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Effect of Coumestrol on Efferent Ductules in Dogs

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ABSTRACT

The present study was conducted on twelve adult stray dogs of age 2 – 3 years, weighing 14 - 18 kg and randomly divided into three groups. Animals in group I (n = 5) and group II (n = 5) were orally given 300 and 500 microgram coumestrol dissolved in di-methyl sulfoxide in the commercial dog treats on days 0, 7, 14, 21 and 28, respectively. Animals in control group III (n = 2) were given DMSO alone as per above schedule. Castration was done one week after the completion of the treatment. Tissues for histology and electron microscopy were fixed in Bouin's fixative and Karvonsky's fixatives, respectively. Epithelial lining of the efferent ductules was composed of cuboidal or columnar epithelium having ciliated and non- ciliated cells. Cellular architecture of the efferent ductules was normal. Scanning electron microscopy revealed normal efferent ductules with presence of cilia on luminal surface. In conclusion, oral feeding of 300 microgram and 500 microgram of coumestrol has no adverse effects on the functioning of efferent ductules in dogs and at this dose rate, this compound cannot be used for population control of stray dogs.

Keywords: Coumestrol, efferent ductules, electron microscopy

Canine is a prolific species and according to one estimate a bitch and its offspring has the potential to produce 67000 new dogs in six years (Pal, 2005). Dog population control such as ovariohysterectomy and orchiectomy are the only methods employed by the NGO's. However, inspite of spending millions of rupees during last decade not much dent has been made in dog population, particularly in India. Nowadays, the use of some phytoestrogens is being investigated for reproductive control (Serrano *et al.* 2007). Coumestrol is one of the phytoestrogen derived from a variety of clovers, alfalfa and other leguminous plants, which are regularly consumed by herbivores in their natural diet. It binds with both α and β estrogen receptors (Kuiper *et al.* 1998). Recently, it has been found that coumestrol could be a good choice for reproductive control of stray dogs (Perez-Rivero *et al.* 2009). It binds to estrogen receptors present in efferent ductules and rete testis (Nie *et al.* 2002). This interaction induces similar changes in reproductive tracts as reported in α Estrogen Receptor Knockout mouse (Perez-Rivero *et al.* 2009). In α Estrogen Receptor Knockout mouse, loss or shortening of microvillus and ciliary borders of efferent ductules occur (Hess *et al.* 1997; 2000) and all these changes cause accumulation of fluid in efferent ductules and subsequently, dilatation of rete testis and efferent ductules, an increase in abnormal spermatozoa, oligospermia and back pressure atrophy of the testis (Eddy *et al.* 1996). Surgical method is expensive, time consuming, require expertise staff, boarding and post surgical care. Development of non surgical methods of sterilization will provide an alternative strategy, particularly in areas where surgical initiative may not be feasible. Therefore the study was planned with the objective to see the effect of oral feeding of coumestrol on efferent ductules to control the stray dog population.

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).

Animals

The present study was conducted on twelve apparently healthy adult stray dogs of age 2 - 3 years having body weight 14 - 18 kg.

Clinical examination

Initially, a general health examination was performed. The scrotum was observed for the presence of lesions, testes were palpated, compressed and compared to each other for their size, shape and consistency. Free movement of the testes into the scrotum was confirmed. Dogs were kept under observation for one week prior to the start of the experiment for treatment of external and internal parasites and immunization against rabies. All the dogs were housed in individual cages. Selected animals were weighed.

Groups of animals

Dogs were randomly divided into three groups. Animals in group I (n = 5) and group II (n = 5) were given 300 and 500 µg coumestrol dissolved in di-methyl sulfoxide (DMSO) orally in the commercial dog treats on days 0, 7, 14, 21 and 28, respectively. Animals in control group III (n = 2) were given DMSO alone as per above schedule. One week after the end of treatment period, dogs were anaesthetized with an intra-muscular injection of Xylazine (2.0mg/kg B. Wt.) and Ketamine HCl (10mg/kg B. Wt.). Testicles were removed and efferent ductules was collected and fixed in Bouin's and Karnovsky's fixatives for histology and electron microscopy, respectively. Tissues were dehydrated in methanol and cleared in xylene, and then paraffin blocks were prepared. Sections were cut at 5 - 7 micron and stained with Harris-hematoxylin and Eosin (H & E). Sections of efferent ductules were examined for microscopic changes in ciliary and microvillus border, types of cells of epithelium and dilatation of tubules, if any. For scanning electron microscopy (SEM) sections of efferent ductules were cut in 0.5 - 1cm size and immediately fixed in Karvonsky's fixative for 6-12 hours at 4ºC. Then three washings in 0.1M phosphate buffer (pH 7.4) at an interval of 15 minutes were given at 4ºC. Finally, these tissues were preserved in phosphate buffer at 4ºC and rest of the processing like dehydration, critical point drying, sputter coating and viewing scanning electron microscope (Leo-435 VP, Japan) was carried out at Electron microscopic facilities, Department of Anatomy, AIIMS, New Delhi.

RESULTS AND DISCUSSION

The testes of dogs of experimental and control group were grossly normal and did not show any swelling, adhesion, ulceration and pain on palpation at the time of castration. Epithelial lining of efferent ductules was composed of pseudo stratified columnar epithelium having ciliated and non- ciliated cells. Ciliary border, microvillus border and cellular architecture were normal. No tubular dilatation was seen (Fig. 1: A, C, E). Electron microscopically, the tubules of efferent ductules were normal in size and shape with intact ciliary border, which is specialized for movement of spermatozoa towards epididymis (Fig.1: B, D, F).

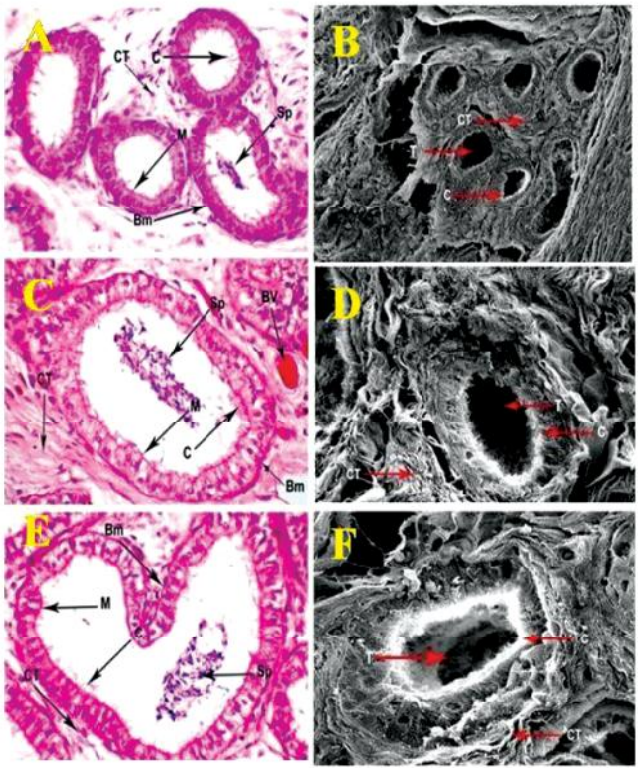

Fig. 1: Histological and electron microscopic examination of efferent ductules of dogs in group-I (A and B), Group-II (C and D), group-III (E and F), respectively (BM: Basement membrane, C: Ciliary border, CT: Connective tissue, M: Microvilli, Sp: Spermatozoa, T: Tubules of efferent ductules)

Histologically and electron microscopically, epithelial lining of the efferent ductules was composed of pseudo stratified columnar epithelium having ciliated and non-ciliated cells. These tubules were normal in size and shape with intact ciliary and microvillus borders. No tubular dilatation was observed (Fig. 1: A-F).

These results cannot be compared because such studies have not been conducted in any species so far. However, Perez-Rivero *et al.* (2009) assumed erroneously that coumestrol may cause dilatation of efferent ductules because of fluid accumulation similar to α Estrogen receptor Knockout mouse (Hess *et al.* 2001 and O' Donnell *et al.* 2001). Moreover, Perez-Rivero *et al*. (2009) found that tubular structure of the gonad is lost and leydig cells are absent or in very low amounts. Since coumestrol is able to bind estrogen receptor β and this interaction produced defective spermatogenesis and spermiogenesis. Furthermore, Serrano *et al*. (2007) fed 200 µg coumestrol in defibrinated blood to wild vampire bats daily for one month. Thereafter, similar to present study, grossly, testes did not show any alteration in structure and shape. However, histology revealed absence of lumens of the seminiferous tubuli and leyding cells in the interstitial spaces. Mature sperm cells were either absent or not detectable in seminiferous tubules. These changes were detected after a long exposure to high pharmacological concentrations of coumestrol. In present study, coumestrol has not acted as an estrogen agonist because of low doses used in this study. Coumestrol does not affect male fertility, as it doesn't bind with estrogen receptors (Rahhal and John, 2011).

In conclusion, oral feeding of 300 and 500µg coumestrol to the dogs did not cause any adverse effects on efferent ductules. So, at this dose rate, coumestrol cannot be used for population control of stray dogs however, the number of animals in the current study was less. Nevertheless, studies are warranted with different dose rate of coumestrol to see the effect on dogs.

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