EFFECTS OF COUMESTROL ON RETE TESTIS IN DOGS

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ABSTRACT

The present study was conducted on ten healthy adult stray male dogs weighing 12-19 kg and randomly divided into two groups. Animals in treatment group (n = 5) were administered coumestrol dissolved in di-methyl sulfoxide (DMSO) orally in dog treats as a single dose @ 1.5 mg/kg body weight. Dogs in control group (n = 5) were given DMSO orally. Castration of the treated dogs was done on 12 hours, 24 hours, 7 days and 15th day post feeding of coumestrol; same castration schedule was also followed for control group. Histopathology revealed normal rete testis epithelium after coumestrol feeding. Therefore, from this study, it was concluded that oral feeding of coumestrol @ 1.5 mg/kg b.wt. has no adverse effects on rete testis histology and therefore, it cannot be used for population control of male dogs.

Key words: Phytoestrogen, Coumestrol, Rete testis, histopathology

Introduction

Phytoestrogens are estrogen-like compounds which can modulate the fertility of herbivores by mimicking reproductive hormone and are supposed to be defensive substances produced by plants (Hughes, 1988). There are three main classes of phytoestrogens: isoflavones, coumestans and lignans, which occur in either plants or their seeds (Murkies *et al.,* 1998). The function of the reproductive system along with gonadotropin levels may be affected theoretically after exposure to high concentrations of any exogenous estrogen (Mitchell *et al*., 2001). The classic scenario of adverse effects to herbivore fertility from the ingestion of phytoestrogen-containing plants is clover disease in sheep in Western Australia. Prolonged ingestion of some varieties of subterranean clover that contain estrogenic isoflavones (Braden *et al*., 1967) caused permanent infertility in sheep (Bennetts *et al*., 1946).

The dogs have been considered an unique member of human family due to either its roles as a companion, guard, hunter, guide to blind persons or for help in detecting narcotics and explosives, nabbing murderers and detecting thieves (Yadav *et al.,* 2015). But, their overpopulation has compelled researchers all over the world for development of an effective contraception drug. Pérez Rivero *et al.* (2009) administered 300 µg/kg coumestrol orally to male dogs, once a week for a 4 week period and concluded that coumestrol induces alterations in the olfactory behavior along with an oligo and terato-spermic effect. The authors speculated that coumestrol feeding in dogs induced a similar state as seen in α- estrogen receptor knockout mice (α-ERKO mice). The male α-ERKO mice were found to be infertile (Lubahn *et al*., 1993) due to lack of estrogen receptor á gene (Lubahn *et al.,* 1993; Eddy *et al*., 1996) raising the possibility that α-ER is required for normal function of the male reproductive system. The rete testis in α-ERKO male was dilated and protruded into the testis (Eddy *et al*., 1996; Hess *et al*., 1997) and the efferent ductules were dilated due to inhibition of fluid reabsorption (Hess, 2003). Therefore, the present study was designed to explicit the effects of coumestrol on dog rete testis and subsequently on their fertility.

Materials and Methods

Study area

The present study was conducted in the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana) from 2011 to 2012.

Animals

The present study was conducted on ten apparently healthy adult stray male dogs weighing 12-19 kg.

Clinical examination

Dogs were housed in an individual cage for one week prior to the start of the experiment. After examination of scrotum; hematological and parasitological examination of these animals were conducted. Selected animals were weighed, treated for parasitic infections and administered prophylactic anti-rabies vaccine (Raksharab, 1 ml, sc, Indian Immunolgicals).

Groups of animals

Dogs were randomly divided into two groups (n = 5). In the treatment group each dog was fed coumestrol @ 1.5 mg/kg b.wt. dissolved in di-methyl sulfoxide (DMSO) in dog treats once only. Dogs in the control group were fed DMSO only. Weight and testicular dimensions of the animals from both the groups were taken before administration of coumestrol or DMSO and before castration.

Castration of three dogs from the treatment group was done at 12 hours, 24 hours and 7 days interval whereas two dogs were castrated on 15th day post feeding of coumestrol, respectively. Similar schedule was followed for the castration of dogs of control group also. Testes were removed after anaesthetizing with an intra-muscular injection of Xylazine

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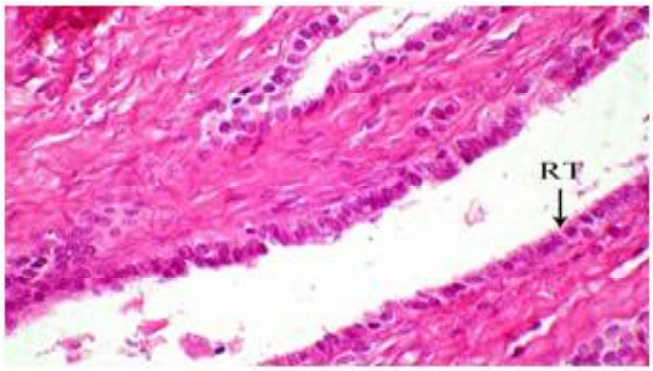

Fig. 1: Photomicrograph of the rete testis of control dog. (RT: Rete testis) H & E 400X

(2 mg/kg b.wt.) and Ketamine HCl (5mg/kg b.wt.). Immediately after castration, tunica vaginalis propria, remnants of the spermatic cord and other extraneous tissues were removed from the testes. Tissue samples for histology examinations were taken from testes. These tissues were sliced into smaller pieces and immediately placed in Bouin's fixative. Fixed tissue were dehydrated in methanol and cleared in xylene. Paraffin blocks were prepared and sections were cut at 5-7 micron. Slides were stained with Harris-hematoxylin and Eosin Y (H & E). Sections of testes were examined under simple microscope & photographs obtained with the help of Olympus digital microscope.

Results and Discussion

Rete testis forms a central channel within the testis and connects with the efferent ductules near the testicular capsule in dogs. It serves as a channel for accommodating sperm release from the seminiferous tubules (Rodriguez-Martinez *et al.,* 1990). The rete testis is lined with simple cuboidal to columnar epithelium in rats, mice, macaque monkeys, baboons, guinea pigs, hamsters, cats, dogs, rams, rabbits, boar and bull (Dym, 1976). The epithelial cells of rete testis are positive for α-ER and weakly positive for β-ER in dog (Nie *et al.,* 2002).

Microscopic section examination revealed rete testis tubules packed in mediastinum testis in dogs of both groups. Epithelial lining of rete testis was composed of simple cuboidal to low columnar epithelium in all dogs of control group (Fig.1). The epithelial lining of rete testis in the treatment group showed no histopathological changes (Fig. 2) and the epithelium type was similar to previous which was reported by Dym (1976). The results of present study can't be compared due to lack of studies with similar designs in literature but the findings are in agreement with the study of Kumar (2012) who reported no change in the histology of the epithelial lining of rete testis in treated dogs after administering 300 (n=5) & 500 µg (n=5) coumestrol to ten dogs at 0, 7, 14, 21 and 28 days. Similarly, in another two studies, coumestrol oral feeding @ 300 and 500 ìg once a week for a 5 week period and 1.5 mg/kg body weight as a single dose had no adverse effects on the histology of efferent ductules in dogs (Kumar *et al.,* 2016; Kumar *et al.,* 2017).

Pérez Rivero *et al.* (2009) speculated that coumestrol feeding in dogs induces a similar state as seen in α-ERKO mice but it seems unlikely because rodent rete testis is surrounded by loose connective tissue, whereas dog rete testis is embedded within the testis and probably it can accommodate any back pressure and thus chances of back pressure atrophy in dog is very less probably. Moreover, Kumar *et al.* (2018) have stated that coumestrol is a complex compound and its effects may vary due to various reasons between species and therefore interpretations and extrapolation of effects between species need to be made with caution and warrants

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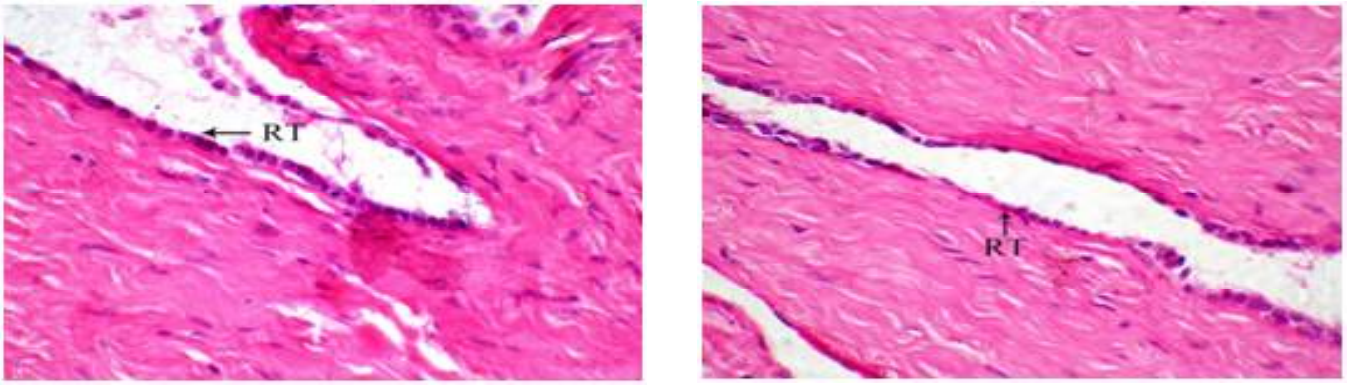

Fig. 2: Photomicrograph of the rete testis of treated dog (Left 7d & Right 15d) (RT: Rete testis) H & E 400X

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