

The relationship between testis size and stimulated plasma testosterone concentrations and its influence on mating performance in Dorper rams

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Testis size, stimulated testosterone concentrations and mating performance (serving capacity, libido score and serve:mount ratio) of 14 Dorper rams were measured in the breeding season (March) in order to determine the influence of testis size and testosterone concentration on mating performance. Results indicated statistically non-significant ($P>0,05$) correlations between testis size and stimulated plasma testosterone concentrations. Live body mass was however significantly ($P<0,05$) negatively correlated ($r=-0,530$) to the post-copulatory mean testosterone level. In only two of three mating performance pen tests significant ($P<0,05$ and $P<0,01$) negative correlations of $-0,577$ and $-0,795$ were obtained between scrotal circumference and serving capacity. Testis volume and serving capacity was however positively correlated ($r=0,593$; $P<0,05$) in the other test. Estimates of repeatability for serving capacity ranged from 0,102 to 0,690 and for libido score from 0,065 to 0,269. The only significant ($P<0,05$ and $P<0,01$) correlations between stimulated testosterone concentrations and mating performance were obtained between the post-copulatory testosterone levels and serving capacity ($r=0,528$ and $0,736$ for tests 2 and 3 respectively) and for the serve:mount ratio in test 1 ($r=0,557$). It may therefore be concluded that serving capacity is the only criterion that offers some advantage as a selection aid to improve efficiency of reproductive performance in pen-tested rams.

Testisgrootte, gestimuleerde testosteroonkonsentrasies en paringsgedrag (dekvermoë, libido en die verhouding aantal dekkings:totaal aantal dek pogings) van 14 Dorper ramme is gedurende die teeltseisoen (Maart) gemeet om die invloed van testisgrootte en testosteroonkonsentrasie op die paringsgedrag te bepaal. Verkreë resultate toon statisties nie-betekenisvolle ($P>0,05$) korrelasies tussen testisgrootte en gestimuleerde plasmatestosteroonkonsentrasies. Liggaamsmassa is egter statisties betekenisvol ($P<0,05$) negatief met die testosteroonkonsentrasie na paring gekorreleer ($r=-0,530$). In slegs twee van die drie paringstoetse is betekenisvolle ($P<0,05$ en $P<0,01$) negatiewe korrelasies van $-0,577$ en $-0,795$ tussen skrotumomvang en dekvermoë aangetoon. Testisvolume en dekvermoë was egter slegs in die oorblywende toets positief gekorreleer ($r=0,593$; $P<0,05$). Herhaalbaarheidsberamings vir dekvermoë het van 0,102 tot 0,690 en vir libido van 0,065 tot 0,269 gevarieer. Die enigste betekenisvolle ($P<0,05$ en $P<0,01$) korrelasies tussen gestimuleerde testosteroonkonsentrasies en paringsgedrag is tussen dekvermoë en die testosteroonkonsentrasie na paring ($r=0,528$ en $0,736$ vir toetse 2 en 3 onderskeidelik) en vir die verhouding aantal dekkings:aantal pogings in toets 1 ($r=0,557$) gevind. Daar kan derhalwe tot die gevolgtrekking gekom word dat slegs dekvermoë 'n nuttige kriterium by die seleksie van ramme is om reproduksieprestasie te verbeter.

Keywords: Testis size, stimulated testosterone concentration, mating performance, Dorper rams.

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Introduction

The ability of rams to serve a large number of ewes in a short time is of great importance in terms of flock fertility. Failure of insemination is an important cause of reproductive wastage (Cahill, Kearins, Blockey & Restall, 1974). Other benefits of increased ram fertility are increased genetic progress through use of fewer rams, decreased ram costs and shortened joining periods.

The proportion of ewes served depends primarily on the libido (sexual drive) and serving capacity (number of successful serves in a period of time) of the ram (Kilgour & Wilkins, 1980). Both vary considerably (Marincowitz, Pretorius & Herbst, 1966; Mattner, Braden & Turnbull, 1967). Marincowitz, *et al.* (1966) and van Wyk, van der Merwe & Slippers (1984) indicated that rams with higher libido impregnated more ewes than did rams with lower libido. However, Mattner, Braden & George (1973) and Crichton & Lishman (1984) found that the majority of low-response rams as tested in pens began to show sexual activity following flock mating.

Changes in photoperiod are related to changes in

blood levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (Schanbacher & Ford, 1976; Pelletier, Garnier, de Riviers, Terqui & Ortavant, 1982) as well as testis size (Islam & Land, 1977). Just prior to the breeding season FSH and LH start to increase in response to LH-RH released by the hypothalamus. Fluctuations in these hormones through the year are also related to changes in sexual performance in sheep (Schanbacher & Lunstra, 1976; Sanford, Palmer & Howland, 1977; D'Occhio & Brooks, 1983a). Artificially stimulated levels of plasma testosterone by means of either LH-RH challenge or natural mating may offer a helpful criterion to identify rams of higher reproductive status (Moore, Whyman & Wilson, 1978). Sanford, Palmer & Howland (1974) reported an increase in testosterone level in rams following natural mating and Illius, Haynes, Lamming, Howles, Fairall & Millar (1983) investigated such a relationship in rams and wild antelope.

The objectives of this study were to compare the relationships between live body mass, testis size and mating performance, testis size and stimulated plasma

testosterone concentration (testosterone response to LH-RH and natural mating respectively) and plasma testosterone concentration and mating performance within the breeding season in young Dorper rams lacking heterosexual experience.

Another objective was to determine the repeatability of mating performance in three independent pen tests.

Procedures

Fourteen single-born, previously inexperienced, unselected Dorper rams aged 18 months, born and reared at the experimental farm of the University of Pretoria were used. Lambs were weaned at 60 days of age and ram and ewe lambs were separated at an age of 3 months. All rams were in good body condition and the mean body mass was $79,0 \pm 6,38$ kg. Data on testicular size, stimulated plasma testosterone concentration and mating performance were collected over a 2-week period during March 1983 which is more or less 2 months before the peak of the breeding season. Thirty days before the tests started, both rams and ewes were placed in pens in which all observations subsequently took place. This permitted the animals to adapt to the testing environment.

Testicular size

Testicular size was determined by measuring scrotal circumference and testes volume as described by both Knight (1977) and Schoeman & Combrink (1987). The latter authors indicated a repeatability estimate of 0,725 and 0,638 for scrotal circumference and testes volume respectively and a correlation coefficient of 0,813 between these two measurements in Dorper rams between 3 and 14 months of age.

Response in stimulated testosterone concentration

Rams were stratified according to testicular size and randomly allocated to two equal groups. Rams in one group were intravenously treated with a synthetic FSH & LH-RH combination (Receptal, Hoechst) at a dose of 30 μ l/kg live body mass (Treatment 1). Rams of the other group were introduced to 70 ewes in oestrous (Treatment 2). Sexual activity acted as a stimulus to increase testosterone concentration in the blood (Sanford, *et al*, 1974). Rams were stimulated to allow each ram the greatest opportunity to express his testosterone secretion capacity. Both procedures were repeated after 7 days but the two groups were interchanged (Periods 1 and 2 respectively).

A blood sample was collected from each ram by jugular venipuncture using vacutainer heparinized tubes before treatment (T_0) and subsequently every hour for a period of 5 h ($T_1 - T_5$). In the case of Treatment 2 the first samples (T_0) were taken prior to the introduction of rams to the ewes. The time each ram served an ewe was recorded, the ram was then removed from the group and other blood samples ($T_1 - T_5$) were taken subsequently every hour after the ram served the ewe. Testis size and

mating performance (libido score, serving capacity and the serve:mount ratio) were correlated with the maximum testosterone or peak level (T_{max}) and the mean testosterone level ($T_1 - T_5$; T_{mean}). Collection of blood samples was carried out 2 days before the sexual performance trials started.

Plasma was separated by centrifugation shortly after collection and kept frozen at -20°C until assayed. Plasma testosterone concentration was measured by radioimmunoassay using the 'Immunochem Covalent-Coat TM RIA Kit' in a medical pathology laboratory (Drs. du Buisson and Partners, Louis Pasteur Building, Schoeman St., Pretoria).

Sexual performance

Sexual performance was determined by introducing the rams into pens containing oestrous ewes. Oestrous was synchronized in ewes with intravaginal progesterone-impregnated sponges (Repromap, Upjohn) for 14 days. Ewes in oestrous were identified by means of vasectomized rams.

Records were kept of the number of mounts and successful serves by each individual ram in three different tests. For the first two tests observations were made by 14 hidden operators. The definitions for a 'mount' and a 'successful serve' as described by Kilgour (1985) was used in this study.

In the first test (Test 1) the 14 rams were introduced to 50 oestrous ewes in a pen of 350 m^2 for a continuous period of 24 h, starting at 07h00. During the night observations were made in dim artificial light. In the second test (Test 2) the same rams were again introduced to 50 oestrous ewes in the same pen for two 1-h periods separated by an 8-h period (night). During the third test (Test 3) each ram was introduced individually to six oestrous ewes in a pen of 50 m^2 for a 30-min period. This procedure was repeated after 2 days. Visual contact between rams was prevented to overcome a possible audience effect (Lindsay, Dunsmore, Williams & Syme, 1976) as a building complex separated the rams in the pens from the other rams.

Libido scores were obtained by adding the number of unsuccessful mounts (M) to the number of successful serves (S) for each individual ram. A serve:mount ratio was also calculated for each ram in all tests.

Statistical analyses

Relationships between testis size, testosterone concentrations and sexual performance were determined by simple correlation coefficients. Data for the number of serves were transformed using the $\sqrt{X} + 1/2$ transformation. Least square analyses of variance and Student's *t* test were used to examine the effect of treatment (LH-RH or natural mating) and period (1 and 2) on stimulated testosterone concentrations at $T_0 - T_5$ and orthogonal contrasts to test for possible interactions between treatment and period at $T_1 - T_5$ (Steel & Torrie, 1980).

Results and Discussion

Stimulated plasma testosterone concentration and testis size

Circulating testosterone concentrations in rams and its relationship with mating activity and testis size have been described previously (Schanbacher & Lunstra, 1976; Sanford, Palmer & Howland, 1977). The present study, however, compared testis size and sexual activity with stimulated values of plasma testosterone concentrations.

The mean plasma testosterone levels increased rapidly after stimulation, reaching a maximum value after 2 h in treatment 1 and a maximum value after only 1 h in treatment 2 followed by a gradual decline (Figure 1). The artificial stimulation (treatment 1) gave statistically significant ($P < 0,05$) higher mean values at $T_1 - T_5$ than for the post-copulatory values (treatment 2). All rams responded better for both treatments during the second period compared to the first. Differences between the two periods were statistically significant ($P < 0,05$ at T_1 and $P < 0,01$ at $T_2 - T_5$). No statistically significant ($P > 0,05$) interactions existed between treatment and period.

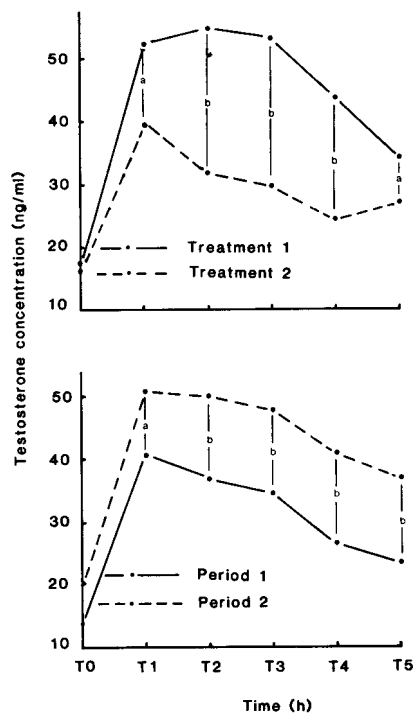


Figure 1 Plasma testosterone concentration stimulated by two treatments (LH-RH and exposed to ewes respectively) and pooled over periods and repeated after seven days (Periods 1 and 2, pooled over treatments) (a, $P < 0,05$; b, $P < 0,01$)

Several other authors also indicated significant increases in testosterone concentrations after natural mating (Sanford, *et al.*, 1974; Illius, Haynes & Lamming, 1976; Moore, *et al.*, 1978) whereas Purvis, Illius & Haynes (1974) and D'Occhio & Brooks (1976) reported no such increase.

Table 1 Correlation coefficients between body mass, testis size (SC = scrotal circumference; TV = testis volume) and stimulated plasma testosterone concentration for two treatments (treatment 1 = LH-RH stimulation and treatment 2 = natural service)

Item	Treatment 1		Treatment 2		Average for T_{mean}
	T_{max}	T_{mean}	T_{max}	T_{mean}	
Live body mass (kg)	-0,303	-0,341	-0,133	-0,530 ^a	-0,582 ^a
Scrotal circumference (cm)	0,193	0,014	-0,108	-0,255	0,241
Testis volume (cm ³)	0,249	0,346	-0,097	-0,128	0,338

T_{max} = maximum plasma testosterone peak reached over 5 h;
 T_{mean} = mean plasma testosterone concentration over 5 h ($T_1 - T_5$);
^a $P < 0,05$

Simple correlation coefficients between live body mass and testis size (scrotal circumference and testes volume) was negative ($P > 0,05$; $r = -0,264$ for scrotal circumference and $r = -0,431$ for testes volume). Live body mass was also negatively correlated ($P < 0,05$) to testosterone concentration ($r = -0,530$) for the mean value (T_{mean}) in treatment 2 as well as with the average value for both treatments ($r = -0,582$). These findings did not agree with the positive correlations obtained by Illius, Haynes, Purvis & Lamming (1976). The reason for this discrepancy is not known. Although all coefficients between testis size and testosterone concentration were non-significant ($P > 0,05$), a positive relationship was indicated in treatment 1 and a negative relationship in treatment 2 (Table 1). Dufour, Fahmy & Minvielle (1984)

Table 2 Number of mounts and serves recorded for 14 rams in three mating behaviour pen tests

Ram	Test 1		Test 2				Test 3			
	M	S	First hour		Second hour		First day		Second day	
			M	S	M	S	M	S	M	S
1	151	12	17	3	7	1	2	4	14	3
2	64	21	8	2	5	7	1	4	9	3
3	103	27	4	1	8	1	3	6	1	3
4	80	13	11	3	3	4	14	4	7	4
5	89	22	5	1	2	0	9	4	23	2
6	53	13	10	2	6	3	14	2	3	4
7	108	30	5	2	22	3	6	3	6	4
8	132	13	14	0	8	4	27	4	9	4
9	127	28	25	0	1	5	2	4	12	4
10	151	13	21	1	5	5	4	5	3	6
11	94	24	15	2	11	3	3	6	1	4
12	68	25	9	2	9	1	1	5	7	3
13	100	17	39	0	10	0	36	2	25	2
14	53	18	2	4	19	4	2	2	5	3
Total	1373	276	185	23	116	41	124	55	125	49

M = Number of mounts; S = Number of serves

indicated that the basal testosterone concentration could account statistically for 65 and 37% of the variation of testicular diameter in Suffolk and Dorset×Leicester×Suffolk crossbred rams respectively during the decreasing day-length period. However, Illius, *et al.* (1983) indicated a highly significant positive relationship ($r=0,87$) between the basal testosterone concentration and LH-RH stimulated testosterone concentration.

Mating performance and testis size

The number of serves (S) and mounts (M) as recorded during the three pen tests are shown in Table 2. No outstanding ram-ram interactions or rams seriously dominating others were observed. Repeatability estimates varied between 0,102 – 0,690 (0,690 between tests 1 and 2; 0,102 between tests 1 and 3; 0,340 between tests 2 and 3; 0,152 within test 2 and 0,272 within test 3) for number of serves. The lower values between and within the later tests may be due to the fact that rams gained sexual experience in later tests, however, no rams could be considered as 'inactive', since all rams successfully served ewes during the first hour of test 1. Estimates of repeatability for libido score varied from 0,065 to 0,269 and for the serve:mount ratio between 0,222 and 0,491

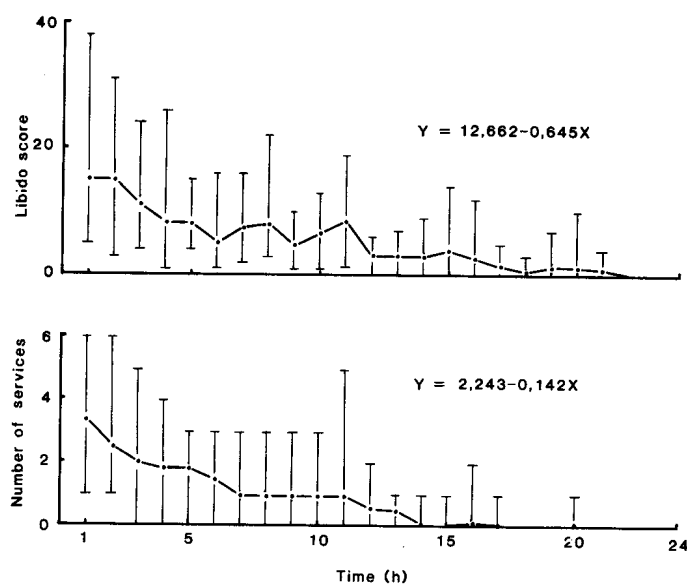


Figure 2 Libido score and number of serves per ram per hour over 24 h. (Vertical bars represent range in number of observations.)

which are lower than that for the number of serves. Mattner, Braden & George (1971) obtained statistically highly significant repeatabilities for serving capacity (0,709 – 0,853) but lower repeatabilities for number of mounts (0,286 – 0,401). These authors furthermore reported that the ratio serves:mounts in pen tests was poorly related to field mating. Kilgour & Whale (1980) also obtained a repeatability estimate of 0,77 for number of serves and Barwick, Kilgour & Gleeson (1985) and Kilgour (1985) reported repeatabilities for serving capacity varying from 0,66 to 0,81. Kilgour (1985) thus stated that serving capacity is a highly repeatable character, the number of mounts moderately repeatable ($0,40 \pm 0,19$) and the ratio mounts:serves poorly repeatable ($0,09 \pm 0,12$).

During the 24 h test (test 1) the libido and number of serves of the rams decreased over time as indicated in Figure 2. After 22 h all rams were exhausted and no longer interested in ewes. Individual rams showed considerable variation in libido score and number of serves per hour. During the first 2-h period sexual activity varied from three to 38 mounts per individual ram and from one to six serves per ram. A non-significant ($P>0,05$) correlation ($r=0,032$) existed between libido score and serving capacity and a highly significant ($P<0,01$) correlation ($r=0,734$) between serving capacity and the serve:mount ratio. Rams with the least number of mounts per mating served the largest number of ewes. However, this ratio seems to be of little practical value (Winfield & Cahill, 1978). Lindsay (1979) is of the opinion that mounting several times prior to ejaculation seems to be part of the normal sexual foreplay in many rams.

The number of serves were negatively correlated to scrotal circumference in test 1 ($r=-0,795$; $P<0,01$) and test 3 ($r=-0,577$; $P<0,05$) while testis volume was positively correlated to serving capacity in test 2 ($r=0,593$; $P<0,05$) (Table 3). Libido scores were non-significantly related to live body mass, scrotal circumference and testes volume. The ratio serves:mounts was also positively correlated to both scrotal circumference ($r=0,541$; $P<0,05$) and testes volume ($r=0,554$; $P<0,05$) in test 2 only. Barwick, *et al.* (1985) reported positive correlations between serving capacity and testis diameter and serving capacity and live mass at 1,5 years of age. In the present study the associations among live body mass and serving capacity in all three tests were, although non-significant ($P>0,05$), a negative one. Barwick, *et al.*

Table 3 Simple correlation coefficients between testis size, body mass, libido score (S+M), number of serves (S), and the serve:mount ratio (S:M) in three pen tests

Item	Test 1			Test 2			Test 3		
	S+M	S	S:M	S+M	S	S:M	S+M	S	S:M
Live body mass	-0,254	-0,323	-0,003	0,030	-0,067	-0,014	0,171	-0,232	-0,114
Scrotal circumference	-0,141	-0,795 ^b	0,049	0,027	0,320	0,541 ^a	-0,294	-0,577 ^a	-0,240
Testis volume	-0,083	-0,080	0,099	0,007	0,593 ^a	0,554 ^a	-0,334	0,205	-0,129

^a $P<0,05$; ^b $P<0,01$

(1985) also reported a negative correlation between live mass at 2,5 years and serving capacity. Differences in body condition between rams (over-fat rams) could be a reason for such negative correlations between live body mass and serving capacity in the present study.

Stimulated plasma testosterone concentration and mating performance

Simple correlation coefficients between mating performance and testosterone concentrations for both treatments are in most cases non-significant (Table 4). Although non-significant ($P > 0,05$), libido scores (S + M) tended to have a positive association with both T_{mean} and T_{max} in treatment 1, while the values were negative in treatment 2. Serving capacity in most cases gave negative correlations with T_{mean} and T_{max} in treatment 1 and positive values in treatment 2. The reason for this discrepancy is not known but it may be related to the treatment itself. The only significant correlations were obtained between serving capacity and testosterone concentrations in treatment 2 for tests 2 and 3 ($r = 0,528 - 0,736$).

According to Howles, Webster & Haynes (1980) and D'Occhio & Brooks (1983a) sexual performance was not related to peripheral testosterone concentration. D'Occhio & Brooks (1976) also reported that the plasma testosterone concentration in rams was not influenced by contact with oestrous ewes and was also not correlated with the rams' reproductive performance. However, Dufour *et al.* (1984) indicated that peripheral testosterone concentration was responsible for 28% of variation in libido. Sanford, *et al.* (1977) also observed a significant ($P < 0,05$) and positive relationship between serving capacity and serum testosterone concentration during the breeding season. The latter authors indicated that this relationship diminished with time as circulating testosterone levels rose. The ram's mating performance is according to Sanford, *et al.* (1977) and D'Occhio &

Brooks (1982) more closely related to serum testosterone levels early in the breeding season. The higher basal testosterone concentrations during the breeding season are brought about by both an increase in the frequency of LH release and an increase in response of the testes to LH. This indicates that concentrations may be below a minimum threshold level required to maintain maximal mating activity earlier in the season, while the higher levels later in the breeding season exceeded this threshold level. This may be a reason for the lower correlations in the present study compared to other literature. This may partly explain why there is no relationship between stimulated plasma testosterone concentration (over-stimulated) and service capacity in treatment 1 and a moderate positive relationship in treatment 2.

The mating response of various breeds may also vary in sensitivity to changes in circulating testosterone (D'Occhio & Brooks, 1983b). These authors were of the opinion that breeds differing in seasonality in mating behaviour may show marked seasonal changes in plasma testosterone profiles.

Conclusions

Results obtained indicated an increase in testosterone levels after stimulation by means of both LH-RH and natural mating. These stimulated testosterone levels were non-significantly correlated with testis size but significantly positively related to serving capacity when stimulated by means of natural mating. Scrotal circumference was also negatively correlated with serving capacity in two of the three mating performance pen tests. Repeatability estimates for mating performance varied considerably but were the highest for serving capacity. It may therefore be concluded that stimulation of testosterone to predict sexual activity and to identify rams of higher reproductive status offers advantages only when stimulated by means of natural mating.

Table 4 Simple correlation coefficients between stimulated testosterone concentration (T_{max} and T_{mean}) in treatments 1 and 2 and sexual performance in three tests (libido score = S+M; number of serves = S; serves;mount ratio = S:M)

Item	Mean testosterone levels (ng/ml)	Test 1			Test 2			Test 3		
		S+M	S	S:M	S+M	S	S:M	S+M	S	S:M
Treatment 1 (LH-RH stimulation)										
T_{max}	57,01 ± 12,96 ^a	0,351	-0,397	-0,412	0,203	-0,240	-0,294	0,114	0,366	0,134
T_{mean}	47,89 ± 13,35 ^b	0,305	-0,352	-0,396	0,184	0,167	-0,183	0,072	0,108	0,222
Treatment 2 (Natural service)										
T_{max}	50,17 ± 17,27 ^c	-0,336	0,376	0,557*	-0,147	0,573*	0,237	-0,275	0,437	0,073
T_{mean}	30,81 ± 11,98 ^d	-0,139	0,335	0,363	-0,384	0,736**	0,355	-0,341	0,528*	0,126

T_{max} = maximum testosterone level observed for each ram or peak value

T_{mean} = mean testosterone level from T_1 to T_5

* $P < 0,05$; ** $P < 0,01$

a,b,c,d = values with the same superscript do not differ significantly ($P < 0,05$)

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