THE RAM EFFECT ON THE REPRODUCTIVE CYCLE OF THE SPRINGBOK EWE (*Antidorcas marsupialis*)

by

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Abstract

Plasma progesterone concentrations were used as a measure to determine the "ram effect" on nine cycling springbok ewes. Blood was collected biweekly, prior to and after ram introduction. Ewes were subjected to the ram for a period of forty two days. Blood was analysed for plasma progesterone by means of radioimmunoassays. No significant changes in the plasma progesterone concentrations were detected. Superficially, a reduction in the variation of the follicular phase of the ewes oestrous cycle was noted for before- to after ram introduction. However, possibly because of a too small sample size, no statistical significance was found. It is suggested that the "ram effect" on aseasonal cycling species may only have a synchronisation effect (reduction in the variation of time between the follicular phase of females) but more individuals should be used in future experiments to make any clear and definite conclusions. Furthermore other hormones such as e.g. luteinizing hormone, which may be less affected by translocation and handling stress, should also be used to determine the "ram effect" on aseasonally breeding wild ungulates.

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INTRODUCTION

Springbok are opportunistic breeders with an unrestricted breeding season (Skinner & Louw, 1996). They may breed throughout the year if physiological and environmental conditions are optimal for producing sufficient energy and resources for the springbok. Breeding is climaticaly sensitive, and the bulk of the lamb crop in the wild are born during the wet season of the year (Van Zyl & Skinner, 1970). This suggests that proximate factors may influence the onset of reproduction in this species. However, the factors involved and the effects they have on reproductive activity, are still unknown.

The current belief regarding the reproduction of springbok is that rain acts indirectly as a proximate factor to stimulate vegetative growth. This leads to the "flushing" of the ewes through an increase in nutritional levels and to optimum physical and physiological conditions that stimulate the onset of reproduction (Skinner & Louw, 1996). The effect the springbok male has on onset and synchronisation of reproduction in the anoestrous ewe has not been clearly defined although evidence for this has been reported (Skinner, Jackson & Marais, 1991; Skinner & Louw, 1996). The role of the male in the reproductive adaptation of springbok may be purely secondary, in order to synchronise births as it has been suggested that a synchronised birth pulse of lambs will reduce the predation pressure and ensure greater survival of the young springbok (Skinner & Louw, 1996).

Depending on resource availability, rams remain territorial throughout the year. The males defend these territorial resources to indirectly attract females during the optimum breeding period. Only territorial males mate, and it has been speculated that the non-territorial males are suppressed at the hypothalamic level (Skinner & Louw, 1996). Territorial males may rut for varying periods at any time during the year. This male rut is activated by an unknown proximate factor. It has been suggested that the effect of this rutting is to induce anoestrous ewes to come into oestrus, synchronising conceptions and later births (Skinner & Louw, 1996). Skinner, van Aarde, Knight & Dott (1996) suggest that in the case of springbok, there could be at least two stimuli, one acting on the male to initiate rutting behaviour, and the other on the female to initiate oestrus.

I suggest that it might be only one factor, such as rainfall, which acts on both sexes. The ram might perceive this factor faster than the female and start rutting. A rut after summer rain and the subsequent increase in plant growth, "flushing", stimulates and synchronises the reproductive state of the ewes. Pheromones produced by both sexes may then stimulate the different sexes and the ewes may, following such fine tuning, become even more synchronised.

There are many ways in which hormones affect the behaviour of animals, but few where behaviour results in changes in hormone secretion, and thus reproductive processes. Heap (1901) was the first to suggest that the presence of the male influences the onset of oestrus. This has subsequently been found to occur in sheep *Ovis aries* (Underwood, Shier & Davenport, 1944), cattle *Bos indicus* (Skinner & Bonsma, 1964), goats *Capra hircus* (Skinner & Hofmeyr, 1969), red deer *Cervus elaphus* (McComb, 1987), impala *Aepyceros melampus* (Marais, 1988; Skinner *et al.*, 1991) and blesbok *Damaliscus dorcas* (Marais & Skinner, 1993).

In sheep it has been recorded that within 10 minutes of introducing rams, the luteinizing hormone (LH) levels of anoestrous ewes increase significantly (Knight, 1983). This is followed by a LH surge 27 to 35 hours later and a silent ovulation within 54 to 72 hours in anoestrous ewes. This surge of LH stimulates the formation of the corpus lutea and production of progesterone. As many as 60 % of the ewes have a premature regression of the corpus luteum (CL) and a second silent ovulation four to six days later (Knight, 1983). This leads to flocks having peaks of first oestrus around days 18 and 24 after ram introduction. Although LH surges in sheep occur within 10 minutes of male introduction, males must remain with the ewe for a longer time period to get an ovulatory response. The oestrous cycle of springbok closely resembles that of sheep, and therefore the same CL regression and LH surge may be expected in the springbok cycle after the male introduction.

During a study on aseasonally breeding blesbok ewes (Marais & Skinner, 1993), the progesterone levels of the ewes increased within a week of ram introduction and synchronous cycling was achieved a month (two cycles) later. Skinner & Hofmeyr (1969) found that the presence of the male is sufficient both to synchronise and produce oestrus in female goats and that hormone treatment is unnecessary. No experimental published evidence that males induce oestrus and ovulation in wild aseasonally breeding ungulates has been reported. Furthermore, no experimental published evidence of the ram effect on cycling females has been reported. The effect of the male of cycling females thus remains unclear.

Progesterone has a dominant role in the regulation of the oestrous cycle (Bearden & Fuquay, 1992) and is thus a good indicator of the reproductive status of an animal. During the luteal phase high concentrations of progesterone inhibit the release of the follicle stimulating hormone (FSH) and luteinizing hormone (LH) through negative feedback control of the hypothalamus and anterior pituitary. Progesterone also indirectly inhibits behavioural oestrus. The decline of progesterone releases the hypothalamus from the hormone's negative feedback inhibition. After removal of this inhibition the concentration of FSH and LH increases gradually, stimulating follicle growth and an increased secretion of estradiol. The presence of the ram may therefore, in cycling ewes, either cause an increase in circulating progesterone levels setting the cycle on a higher level, or may cause a premature regression of the corpus lutea that will lead to decreased plasma progesterone concentrations. The decrease in plasma progesterone, together with the increase in luteinizing hormone (LH) and oestrogen, will cause the female to come into oestrus.

The "ram effect" in sheep occurs due to pheromones which are present in the urine, faeces and wax of the wool and skin (Knight, 1983). It is postulated that pheromones may also play a role in the springbok where cutaneous secretions are present on the dorsal fan of white hairs (Burger, le Roux, Spies, Truter & Bigalke, 1978), faeces and urine of the springbok ram. Knight & Lynch (1980) state that urine stimulated 22 %, and wool and wax 48 %, of anoestrous sheep ewes to ovulate. Pheromones consist mainly of fatty acids and are believed to be intraspecific due to variation in chemical composition between species (Burger, le Roux, Spies, Truter & Bigalke, 1978). Pheromones are produced by both sexes and production is stimulated by androgens and oestrogens (Knight, 1983). It thus seems likely that both sexes are responsible for increased pheromone production in the opposite sex. Whether such increased pheromone production in both sexes has an influence on the hormonal status of the springbok is still debatable, and if it has, who affects who first ?

The "ram effect" has been intensively researched and documented in seasonally breeding anoestrous sheep (Underwood, Shier & Davenport, 1944; Knight, 1983). The "ram effect" in above mentioned studies refers to the ram stimulating oestrus activity in anoestrous seasonally breeding ewes. To what degree will the "ram effect" therefore have an effect, if any, on aseasonal cycling females like springbok ewes ?

In accordance with the above mentioned factors, the following question needs to be addressed :

- What effect does the male springbok play in the reproductive adaptation of the aseasonal opportunistic breeding springbok ewe ?

  → Does the introduction of the ram significantly alter the blood progesterone levels of the ewe ?

  The **null hypothesis** would thus state that there is no significant difference between blood progesterone levels of springbok ewes prior to, and after ram introduction.

  (H_0 : prog. conc. without ram = prog. conc. with ram).

In order to answer one of these questions the following study, to determine the role of the springbok ram ("ram effect") on the progesterone concentrations of the ewe, was carried out.

MATERIALS AND METHODS

1. Animals

Two stage sampling was used to select the springbok ewes. First, nonprobability sampling was used since nine springbok ewes were already available on the experimental farm of the University of Pretoria. All nine ewes were between the age of two years six months and two years eight months and were hormonally active, cycling prior to and during the time of the experimental procedures. These ewes were hand-reared and had not produced offspring prior to this experimental study. The ewes were divided into two groups consisting of a randomly selected group of 4 ewes (group 1), and a group of 5 ewes (group 2). The springbok ram, which had sired offspring, was vasectomised (surgical ligation of the vas deferens) 28 days prior to introduction to group 1. The 4 randomly selected ewes of group 1 were kept with the other 5 ewes of group 2 prior to the introduction to the ram. They were captured by hand and restrained for a 6 minute timespan, translocated and introduced to the ram that was housed as mentioned in 2.1. After the introduction the ram was kept with these ewes for a period of 42 days. This period allowed for 2 oestrous cycles of 16 days each and an extra time of 10 days to allow for error. After this period, the ram was captured by hand and moved to the next ewe enclosure and placed with the remaining 5 ewes that were housed as mentioned in 2.1. Physical restraining of the ram was used for a period of approximately 6 minutes in order to translocate the ram to the next enclosure.

2. Husbandry of animals

2.1. Housing

The housing and locality of the ram was on the experimental farm of the University of Pretoria, Pretoria (25° 45" 19' S & 28° 15" 14' E). The ram was kept in a 198 m^2 enclosure constructed of 1.5m high mesh fencing and 3m high game capture tarpoulin (Vynatarp, Pietersburg, RSA). Group 1 consisting of the 4 randomly selected ewes were introduced to the ram in this enclosure. The ram and group 1 were kept together for a period of 42 days, whereafter the ram was moved to another enclosure where the 5 ewes of group 2 were kept.

Group 2 was housed 1700m NE of the experimental group 1 (25° 44" 75' S & 28° 16" 01' E). These 5 ewes were kept in a 500 m^2 enclosure. The enclosure had 3 m high sides constructed of gum poles. The housing and locality of group 2 was also on the experimental farm of the University of Pretoria, Pretoria. No prevailing wind direction was observed on the experimental farm during the period of study.

2.2. Feeding

The springbok were fed daily on lucerne, cotton seed and antelope pellets (Epol Ltd, Pretoria, RSA.). This diet was supplemented with freshly cut *Salix mucronata* and/or *Morusmesozygia* leaf. Care was taken to ensure that the two groups received the same diet. They all had free access to clean drinking water.

3. Sampling

3.1. Capture of springbok

Both springbok groups were driven into a corner of their enclosure and surrounded with game capture tarpaulin (Vynatarp, Pietersburg, RSA.). The springbok ewes were familiar with this procedure as they had been captured in this way in a previous study to determine the oestrous cycle. Each ewe was physically restrained by two persons, blindfolded and held for approximately 1 minute during blood sampling.

3.2. Blood sampling

Blood was taken from the jugular vein, collected in a heparinised venoject tubes (Becton Dickinson, Meylan Cedex, France) and kept on ice for approximately half an hour until centrifuged. Plasma was harvested and frozen at -20°C for later radioimmunoassay analysis. Sampling was undertaken biweekly between 8 & 10 am for the duration of the experiment. Different clothing, boots and an antelope blindfold were used in each of the enclosures to avoid transmitting any smells or objects between enclosures. The average oestrous cycle of the springbok ewe is approximately 16 days (range: 14-17) (Liversidge & De Jager, 1984; Skinner & Louw, 1996; Richter, 1997). Sampling twice weekly ensured that the different stages or changes in the progesterone levels would be detected. The duration of the follicular phase, when progesterone levels are low is approximately 3-4 days (Richter, 1997).

4. Radioimmunoassays

Progesterone was extracted from plasma samples prior to radioimmunoassay. The extracts were assayed using the standard radioimmunoassay techniques as done by Pelletier, Kann, Dolias & Rosselin (1968), and in the same manner as Richter (1997).

Frozen plasma samples was defrosted before 100 µl of plasma per sample was pipetted into glass tubes for progesterone extraction. Each sample was pipetted in triplicate. Progesterone was extracted from the defrosted plasma samples by adding 4 ml of petroleum ether (AR 40-60°C), an organic solvent that selectively absorbs progesterone, to each glass tube containing 100 µl of plasma. The samples were shaken for 10 min and frozen for 1 h at -8°C, then for 10 min at -70°C and then thawed. The ether was decanted into clean glass tubes and evaporated with nitrogen gas, leaving the ether solute substance and progesterone in the tube. This was reconstituted in a buffer medium (PBS, pH 6.8 - 7.0). Progesterone antibody (100 µl) was added to each sample tube, shaken and incubated for 10 min at 25°C. During this period the antibody binds to the free natural progesterone in the sample. After 10 min, 100 µl tritiated progesterone (*c.* 20 000 dpm/tube) was added. This allowed for the remaining unbound progesterone antibody to bind to the tritiated progesterone. The tubes were then shaken and kept at 4°C for between 12 and 18 hours. Thereafter, unbound steroids were exposed to dextran-coated charcoal (0.156%) (Merck, Darmstadt, Germany) for 12 min. This allowed for the smaller unbound steroids to be absorbed by the charcoal. The bound and unbound phases were separated by centrifuging at 2500 rpm for 15 min. The supernatant was then decanted into 8 ml hinge cap scintillation vials (Packard Instruments, Cape Town, South Africa) and 4 ml of scintillation cocktail (Ultima Gold XR, Packard Instruments) added. After 3 - 4 hours, radioactivity was determined using a Packard 1500 liquid scintillation counter. The cross-reactivity of the antibody for progesterone (# 1529, supplied by R.P. Millar, Department of Chemical Pathology, University of Cape Town, South Africa) was as follows: Progesterone 100%, 11α-hydroxyprogesterone 85%, 17α-hydroxyprogesterone 12.5% and 5β-prenane-3,20-dione 12.5%.

Each sample was assayed in triplicate and the average result (ng/ml) taken.

A range of standards with concentrations of 0, 0.0156, 0.0312, 0.0625, 0.125, 0.250, 0.500, 1 and 2 ng/ml unlabelled progesterone were used to determine a "standard" sigmoidal curve. The sigmoidal curve indicated the "best response" of the dose responsive curve between percentage labelled bindings and the range of standard concentrations (ng/ml) used. Each standard was assayed in triplicate and average result (ng/ml) was taken.

Control samples of concentration of 0, 0.0312 and 0.500 ng/ml were used to determine the level of precision and the level of variation in and between assays. The control sample range were placed between the standards and unknown samples of each analysis and were treated identically to the unknown samples.

Sensitivity was defined as the minimal detection limit of an assay. The sensitivity varied between the assays; ranging from 0.0189 to 0.078 ng. progesterone/ml (n=3). The average non-specific binding was 9.35% and specific binding of the antiserum was 37.81%.

5. Statistical analysis

Due to the large variation between individual springbok ewes and the non-normal frequency of progesterone concentration distributions of individual springbok, non-parametrical statistical testing methods were used. Non-parametric tests may be used with observations on nominal, ordinal and interval data. These non-parametric tests do not require data to be normally distributed or to have a homogeneous variance and can also be used for smaller sample sizes.

Comparing two groups, experimental group 1 versus the control group 2.

5.1. Mann-Whitney U-test.

This test compares the medians of two unmatched samples, and is equivalent to the parametrical Wilcoxon's rank sum tests and the pooled t-test. Data used were those from means of group 1 of 4 ewes (no: 11, 16, 17, 20) versus the group 2 of 5 ewes (3, 9, 14, 18, 21) for the period of 5 August to 12 September. Group 1 was taken as the experimental group and group 2 as the control group. Except for ram introduction to the experimental group 1, the control group (group 2, n=5) was subjected to the same conditions as the experimental group (group 1, n=4).

5.2. Wilcoxon's rank sum test.

This test was used to compare the minimum, middle and maximum progesterone concentration values between the two springbok groups for the period of 05/08 to 12/09 (n=12/individual springbok & n=112 for all the ewes). Group 1 was taken as the experimental group (with ram) and group 2 (without ram) as the control group. Progesterone concentrations within this data period were sorted into minimum (n=4), middle (n=4) and maximum (n=4) values for each individual ewe. These divisions of minimum, middle and maximum values were then compared between the two groups; with the ram and without the ram. Sorted data are summarised in appendix 2.

Comparing within each individual ewe, prior and post ram introduction.

5.3. Wilcoxon's test for matched pairs.

This test is a test for comparing the medians of two matched samples.
Each individual ewe was used to compare values prior to ram introduction (12 observations) to values when the ram was present (12 observations). Values used were C-, E-, F-, H-, 1-12 (prior) & 13-24 (with ram) and A-, B-, D-, G-, I-, 13-24 (prior) & 25-36 (with ram) for the two groups separately. (Appendix 1).

Comparing within individual ewes, ram removal.

5.4. Wilcoxon's test for matched pairs.

To examine the effect of ram removal on the progesterone concentrations of the springbok ewes, the Wilcoxon's matched pairs test was done on the four springbok ewes of group 1. Values used were C-, E-, F-, H-, 13-24 (with the ram) compared to C-, E-, F-, H-, 25-36 (without the ram and after the ram had been removed) (Appendix 1).

Comparing variation between before- and after ram introduction.

5.5. Friedman two way ANOVA test.

To compare the degree of time (date) variation of the follicular phase of the ewes, for before and after ram introduction of both experimental groups, the Friedman test was used. Dates used were those were the ewe's progesterone was at the lowest value; the average follicular phase. The average period for cycles was taken as 15 days to determine the intervals of each follicular phase.

RESULTS

The results of the radioimmunoassays (RIA) are tabulated in appendix 1.

Probabilities of less than 0.05 were considered significant.

Rainfall data during the year and period of study are illustrated graphically in appendix 3.

Results of the Mann-Whitney U-test, comparing plasma progesterone concentrations of the two groups - experimental versus control group are summarised in Table 1.

Table 1: Mann-Whitney U-test, experimental group 1 (with ram) versus the control group 2 (without ram) (ng/ml).

	Group 1 (Experimental) (n=4)	**Group 2 (Control) (n=5)**
Mean	3,692	3,668
Variance	9,51	12,22
Std.deviation	3,08421	3,49632
z-value	-0,587	
p-value	0,557	

The mean progesterone concentrations, standard deviation and variance for group 1 and group 2 were calculated for before- and after ram introduction. Results are summarised in Table 2 and mean progesterone concentrations for before- and after ram introduction for both groups are graphically illustrated in Figure 1. The trends in progesterone concentration prior to and after ram introduction of all nine springbok ewes are graphically illustrated in Figures 3a-i.

Table 2. Mean progesterone concentrations, standard deviation and variance for group 1 and group 2 for before- and after ram introduction (ng/ml).

		Group 1 (n=4)	Group 2 (n=5)
Before ram introduction (Without ram)	Mean	2,659	3,668
	Std.deviation	3,01453	3,49632
	Variance	9,08	12,22
After ram introduction (With ram)	Mean	3,692	3,537
	Std.deviation	3,08421	3,10860
	Variance	9,51	9,66

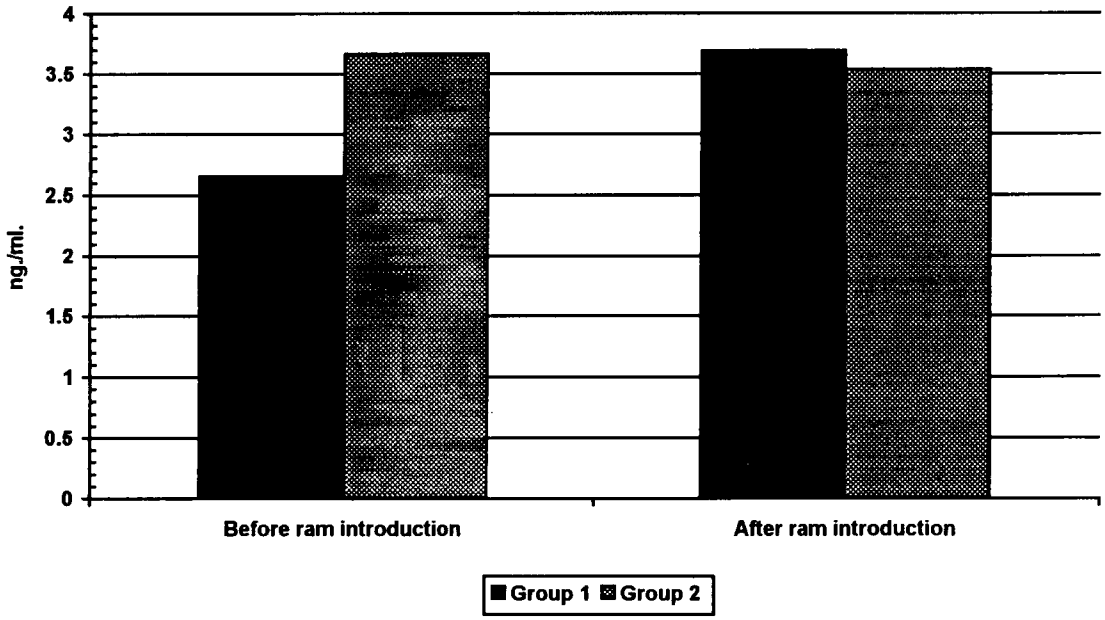

Fig.1: Mean progesterone concentrations for group 1 and group 2, before- and after ram introduction.

Results of the Wilcoxon's test for matched pairs, comparing within each individual ewe, for all ewes, prior and post ram introduction, are summarised in Table 3. Results are graphically illustrated in Figure 2.

Table 3: Results of Wilcoxon's matched pairs test of plasma progesterone for all individuals ewes for both group 1 and group 2 prior to-, and after ram introduction.

Springbok number:									
	Group 1				Group 2				
ng./ml.	11	16	17	20	3	9	14	18	21
Mean no ram (1)	1.24830	2.15000	4.74670	2.49000	3.02420	3.80670	2.42670	6.92670	2.15420
Mean with ram (2)	2.99920	3.78330	4.90410	3.08170	3.09580	3.71330	2.26830	6.39670	2.20830
Std.deviation (1)	0.71357	1.19800	5.12699	1.83253	1.27695	3.02948	1.46757	5.89772	1.25769
Std.deviation (2)	1.68018	2.02606	5.34670	1.60825	2.01359	2.79940	1.31934	4.89174	1.19203
Variance (1)	50.9179	143.5218	2628.606	335.8182	163.069	917.7733	215.497	3478.304	158.1772
Variance (2)	282.299	410.4915	2858.721	258.647	405.454	783.6642	174.067	2392.913	142.0942
Pearson's correla.	-0.24057	0.043285	0.379576	-0.39876	-0.03296	-0.1115	-0.49555	0.483303	-0.27589
Z - value	2.431840	2.118054	0.862911	0.706018	0.078446	0.235339	0.078446	0.156893	0.156893
P - value	0.015028	0.034178	0.388193	0.480182	0.937473	0.813947	0.937473	0.875330	0.875330

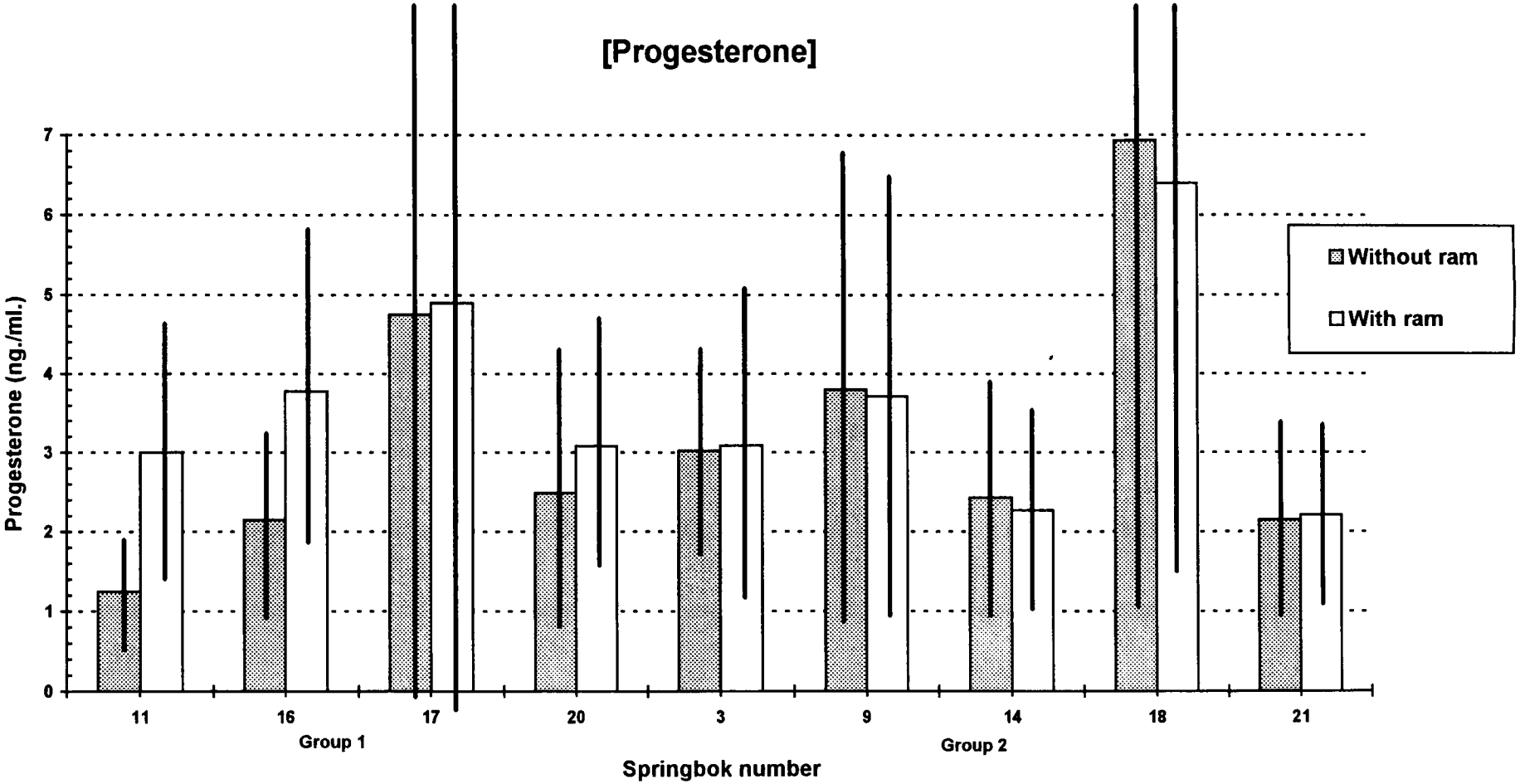

Fig.2: Graphical illustration of plasma progesterone concentrations, comparing each individual ewe for before- and after ram introduction.

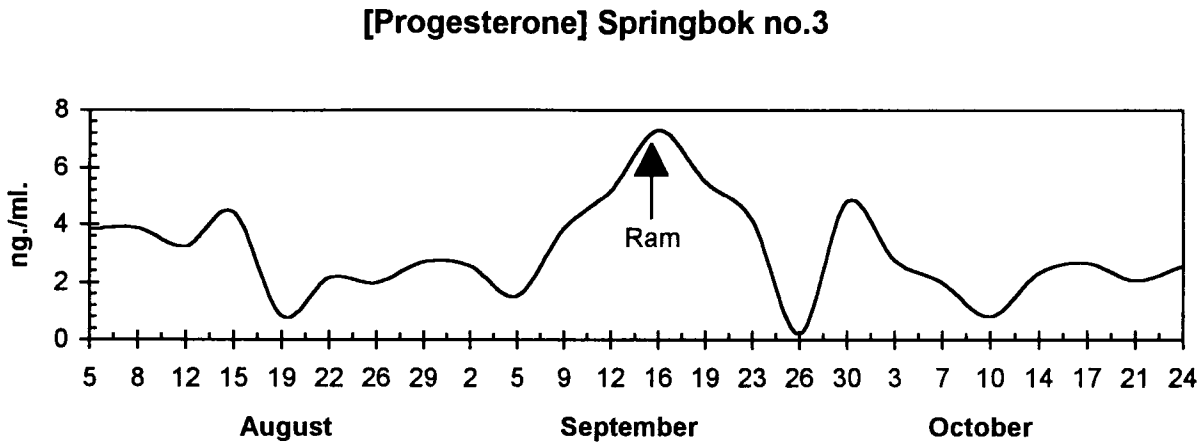

Fig. 3a: Trend in progesterone concentration prior to, and after ram introduction.

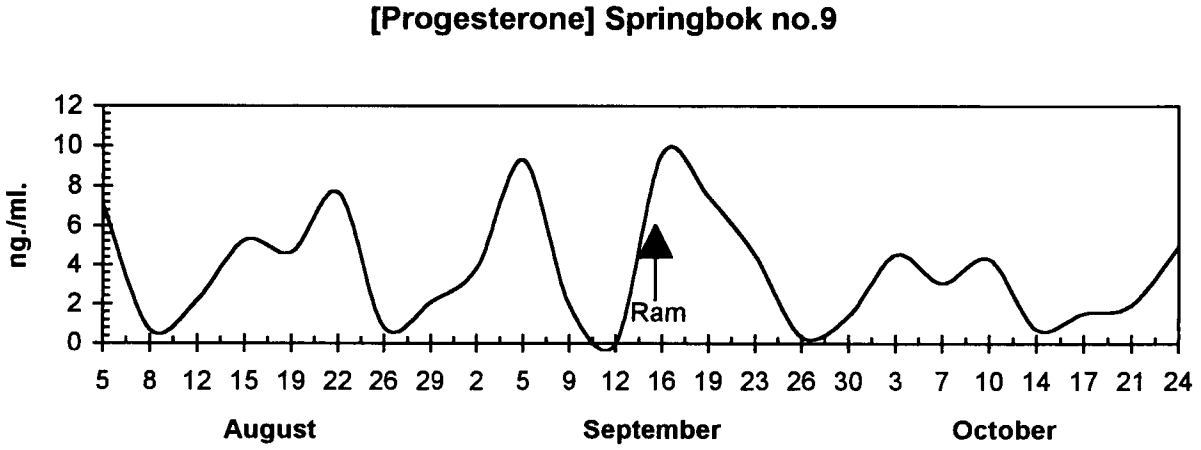

Fig. 3b: Trend in progesterone concentration prior to, and after ram introduction.

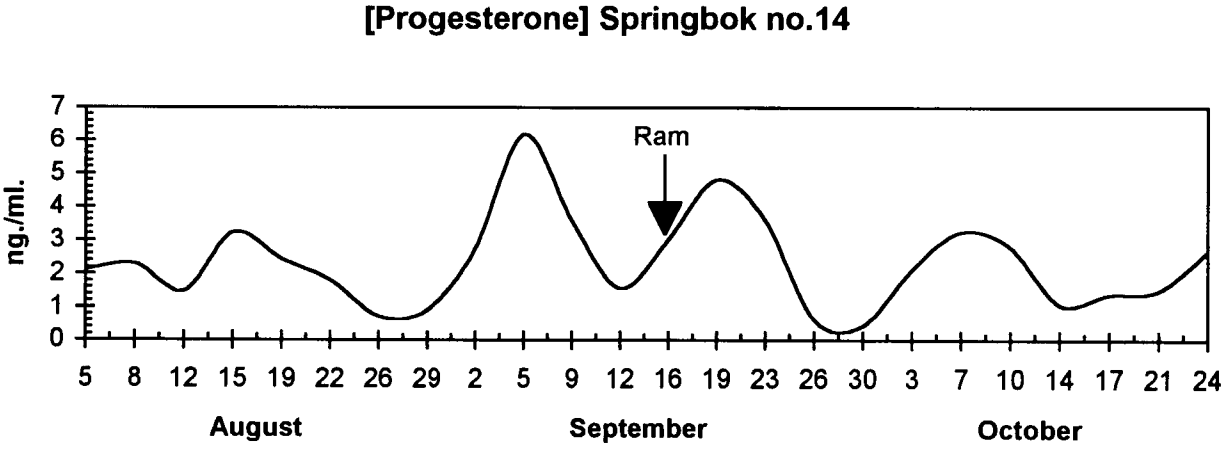

Fig. 3c: Trend in progesterone concentration prior to, and after ram introduction.

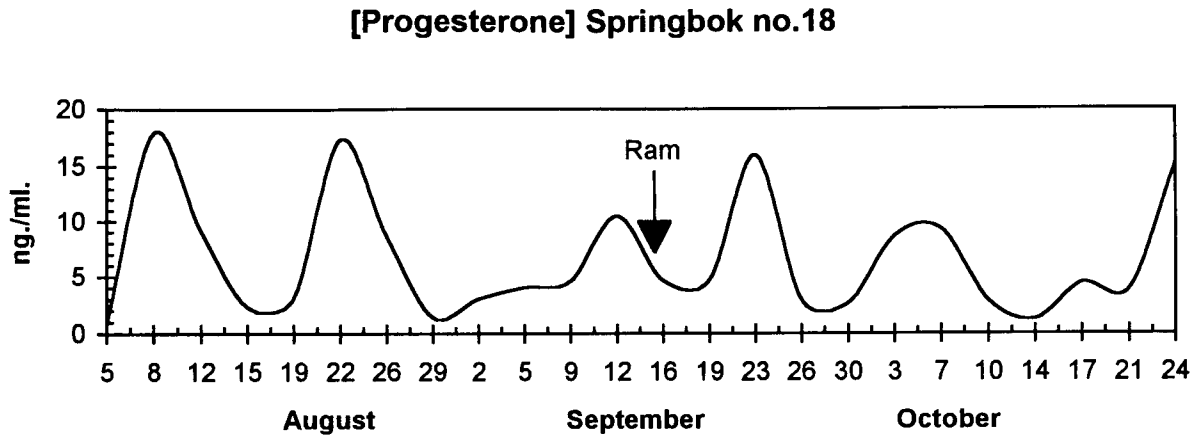

Fig. 3d: Trend in progesterone concentration prior to, and after ram introduction.

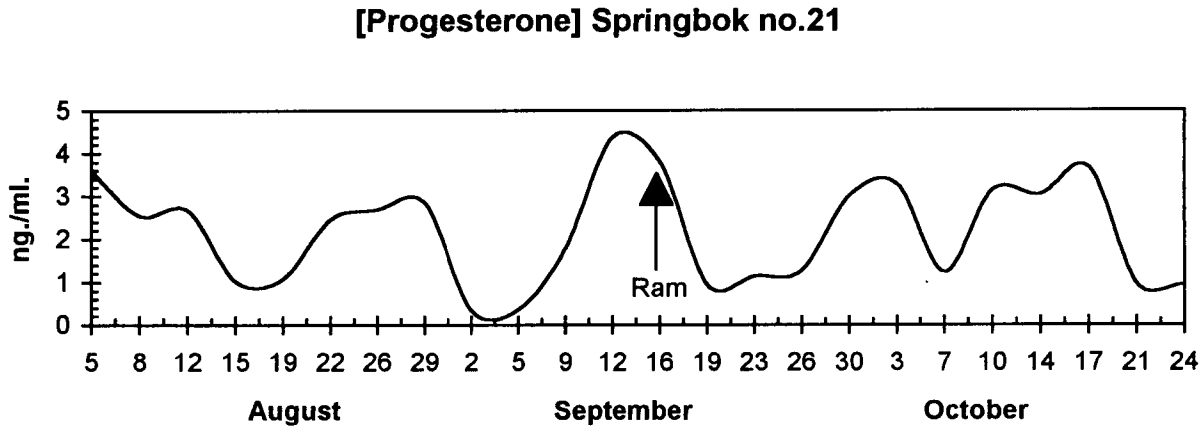

Fig. 3e: Trend in progesterone concentration prior to, and after ram introduction.

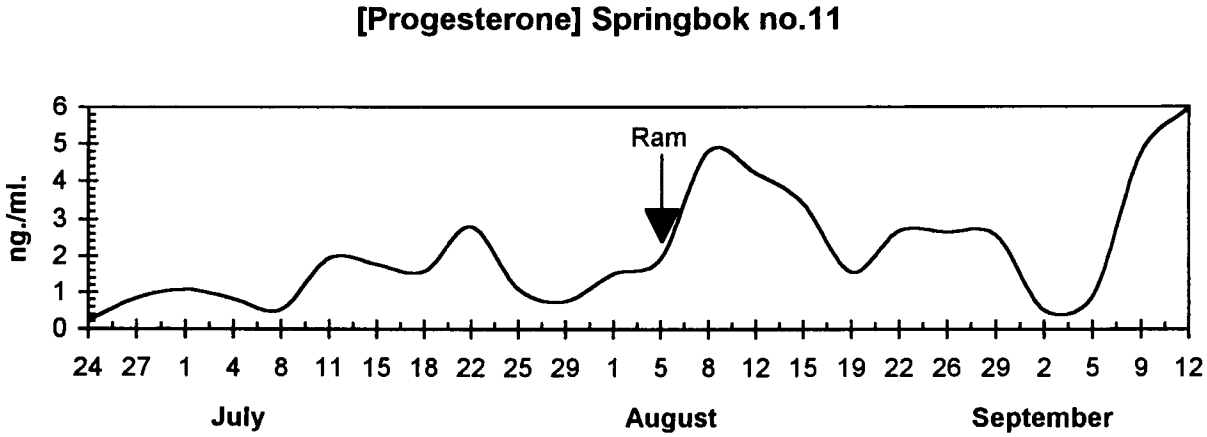

Fig. 3f: Trend in progesterone concentration prior to, and after ram introduction.

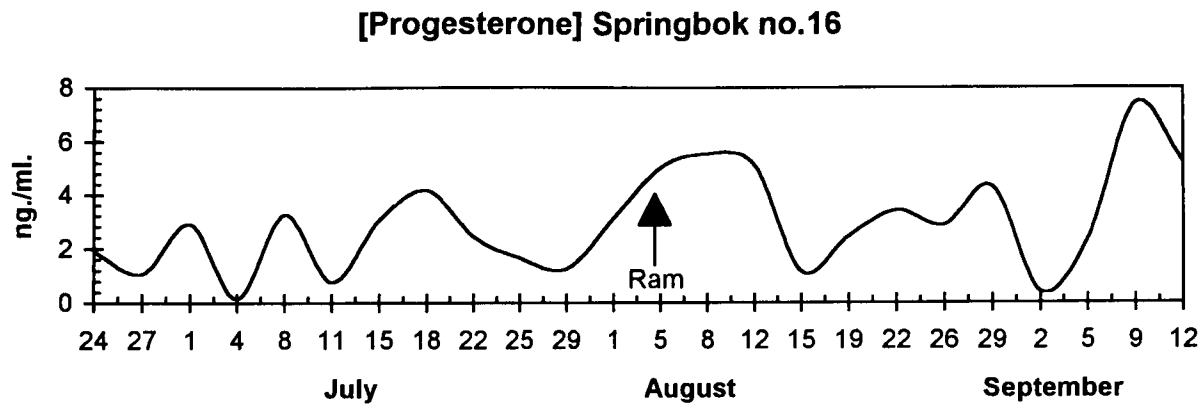

Fig. 3g: Trend in progesterone concentration prior to, and after ram introduction.

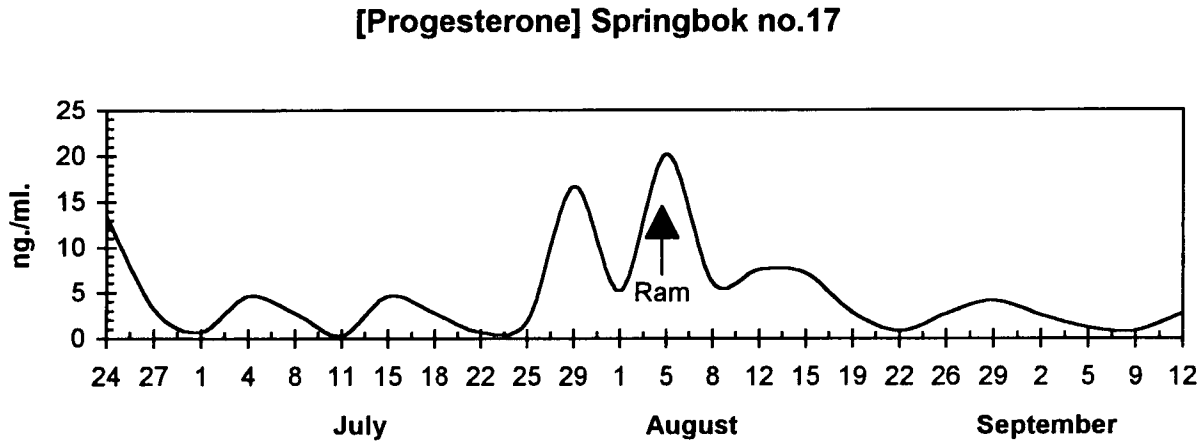

Fig. 3h: Trend in progesterone concentration prior to, and after ram introduction.

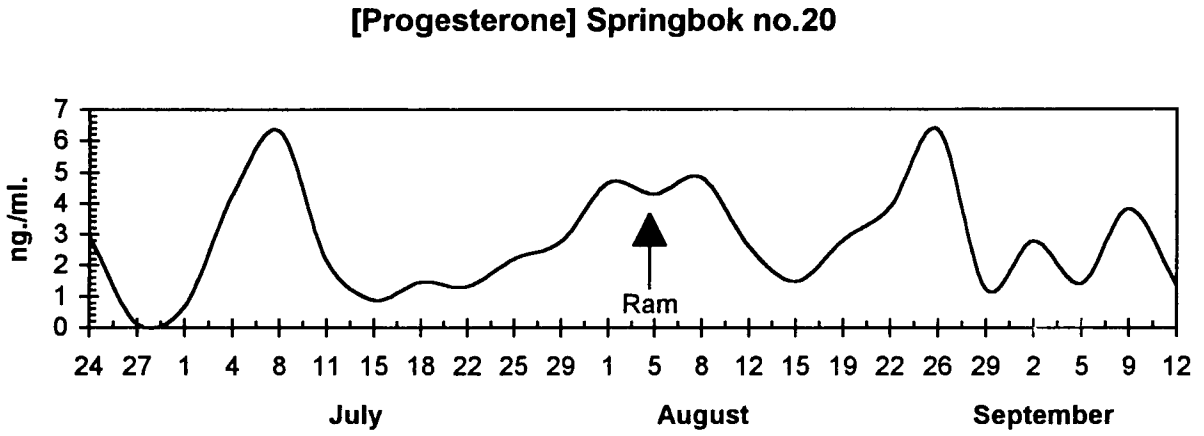

Fig. 3i: Trend in progesterone concentration prior to, and after ram introduction.

Results of the Wilcoxon's test for data with ram, and ram removed for group 1 are summarised in Table 4.

Table 4: Wilcoxon's test results for plasma progesterone concentrations of group 1 while with the ram and after the ram has been removed (ng/ml).

	Springbok number			
	11	**16**	**17**	**20**
Mean with ram present (1)	2.999	3.783	4.904	3.082
Mean after ram removal (2)	1.263	2.268	2.873	2.052
Std.deviation (1)	1.680	2.026	5.347	1.608
Std.deviation (2)	0.806	2.057	2.631	1.069
Variance (1)	2.82	4.10	28.59	2.59
Variance (2)	0.65	4.23	6.92	1.14
Z - value	2.274947	1.333590	1.647376	1.961161
P - value	0.022916	0.182348	0.099491	0.049869

The trends in plasma progesterone concentration after the removal of the ram for springbok ewes of group 1 (11, 16, 17, and 20) are illustrated graphically in figures 4a-d.

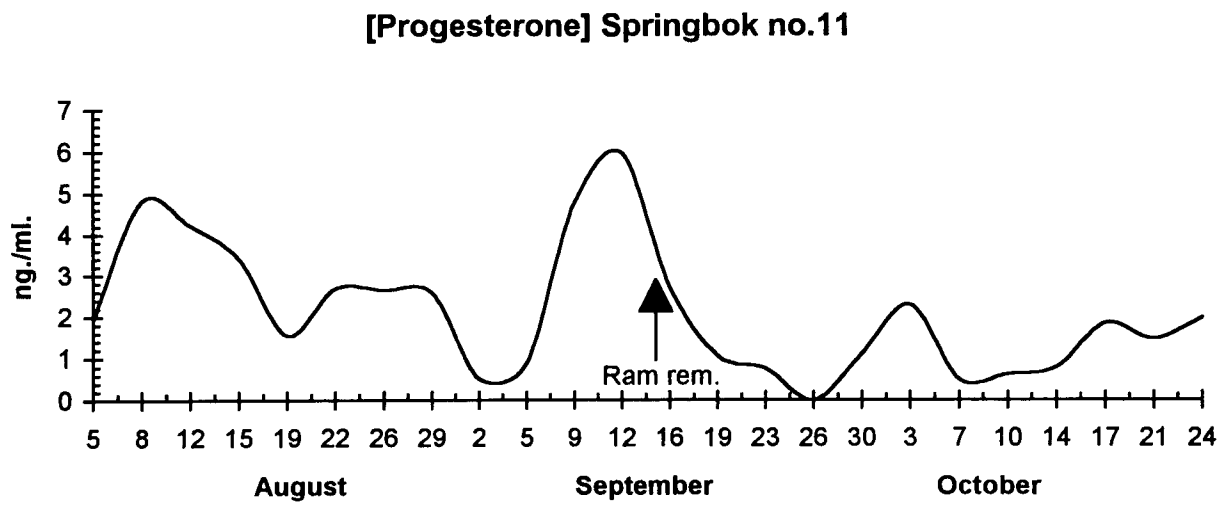

Fig. 4a: Trend in progesterone concentration after the removal of the ram.

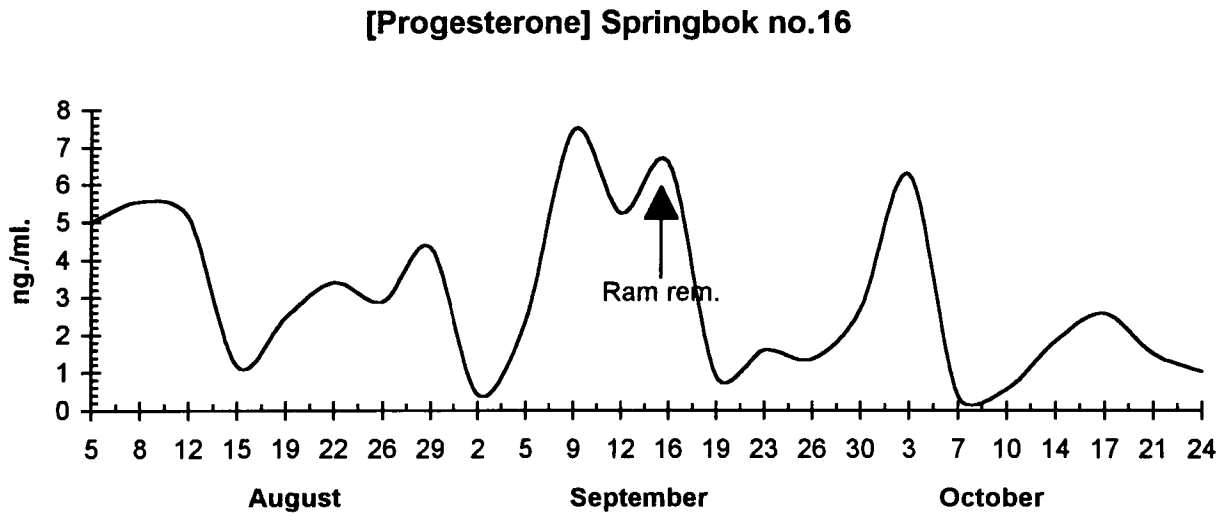

Fig. 4b: Trend in progesterone concentration after the removal of the ram.

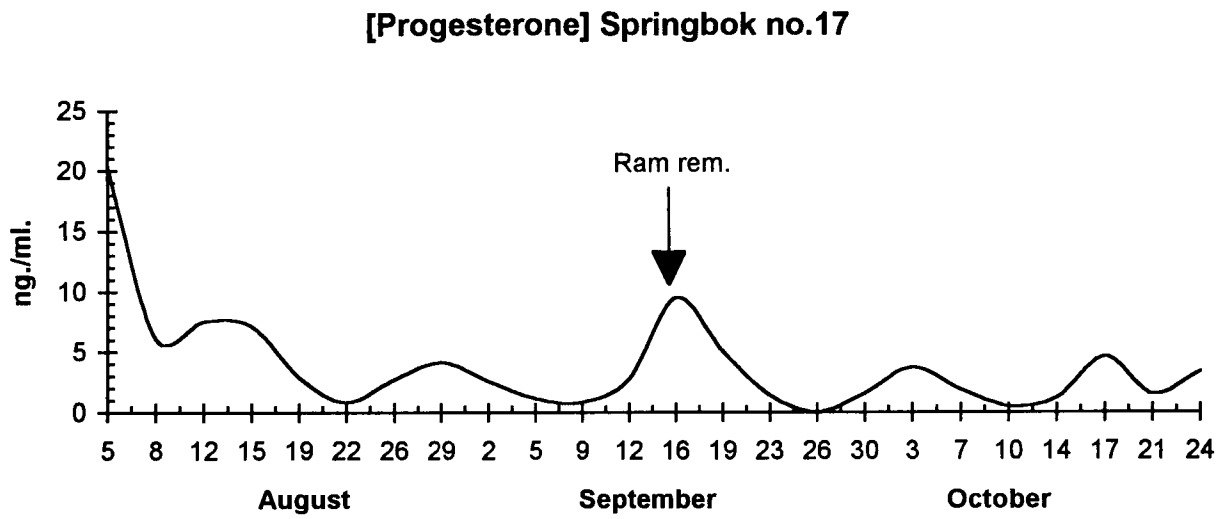

Fig. 4c: Trend in progesterone concentration after the removal of the ram.

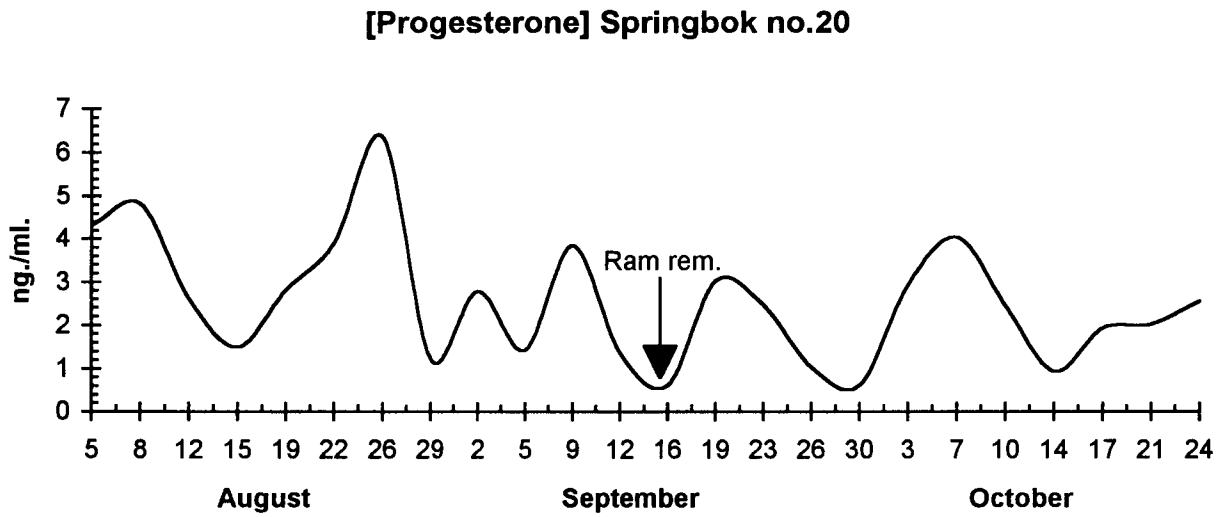

Fig. 4d: Trend in progesterone concentration after the removal of the ram.

Results of Wilcoxon's test: Comparing minimum, middle and maximum plasma progesterone concentrations (ng/ml) of group 1 and group 2 between for the period 05/08 to 12/09 are summarised in Table 5.

Table 5: Results of Wilcoxon's test; comparing sorted data of plasma progesterone concentration of group 1 and group 2 (ng/ml).

	Group 1 (n=16)		**Group 2** (n=20)		
	Mean	**Std.dev.**	**Mean**	**Std.dev.**	**p-value**
Minimum	1.38188	0.64562	1.256	0.75828	0.897
Middle	3.21875	0.73675	3.197	1.56618	0.955
Maximum	6.46313	3.83833	6.548	4.45269	0.569

To compare the degree of time (date) variation of the follicular phase of the ewes, for before and after ram introduction of both experimental groups, the Friedman two way ANOVA test was used. This method will detect any significant change within and between the 4 periods [Before ram (2) & after ram (2)] used. Results are summarised in Table 6.

Table 6: Mean dates of follicular phase, variance and standard deviation of the follicular phase for groups 1 & 2, used to determine synchronisation of the "ram effect" before and after ram introduction.

		Follicular phase			
		Before ram		**After ram**	
Springbok No.		**July**	**July**	**Aug**	**Sept**
Group 1	11	8	29	19	3
	16	11	28	15	2
	17	10	24	22	7
	20	15	(30)	15	30 aug
	Variance	**8.67**	**6.92**	**11.58**	**10.91**
	Std.dev	2.94	2.63	3.40	3.30
Friedman p-value		0.8073			
	No.	**Aug**	**Sept**	**Sept**	**Oct**
Group 2	3	19	5	26	10
	9	27	11	27	14
	14	27	12	28	15
	18	17	30 aug	28	14
	21	17	3	23	7
	Variance	**26.80**	**30.0**	**4.3**	**11.5**
	Std.dev.	5.18	5.48	2.07	3.39
Friedman p-value		0.7819			

Table 7: T-test assuming unequal variance to compare the variation for before- and after ram introduction. Dates used are the period of follicular phase before- and after ram introduction.

Springbok number	Before ram introduction Dates		After ram introduction Dates	
3	19	5	26	10
9	27	11	27	14
14	27	12	28	15
18	17	30 (aug)	28	14
21	17	3	23	7
Diff.(days)	10	13	5	8
Std. dev.	5.18	5.48	2.07	3.39
11	8	29	19	3
16	11	28	15	2
17	10	24	22	7
20	15	30	15	30 (aug)
Diff.(days)	7	6	7	8
Std. dev.	2.94	2.63	3.4	3.3
Average diff. (days)	**9**		**7**	
Average std.dev.	**3.16**		**1.41**	
p-value (one tail)	**0.156**			
p-value (two tail)	**0.313**			

DISCUSSION

There is great variation in plasma progesterone concentrations between individual springbok ewes. This variation can be seen in the degree of variation between the means and variances of individual springbok ewes as indicated in Table 3. The distribution of plasma progesterone concentrations within individuals was found not to have a normal frequency, tending towards a bimodal graphical curve for each individual springbok ewe. Due to the large variation between individual springbok ewes and the non-normal frequency of progesterone concentration distributions of individual springbok, non-parametrical statistical testing methods were preferred to parametrical statistical testing methods.

In a study of the "ram effect" in seasonally breeding anoestrous blesbok (*Damaliscus dorcas*) it was found that the presence of the ram dramatically increased the progesterone concentrations in all the ewes within a week (Skinner *et al.*, 1991). The "ram effect" in the seasonally breeding animals may, depending on the female's hormonal state, refer to first: activation of the oestrous cycle, secondly: synchronisation of oestrus within a group of female individuals. The springbok ewes in this study, being opportunistic and being well fed (good body condition), cycled prior to ram introduction. In springbok there may thus be two possibilities of a possible "ram effect", first: his introduction to the cycling springbok ewes may cause a significant change in the levels of plasma progesterone concentrations of the ewes. The difference between the minimum and peak progesterone concentrations of the ewes may be significantly greater after introduction of the ram, or the progesterone cycle may change to a higher level. Secondly, his introduction may cause an oestrus synchronisation (follicular phase) or "fine tuning" between ewes.

The Mann-Whitney U-test was used to compare the mean plasma progesterone concentration of the experimental group 1 of 4 ewes (with the ram) with that of the control group 2 of 5 ewes (without ram). This test may be used with as few as four observations in each sample as well as sample sizes that are unequal (Fowler & Cohen, 1994). No significant difference was measured between the mean plasma progesterone concentrations of the experimental group 1 and the control group 2 ($p=0,557$) (Table 1).

After both groups had been exposed to the ram, means and variances were determined for before- and after ram introduction (Table 2). Although the mean progesterone concentration of group 1 (n=4) increased from 2,659 ng/ml to 3,692 ng/ml plasma progesterone after the ram had been introduced (Figure 1), the large variation within the sample groups may have caused a non-significant statistical difference. Two ewes of group 1, ewes 11 & 16 had a significant increase in mean plasma progesterone concentration after ram introduction (Table 3). This increase could be ascribed to the effect of translocation and handling stress on circulating progesterone as later described in this document. A nonsignificant decrease from 3,668 ng/ml to 3,537 ng/ml in the mean plasma progesterone of group 2 for before- and after ram introduction was detected (Table 2, Figure 1).

Progesterone data were sorted (Appendix 2) into minimum, middle and maximum plasma progesterone values. These sorted values of group 1 (with ram) and group 2 (without ram) were compared statistically. This was done in order to compare the sorted values, irrespective of the asymmetry of the progesterone cycles of the ewes. Maximum plasma progesterone values should thus not cancel the minimum progesterone values, and vice versa, within a group of ewes. Neither minimum, middle or maximum progesterone concentrations differed significantly between group 1 (with ram) and group 2 (without ram) (Table 5). This indicates that there was no significant change in plasma progesterone concentrations between the two groups after ram introduction. The null hypothesis can therefore not be rejected, as there was no significant change in progesterone concentration, and it may be stated that the ram did not have a significant influence on plasma progesterone levels of the springbok ewes after he was introduced.

Watson & Radford (1960) have demonstrated that the visual and physical components of perception of the ram are not essential to the induction of ovulation in sheep. They do however suggest that the importance of ram perception should not be ignored. The perception of the springbok ram by the springbok ewes may thus also be very important. Pearce & Oldham (1984) mention that in a study done on the ram effect on a group of male-isolated female sheep, and another group of females kept next to rams, the percentage ewes ovulating in the first group of isolated ewes were significantly less than those of the other group that were kept next to the ram enclosure. The visual and physical components of perception and the presence of different pheromones may be more important in the response of ewes than has previously been recognised (Pearce & Oldham, 1984).

The springbok ewes were hand reared, and had been isolated from a mature social structure and the presence of a mature springbok ram before puberty. This may have affected their perception of the ram. Bearden & Fuquay (1992) mention that sight, smell, sound and experience are very important in the sexual cycle and female perception of especially males. This may also be the case in springbok, where mature adults should be present to "teach" the young. Auditory effects (grunting or "roaring") during a rut also induce ovulation (McComb, 1987; Skinner *et al.*, 1991). The absence of auditory effects during rearing might thus also contribute towards a skewed perception of the ram. Due to these factors, the springbok ewes in this study might not have had enough time to perceive the ram properly, and that this therefore resulted in no significant response in progesterone levels after the introduction of the ram.

Under closer observation where Wilcoxon's test for matched pairs was done comparing each of the nine springbok prior to, and after ram introduction, I found that only two of the nine springbok ewes showed significant progesterone differences prior to, and after ram introduction. The mean progesterone concentration in springbok 11 increased from 1,248 ng/ml to 3,00 ng/ml (Table 3) after the ram had been introduced ($p<0.05$). Similarly the plasma progesterone concentration in springbok 16 increased from 2,150 ng/ml to 3,783 ng/ml (Table 3) after the ram had been introduced ($p<0.05$). A significant change of progesterone concentration in 2 out of 9 (22.2%) springbok ewes is not enough to conclude that the ram has an effect on the ewes. According to the exact one-sided F-table (Fowler & Cohen, 1994), in a small sample size of 9 individuals it is expected that there be a significant response of 7 individuals before a group significance may be stated. Both ewes (11 & 16) showing a significant increase in progesterone concentrations were in group 1 that were translocated to the ram. The translocation may have served as a stressor which could have increased the progesterone concentrations of these two ewes. The effects of a particular stressor on different individuals appears to be variable (Kant, Bauman, Anderson, & Mougey. 1992). Anderson, Saviolakis, Bauman, Chu, Ghosh & Kant (1996) state that uncontrollable stress in general, such as translocating or handling animals, causes greater physiological disruptions as assessed by changes in plasma hormones and body weight. The weights of the nine springbok used in this study did however not change dramatically over the study period (±2 kg.). One of the factors that appears to be important in determining the effects a stressor has upon an individual is the degree of control that the individual perceives himself/herself to have over the stressor (Fisher, 1986).

In a study done on the effects of chronic stress on the plasma hormones of female rats (Anderson *et al,* 1996) it was found that progesterone concentrations increased as a result, but that the increase was not statistically significant. On the other hand, Marchlewska-Koy, Pochron, Galewicz-Sojecka & Galas (1994) reported elevated levels of plasma progesterone in female rodents exposed to stress. Springbok ewes (11, 16) might have experienced the translocation as a stressor, and this may have influenced the increase in plasma progesterone concentrations detected. The other seven springbok ewes (3, 9, 14, 17, 18, 20, 21) showed no significant change in their progesterone levels after the ram had been introduced ($p>0.05$) (Table 3). In contrast to the significant increase in plasma progesterone concentrations in springbok ewes 11 & 16, the remaining ewes (3, 17, 20, & 21) showed only a slight and non-significant increase in the mean progesterone concentrations after ram introduction. Springbok ewes 9, 14, 18 however were found to have a non-significant decrease in the mean progesterone concentration prior to, and after ram introduction (Table 3).

Signoret, Fulkerson & Lindsay (1983) found that the proportion of sheep ovulating after the introduction of rams was highest after four days of introduction and exposure to rams. However, these findings were on sheep and because of the fact that species specific reactions to rams are likely, the reaction time of springbok ewes might be much longer. If the presence of rams directly affects the secretion of LH, the subsequent withdrawal of rams should reduce the secretion of LH (Pearce & Oldham, 1984), and thus progesterone secretion should also eventually increase. Pearce and Oldham (1984) found that the continued presence of rams is necessary to stimulate the preovulatory surge of LH since their early withdrawal reduces the incidence of ovulation. The "ram effect" might have been confounded by a too short time period during which the ram was with the ewes, or by a difference of ram perception by different individual ewes.

Results of the effect of ram removal on the progesterone concentrations of four springbok ewes of group 1 indicated a significant difference of progesterone concentration in springbok 11 and 20 after ram removal ($p<0.05$) (Table 4). The mean progesterone concentration significantly decreased in these two ewes while there were a non-significant decrease of progesterone concentrations in the other two ewes (Table 4). It could be expected that if there is a "ram effect", the progesterone concentration may increase, depending on the hormonal state of the ewes, and shift the oestrus cycle to the luteal phase after the removal of the ram.

Pheromones are present in the urine and faeces of sheep (Knight, 1983). If this is also true in springbok, the presence of pheromones in the ram's faeces and urine may have had an effect on the hormonal responses of the ewes. The pheromones present in the ram's faeces and urine left in the enclosures after the ram had been removed, might therefore still stimulate a hormonal response on the springbok ewes. This might have confounded results found during this study. Overall, group 1 showed no significant change in plasma progesterone concentrations after the ram had been removed. The effect of ram removal in group 2 was not measured.

Through the above analysis of the "ram effect" of changes of the progesterone concentrations, it can be concluded that the ram does not have a significant effect on circulating progesterone or the plane/level of progesterone in cycling springbok ewes. In future, it may be useful to include other hormones such as luteinizing hormone (LH), which are less affected by stress, in these studies on the "ram effect".

In order to look at the synchronisation aspect of the "ram effect", time variation of the mean follicular phase before- and after ram introduction were measured. Oestrous cycles were subjectively determined for each ewe at approximately 14-17 day intervals. The lowest levels of analysed progesterone data, the average time/date of the follicular phase, were used to determine the variation before- and after ram introduction. Dates used, and results are summarised in Table 6. In group 1, the variance in both cycles for before ram introduction increased to a larger variance after ram introduction. In group 1 the average variation for two cycles before ram introduction was 7.80 increasing to 11.25 after ram introduction. However, no significant differences between any of these cycles were measured (Table 6). Since group 1 was translocated to the ram enclosure, stress as previously described, may have confounded or altered the results and degree of variation. In group 2 the variation in both cycles before ram introduction decreased considerably after the introduction of the ram. The average variation of 28.4 before ram introduction decreased to an average variation of 7.9 after ram introduction. Although superficially the decrease in variation looks significant, it was found to be non-significant (Table 6). The translocation stress factor in group 1 and the small group size might have confounded the synchronisation effect of the springbok male on the ewes. The t-test (Table 7) also indicated no significant differences in the variation and follicular phase's standard deviation of the ewes before- and after ram introduction. The considerable but non-significant decrease in variation after ram introduction might suggest that more individuals be used to determine the synchronisation effect of the male sufficiently before any definite conclusions can be made.

Although the "ram effect" on anoestrous, non cycling animals such as the sheep (Knight, 1983; Pearce & Oldham, 1984) has been studied extensively, the "ram effect" on cycling animals, especially aseasonally breeding wild ungulates such as the springbok is still unknown. Pearce and Oldham (1984) mention that one of the potential limitations of the "ram effect" in sheep is where a portion of ewes in a flock may cycle spontaneously and are therefore unavailable to be induced to ovulate by the introduction of rams. The fact that the springbok ewes were cycling (fluctuations in measured plasma progesterone concentrations) prior to ram introduction might thus also have confounded the "ram effect" or the detection of a significant change in progesterone concentrations of the ewes. The springbok ewes used in this study may have been cycling because of supplementary feeding, thus being in a good body condition, contrary to female springbok in an arid environment with unpredictable environmental conditions.

The "ram effect" in cycling individuals should therefore refer to the synchronisation of the follicular phase after ram introduction. The "ram effect" in such cases might therefore be only to "fine tune" the ewes into synchronised conceptions and later births of lambs. Skinner & Louw (1996) state that such synchronisation of oestrus and subsequently parturition occurs widely in ungulates with a restricted breeding season. The birth pulses in springbok could be of similar value but occur in a more opportunistic manner because of the unpredictability of the arid environment where springbok occur (Skinner & Louw, 1996).

CONCLUSION

The findings in this experiment of the "ram effect" on the progesterone concentrations of springbok ewes under the described conditions were that the ram did not have a significant effect in changing the progesterone concentrations or the level/plane of the progesterone cycle in the nine cycling ewes used. In accordance with the above findings, the initial null hypothesis (prog. conc. without ram = prog. conc. with ram) can not be rejected at the 5 % level of significance.

The "ram effect" in already cycling ewes should refer to the synchronisation effect on the oestrous cycle. The results found in this regard must be evaluated with caution as external factors may have confounded the "ram effect". These factors include stress (which is individually specific and might lead to change in progesterone levels), the perception of the ram by the ewes (which might also be different between individuals), the fact that the springbok were hand reared (might delay the response to the ram), and the fact that the springbok ewes, although mature and cycling, have not mothered young before and were not in the constant vicinity of a ram during their up-bringing. The duration that the ram was kept with the ewes might have been too short to allow for a significant change in plasma progesterone concentration when the ram was introduced, thus no significance was detected.

Synchronisation of the oestrous cycle through the "ram effect" superficially appears significant but isn't. More individuals should be used before any reasonable conclusions can be made. Speculatively if the experimental group was to be increased in quantity, it may be found that the ram synchronises ("fine tunes") the oestrous cycle of ewes significantly. Other hormones such as luteinizing hormone and oestrogens, which may be less affected by stress, should also be analysed to illustrate and examine the "ram effect" on the oestrous cycle of an aseasonal breeder properly.

Determining the "ram effect" in an aseasonal cycling breeder is thus not as clear as it seems. There may be a great number of confounding effects, especially where a wild ungulate such as the aseasonally breeding springbok is concerned and where females are already cycling.

FUTURE RESEARCH.

It would be interesting to compare these progesterone trends of captive and well fed springbok ewes (n=9) (kept in a mesic environment), with captive springbok "environmentally fed" and in their natural habitat, with each other (individuals of the same age and weight).
Out of this experiment, other questions come to mind:

- Do the progesterone concentrations of wild springbok ewes of the same age but in a natural ("drier") environment with the natural availability of food, differ much from the progesterone concentrations attained in the above experiment ?

- Do springbok ewes in a drier environment held under the same conditions as this experiment, cycle throughout the year ?

- What role does supplemental feeding play on the oestrous cycle of springbok ?

- To what extent does the springbok ram fine tune the oestrous cycle and oestrus of the ewe ?

A more in depth/detailed examination of the blood chemistry with ram introduction might be worthwhile. Include measurements of other hormones such as luteinizing hormone and oestrogens, which may be less affected by stress, to illustrate and examine the oestrous cycle of an aseasonal breeder properly. Including more or a longer period of data collection would also be more significant to get to an in depth view of the hormonal changes of the "ram effect" and the extent (if any) on oestrus synchronisation/finer tuning of springbok ewes.

SUMMARY

The purpose of this experiment was to study the effect of the springbok ram, the so-called "ram effect" on the progesterone concentrations of the springbok ewe (*Antidorcas marsupialis*). The "ram effect" of and on aseasonally breeding wild ungulates and already cycling females has not been studied in detail yet and this study might contribute towards a clearer understanding thereof. Results and conclusions might contribute towards management plans for intensive aseasonal and wild ungulate breeding programmes.

Plasma progesterone concentrations were used as a measure to determine the response of the ewes to the ram. The question addressed was: Does the introduction of the male springbok to a group of springbok ewes significantly alter the blood progesterone concentrations of the ewes ?

Nine mature (2 years six months ± 2 months) springbok ewes were used to determine whether this "ram effect" applies to an aseasonal cycling opportunistic breeder. Ewes were randomly divided into two groups of four and five individuals to each group. Blood was collected biweekly from all ewes prior to and after ram introduction. Each group was subjected to the ram for a period of forty two days. Radioimmunoassays were used to determine the plasma progesterone concentration for all the ewes. No significant difference (using the Mann-Whitney U-test) in the change of progesterone concentrations after the introduction of the ram was detected in both ewe groups. Under closer individual observation using the same animal for before- and after ram introduction (n=9, Wilcoxon's test), only 2 springbok ewes showed a significant increased difference in progesterone concentration ($p<0.05$). However, this change in plasma progesterone concentration could have been artificial as a consequence of the stress of handling and translocation. It is concluded that the ram had no significant effect on the progesterone concentration of the nine cycling springbok ewes.

In already cycling female individuals no significance in progesterone concentration and/or the plane of progesterone for before- and after ram introduction was detected. The effect of follicular phase synchronisation superficially looked significant, Follicular phase variation decreased after ram introduction, but statistically no significance was found.

The results should however be considered with caution as several factors might have confounded the "ram effect". Such factors include stress, the perception of the ram by the ewes, and social organisation and rearing of, especially a wild ungulate. It is suggested that other hormones such as e.g. luteinizing hormone, which may be less affected by stress, also be used to determine the "ram effect" on aseasonally breeding wild ungulates. Where cycling females are used, variation in the oestrous cycle, prior to and after ram introduction should be measured.

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APPENDIX 1

PROGESTERONE CONCENTRATIONS FOR STATISTICAL USE (ng/ml)

Springbok number

		3 (A)	9 (B)	11 (C)	14 (D)	16 (E)	17 (F)	18 (G)	20 (H)	21 (I)
24\06	1	0.86	2.42	0.22	1.78	1.89	13.42	6.99	2.93	1.14
27\06	2	3.33	2.49	0.84	2.25	1.08	3.19	10.35	0.13	0.14
01\07	3	1.35	2.94	1.08	2.12	2.9	0.76	1.19	0.68	0.73
04\07	4	1.09	2.6	0.83	1.25	0.17	4.61	1.02	4.3	0.54
08\07	5	0.66	1.26	0.53	1.38	3.24	2.75	10.75	6.32	3.63
11\07	6	1.7	4.13	1.95	1.96	0.77	0.34	4.8	2.16	1.38
15\07	7	1.96	3.96	1.77	2.78	3.07	4.63	1.47	0.9	1.21
18\07	8	2.15	3.76	1.58	1.71	4.18	2.79	5.3	1.47	3.41
22\07	9	2.41	0.62	2.81	2.26	2.46	0.67	15.42	1.32	1.23
25\07	10	1.12	0.79	1.1	1.07	1.66	1.89	2.21	2.22	3.04
29\07	11	0.77	4.86	0.75	2.11	1.27	16.72	2.56	2.78	2.84
01\08	12	1.57	3.98	1.52	3.32	3.11	5.19	3.1	4.67	1.2
05\08	13	3.85	7.04	1.93	2.11	5	20.21	0.82	4.31	3.6
08\08	14	3.9	0.7	4.82	2.3	5.55	8.09	17.95	4.83	2.54
12\08	15	3.25	2.22	4.22	1.48	5.13	7.54	9.16	2.61	2.68
15\08	16	4.43	5.29	3.41	3.24	1.17	7.14	2.32	1.49	1.03
19\08	17	0.84	4.68	1.56	2.44	2.46	2.87	3.19	2.82	1.09
22\08	18	2.17	7.68	2.69	1.81	3.41	0.83	17.38	3.9	2.45
26\08	19	1.98	0.83	2.64	0.72	2.88	2.72	8.7	6.35	2.69
29\08	20	2.74	2.09	2.57	0.91	4.33	4.14	1.44	1.24	2.86
02\09	21	2.57	3.86	0.52	2.77	0.42	2.55	3.04	2.79	0.35
05\09	22	1.52	9.34	0.9	6.2	2.38	1.15	4.05	1.43	0.37
09\09	23	3.87	1.95	4.77	3.58	7.45	0.83	4.62	3.84	1.79
12\09	24	5.17	0	5.96	1.56	5.24	2.78	10.45	1.37	4.4
16\09	25	7.3	9.65	2.71	3.04	6.56	9.56	4.67	0.62	3.8
19\09	26	5.51	7.48	1.07	4.82	0.91	5.04	4.73	3.02	0.66
23\09	27	4.16	4.52	0.77	3.56	1.6	1.44	15.97	2.46	1.14
26\09	28	0.21	0.35	0	0.63	1.36	0.07	2.98	1.04	1.28
30\09	29	4.8	1.42	1.12	0.43	2.68	1.56	2.67	0.62	3.01
03\10	30	2.77	4.54	2.29	2.12	6.26	3.72	8.63	2.92	3.28
07\10	31	2	3.09	0.5	3.24	0.39	1.9	9.33	4.03	1.24
10\10	32	0.8	4.3	0.62	2.79	0.55	0.52	2.88	2.45	3.15
14\10	33	2.32	0.75	0.8	1.06	1.82	1.2	1.28	0.94	3.64
17\10	34	2.67	1.54	1.85	1.37	2.57	4.61	4.48	1.93	3.67
21\10	35	2.06	1.96	1.46	1.49	1.5	1.51	3.9	2.03	0.97
24\10	36	2.55	4.96	1.97	2.65	1.02	3.34	15.24	2.56	0.96

Without ram

With ram present

Group 1: 11, 16, 17, 20.

Group 2: 3, 9, 14, 18, 21.

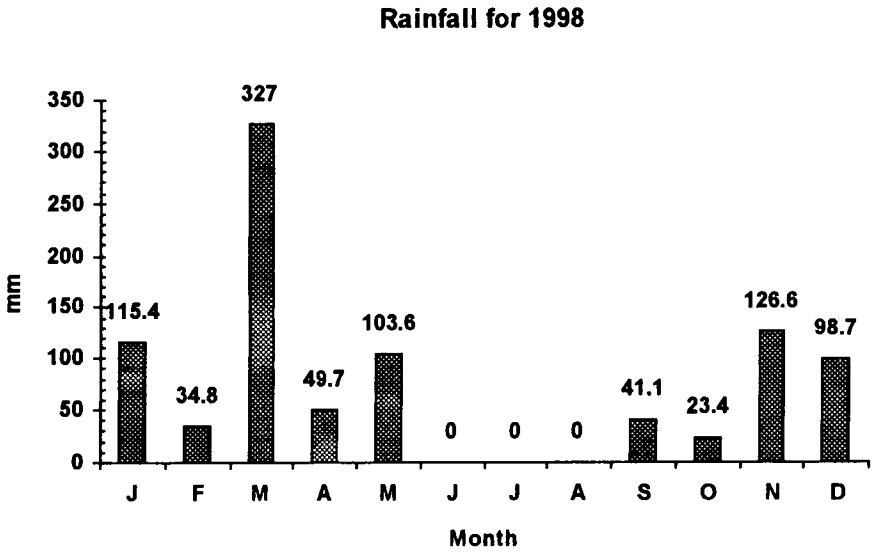
APPENDIX 2

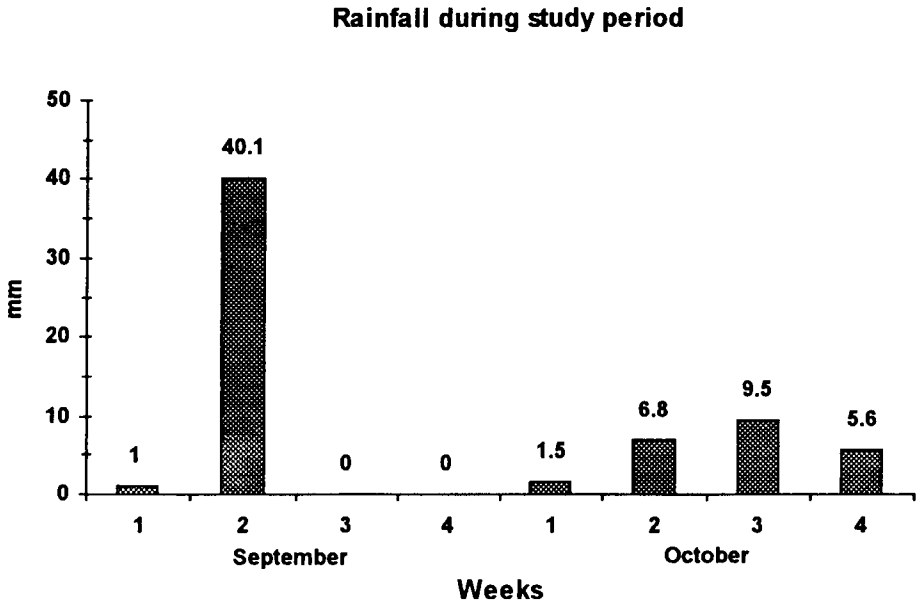
Values used in Wilcoxon's test for comparing minimum, middle and maximum progesterone concentratins (ng/ml) of group 1 and group 2 between the period 05/08 to 12/09.

Group 1 (with ram)					Group 2 (without ram)				
Springbok number									
Value	**11**	**16**	**17**	**20**	**3**	**9**	**14**	**18**	**21**
Min.	1.93	1.17	0.83	1.49	0.84	0.70	1.48	0.82	1.03
	1.56	2.36	2.55	1.24	2.17	0.83	0.72	2.32	1.09
	0.52	0.42	1.15	1.43	1.98	1.95	0.91	1.44	0.35
	0.90	2.36	0.83	1.37	1.52	0	1.56	3.04	0.37
Mid.	3.41	5.00	2.87	2.61	3.85	2.22	2.11	3.19	2.54
	2.69	3.41	2.72	2.82	3.25	4.68	2.30	8.70	2.68
	2.64	2.88	4.14	2.79	2.74	2.09	2.44	4.05	2.45
	2.57	4.33	2.78	3.84	2.57	3.86	1.81	4.62	1.79
Max.	4.82	5.55	20.21	4.21	3.90	7.04	3.24	17.95	3.60
	4.22	5.13	6.09	4.83	4.43	5.29	2.77	9.16	2.69
	4.77	7.45	7.54	3.90	3.87	7.64	6.20	17.38	2.86
	5.96	5.24	7.14	6.35	5.17	9.34	3.58	10.45	4.40

APPENDIX 3

(Rainfall during the year and time of study period)





"From now on, you, springbok, will be known as the animal of light, faith and reliability"
"You are the faithful one, the reliable one, the "shining tassel", because of the fringe of hair near your rump that gives you a strange appearance as you leap in front of the sun."

Credo Mutwa