Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5453

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 178-182 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Effect of Coumestrol Feeding on Sterilization of Male Dog

Rakesh Kumar[1*], A. K. Sharma[2] and Anand Kumar Pandey[3]

[1]Government Veterinary Hospital Kirmara, Hisar, Haryana, India

[2]Department of Veterinary Gynaecology and Obstetrics,

[3]Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana), INDIA

*Corresponding Author E-mail: dr.r.k.duggal@gmail.com

Received: 16.08.2017 | Revised: 28.08.2017 | Accepted: 1.09.2017

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 were given coumestrol @ 1.5 mg/kg body weight (n=5) dissolved in di-methyl sulfoxide orally in a treat, once only. Dogs in control group (n=5) were given DMSO only. Castration of one dog was done on 12 hours, 24 hours and 7 days post feeding of coumestrol, respectively whereas two treated dogs were castrated on 15th day post feeding; same castration schedule was followed for control animals. Tissue from different parts of the testes were incised and fixed in Bouin's fixative. Stages of seminiferous epithelial cycle and spermiogenesis were not identifiable in the treated dogs. However, no histopathological lesions were observed on structure of spermatogonial and sertoli cells. Therefore, it was concluded that oral feeding of coumestrol @ 1.5mg/kg b.w. cannot be used for sterilization of male dogs.

Key words: *Coumestrol, Seminiferous epithelial cycle, Testes.*

INTRODUCTION

Stray dog population poses a huge threat in many developing countries including India due to Rabies, traffic accidents and environmental pollution because of open defecation. 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, to achieve negative population growth it is necessary that the method used should be non-surgical. Various chemicals/agents that have been used for sterilization of male dogs are: 10% silver nitrate, 3.6% formaldehyde[7]; methallibure, dexamethasone, metopiron, niridazole and α-chlorohydrin[5]; cadmium chloride[10]; calcium chloride[9]; chlorhexidine gluconate[1] and zinc arginine[13]. However, out of all these compounds only zinc arginine was marketed as Neutersol in USA for population control of dogs and that too was subsequently withdrawn from the market just after two years[4]. Therefore, search for a chemical/drug/ hormone that can cause sterility in male dogs after one time exposure is still going on.

Cite this article: Kumar, R., Sharma, A.K. and Pandey, A.K., Effect of Coumestrol Feeding on Sterilization of Male Dog, *Int. J. Pure App. Biosci.* **5(5):** 178-182 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5453

Recently, coumestrol, a phytoestrogen has been tried for population control of stray dogs in Mexico, a Latin American country suffering from stray dog problem like India[12]. Ejaculates from treated dogs showed fewer spermatozoa than controls two weeks after the treatment. Coumestrol acts as an anti-estrogenic compound by inhibiting the aromatase activity, and thus reduces the concentration of estrogens in the blood[15, 8]. Therefore, the present study was designed to explicit coumestrol effects on fertility in male dogs.

MATERIALS AND METHODS

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). The study included ten apparently healthy adult stray male dogs weighing between 12-19 kg. The dogs were housed in individual cages for one week prior to the start of the experiment. After examination of scrotum, hematological and parasitological examination of these animals was conducted. Selected animals were weighed, treated for parasitic infections and administered prophylactic antirabies vaccine.

Animals were randomly divided into two groups (n=5). Each dog in treatment group was fed coumestrol @ 1.5 mg/kg body weight dissolved in di-methyl sulfoxide (DMSO) in dog treats once only. Dogs in the control group were fed DMSO only.

Castration of one dog from the treatment group was done at 12 hours, 24 hours and 7 days post feeding of coumestrol, respectively whereas two treated dogs were castrated on 15th day post feeding. Similar schedule was followed for the castration of control group. Weight and testicular dimensions of the animals from both the groups were taken before administration of coumestrol/DMSO and before castration.

Testes were removed after anaesthetizing with an intra-muscular injection of Xylazine (2 mg/kg b.w.) and Ketamine HCl (4 mg/kg b.w.) intravenously. 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 were placed immediately 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. The criterion described by Foote[6] was used in scoring the stages of the seminiferous epithelial cycle and for scoring the steps of the development of acrosomic system the criterion described by Clermont and Leblond[2] was used. Harris-hematoxylin and Eosin Y stained slides were photographed with Olympus digital microscope. PAS stained slides were photographed with Nikon microphotography camera.

RESULTS AND DISCUSSION

Gross appearance of the testes was normal on palpation from day of drug administration till day of castration in all the dogs. No significant difference was observed in the body weight and testicular dimensions of dogs after treatment ($P<0.05$) within and between the groups. Microscopic examination of cross sections of testicular tissues from both groups revealed seminiferous tubules with intact basement membrane in all dogs. Spermatogonial, sertoli cells and Leydig cells with normal structure were present in all the dogs of both groups.

Microscopic examination of the testicular tissues of control group revealed eight stages of seminiferous epithelial cycle and all the four stages of spermiogenesis in all the dogs (Fig. 1-2). Stages of seminiferous epithelial cycle were not identifiable in treated dogs (Fig. 3-6). Similarly, Pérez–Rivero[12] observed meiotic progress restricted to as much as having few round spermatids and no mature spermatozoa in seminiferous tubules of treated dogs after coumestrol administration @

300µg/kg b.w. on 0, 7, 14, 21 and 28 days. In the present study, absence of acrosome development steps was also observed after administration of coumestrol (Fig. 7, I-IV). Similarly, Pérez–Rivero[12] also observed defective spermiogenesis in their study and authors stated that coumestrol binding to βER in mammalian testis could affect spermiogenesis. However, Rajesh Kumar[14] observed normal spermatogenesis and spermiogenesis in treated dogs after coumestrol feeding which may be due to low dose of coumestrol used in his study.

Coumestrol feeding @ 1.5 mg/kg b.w. affected spermatogenesis in the present study severely but, as spermatogonial cells were unaffected, therefore, the effects observed on spermatogenesis in the present study would be of temporary nature. Noakes[11] has stated if stem cells and sertoli cells are undamaged and provided lumen is not blocked with cellular debris, regeneration can place in cases of testicular degeneration. Similarly, Corrada[3] reported increased volume of the spermatic fraction of the ejaculate to pre-treatment volumes in treated male dogs after administration of Tamoxifen, a compound which is more specific estrogen receptor antagonist than coumestrol.

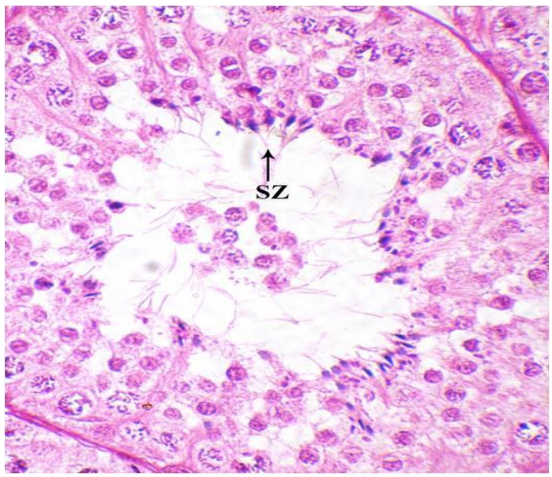

Fig. 1: Cross section of the seminiferous tubule of control dog showing stage VIII of seminiferous epithelial cycle (SEC). Fully formed spermatozoa (SZ) are lining the lumen (H & E 400X).

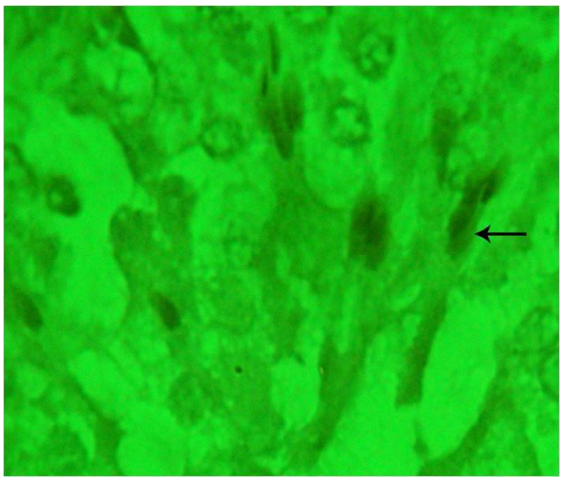

Fig. 2: Cross section of the seminiferous tubule of control dog showing maturation phase of spermiogenesis (Arrow Head; PAS 1000X).

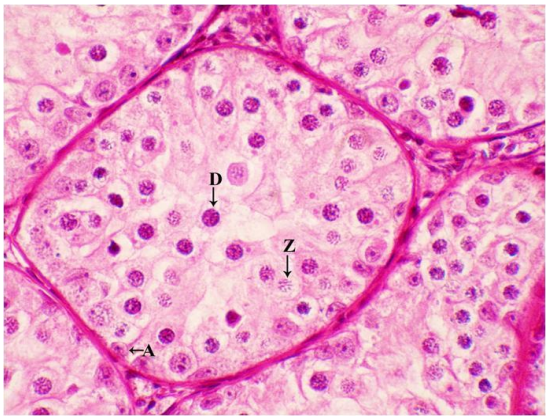

Fig. 3: Cross section of the seminiferous tubule of treated dog showing absence of seminiferous epithelial cycle at 12 hours {D: Diplotene, Z: Zygotene, A: Spermatogonia A} (H & E 400X).

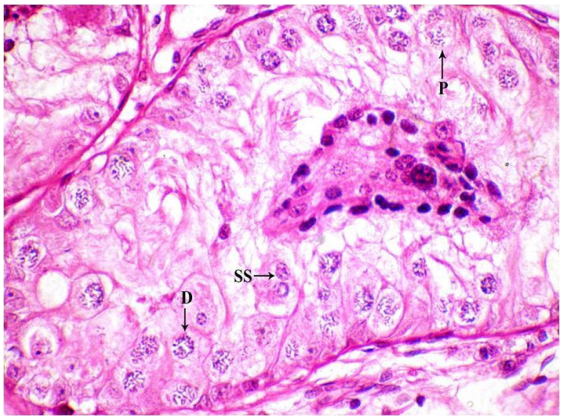

Fig. 4: Cross section of the seminiferous tubule of treated dog showing absence of seminiferous epithelial cycle at 24 hours {D: Diplotene, SS: Secondary spermatocytes} (H & E 400X).

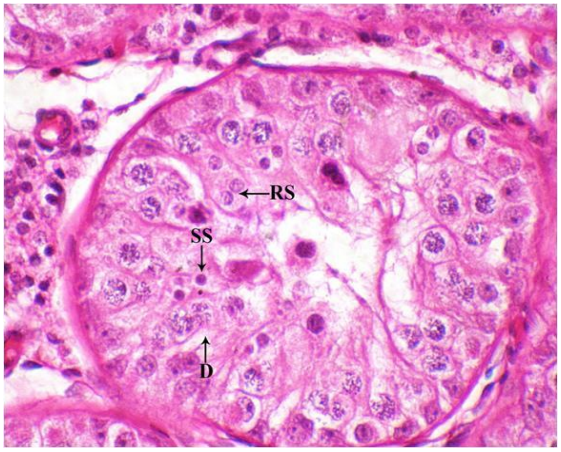

Fig. 5: Cross section of the seminiferous tubule of treated dog showing absence of seminiferous epithelial cycle at 7 days {D: Diplotene, SS: Secondary spermatocytes, RS: Round spermatids} (H & E 400X).

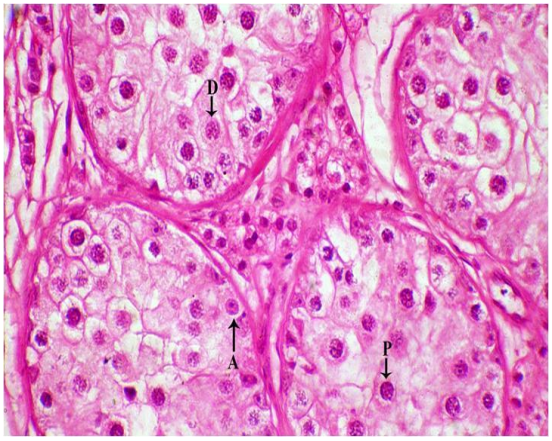

Fig. 6: Cross section of the seminiferous tubule of treated dog showing absence of seminiferous epithelial cycle at 15 days {D: Diplotene, A: Spermatogonia A, P: Pachytene} (H & E 400X).

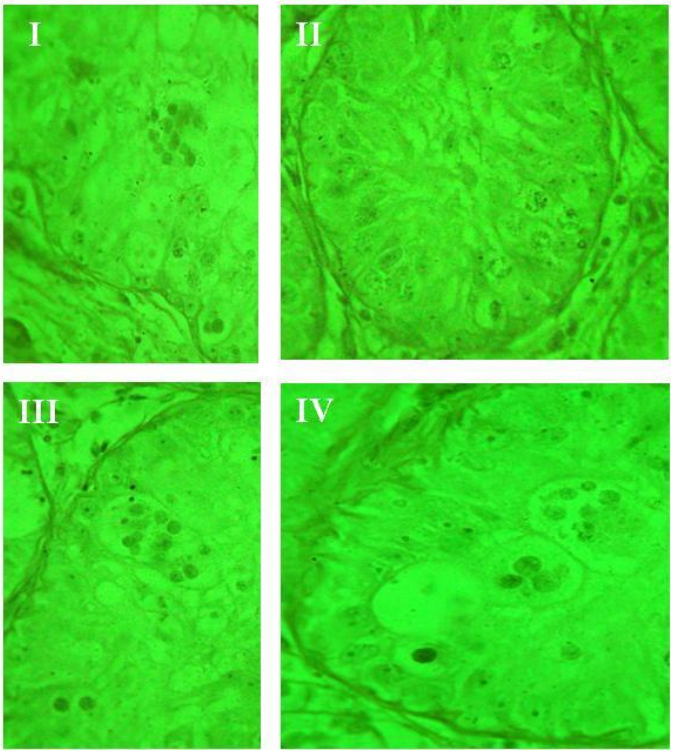

Fig. 7 (I-IV): Cross section of the seminiferous tubule of treated dog showing absence of spermiogenesis at 12 (I) and 24 (II) hours, 7 (III) and 15 (IV) days (PAS 1000X).

CONCLUSION

In conclusion, coumestrol @ 1.5 mg/kg b.w. cannot be used for sterilization of male dogs because spermatogonial and sertoli cells were unaffected histologically but as number of animals were less in present study and large volume of confusing data is available on the effects of phytoestrogen on fertility in other species so, further research is warranted with increased dose rate to see the effects of coumestrol on fertility in male dogs.

Acknowledgements

The authors fully acknowledge the help provided by Rex A. Hess, Professor Emeritus, Comparative Biosciences, College of Veterinary Medicine, University of Illinois on various occasions during this study.

REFERENCES

1. Brij Lal., Studies on the effects of Chlorhexidine gluconate on epididymis and spermatogenesis in dogs, M.V.Sc. Thesis, Chaudhary Charan Singh Haryana Aricultural University, Hisar, India (2002).
2. Clemont, Y. and Leblond, C.P., Spermatogenesis of men, monkey, ram and other mammals as shown by periodic acid Schiff Technique. *Am. J. Anat.,* **96:** 229-253(1955).
3. Corrada, Y., Arias, D., Rodrı´guez, R., Spaini, E., Fava, F. and Gobello, C., Effect of tamoxifen citrate on reproductive parameters of male dogs, *Theriogenology.*, **61:** 1327–1341 (2004).
4. Dinnage, J., Getting beyond surgical contraception: Nonsurgical alternatives explored as possible answer to dog and cat overpopulation, *J.A.V.M.A.*, **230(2):** 168-170 (2007).
5. Dixit, V.P., Chemical sterilization of male dogs (Canis familiaris) after single intratesticular administration of methallibure (ICI-33828), dexamethasone, metopiron (SU-4885, Ciba), niridazole (33644-Ba, Ciba), alpha-chlorohydrin (U-5897) & danazol, *Ind. J. Exp. Biol.*, **17:** 937-940 (1979).
6. Foote, R.H., Swierstra, E.E. and Hunt, W.L., Spermatogenesis in the dogs, *Anat. Rec.*, **173:** 341-352 (1972).
7. Freeman, M.D. and Coffey, D.S., Sterility in male animals induced by injection of chemical agents into the vas deferens. *Fertil. and Steril*., **24:** 884 (1973).
8. Hong, Y., Cho, M., Yuan, Y.C., Chen, S., Molecular basis for the interaction of four different classes of substrates and inhibitors with human aromatase, *Biochem. Pharmacol.*, **75:** 1161–1169 (2008).
9. Koger, L.M., Calcium chloride castration. *Mod. Vet. Pract.,* **59(2):** 119-121 (1978).
10. Murty, T.S. and Sastry, G.A., Effect of cadmium chloride (CdCl2) injection on the histopathology of the testis and the prostate in dogs. I. Intratesticular procedure. *Indian Vet. J.,* **55(5):** 368-371 (1978).
11. Noakes, David E., Parkinson, Timothy J., England, Gary C. W., Veterinary Reproduction and Obstetrics, 9th ed., Saunders Elsevier, Edinburgh, 2009, 742.
12. Pérez-Rivero, J.J., Martinez-Maya, J.J., Perez-Martinez, M., Aguilar-Setien, A., Garcia-Suarez, M.D. and Serrano, H., Phytoestrogen treatment induces testis alterations in dogs. Potential use in population control. *Vet. Res. Commun.*, **33:** 87-95 (2009).
13. Rajan Chaudhary., Effects of intra-epididymal injection of zinc arginine on epididymis and spermatogenesis in pre-pubertal dogs, M.V.Sc. Thesis, Chaudhary Charan Singh Haryana Aricultural University, Hisar, India (2006).
14. Rajesh Kumar., Effects of coumestrol (phytoestrogen) on spermatogenesis in dogs, M.V.Sc. Thesis, Chaudhary Charan Singh Haryana Aricultural University, Hisar, India (2012).
15. Wang, C., Makela, T., Hase, T., Adlercreutz, H. and Kurzer, M.S., Lignans and Xavonoids inhibit aromatase enzyme in human pre adipocytes., *J. Steroid Biochem. Mol. Biol.,* **50:** 205–212 (1994).