were subjected to bilateral orchiectomy, the deferent ducts were sectioned and tied and the two testes were removed the other group of rabbits was used as intact group. Each group was further divided randomly to four sub-group (6 for each sub-group).Three sub-groups of intact and castrated animals were treated once each alternative day with the intramuscular injections of estradiol benzoate (Estradol® Animal Health Care Australia) at a doses of (40, 80 and 120 µgm/ rabbit), respectively for thirty days, whereas the fourth group of each receive no estradiol and act as control group..

Blood collection: After 30 days of treatment, a five ml of blood samples were collected into plan plastic containers using disposable syringe from the heart, and the serum samples were being harvested into Eppendorf tubes, deep-frozen for later analysis of LH, FSH and testosterone hormones.

Hormones assay: Serum LH, FSH, and Testosterone levels were performed by immunoenzymometric assay (mouse anti-LH monoclonal antibodies, mouse anti-FSH monoclonal antibodies and mouse anti-Testosterone monoclonal antibodies; TOSOH Corporation Japan) in an automatic analyzer: TOSOH AIA TOSOH Corporation, Japan).

Tissue Preparation: The animals were scarified, and about one cm3 of prostate tissue specimens were collected from all groups immediately by using a sharp knife or a razor blade, and fixed immediately in 10% formal saline, the volume ration of tissue to fixative was 1:10. The tissues were left at room temperature for fixation before they were processed. Tissue processing method was made by automatic tissue processor (LEICATP1020), and then embedded in paraffin wax. Sections of 5µ thick were cut by a rotary microtome, stained in Mayer's haematoxylin and Eosin H&E (Bancrof et al., 1996). All sections were examined under the light microscope (Olympus) to describe the microscopic changes and imaged by using digital camera (Dewinter- DigiEye).

Statistical analysis: The results were expressed in form of mean±standard deviation. The difference between the mean in LH, FSH, and testosterone in the study of different groups were assessed by independent T- test. Results were considered statistically significant when the P value was less than 0.05. The statistical analyses were done using the SPSS statistical program, version 20 for Windows (IBM SPSS Statistics 20 IL, USA).

RESULTS

LH, FSH and testosterone of treated and untreated intact male rabbits with different doses of estradiol are shown in **Table 1**. It shows that testosterone was increased significantly with increase of estradiol. LH decrease insignificantly between treated and untreated subgroup. However there were no significant (P>0.05) changes in FSH concentration between treated and untreated subgroup. **Table 2** shows the mean of serum LH, FSH and testosterone hormones in castrated male rabbits injected with different doses of estradiol. None of the hormones FSH, LH and testosterone showed significance difference between the sub groups (P>0.05). The effects of estradiol benzoate in prostate histology of intact group were ranged from hyperplasia with dysplasia or dysplasia only (**Figure 1**). In the other hand the effects of estradiol benzoate in prostate of castrated groups shown the presence of hyperplasia representing by papillary projection (**Figure 2**).

Table 1. Mean±SD of LH , FSH, and testosterone hormones of intact group

parameters	Estradiol benzoate Doses				*P*-value
	0 µg	40 µg	80 µg	120 µg	
FSH mIU/mL	00. 55±0.31	00.68±0.23	00.55±0.16	00.86±0.12	0.41
LH mIU/mL	00.12±0.06	00.09±0.04	00.04±0.02	00.06±0.03	0.36
Testosterone ng/ml	17.00±8.32	60.14±1.22	42.75±2.81	63.94±2.41*	0.03

Mean with superscript stars within the row was significantly different (P<0.05).

Table 2. Mean±SD of LH and FSH and testosterone hormones of castrated group

Parameters	Estradiol benzoate Doses				*P*-value
	0 µg	40 µg	80 µg	120 µg	
FSH mIU/mL	00. 54±0.30	00.85±0.08	00.79±0.14	00.75±0.13	0.25
LH mIU/mL	00.12±0.07	00.11±0.04	00.09±0.02	00.57±0.31	0.86
Testosterone ng/mL	16.00±8.03	59.79±1.08	58.50±1.31	58.58±5.89	0.22

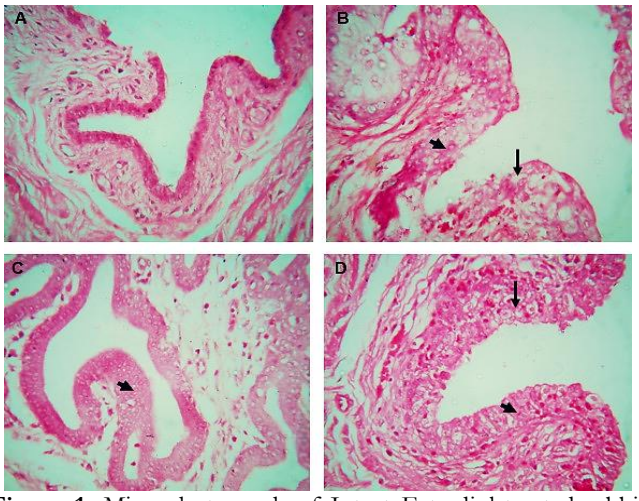

Figure 1. Microphotograph of Intact Estradiol-treated rabbit prostate tissues, magnification ×400. (A) No obvious histological change was observed in the prostate tissues of the control . (B-D) 40, 80 and 120µg estradiol benzoate treated demonstrating the presence of hyperplastic (*short arrows*) and dysplastic (*long arrows*) lesions.

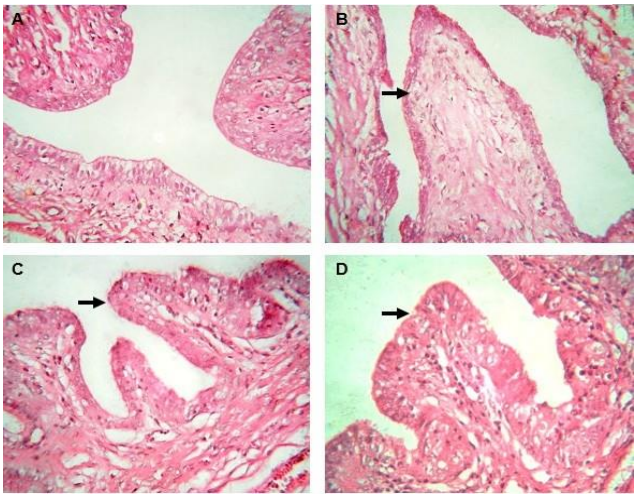

Figure 2. Microphotograph of Castrate estradiol treated prostate tissue, magnification X400 . (A) Representative H&E of prostate section of Control showed no histological changes. (B-D) 40, 80 and 120µg estradiol treatment showing the presence of hyperplasia representing by papillary projection (*arrow*) in epithelia.

DISCUSSION

Study in adult male rat shown that 17 beta estradiol have a dose-related effects on LH, FSH, and testosterone (Gill-Sharma et al., 2001), at low dose of 0.1 µg/kg/day estradiol have not any effects in serum level of LH, FSH and testosterone, but at high doses of 10 and 100-1000 µg/kg/day, significantly reduced serum level of LH, FSH and testosterone. However in this study there were no significant (P>0.05) changes found in FSH and LH of groups under study. While testosterone show different responses; ranged from significant (P<0.05) increase in high dose 120 µg, to insignificant (P>0.05) increase in a doses 40 µg and 80 µg of estradiol benzoate in intact male rabbits, while in castrated male rabbits show insignificant (P>0.05) increase. the result of FSH and LH in intact and castrated groups and testosterone in intact group were agree with that obtained by (Gill-Sharma et al., 2001), but the result of testosterone in castrated group was disagree.

The effects of estradiol in prostate of intact rabbits were ranged from hyperplasia with dysplasia or dysplasia only (**Figure 1**). A similar prostatic dysplasia result to estrogen treatment was found in mice (Pylkkanen et al., 1993), in dogs (Winter et al., 1995), Noble rat (Lou et al., 1998), and in the rodent Mongolian gerbil (Wellerson et al., 2008). In the other hand the effects of estradiol benzoate in prostate of castrated male rabbits showing the presence of hyperplasia representing by papillary projection (**Figure 2**). The hyperplasia of epithelial cells in castrated animals due to administration of estrogen suggests that estrogens can directly stimulate the activity of epithelial cells. These results are in agreement with several studies indicating that administration of estrogen to castrated animals could exert a stimulatory action on prostate epithelial cells as in castrated rat (Pelletier, 2002) and castrated dogs (Rhodes et al., 2000).

CONCLUSION

In conclusion, our study did not demonstrate different serum levels of LH, FSH, and Testeosterone hormone, in intact or castrated rabbits injected with different doses of estradiol, except Testeosterone hormone in intact group injected 120 µg estradiol. In the other hand the effect of estradiol on prostate histology were ranged from hyperplasia with dysplasia or dysplasia only in intact rabbit or hyperplasia representing by papillary projection in castrated rabbit.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENT

Authors are grateful to the authority of the University of Nyala and DAAD for their supports in conducting the study.

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