Testes Growth, Semen Characteristics and Hormone Levels in Hemiorchidectomized West African Dwarf Rams[1]

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ABSTRACT

Fifteen West African dwarf lambs castrated at six weeks *post partum* were assigned randomly to three treatments with five successive replications: a) Left testis orchidectomy (castration of the left testis, LC); b) Intact testis (zero castration, IN); and right testis orchidectomy (castration of the right testis, RC). Between 20 and 24 months *post partum*, collection of data from the treatments on all the variables began. The variables evaluated were: 1) semen characteristics; 2) seminiferous tubule diameter; 3) testis circumference; 4) plasma LH, FSH and prolactin levels. Semen was collected by electroejaculation from five rams per treatment. Semen characteristics measured were volume of semen, percentage progressive motility, total abnormal sperm percentage, live sperm and sperm concentration. There was no significant difference in the semen characteristics evaluated except for sperm concentration, in which both LC and RC had twice the sperm concentration of IN testis. The seminiferous tubular diameter of LC did not differ from RC, while both were greater than the left and right intact testes. There was no significant difference in testis circumference between LC and RC, although LC and RC differed from mean testis circumference of IN. There was no significant difference from the effect of castration on normal plasma levels of LH, FSH and prolactin. Libido in hemiorchidectomized rams, though not measured, appeared higher than in the intact rams.

COMPENDIO

Quince carneros enanos del Africa Occidental, castrados seis semanas *post partum*, fueron sometidos al azar, en cinco etapas sucesivas, a tres tratamientos: (a) Orquidectomía del testículo izquierdo (castración del testículo izquierdo LC), (b) Testículo intacto (castración zero, IN), y (c) Orquidectomía del testículo derecho (castración del testículo derecho RC). Entre los 20 y 24 meses *post partum*, se inició el registro de datos resultantes de los tratamientos sobre todas las variables. Se estudiaron las siguientes variables: 1) Características del semen, 2) Diámetro del tubo seminífero, 3) Circunferencia del testículo, 4) Plasma LH, FSH y los niveles de prolactina. El semen fue colectado mediante electroeyaculación de cinco carneros por tratamiento. Las características observadas en el semen fueron: volumen del semen, porcentaje de mobilidad progresiva, porcentaje total de esperma anormal, espermas vivos y concentración de esperma. No hubo diferencia significativa entre las características evaluadas, salvo concentración de esperma, en que LC no fue diferente de la de RC, la cual era dos veces la concentración de esperma del testículo IN. El diámetro del tubo seminífero de LC no era diferente del testículo intacto derecho. No hubo diferencia significativa en circunferencia del testículo entre LC y RC, aunque la de LC o la RC fue diferente de la circunferencia media del testículo IN. No hubo diferencia significativa del efecto de la castración sobre los niveles normales de plasma de LH, FSH y prolactina. El líbido en carneros semi-orquidectomizados, aunque no fue medido, resulta ser más alto que en carneros intactos.

INTRODUCTION

Hemiorchidectomy has been shown (6) to result in hypertrophy of the remaining testis and elevation in the plasma luteinizing hormone (LH) in Blackface, Finn and Merino rams; these effects were breed-dependent. Boockfor (4), working with bulls, found that testis weight, seminiferous tubular diameter, epithelial cell height and the ratio of testis to body weight were greater in the unilaterally castrated bulls than those of intact bulls or bulls surgically rendered unilaterally cryptorchid. Bookfor (4) also found that unilateral castration at 3 months caused greater compensatory hypertrophy and associated changes relative to comparable measures in intact and unilaterally cryptorchid bulls of the same age group vis-à-vis bulls of six and nine months of age subjected to similar surgical treatments. The hypothesis to be tested here was whether hemiorchidectomy in West African dwarf rams at six weeks *post partum* could lead to compensatory testis growth, increased semen production and maintenance of normal plasma FSH, LH and prolactin levels by the remaining testis.

MATERIALS AND METHODS

Fifteen West African dwarf ram lambs were assigned randomly to three treatments in five successive replications, as follows: a) left orchidectomy (castration of the left testis, LC); b) intact (zero castration,

1 Received for publication 9 October 1986.

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IN); and c) right orchidectomy (castration of the right testis, RC). The ram lambs were orchidectomized at six weeks **post partum** by crushing the spermatic cord with burdizzo and the area of the skin overlying the cord was sterilized with penicillin antibiotic spray to prevent infection through bruises. At between 20 and 24 months of age, semen was collected from five rams in each treatment by electroejaculation and the following semen characteristics measured: total volume (ml), percentage progressive motility, percentage abnormality, percentage live sperm and sperm concentration ($x10^9$/ml). The methods of semen harvest and estimation of semen characteristics were as described by Chiboka (2, 3). Horizontal axis testis circumference was taken to be the horizontal section of the testis with the greatest girth. The mean testis circumference of the intact testis was used to compare the circumference of the single left or right testis castrates.

Histology: Two rams from each treatment were sacrificed and testes removed for paraffin embedding. Five micrometer-thick serial sample sections were obtained in a rotary microtome and stained routinely for haematoxylin and eosin.

Measurement of seminiferous tubules: The diameter of at least one hundred seminiferous tubules in each section of at least five different paraffin blocks of each ram was measured using the micrometer eye piece and stage according to Carleton, Drusy and Wellington (1). The design of the trials was considered as a one way analysis of variance and was analysed as such, as described by Steel and Torrie (10).

Biochemical Estimations: Blood was taken by jugular vein puncture from each of five rams in each treatment. The blood was put in predried heparinized tubes and gently shaken to redesolve the heparin. The heparinized blood samples were centrifuged for 10 minutes at 3000 rev/minute and the plasma separated for LH, FSH and prolactin assay. The method of analysis used was as described in kits for estimation of plasma LH, FSH and prolactin levels from Radio Amersham, England. The rams used in this research were kept in the University of Ife Teaching and Research Farm.

RESULTS

Table 1 shows semen characteristics of intact and unilaterally orchidectemised West African dwarf rams. There is no significant ($P > 0.05$) effect of hemiorchidectomy in semen volume, motility, total abnormality and live sperm in LC, IN and RC. LC and RC did not differ ($P > 0.01$) in sperm concentration, but each showed significantly ($P < 0.05$) higher sperm concentration than the intact rams (IN). Table 2 shows the analysis of variance in the semen characteristics. Excepting sperm concentration ($P < 0.01$), no other variable showed treatment effect ($P > 0.05$). Table 3 shows significant ($P < 0.01$) treatment effect and Duncan's New Multiple Range Test indicates that LC did not differ from RC ($P > 0.05$), but both LC and RC were significantly ($P < 0.01$) greater in seminiferous tubular diameter than left intact testis (LI) or right intact testis (RI). Table 4 shows significant ($P > 0.01$) treatment effect on testis circumference, and Duncan's test indicates no significant ($P > 0.05$) difference between LC and RC. Left castrate or RC differed ($P < 0.01$) from mean testis circumference on IN. Table 5 shows no significant ($P > 0.05$) difference of the effect of type of castration on the levels of FSH, LH or prolactin. Also, Duncan's test shows no significant ($P > 0.05$) differences in the mean values of each of the three hormones measured within each treatment.

DISCUSSION

The results presented in Table 1 indicate that the effects of orchidectomy of either the left or right testis did not lead to statistical differences when compared with intact testis in semen volume, percentage motile sperm, percentage total sperm abnormality, and percentage live sperm. However, the rams with only one testis, left or right, significantly produced higher sperm concentration than the intact rams. This clearly shows that when one testis is removed in these rams by six weeks *post partum*, the remaining testis compensates for the lost one.

Table 1. Mean semen characteristics of unilaterally orchidectomized WAD rams.

Variables	State of Testis		
	LC	IN	RC
Volume (ml)	0.96[a]	0.89[a]	1.01[a]
Motility (%)	76.32[a]	75.9 [a]	75.66[a]
Total Abnormality (%)	9.84[a]	10.08[a]	9.68[a]
Live Sperm (%)	86.30[a]	85.38[a]	84.74[a]
Sperm Concentration ($x10^9$/ml)	1.73[a]	0.78[b]	1.61[a]

LC = Left testis castrated; IN = Intact; RC = Right testis castrated.

Within a variable, a row of means with the same superscript are not significantly ($P > 0.05$) different.

Table 2. Analysis of variance: Semen characteristics of unilaterally orchidectomized West African dwarf rams.

Source	df	Mean Squares														
		Volume (ml)			Motility %			Total Abnormality			Live Sperm			Sperm Cone x10^9ml		
		LC	IN	RC	LC	IN	RC	LC	IN	RC	LC	IN	RC	LC	IN	RC
Type of castration (treatment effect)	2	–	0.02^{ns}	–	–	0.56^{ns}	–	–	–	–	–	11.29	–	1.36**	–	–
Error	12	–	0.057	–	–	10.27	–	–	0.19	–	–	8.50	–	0.02	–	–
Mean	–	0.96^a	0.89^a	1.01^a	76.32^a	75.9^a	75.68^a	9.84^a	10.08	9.68^a	86.30^a	85.38^a	84.74^a	1.73^a	0.78^b	1.61^a
SEM	–	–	0.11	–	–	1.43	–	–	0.19	–	–	1.30	–	0.06	–	–

ns = Not significant ($P > 0.05$): ** = significant ($P < 0.01$).

a Within a variable, a row of means with the same superscript are not significantly ($P > 0.05$) different. SEM = Standard error of the mean.

Table 3. Analysis of variance: Seminiferous tubular diameter in micrometer and comparison of means by Duncan's Test.

Source	df	Mean	Squares	Duncan's Test Means			
				LC	LI	RI	RC
Type of castration (treatment effect)	3	–	66.59**	–	–	–	–
Error	–	36	0.84	–	–	–	–
Mean (um)	–	–	–	6.82[a]	2.53[b]	3.01[b]	7.57[a]
SEM	–	0.41	–	–	–	–	–

** = significant ($P < 0.01$).
Means with the same superscript are not significantly ($P > 0.05$) different.

Table 4. Analysis of variance and Duncan's Test on effect of hemiorchidectomy on testis circumference (cm) in West African dwarf rams.

Source	df	ms	Duncan's Test Means		
			LC	IN	RC
Type of castration (treatment effect)	2	59.84**	–	–	–
Error	12	2.44	–	–	–
Mean	–	–	17.94[a]	12.4[b]	18.76[a]
SEM	0.69	–	–	–	–

** = significant ($P < 0.01$).
Means with the same superscript are not significantly ($P > 0.05$) different.

dectomized rams than the intact or control rams. Above all, it seems that the removal of one testis reduces the operation of negative feedback mechanisms, producing sustained effects of the gonadotrophins and endogenous androgens on testis growth.

This is also in keeping with the work of Moger (8) on endocrine responses of the pubertal male rat to hemiorchidectomy. The rats were hemiorchidectomized at 25 days *post partum* and blood samples for LH, FSH and testosterone taken at 30-55 days *post partum*. He found that serum LH concentrations were significantly increased by hemiorchidectomy. The increase in LH, he found, was less pronounced than that of FSH. He also found that testicular testosterone and estradiol concentrations were unaffected by the operation. He concluded that the experiment indicated that hemiorchidectomy caused a disturbance of the hypothalamic-pituitary-testicular axis, resulting primarily in elevated serum FSH concentrations, but that this disturbance has little effect on the endocrine changes associated with puberty in the male rat.

The high output of sperm cells (see Figs 1 and 2) by the rams with one testis appears to be very crucial in artificial insemination programmes in which potential studs could be hemiorchidectomized to increase sperm yield for insemination of more females, in this case ewes. The results are in keeping with the findings of Hochereau de Reviers and Pelletier (5) who found that, following hemiorchidectomy in rams, there was testicular hypertrophy and an increase in the yield of spermatogonial divisions while spermatogenic activity was low in normal animals.

Though not measured, libido was observed on the average to be higher in the hemiorchidectromized rams than the normal or intact rams. The sperm concentration aspect of this work is also in agreement with the report of Boockfor (4), who found an increase in sperm concentration in unilaterally castrated bulls compared to intact or normal bulls; he also noted that the earlier the hemiorchidectomy, the greater the effect of compensatory hypertrophy.

Table 3 shows that there is no statistically significant difference between the mean seminiferous tubular diameter of specimens taken from left or right testis orchidectomy. But rams with single testis, left or right, showed a significantly higher seminiferous tubular diameter when compared with seminiferous tubules from left or right side of the normal or intact testis (see Figs 1 and 2). This is in accordance with the findings of Boockfor (4) in bulls in which unilateral castration was at three, six and nine months *post partum* and samples taken in all groups at 11 months *post surgery*. Greater compensatory growth occurred the earlier the hemiorchidectomy. Land and Carr (6) found that the diameter of these testes increased following hemiorchidectomy in sheep compared to normal testis when they unilaterally castrated rams at 12 and 16 weeks *post partum* and took samples at 25 weeks of age.

Table 4 shows that testes circumference in the hemiorchidectomized rams was significantly higher than in the normal testes. Lunstra *et al.* (7) and Osinowo *et al.* (9) reported that scrotal circumference was a good indicator of testicular development as it related to gonadal sperm reserve, while Land and Carr (6) observed that, in sheep, testis diameter was highly correlated with testis weight, and that a single curve was adequate to describe the relationships between the two variables for each of the breeds and combinations used. Their trial confirms the use of testis diameter as an estimate of testis size. Their observation is in accord with the findings in this experiment since, mathematically, the larger the circumference, the larger the diameter; hence the significant larger diameter in the hemiorchidectomized testis as reported by Land and Carr (6) implied a larger testis circumference.

Table 5 shows no significant treatment effect in mean hormonal levels. Blood samples for this experiment were taken when the rams were between 20-24 months of age. The experiment did not set out to monitor changes in these hormones with growth. Land and Carr (6) and Lunstra *et al.* (7) found that the gonadotropins increased in concentration with age. However, at adult age some of the values in this trial agreed with some of the values in rams used by Lunstra *et al.* (7), though the rams used by these workers were much younger (7 – 13 months) than the rams used in this trial at the start of the experiment. Though libido was not measured, we observed a significantly higher sexual aggressiveness in the hemiorchi-

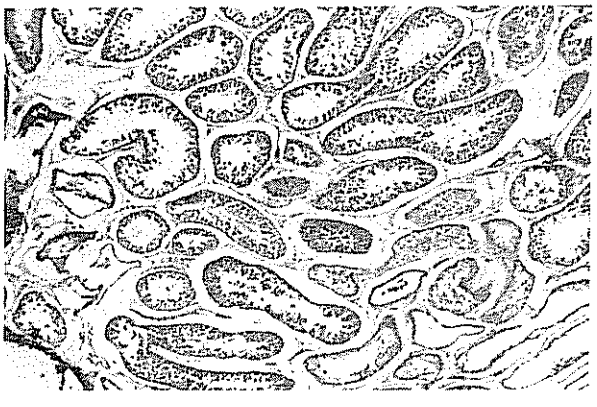

Fig. 1. Control sample, same magnification (12 500). Picture shows smaller seminiferous tubular diameter whose lumen is not filled completely with young sperm cells.

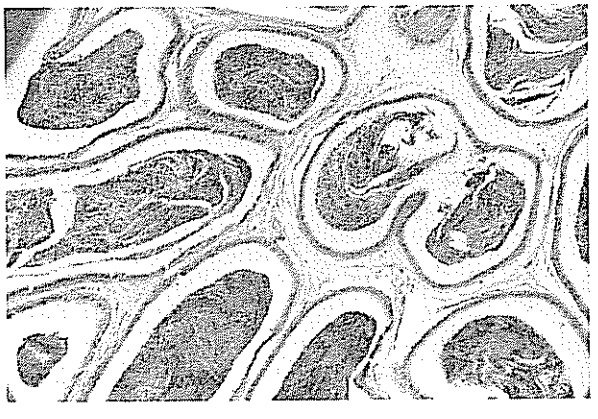

Fig. 2. Test sample. Same magnification (12 500). Picture shows larger seminiferous tubular diameter whose lumen is filled with young sperm cells.

Table 5. Analysis of variance and Duncan's Test on the effect of hemiorchidectomy in West African dwarf rams on the plasma levels of LH (ng/ml), FSH (ng/ml) and prolactin.

Source	df	Mean Square								
Type of castration (treatment effect)	2	LH (ng/ml)			FSH (ng/ml)			PROLACTIN (miu/l)		
		LC	IN	RC	LC	IN	RC	LC	IN	RC
		–	0.01^{ns}	–	–	0.003^{ns}	–	–	13.4^{ns}	–
Error	12	–	19.89	–	–	0.014	–	–	17.23	–
Mean	–	1.72^{a}	1.82^{a}	1.80^{a}	0.24^{a}	0.24^{a}	0.28^{a}	58^{a}	61.2^{a}	59.1^{a}
SEM	–	–	2.00	–	–	0.05	–	–	0.45	–

ns = Not significant (P > 0.05).

a In a given row within a given variable, means with the same superscript are not significantly (P > 0.05) different (miu/l = milli -international units/litre)

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