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Original Article

EFFECT OF IVERMECTIN (MECTIZAN(R)) ON SEMEN AND TESTICULAR CHARACTERISTICS IN YANKASA RAMS

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Abstract

Ivermectin (Mectizan(R)) is a well-tolerated acaricide and anthelmentic produced by *Streptomyces avermitilis* cultures, with no side-effects at pharmacological doses. The aim of the study was to examine the effect of Mectizan(R) on semen parameters and testicular characteristics of Yankasa rams. Twenty, apparently, healthy rams aged 1½-2 years and weighing 25-30 kg were used for this study. The rams were housed in standard penwith access to feed and water provided *ad libitum*. Mectizan(R) was administered orally eight times at 14 days' intervals for 16 weeks at 200 mg/kg to all the rams. Semen was collected using electro-ejaculator once weekly for three weeks before the treatment and throughout the treatment period. Semen parameters before and after treatment were evaluated for volume, density, colour, motility, pH, live spermatozoa and sperm morphology. Five pairs of testes were removed through surgical castration from five rams randomly selected five days after the last treatment for histological examination using haematoxylin and eosin stain. There was no significant ($P > 0.05$) difference in the semen parameters of all the rams before and after the treatment. The volume of ejaculates was higher ($P < 0.05$) from weeks 4 to 16 compared to pre-treatment values. The seminal pH values were not significantly different. No lesions were observed on the histological section of the testes. It was concluded that repeated use of Mectizan(R) at the recommended dosage of 200 mg/kg did not alter semen parameters and testicular characteristics, and may not impair reproduction in Yankasa rams.

Key words: Mectizan(R),Yankasa ram, Semen characteristics, Safety, Testes, Histology.

Introduction

Mectizan(R),a brand name for Ivermectin(Merck Company, Canada), is an acaricide and anthelmentic drug of the family of avermectins, produced by *Streptomyces avermitilis* cultures. It is a well-tolerated drug with no side-effects in mammals at pharmacological doses. Ivermectin has been used against nematodes and ectoparasites (Chauasse *et al.*, 1992; WHO, 2019), the drug diffuses to all tissue compartments, except the

centralnervous system after being taken orally or by other routes (Daurio *et al.,* 1987; Maheu-Groux and Joseph, 2018). The reproductive activity of rams appears to be influenced by breeds, age, nutrition, geographical location, season and especially photoperiod, being the key environmental signal timing the reproductive cycle (Karagiannidis *et al.,* 2000; Kofi *et al.,* 2004; Azawi and Ismaeel, 2012; Al-Anazi et *al.,* 2017; Benia *et al.,* 2018). Ivermectin influences the reproductive potential in domestic animals without deleterious effects on semen quality or sexual desire in stallions (Janett *et al.,* 2001), rams (Schroder *et al.,* 1986), bucks (Onakpa *et al.,* 2010) and cattle (Leaning *et al.,* 1983). It improves semen quality (Wrona, and Krzyzanowski, 1995; Bearden *et al.,* 2004) and reproductive potential in ewes (Sania *et al.,* 2014; Benmoula *et al.,* 2017). Other studies reported deleterious effects of ivermectin on semen characteristics and hyaluronidase in rams (Tanyildizi and Bozkurt, 2002), and reproductive characteristics in cows (Sadek and Shaheen, 2015). The aim of this study was to investigate the effects of repeated orally-administered Mectizan(R) on semen parameters and testicular characteristics of Yankasa rams.

Materials and methods

Study location

The research was carried out at the National Animal Production Research Institute Shika, Ahmadu Bello University, Zaria, situated in the Northern Guinea Savannah zone of Nigeria and lying between latitudes 11° and 12°N andbetween longitude 7° and 8°E, at an elevation of 650m above sea level. The area has an average annual rainfall of 1100mm (Mortimore, 1975).

Experimental animals

Twenty (20), apparently, healthy rams aged 1½ -2 years and weighing 25-30 kg with clinically normal genitalia were used for this study. The rams were housed in standard pen. Theywere given access to *Digitaria* hay, supplementary concentrate and water *ad libitum*

Experimental design and treatment

The rams were acclimatised for one week prior to the commencement of the study. Thereafter, pretreatment semen was collected for three weeks, followed by administration of Mectizan(R). The drug was administered orally to all the experimental rams (n = 20) eight times at 14 days intervals for 16 weeks, at 200 mg/kg to all the rams.

Semen collection and evaluation

Semen collection was carried out on weekly basis from each ramduring the experiment by means of a hand-held electro-ejaculator (Electrojet®, Electrovet, Sao Paulo, Brazil). The semen was collected once weekly for three weeks before and throughout the treatment period. The electro-ejaculator consisted of a bipolarelectrode and variable source of alternating electric current. Semen was collected in the morning between 8:00am and 10:00am once weekly. Before semen collection, the animals were adequately restrained and the prepuce disinfected using 4.8% chloroxynol (Detol®) diluted with water. Prior to the rectal insertion of the probe, theelectrode was lubricated with petroleum (KY(R)) jelly to ease insertion. The lubricated probe of the electro-ejaculator was then inserted into the rectum and switched on, to produce an erection. Subsequently, ejaculation was induced using the manual button of theswitch on by pressing and holding it for 2 to 3 seconds. The power output was againpressed to cut off the output. This procedure was repeated after a rest period, equal to the duration of electrical stimulation, by increasing the duration of power output by one second onevery attempt until ejaculation recurred. The urethral process and end of the penis was held in a semen collection tube for the ejaculate to be collected in graduated plastic tubes for evaluation.

Semen examination and evaluation

Semen parameters evaluated were: semen volume (mL), semen colour, semen pH, sperm concentration ($x10^6$/mL), sperm motility (%), percentage live (%) and morphological defects (%).

Gross examination of semen

The procedure used for evaluation of semen quality traits was that described by Singh *et al.* (1987). The ejaculated semen was collected in calibrated glass tube, the volume and colour were immediately recorded before the tube was placed in a water bath at 37°C. The colour was read from the graduated collecting tube. For colour determination, a score of 4, 3 and 2was used for creamy, milky and watery/colourless, respectively. The pH was determined using pH-indicator strips (Neutralit®, Merck, Bucharest, Romania). Gross sperm motility wasassessed immediately by examining a drop of raw undiluted semen on a pre-warmed slide under a field microscope at the magnification of x 10 (Rota *et al.,* 1995). Semen motility was graded from 0 -100%; 0-20 very poor, 20-40 poor, 40-50 fair, 50-80 good, 80-90 very good and 90-100 excellent, with 100 for those that showed rectilineal movement and wave pattern. Sperm concentration was determined using the improved Neubauer haemocytometer after dilution in 0.05% formalsaline.

Microscopic examination of the semen

Sperm morphology was determined by methods described by Zemjanis (1970). The percentage live sperm and morphological sperm abnormalities/ defects were determined by examining semen smears, stained with eosin-nigrosin on a glass slide (Vilakazi and Webb, 2004; Michael *et al.,* 2008). Sperm abnormalities were classified as described by Blom (1972). The live-dead staining principle was based upon the observation that eosin B penetrated and stained the dead sperms, whereas the viable cells repelled the stain. The staining mixture consisted of 1% eosin B and 5% of nigrosin in 3% sodium citrate dehydrate solution. One drop of raw semen was added to one drop of the stain, mixed thoroughly and a fresh smear was made. The slide was then examined under high power (x 100) and at least 100 cells (both stained and unstained) were counted, and a percentage of each was estimated.

Testicular histology

Five pairs of testes were removed through surgical castration from five rams, randomly selected 5 days after the last treatment for histological examination. The slides were stained using Haematoxylin and Eosin and examined at x400

Data analyses

Data were expressed as means and standard error of the mean ± SEM. Data were analysed using paired Student's *t-test* with SPSS/PC Computer Programme, (Version 20.0, SPSS®, Chicago IL, USA). Differences with confidence values of $P < 0.05$ were considered significant.

Results

Semen characteristics

The result obtained from this study revealed no significant ($P > 0.05$) difference in the semen parameters (motility, density, morphology, percentage live and abnormality of all the rams before and after the treatment. However, the volume of ejaculates was higher ($P < 0.05$) from weeks 4 to 16 compared to pre-treatment values, but the seminal pH was not significantly ($P > 0.05$) lower than the pre-treatment values (Table 1).

Testicular histology

No histological changes were observed in the testicular tissue. The seminiferous tubules contained spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, spermatozoa and Sertoli cells. No signs of testicular degeneration wereobserved in the testicles (Plate I)

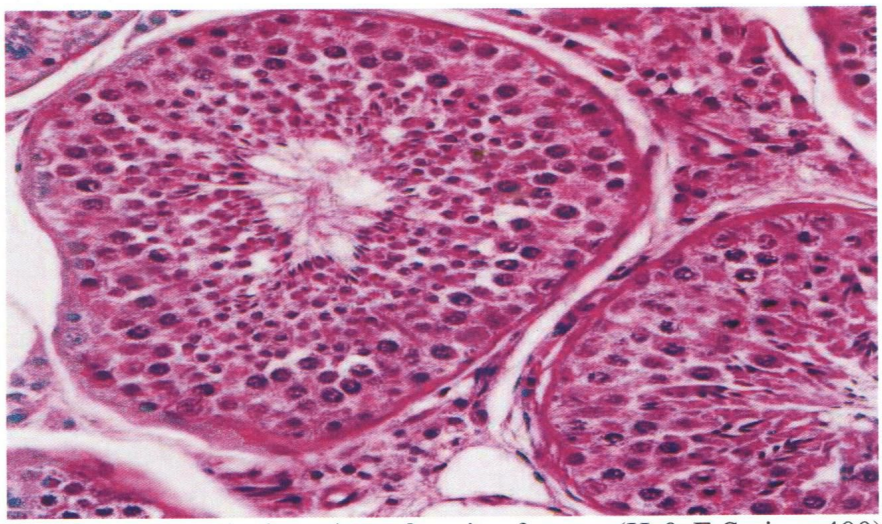

Plate I: Histological section of testis of a ram (H & E Stain x 400)

Discussion

The study revealed that repeated use of oral ivermectin has no deleterious effects on the spermiogram and testicular characteristics of Yankasa rams. Semen motility, concentration, live spermatozoa and spermatozoa abnormality were not significantly different in both pre- and post-treatment values. This finding agreed with the result obtained by Schroder et al. (1986) and Benmoula *et al.* (2017) in rams, but disagreed with the reports of Naoman (2012) and Tanyildizi and Bozkurt (2002), who showed a significant difference in semen characteristics of rams treated with ivermectin. Variable volumes of ejaculates (0.5-2.5 mL) in rams has been reported using electroejaculation method (Van Tonder, 1977). The result obtained from this work showed no differences in semen volume from week 1 to 4. Thereafter, a significant increase was observed throughout the post-treatment period. This finding agreed with the result obtained by Schroder *et al.* (1986) and Tanyildizi and Bozkurt (2002) in rams, and disagreed with the findings of Onakpa *et al.* (2010), who reported a decrease in semen volume in bucks treated with ivermectin. The seminal pH was found to decrease consistently, though not significantly different from the pre-treatment values. This finding agreed with the work of Schroder *et al.* (1986), who reported a decrease in seminal pH following ivermectin treatments in rams. The pH decrease could be attributed to increase fertility (Sumington, 1961). The overall effect of Mectizan$^{(R)}$ on spermiogram could be due to increase in stimulation of accessory glands, increase in testosterone level and increase in serum hyaluronidase activity which account for increased semen volume and decrease in pH (Hirayama *et al.,* 1989; Tanyildiziand Bozkurt, 2002). No histological abnormality was observed in the testicles of the rams; and this result agreed with the finding of Schroder *et al.* (1986),who reported that ivermectin has no effect on the testicular architecture in rams.The finding may be due to wider margin of safety of the drug at therapeutic dose (Maheu-Groux and Joseph, 2018).

It was concluded that repeated use of Mectizan$^{(R)}$ at the recommended dosage of 200 mg/kg did not alter significantly the semen parameters and testicular characteristics of Yankasa rams.

Table I: Effect of oral Mectizan(R) on semen characteristics (Mean ± SEM)

		Weeks of semen collection in Yankasa rams (n = 20)				
Semen characteristic	Groups	1 – 4	5 - 8	9 – 12	13- 16	Mean ± SEM
Volume (ml)	Post-treatment	0.7±0.23 - 0.8±0.4 [a]	0.8±0.3 [a]- 0.8±0.1 [a]	0.8±0.5 [a] -0.8± 0.3 [a]	0.8±0.2 [a] - 0.7±0.0 [a]	0.8±0.3
	Pre-treatment	0.7±0.2 [b] - 0.7±0.2 [b]	0.7±0.2[b] -0.7±0.2 [b]	0.7±0.2 [b]-0.7± 0.24 [b]	0.7±0.2 [b] -0.7± 0.24 [b]	0.7±0.2
Gross Motility (%)	Post-treatment	80±0.5- 80±0.2	76±2.6-77± 6.5	80±0.1-72±5.60	70±7.30 -64±2.4	74±4.3
	Pre-treatment	80± 0.6 ¯ 78±0.3	80±1.2¯78±0.3	80±0.1¯ 80± 0.91	81±0.22¯82±0.1	80±0.5
Individual Motility (%)	Post-treatment	88±0.2- 86±0.9	90±0.1-80±0.9	85±0.1- 87±0.12	80± 3.91-86 ± 0.4	86±0.4
	Pre-treatment	90±0.45-88±0.4	90±0.2-84±0.2	87±0.2- 90 ± 0.21	85± 0.52-89± 0.6	88±0.4
Sperm concentration ($\times 1^6$/ml)	Post-treatment	300±52. - 305±43	300±3-297±82	295±88- 289±32.2	287±62- 280±52.6	290±40
	Pre-treatment	300±54-300±54	300±54-300±54	300±54-300±54.51	300±54- 300±54.5	300±54
Live spermatozoa (%)	Post-treatment	86±1.93- 88±6.3	87±8.9-87±6.3	88±62 - 86.5±43.3	87±32.3-81.2±55	84±34
	Pre-treatment	88± 2.93-88±2.3	88±2.9-88±2.9	88±2.9-88.5 ± 2.93	88.5±2.9-88.5±2.9	88±2.4
Sperm abnormality (%)	Post-treatment	9.2±1.55-9.9±6.1	11±0.1- 9.8±0.8	9.5±1- 10.5 ± 0.32	10.1±0.1-10.9 ± 0.7	10±0.8
	Pre-treatment	10.8±0.6-11±0.6	11±0.6-11±0.5	10±0.6-10.8 ± 0.55	10.8±0.6-10.8± 0.6	10.5±0.5
Sperm pH	Post-treatment	5.9±1.2[a]-5.9±0.1 [a]	5.7±0.3-5.1±0.6	5.2±0.0-5.9 ± 0.5	6.1±0.4-5.0±1.02	5.5±0.3
	Pre-treatment	6.9 ±0.2[a] - 6.1±0.2	6.1±0.2- 6.1±0.2	6.1±0.2-6.1±0.2	6.1±0.2-6.09± 0.2	6.1±0.2

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Conflict of interest statement
No conflict of interest

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