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SHORT COMMUNICATION

Effects of Oral Feeding of Coumestrol on Efferent Ductules 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 given coumestrol dissolved in di-methyl sulfoxide (DMSO) orally as single dose @ 1.5 mg/kg body weight. Dogs in control group (n = 5) were given DMSO only. 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 followed for control group also. Histopathology revealed normal efferent ductules epithelium after coumestrol feeding. There were no any changes seen that would be responsible to lead infertility in male dogs. Therefore, from this study, it was concluded that oral feeding of coumestrol @ 1.5 mg/kg b.w. has no adverse effects on efferent ductules and therefore it cannot be used for population control of dogs.

Keywords: Coumestrol, Efferent ductules, Histopathology

Efferent ductules are the small tubules that run between the rete testis and epididymis. Their number can vary from 2 to 33, depending on the species (Ilio and Hess, 1994) and one of their main functions is to reabsorb the fluid secreted by the seminiferous epithelium (Hess, 2000). Efferent ductules involve parallel coils of ductules which are observed in large mammals, including dogs (Ilio and Hess, 1994). Role of efferent ductules came into the light due to α estrogen receptor knockout male mice (αERKO mice) which were found to be infertile (Lubahn *et al.,* 1993). The efferent ductules were dilated in αERKO male (Hess *et al.,* 1997) due to inhibition of fluid reabsorption (Hess, 2003).

Recently, coumestrol, a phytoestrogen was tried for population control of stray dogs in Mexico, a Latin American country suffering from stray dog problem like India. Ejaculates from treated dogs showed fewer spermatozoa than controls two weeks after the start of treatment (Pérez-Rivero *et al.,* 2009) and further suggested that as in αERKO mice, coumestrol may cause alteration in fluid resorption in efferent ductules resulting in accumulation of fluid in the efferent ductules (O' Donnell *et al*., 2001) in dog also. Therefore, the present study was designed to explicit effect of coumestrol on efferent ductules based on suggestion of Perez-Rivero *et al.* (2009) to control the stray dog population in India through estrogenic compound that affect male dog fertility.

The present study was carried out in the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana). Trial was conducted on ten apparently healthy adult stray male dogs weighing 12-19 kg.

Dogs were housed in individual cages for one week prior to the start of the experiment. After examination of scrotum; hematological and parasitological examination was carried out. Selected animals were weighed, treated for parasitic infections and administered prophylactic antirabies vaccine.

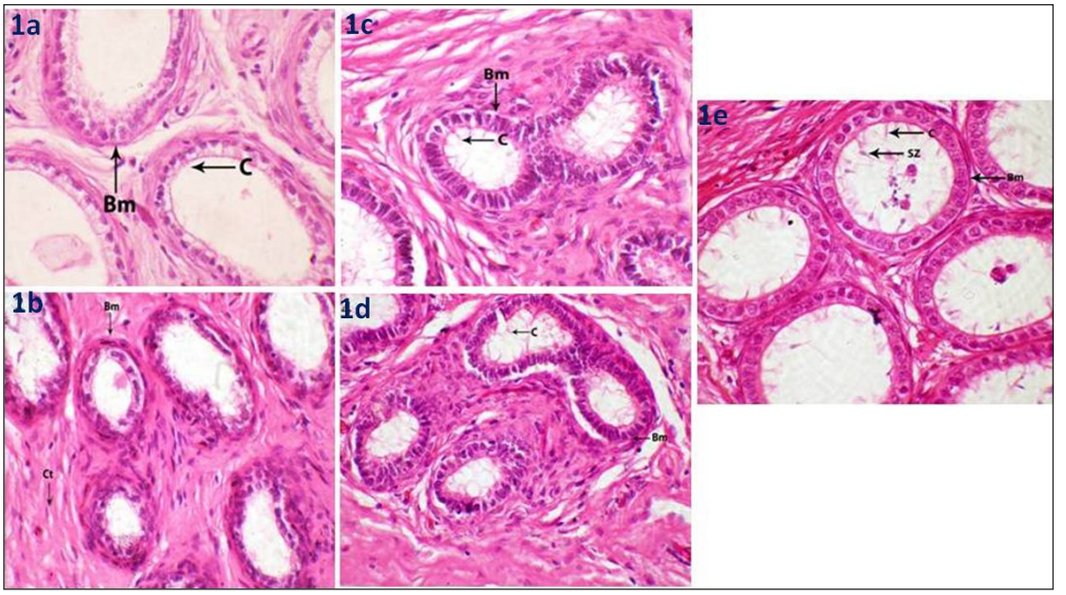

Fig. 1: Histological examination of efferent ductules of dogs in treatment (Fig. 1a-1d) and control (Fig. 1e) group, respectively (Bm: Basement membrane, C: Cilia, Ct: Connective tissue, SZ: Spermatozoa)

Dogs were randomly divided into two groups (n = 5). In treatment group each dog was fed coumestrol @ 1.5 mg/kg body weight dissolved in di-methyl sulfoxide (DMSO) 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/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 (2 mg/kg b.w.) and Ketamine HCl (5 mg/kg b.w.). 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, 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) and periodic acid Schiff's (PAS) reagent and counter stained with Harris-hematoxylin. Sections of efferent ductules were examined under simple microscope & photographed with Olympus digital microscope.

Epithelial lining of the efferent ductules was composed of simple cuboidal to low columnar epithelium. Similar type of epithelium has been observed in efferent ductules of dogs by Schimming and Abreu (2001). No histopathological changes were observed in the efferent ductules epithelium in this study in treatment (Fig. 1; 1a-1d) and control (Fig. 1e) group. Similarly, Kumar *et al*. (2016) also did not observe any change in epithelium of efferent ductules of the coumestrol treated dogs. However, Perez-Rivero *et al.* (2009) suggested that coumestrol may cause alteration in fluid resorption in efferent ductules in dogs also resulting in accumulation of fluid in the efferent ductules (O' Donnell *et al*., 2001) as in αERKO mice. This fluid accumulation in αERKO mice resulted into changes in ciliary and microvillus borders and ultimately dilation of efferent ductules (Hess *et al*., 2001). Nevertheless, such types of changes were not observed in the present study and probably these effects cannot be produced in dogs because of the difference between the basic design of efferent ductules in mouse and dog. Efferent ductules of Mouse merge into a common duct and thus act like a

funnel into epididymis and a funnel like efferent duct is more susceptible to back pressure than would be efferent ductules of dog which have multiple openings into the epididymis (Hess *et al*., 2001) and thus effect observed with anti-estrogen treatment in the rodent species is unlikely to occur in dog.

In conclusion, coumestrol @ 1.5 mg/kg b.w. to male dogs cannot be used for population control of dogs as it did not affect the histology of efferent ductules. However, furthermore study is needed with different dose rate and increased number of experimental dogs to see the effects of coumestrol on male fertility.

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