COUMESTROL FEEDING - AN ATTEMPT TO DEVELOP A TOOL FOR STERILIZATION OF MALE STRAY DOGS

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ABSTRACT

Coumestrol dissolved in di-methyl sulfoxide (DMSO) was fed (50 mg, p.o., single dose) to a dog, whereas, the other dog was fed DMSO only. This followed castration of both the dogs on day 30 post-feeding. Microscopic examination revealed normal rete testis, efferent ductules, seminiferous epithelial cycle and spermiogenesis in both the dogs suggesting that coumestrol had no impact. Thus, 50 mg coumestrol as a single per-oral dose cannot be used for the sterilization of male dogs.

Keywords: Coumestrol, Dog, Spermatogenesis, Sterilization, Stray

Stray dog population related problems like rabies, traffic accidents and other zoonotic disease transmission poses a huge challenge in many developing countries including India where dog population is estimated to 11.67 million (Livestock census of India, 2012). Rabies takes life of about 20,000 people every year in India and 61,000 at global level, respectively (WHO, 2013; Sudarshan *et al*., 2007). Dog population control by surgical method has been tried and still in use by several NGO's but its impact on stray dog population is not appraisable. Therefore, researchers are exploiting other non-surgical methods to control their population by fertility impairment of male dogs and coumestrol appeared to be a promising compound. In a study, coumestrol feeding to experimental dogs reported fewer spermatozoa in ejaculates (Pérez-Rivero *et al*., 2009). Therefore, the present experiment was designed to explicit coumestrol effects on fertility in stray male dogs.

In the present study, 50 mg coumestrol was fed once to a dog after dissolving in di-methyl sulfoxide (DMSO) and another dog was fed DMSO only. Both the dogs were castrated 30 days post-feeding of coumestrol or DMSO. 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.). Testes samples for histology examination were sliced and placed immediately in Bouin's fixative. Fixed tissue were dehydrated in methanol and cleared in xylene. The paraffin blocks were prepared and sections were cut at 5-7 micron and the slides were stained.

On palpation, the gross appearance of testes was normal starting from the day of drug administration till the day of castration in both dogs. The microscopic examination of testes sections from both dogs revealed round seminiferous tubules with intact basement membrane. All the cellular elements that are spermatogonia (type A and type B) and primary spermatocytes were normal and did not reveal any qualitative change. Leydig cells with normal structures were present. Seminiferous tubules were lined with several layers of different generations of germ cells. Eight stages of seminiferous epithelial cycle were present. Various cellular associations in these eight stages consisted of one or two generations of spermatogonia, spermatocytes were observed.

Microscopic examination of section stained with PAS revealed all the four stages of spermiogenesis (Golgi phase, Cap phase, Acrosome phase and Maturation phase) in both dogs. Microscopic

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examination of the sections of both dogs stained with H&E revealed simple cuboidal to low columnar epithelium in efferent ductules. Epithelium was observed to be resting on a well-defined basement membrane. Ciliated and non-ciliated, both types of cells were observed in epithelium. Microscopic examination of the sections of both dogs stained with H&E revealed simple cuboidal to low columnar epithelium in rete testis tubules. Epithelial cytoarchitecture was intact and no histopathological lesions were observed in rete testis epithelium. Epithelial lining of the efferent ductules was composed of simple cuboidal to low columnar epithelium. Similar type of epithelium was observed in efferent ductules of dogs earlier (Schimming and Abreu, 2001). No histopathological changes were observed in the efferent ductules epithelium in this study in treatment and control group.

Similarly, others did not observe any change in epithelium of efferent ductules of the coumestrol treated dogs (Kumar *et al*., 2016). Nevertheless, they did not observed the effect of coumestrol on seminiferous epithelial cycle, spermiogenesis, rete testis and efferent ductules in treated dogs. However, Pérez–Rivero *et al.* (2009) observed meiotic progress restricted to as much as having few round spermatids with no mature spermatozoa in seminiferous tubules and defective spermiogenesis in treated dogs after coumestrol administration @ 300 µg/kg b.w. on day 0, 7, 14, 21 and 28 and suggested that coumestrol binding to β-estrogen receptor in mammalian testis could affect spermatogenesis and spermiogenesis but these effects could not be produced in the current study which might be due to breed or individual difference in dogs and also β-estrogen receptor role in male reproduction has not been revealed yet as β-estrogen receptor Knockout Mice itself is fertile (Walker and Korach, 2004).

In conclusion, coumestrol had no effect on spermatogenesis, rete testis and efferent ductules in the present study, therefore, it cannot be used for sterilization of male dogs but as this study involved only one treated animal and large volume of confusing/ contradictory data is available on the effects of phytoestrogen on fertility in other species further research is needed to conclude effects of coumestrol on fertility in male dogs.

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