

Effects of Enrofloxacin Administration on Semen Quality of Barbari bucksChandrima Sinha¹, Sarvajeet Yadav², Brijesh Yadav^{3*}, Krantidev Singh⁴¹Research Scholar, Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India, 281001.²Professor, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India, 281001.³Assistant Professor, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India, 281001.⁴Dean, College of Biotechnology, DUVASU, Mathura, Uttar Pradesh, India, 281001.

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Abstract

The present study was undertaken to study the effect of enrofloxacin administration on semen quality of Barbari buck. Six normal, healthy adult Barbari bucks (25-30 Kg) aged between 2 to 2.5 years were used as semen donors. The bucks were administered enrofloxacin at the dose of 5mg/kg body weight intra-muscularly daily for 7 days. From each buck biweekly eighteen semen ejaculates (1st to 18th) were collected and one sample (0th) from each buck was collected before administration of enrofloxacin, using a non-oestrous doe as dummy and investigated for semen quality parameters. There was no significant difference ($P \geq 0.05$) in seminal volume between different ejaculates. Mass motility decreased significantly ($P \leq 0.01$) and was lowest in 4th ejaculate as compared to mass motility prior to drug administration. Highest significant ($P \leq 0.01$) decrease in progressive motility was seen in 9th ejaculate as compared to progressive motility before drug administration. Sperm concentration decreased significantly ($P \leq 0.01$) and was found to be lowest in 4th ejaculate as compared to sperm concentration before enrofloxacin administration. Percent live spermatozoa decreased significantly ($P \leq 0.01$) and was found to be minimum in 12th ejaculate as compared to sperm concentration prior to drug administration. Highest significant increase ($P \leq 0.01$) in per cent head, mid-piece and tail abnormalities of spermatozoa was observed in 4th, 3rd and 6th ejaculate respectively following parenteral administration of enrofloxacin as compared to ejaculate before drug administration. Following drug administration, the semen quality decreased progressively and then became slowly normal after 14th ejaculate.

Keywords: Buck; Enrofloxacin; Semen quality

Introduction

In spite of best management practices at organized farms where breeding bucks are reared, the diseases are bound to occur and the use of various chemotherapeutic agents is very common. In breeding bucks, the ailments can be from mild trauma, metabolic diseases to severe infections. The animals with acute/chronic bacterial infection require the systemic administration of antibiotics. Repeated administration of chemotherapeutic agents affect the testicular cells, accessory sex glands and may influence the andrological status of male animals. Various biochemical alterations in semen following repeated administration of antibiotics have also been reported (Timmermans, 1974).

It has been reported that enrofloxacin has tendency to get accumulated in the germ cells, spermatogonia or spermatids and accessory sex glands and may exert chemo-sterilizing effect on testes (Verma *et al.*, 1999). Further, enrofloxacin was reported to have inhibitory effect on libido and service behaviour while the reaction time was increased after parenteral administration of the drug in buffalo bulls.

Enrofloxacin is a quinolone carboxylic acid derivative with antimicrobial action. Enrofloxacin is most effective against gram-negative bacteria and can also be used against gram-positive as well as anaerobic bacteria. The male sexual function is very sensitive to pharmacological agents of all sorts. Multidisciplinary effort is needed to analyse and establish the mode of action of antispermatic, antiandrogenic, spermiostatic and spermicidal agents on male reproductive function and semen (Mann and Mann, 1981). There are some re-

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ports on the effect of chemotherapeutic agents on semen quality in human (White, 1954); male rats (Timmermans, 1974); bulls (Abbit *et al.*, 1984); bovine (Ahmad and Foote, 1986); buck (Fattouh *et al.* 1992); albino rats (Vijaya *et al.* 1995); male fowl (Mohammed, 2008) and male mice (Aral *et al.*, 2008); however, such informations are lacking in ruminants especially in bucks. Hence the present study was undertaken with the objective to study the effect of enrofloxacin administration on semen quality of Barbari buck.

Materials and methods

Six normal, healthy adult Barbari bucks (25-30 Kg) aged between 2 to 2.5 years stationed at the experimental sheds of Department of Veterinary Physiology, DUVASU, Mathura (U.P) were used as semen donors. The animals were grazed in a flock in institute's pasture daily from 9.00 AM to 3.00 P.M. and 250g concentrate mixture having DCP 13% and TDN 69% was offered per animal daily with ad libitum watering. All the experimental animals were regularly dewormed for internal parasites.

The bucks were administered enrofloxacin at the dose of 5mg/kg body weight intra-muscularly daily for 7 days. From each buck biweekly eighteen ejaculates (1st to 18th) were collected and one sample (0th) from each buck was collected before administration of enrofloxacin using a non-oestrous doe as dummy. The doner bucks were trained for semen collection prior to conduction of the experiment.

Evaluation of Semen: Volume of each ejaculate was directly measured in milliliters (ml) in graduated collection cup with an accuracy of 0.10ml. Gross motility of the neat semen was assessed by placing a small drop of neat semen on a clean, grease free glass slide kept on thermostatically regulated stage at 37°C and examined under low power objective of the microscope. Depending on the appearance of wave motion, swirl's and vigour, the gross motility is graded on 0-5 scale (Graham *et al.*, 1970). The progressive motility of spermatozoa was estimated using a 200x of phase contrast microscope. A small drop of diluted (1: 100) semen was put on a clean, grease free glass slide and a cover slip was placed over it. The fields were examined to obtain the percentage of progressively motile spermatozoa by constant visual observation.

Sperm concentration was estimated by the haemocytometer method. To ascertain the percentage of live or dead spermatozoa and morphological abnormalities in a semen sample, eosin-nigrosin staining technique was used and the smear was observed under 400x (Hancock, 1952). Abnormal sperms were counted in the same slides prepared for live and dead count. Sperms with head, mid-piece and tail abnormalities were counted in 200 live spermatozoa from different fields and the percentage of abnormalities was estimated.

Analysis of variance (ANOVA) was employed using computer software SPSS 13.0 to compare semen quality before and after enrofloxacin administration in Barbari bucks.

Results

The volume (mean±standard error mean (SEM)) of semen of Barbari bucks prior to drug administration was 0.75±0.02 ml and following enrofloxacin administration it ranged between 0.67±0.10 and 1.05±0.15ml in different ejaculates (Fig. 1).

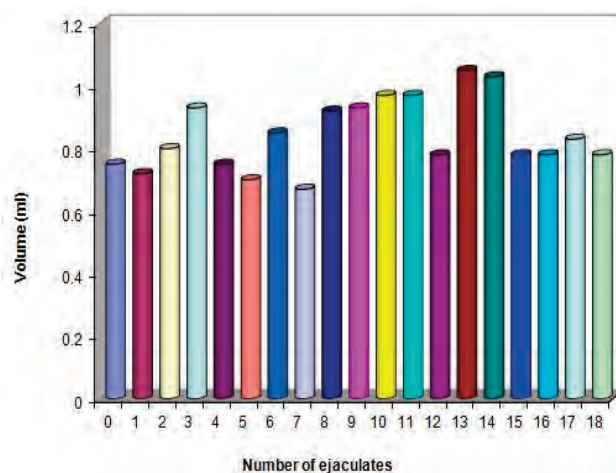


Fig. 1. Effect of parental administration of enrofloxacin on semen volume.

There was no significant difference ($P \geq 0.05$) in seminal volume between different ejaculates. The mass motility (mean±SEM) (0-5 scale) of semen before drug administration was 3.20±0.09 and following enrofloxacin administration it ranged between 1.83±0.11 and 3.75±0.11 in different ejaculates (Fig. 2). Mass motility decreased significantly ($P \leq 0.01$) and was lowest in 4th ejaculate as compared to mass motility prior to drug administration. The progressive motility (mean±SEM) (percent) of semen of Barbari bucks prior to drug administration was 65.12±1.74 % and ranged between 38.33±1.05 and 78.33±1.05 in different ejac-

ulates following enrofloxacin administration. Highest significant ($P \leq 0.01$) decrease in progressive motility was seen in 9th ejaculate as compared to progressive motility prior to drug administration (Fig. 3).

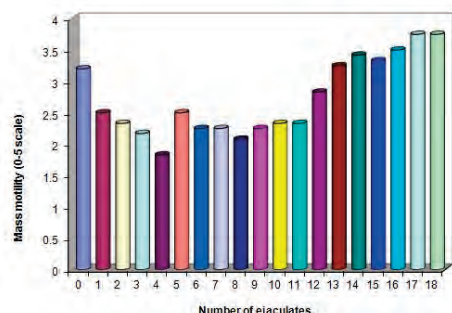


Fig. 2. Effect of parental administration of enrofloxacin on mass motility of spermatozoa.

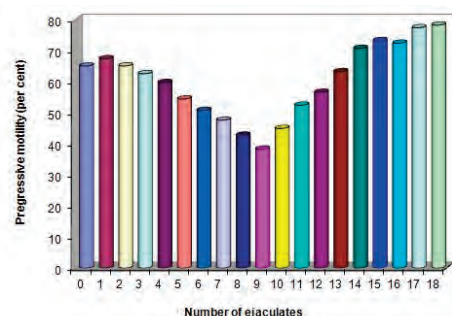


Fig. 3. Effect of parental administration of enrofloxacin on progressive motility.

The sperm concentration (mean±SEM) (millions/ml) of semen prior to drug administration was 3503.10 ± 99.85 and ranged between 2955 ± 35.66 and 3488.33 ± 126.74 in different ejaculates following enrofloxacin administration. Sperm concentration decreased significantly ($P \leq 0.01$) and was found to be lowest in 4th ejaculate as compared to sperm concentration prior to drug administration (Fig. 4).

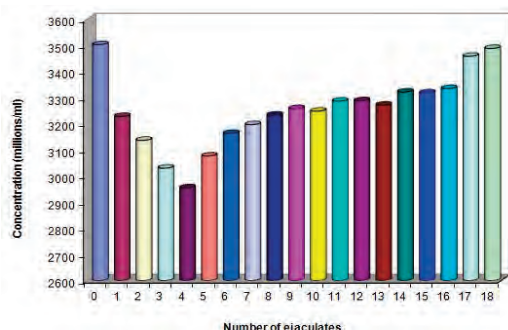


Fig. 4. Effect of parental administration of enrofloxacin on sperm concentration.

Prior to drug administration, the percent live spermatozoa (mean±SEM) in semen was $77.36 \pm 0.57\%$ and ranged between 31.75 ± 3.29 and 77.70 ± 0.75 percent in different ejaculates follow-

ing enrofloxacin administration. Percent live spermatozoa decreased significantly ($P \leq 0.01$) and was found to be minimum in 12th ejaculate as compared to percent live spermatozoa prior to drug administration (Fig. 5).

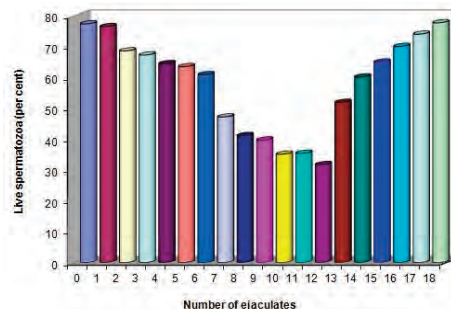


Fig. 5. Effect of parental administration of enrofloxacin on live spermatozoa.

The per cent head, mid piece and tail abnormalities of semen prior to drug administration was 1.80 ± 0.01 , 0.86 ± 0.00 and 1.66 ± 0.00 respectively and following enrofloxacin administration it ranged between 1.82 ± 0.00 and 4.11 ± 0.01 ; 0.87 ± 0.00 and 1.92 ± 0.01 ; 1.64 ± 0.00 and 3.87 ± 0.01 respectively in different ejaculates. Highest significant increase ($P \leq 0.01$) in per cent head, mid-piece and tail abnormalities of spermatozoa was observed in 4th, 3rd and 6th ejaculate respectively following parental administration of enrofloxacin as compared to ejaculate before drug administration (Fig. 6,7,8).

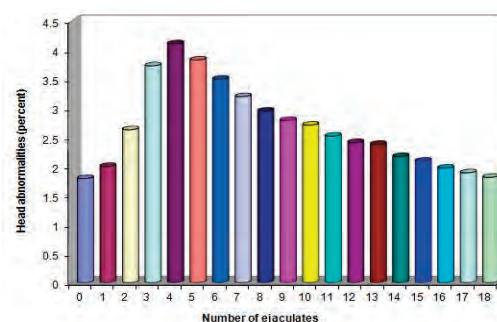


Fig. 6. Effect of parental administration of enrofloxacin on head abnormalities.

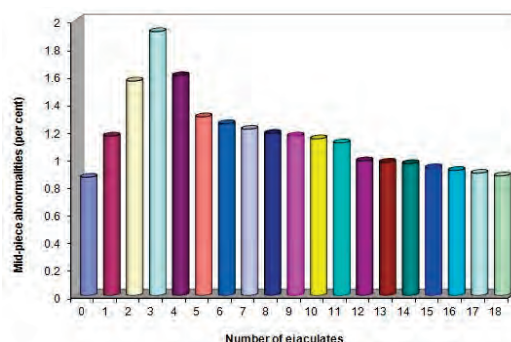


Fig. 7. Effect of parental administration of enrofloxacin on mid-piece abnormalities.

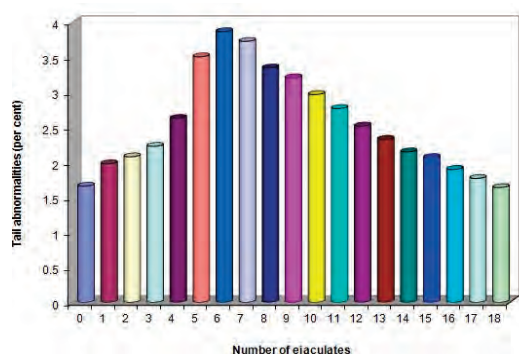


Fig. 8. Effect of parental administration of enrofloxacin on tail abnormalities.

Discussion

Antimicrobial therapy has been shown to significantly affect semen parameters in human and animal models (Schlegel, 1991). This effect on spermatogenesis may have a significant impact on animals treated with these agents. In present study, enrofloxacin administration had adverse effect on semen quality parameters. The present results indicated that, administration of enrofloxacin for 7 consecutive days, results in a marked reduction in mass motility, progressive motility, sperm concentration, viability and morphologically normal spermatozoa as compared to respective parameters prior to administration of the drug. This is in agreement with that of Abd-Allah *et al.* (2000) who reported that ofloxacin administration impaired testicular functions in rats. In addition, AndreeBen *et al.* (1993) reported that sperm concentration was significantly decreased after 50 days when ofloxacin was administered to patients daily for 20 days. On the other hand, Crotty *et al.* (1995) shown that ofloxacin at a dose of 10 mg/kg/day for 10 consecutive days in rats revealed testicular impairment, indicated by decreased haploid cells at 11 day after the treatment. Fattouh *et al.* (1992) and Berndtson and Foote (1976) reported a decrease in percent live spermatozoa following enrofloxacin administration in bucks and bovines respectively which is comparable to our study. Mass motility, progressive motility, sperm concentration, live spermatozoa and number of normal spermatozoa decreased significantly in buffalo bull (Verma *et al.*, 1999), epididymal sperms of male mice (Aral *et al.*, 2008) and chicken (Mohammed, 2008) following administration of enrofloxacin. The decrease in sperm motility could be due to direct and detrimental effects of drug on sperm physiology (Aral *et al.*, 2008). Induced sperm abnormalities in-

dicating point mutation in germ cells (Acharya *et al.*, 2004) which might have triggered structural changes in the cell organelles involved in head and tail formation, leading to sperm abnormality. In present study, the semen volume was not affected by enrofloxacin which may be attributed to less/no effect of drug on the secondary sex glands which secrete the major portion of the seminal fluid. This is in agreement with Verma *et al.* (1999) study in buffalo bull. These results indicate that enrofloxacin may directly interfere in the process of spermatogenesis. The results of our study also indicated a differential response of enrofloxacin administration to different seminal parameters in different ejaculates which may be a subject of further investigation. But the semen quality became normal after 14th ejaculate which may be attributed to spermatogenic length in goats that is estimated to last for 47.7 days (França *et al.*, 1999). Once the spermatogenic cells affected with drug were ejaculated after their development which lasted for 47.7 days, the semen quality became normal.

Conclusion

Following parental administration of enrofloxacin, there was no significant change in semen volume, whereas mass motility, progressive motility, sperm concentration, percent live spermatozoa decreased and percent sperm abnormality increased significantly in different ejaculates variably as compared to ejaculate before drug administration. The semen quality decreased progressively and then became slowly normal after 14th ejaculate.

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