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Effects of Diminazene aceturate and Ivermectin on Semen and Serum parameters of the Red Sokoto buck

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Abstract: The effects of diminazene aceturate and ivermectin on semen parameters, serum testosterone and serum follicle stimulating hormone levels of the red Sokoto buck was investigated. Twenty seven red Sokoto bucks, at the age of 2 years and weighing between 32 – 34kg were used. After administration of the drugs, semen and sera samples were collected 1, 24, 72, and 192 hours for analysis. The parameters studied viz semen volume, percentage motility of sperm, sperm concentration, live sperm percentage, semen glucose level, serum testosterone and serum follicle stimulating hormone were found to decrease significantly ($P<0.05$) when compared with the control group throughout the collection period. However, the drugs did not affect the live sperm percentage and ivermectin did not affect semen glucose level. A relationship was established between spermatological characteristics and serum testosterone and follicle stimulating hormone levels. These findings indicate that the drugs investigated in this study decreased semen parameters and serum testosterone and follicle stimulating hormone. It was concluded that diminazene aceturate and ivermectin should be used cautiously in red Sokoto bucks meant for breeding due to the deleterious effects they were observed to have on fertility parameters.

Keywords: Red Sokoto Buck, Diminazene aceturate, Ivermectin, Fertility.

Introduction

Goats are among the most important domestic farm animals in the world as a source of meat, milk, skin and wool (Guss, 1977). In Nigeria, it has been estimated that there are about 34.5 million goats. The goats' population in Nigeria makes it the second most important livestock specie (Adu *et al.*, 1979). Little information is available on the diseases of goats (Nelson, 1980).

Many of the drugs used in goats have been approved for use in cattle and sheep, such drugs may not have been approved for use in goats. However, goats have many of the attributes of sheep (multiple births) and cattle (milk production) and many of the diseases seen in goats are similar to those seen in sheep and cattle (Lloyd, 1982).

Three main varieties of goats are recognized in Nigeria, the Sahel, Desert or West African long-legged Goat, the Red Sokoto Goat and the West African Dwarf Goat (Aliyu, 1990).

Research findings have shown that drugs, both synthetic and natural products have considerable effects on the male reproductive system, especially the

spermatozoa of domestic animals and man. The bark of *Corynanthe yohimbe* (*Yohim-be*) and *Pausinystalia johimbe* (*Rubiaceae*), are used to improve fertility, body building and performance in humans, and in captive breeding program of wild animals (Abdulhakeem *et al.*, 2006). Kuncheva *et al.,* 1981 reported that, toxicity of yohimbe causes stimulation of the mitotic activity of spermatogonia in mature male rats and increases spermatozoa counts while Smith *et al.,* 1987 reported that yohimbine increased the rate of copulation and reduces the intercopulatory interval in rats. When administered in dogs yohimbe increased the spermatozoal output and prevented the decrease in volume of ejaculates (Yonezewa *et al.,* 1991). A significant improvement in semen volume, sperm density, and sperm motility was noticed in men treated with Clomiphene citrate (Micic and Dotlic, 1985, Soler *et al.,* 1980).

For several animal species (Amann, 1970; Amann *et al.,* 1974; Amann, 1981; Gebaver *et al.,* 1974; Holtz and Foote, 1972; Olar *et al*., 1983; Swierstra, 1966) and man (Johnson, 1982), the daily spermatozoal output (DSO) is consistently lower than the daily spermatozoal production (DSP). Several factors, including spermatozoal losses in the collection equipment (Amann, 1970; Amann *et al.*, 1974), phagocytosis and epididymal absorption of spermatozoa (Amann *et al.,* 1974; Bedford, 1976), or overestimation of DSP (Amann, 1970; Amann, 1981) which have been studied, failed to account for the quantitative differences between DSO and DSP.

In veterinary practice, several chemotherapeutic and other chemical agents, both synthetic and natural are administered to animals to treat infectious diseases and/or to achieve predetermined physiological modifications such as anesthesia or smooth muscle contraction amongst others (Benson, 2000 and Abdulhakeem *et al.,* 2006). These drugs may have beneficial or deleterious effects on the fertility of the animals.

The aim of this research therefore is to investigate the effects of diminazene aceturate or ivermectin on some reproductive parameters of the red Sokoto buck.

Materials and methods

Experimental animals

Twenty seven randomly sourced adult red Sokoto bucks, between the ages of 2- 2.5 years and weighing between 32 to 34Kg were selected for this study on the basis of their soundness for breeding purposes. (McEntee, 1970 and Roberts, 1971).

During the study, bucks were housed in brick pen houses made of concrete floors in the large animal unit of the Usmanu Danfodiyo University Veterinary Teaching Hospital (UDUVTH), Sokoto, in groups of nine bucks per group. They were fed with wheat bran and bean husks twice daily, allowed some level of free grazing and tap water provided *ad libitum*.

Experimental design

Bucks were randomly assigned in a block design to one of three groups comprising of one negative control and two treatment groups. Each of the three groups comprised of nine bucks each. The nine bucks in each group were further re grouped into two (one having 5 bucks and the other 4 bucks) from which semen and sera samples were collected simultaneously from the first five bucks in all the three groups but alternately (purposive sampling) (Olowu, 2004) for the remaining four bucks in the groups on the next sampling. Samples were thus collected from the first 5 bucks of the control and the two treatment groups concurrently and the same was done for the remaining 4 bucks of the control and the two treatment groups on the following collections.

Administration of test drugs

The treatment groups comprised of diminazene aceturate treated and ivermectin treated group while bucks of the control group were not administered any of the drugs. The diminazene aceturate treated group was given an intramuscular injection of a solution of 188.8mg/ml of diminazene aceturate (Berenil®) (FARVET Bladel, Holland) at a dose of 3.5mg/kg body weight while the ivermectin treated group was given a subcutaneous injection of a 1% w/v solution of invermectin (V.M.D. Ltd, Arendonk, Belgium) at a dose of 0.2mg/kg body weight.

Sample Collection and Analysis

Semen and sera samples were collected from the control group, diminazene aceturate treated and ivermectin treated groups after 1, 24, 72 and 192 hours post drug administration. To obtain semen samples, bucks were restrained in a standing position and semen was collected by electro ejaculation as described by Baracaldo *et al*., 2006. Immediately following collection of semen, evaluation for percentage motility of sperm was carried out. This was determined by the progressive and non-progressive movement of sperm observed under a compound microscope (Laborlux II, Leitz Germany) as described by VijayKumar *et al.,* 2004 and Anderson *et al.,* 1983. The sperm count was determined under a Neuber haemocytometer (Superior, Marienfeld, Germany) as described by Al-Shabanah, 1997. To evaluate for the dead and live sperm percentage the sperm suspension was stained with eosin negrosin; smears were made on slides, air dried and made permanent. The slides were examined by bright field as described by Al-Shabanah, 1997 and Anderson *et al.,* 1983.

Blood samples collected by jugular venipuncture were centrifuged at 3000rpm for 15 minutes and sera harvested for assessment of testosterone and follicle

stimulating hormone concentration by radioimmunoassay using a testosterone and follicle-stimulating hormone EIA test kits respectively (Clinotech® Diagnostics and Pharmaceuticals, Inc., Canada).
The determination of the semen glucose level was done by the glucose oxidase method which employed the use of a digital photo colorimeter.

Statistical Analysis
Statistical analysis of the data obtained before and after the drugs treatment was performed using the independent Student's *t*-test. All results are expressed as means ± standard deviation. Results were considered to be statistically significant at $P < 0.05$ as previously described by Norusis, 1986.

Results

Effects of diminazene aceturate on semen and serum parameters
The volume of semen obtained for the control group was 0.36 ± 0.06ml and values obtained at 1, 24, 72 and 192 hours after administration of diminazene aceturate (0.28 ± 0.16ml, 0.27 ± 0.18ml, 0.27 ± 0.11ml, 0.28 ± 0.11ml respectively) were significantly ($P<0.05$) lower (Table 1). Also the values obtained for sperm motility 1 hour, 24 hours, 72 hours and 192 hours after administration of diminazene aceturate were 32 ± 11.35%, 33 ± 21.33%, 34 ± 31.21% and 34 ± 21.31% respectively but the control group value was 70 ± 0.0%. The control group value for sperm concentration was 332.27 ± 133.59million/ml but were found to be 50.10 ± 17.58million/ml, 66.30 ± 11.30million/ml, 76.10 ± 23.10million/ml, 79.00 ± 23.61million/ml at 1 hour, 24 hours, 72 hours and 192 hours after administration of diminazene aceturate. The values obtained for the control group live sperm cells percentage was 85.90 ± 5.39 and at 1 hour, 24 hours, 72 hours and 192 hours after administration of diminazene aceturate values obtained were 88.50 ± 3.37, 85.91 ± 2.10, 86.00 ± 2.30, and 85.60 ± 2.22 respectively. Semen glucose level before administration of diminazene aceturate was 3.50 ± 0.23mmol/litre. The values obtained at 1 hour, 24 hours, 72 hours and 192 hours after administration of diminazene aceturate were 2.24 ± 0.40mmol/litre, 2.31 ± 0.31mmol/litre, 3.40 ± 0.26mmol/litre, and 3.50 ± 0.31mmol/litre respectively. The control group values for serum testosterone level was 9.10 ± 0.42ng/ml but were observed to be 1.75 ± 1.77ng/ml, 1.77 ± 1.61ng/ml, 2.132 ± 0.60ng/ml, and 3.31 ± 0.71ng/ml at 1 hour, 24 hours, 72 hours and 192 hours respectively after diminazene aceturate administration. In the case of follicle stimulating hormone levels, the control group value was 367.35 ± 23.96mIU/ml and 1 hour, 24 hours, 72 hours and 192 hours after administration of diminazene aceturate the values were 6.25 ± 1.77mIU/ml, 9.31 ± 1.89mIU/ml, 12.21 ± 3.10mIU/ml and 12.13 ± 3.36mIU/ml respectively.
When compared with the values of the control group, significant differences ($P<0.05$) were observed in the values of semen volume, percentage motility, sperm concentration per ml and semen glucose levels throughout the collection period. Serum testosterone and follicle stimulating hormone levels also differ significantly ($P<0.05$) throughout the collection period after administration of diminazene aceturate (Table 1).

Effects of ivermectin on semen and serum parameters
The volume of semen obtained for the control group was 0.36 ± 0.06ml and values obtained 1 hour, 24 hours, 72 hours and 192 hours after administration of ivermectin were 0.23 ± 0.07ml, 0.24 ± 0.06ml, 0.27 ± 0.03ml, 0.35 ± 0.07ml respectively (Table 2). The values obtained for sperm motility 1 hour, 24 hours, 72 hours and 192 hours after administration of ivermectin were 60 ± 10.54%, 63 ± 9.32%, 69 ± 1.35% and 69 ± 1.66% respectively but the control group value was 70.0 ± 0.0%. The control group value for sperm concentration was 332.27 ± 133.59million/ml but was found to be 250.4 ± 84.08million/ml, 252.6 ± 73.03million/ml, 255.3 ± 62.03million/ml, 257.4 ± 61.3million/ml at 1 hour, 24 hours, 72 hours and 192 hours after administration of ivermectin. The value of the control group live sperm cells percentage was 85.90 ± 5.39 but at 1 hour, 24 hours, 72 hours and 192 hours after administration of ivermectin values obtained were 87.5 ± 3.69, 86.31 ± 2.41, 85.56 ± 3.26, 85.71 ± 3.11 respectively. Semen glucose level value for control group was 3.5 ± 0.23mmol/litre, values obtained at 1 hour, 24 hours, 72 hours and 192 hours after administration of ivermectin were 3.38 ± 0.24mmol/litre, 3.43 ± 0.13mmol/litre, 3.47 ± 0.22mmol/litre, and 3.49 ± 0.11mmol/litre respectively. The control group value for serum testosterone level was 9.10 ± 0.42ng/ml but were observed to be 3.80 ± 1.13ng/ml, 3.71 ± 2.11ng/ml, 3.77 ±12.01ng/ml, and 3.88 ± 0.11ng/ml at 1 hour, 24 hours, 72 hours and 192 hours respectively after ivermectin administration. The value obtained for follicle stimulating hormone level before ivermectin administration was 367.35 ± 23.96mIU/ml but at 1 hour, 24 hours, 72 hours and 192 hours after administration of ivermectin the values were found to be 22.5 ± 20.51mIU/ml, 33.31 ± 21.21mIU/ml, 39.32 ± 30.13mIU/ml and 41.3 ± 33.12mIU/ml respectively.
When compared with the values of the control group, significant differences ($P<0.05$) were observed in the values of semen volume, percentage motility, and sperm concentration per ml throughout the collection period. Serum testosterone and follicle stimulating hormone levels also differ significantly throughout the collection period after administration of ivermectin (Table 2).

Table 1: Mean (±SD) semen and serum parameters in bucks before and after diminazene aceturate treatment

Parameters	Control	1hr	24hrs	72hrs	192hrs
Volume of semen (ml)	0.36±0.06	0.28±0.16*	0.27±0.18*	0.27±0.11*	0.28±0.11*
Percentage motility	70±0.0	32±11.35*	33±21.33*	34±31.21*	34±21.31*
Concentration/ml (millions)	332.27±133.59	50.10±17.58*	66.30±11.3*	76.10±23.1*	79.00±23.61*
Live sperm %	85.90±5.39	88.50±3.37	85.91±2.10	86.00±2.30	85.60±2.22
Semen glucose mmol/litre	3.5±0.23	2.24±0.40*	2.31±0.31*	3.40±0.26	3.50±0.31
Serum testosterone (ng/ml)	9.10±0.42	1.75±1.77*	1.77±1.61*	2.13±0.60*	3.31±0.719*
Serum FSH (mIU/ml)	367.35±23.96	6.25±1.77*	9.31±1.89*	12.21±3.10*	12.13±3.36*

* Significant difference at $P<0.05$ compared to value obtained before treatment

Table 2: Mean (±SD) semen and serum parameters in bucks before and after ivermectin treatment

Parameters	Control	1hr	24hrs	72hrs	192hrs
Volume of semen (ml)	0.36±0.06	0.23±0.07*	0.24±0.06*	0.27±0.03*	0.35±0.07*
Percentage motility	70±0.0	60±10.54*	63±9.32*	691±1.35	69±1.66
Concentration/ml (millions)	332.27±133.59	250.40±84.08*	252.60±73.03*	255.30±62.03*	257.40±61.3*
Live sperm %	85.90±5.39	87.50±3.69	86.317±2.41	85.56±3.26	85.71±3.11
Semen glucose mmol/litre	3.5±0.23	3.38±0.24	3.43±0.13	3.47±0.22	3.49±0.11
Serum testosterone (ng/ml)	9.10±0.42	3.80±1.13*	3.71±2.11*	3.77±1.01*	3.88±0.11*
Serum FSH (mIU/ml)	367.35±23.96	22.50±20.51*	337.315±21.21*	39.32±30.13*	41.30±33.12*

* Significant difference at $P<0.05$ compared to value obtained before treatment

Discussion

The effect of diminazene aceturate and ivermectin on semen and serum parameters of the red Sokoto buck was studied.

The significant ($p<0.05$) decrease in volume of semen observed in the diminazene aceturate treated group is in agreement with a similar study on rams which reported that diminazene aceturate caused significant decrease in semen volume compared to the control group (Tanyildizi and Turk, 2004). A decrease in volume was also observed in the ivermectin treated group; this is in disagreement with findings of a similar study using ivermectin on rams where Tanyildizi and Bozkurt, 2002 reported that the levels of semen volume increased significantly ($p<0.01$) in comparison with the control group. The discrepancy may be attributed to the species or breed variation of animals used and also the dosage of drugs administered. Climatic variations may also be a factor responsible for the differences in findings.

Decreased percentage motility was also observed in the diminazene aceturate and ivermectin treated groups of the present study. The results obtained in these treatment groups are in agreement with similar studies using diminazene aceturate on ram (Tanyildizi and Turk, 2004) and ivermectin in sheep (Tanyildizi and Bozkurt, 2002).

A decrease in sperm concentration was observed in the diminazene aceturate and ivermectin treated groups; this may be attributed to the corresponding decrease in serum testosterone and follicle stimulating hormone levels caused by these drugs. Follicle stimulating hormone is necessary to increase the level of the androgen binding protein production by sertoli cells and to develop the blood-testis barrier and other functions of the cells. Once the sertoli function is developed, testosterone alone will maintain spermatogenesis. The yield of spermatozoa, however, is increased if follicle-stimulating hormone is present.

Follicle stimulating hormone is known to increase the yield of spermatogonia by preventing atrasia of differentiating type spermatogonia. Therefore, the decreased sperm concentration noted in the present study, may be attributed to the corresponding decrease in the levels of follicle stimulating hormone and testosterone noted. The results of this study is in agreement with the findings of Doshi *et al.,* 1994 who obtained a positive correlation between testosterone

concentration, and total sperm count and sperm motility in buffalo-bulls treated with clomiphene citrate, indicating that low levels of testosterone was always associated with low values of semen characteristics.
In a similar study with ivermectin in sheep (Tanyildizi and Bozkurt, 2002), the values of sperm concentration were established to decrease highly significantly ($P<0.001$) when compared with the control group. This is in agreement with the results obtained in the present study. Also in agreement with the present study are the findings of Tanyildizi and Turk, 2004 where results using diminazene aceturate on ram caused a significant ($P<0.01$) decrease in sperm concentration, volume and motility compared to the control group.

Conclusion and Recommendation

In conclusion, the drugs diminazene aceturate and ivermectin investigated in this study should be used cautiously in red Sokoto bucks meant for breeding purposes; this is because these drugs have been found in this study, to decrease ejaculate volume and semen glucose level, sperm motility and concentration, serum testosterone and follicle-stimulating hormone levels in the red Sokoto buck. These parameters are indices of fertility in the male animal; there is therefore a tendency to decrease fertility when these drugs are administered to bucks meant for breeding purposes. Compliance with withdrawal time is recommendable where use of any of these drugs is unavoidably administered to bucks meant for breeding.

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