THE SEXUAL CYCLE OF THE IMPALA RAM *AEPYCEROS MELAMPUS* LICHTENSTEIN

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Little is known of the cyclical nature of the reproductive process in seasonal breeding antelope in Africa. The mechanisms affecting testicular endocrine and exocrine function are of fundamental interest in the ecology of these animals and alteration of these could lead to the manipulation of breeding patterns in such animals. Moreover, the mating season (rut) takes place over a four-week period, from mid-May to mid-June, and it is of some interest to establish whether this is dependent on male sexual activity. A pilot investigation was therefore planned to study the reproductive cycle of the impala ram.

MATERIAL AND METHODS

Two fully grown impala rams were shot every second month from January to November, 1969, a total of 12 rams, on the S.A. Lombard Nature Reserve near Bloemhof in the Western Transvaal. The weights of the animals and the dates when they were shot are listed in Table 1. The animals were shot in the neck with a high velocity rifle and immediately afterwards their throats were cut and the animals were bled. The whole carcass was then taken to the laboratory, weighed and dissected.

The testes, epididymides, ampullae of the vasa deferentia, seminal vesicles, bulbo-urethral glands and pituitary gland were dissected out and weighed individually. Sections were taken for histology and the ampullae, seminal vesicles and bulbo-urethral glands were then stored over solid CO_2. The amount of fructose and citric acid was estimated later.

Epididymal Sperm

Immediately following dissection, a droplet of sperm was extruded from the cauda epididymis at the junction with the vas deferens and placed on a warm (37°C) microscope slide. Wave motion of the sperm was then examined under the low power of a microscope and the percentage motile sperm assessed subjectively. A smear of epididymal sperm was also made using nigrosin : eosin to determine the percentage live and morphologically abnormal sperm. The number of sperm in the epididymides was estimated using the method of Dott and Skinner (1967).

Fructose and citric acid assay

The ampullae, seminal vesicles and bulbo-urethral glands were analysed for fructose and citric acid as described by Lindner and Mann (1960). The whole gland was cut into small pieces while still frozen, thoroughly mixed and a random sample taken for analysis.

Histological and histochemical techniques

Pieces of testes were fixed in Bouin's fluid or Zenker-formol solution. Pieces of seminal

TABLE 1

WEIGHTS OF IMPALA RAMS

Animal	*No. and Date*	*Weight (kg)*
1.	22. 1.69	58·2
2.	22. 1.69	70·5
3.	18. 3.69	78·6
4.	18. 3.69	72·7
5.	5. 5.69	65·4
6.	5. 5.69	61·8
7.	22. 7.69	60·9
8.	22. 7.69	65·5
9.	23. 9.69	52·3
10.	23. 9.69	59·1
11.	17.11.69	57·3
12.	17.11.69	57·7
		$\bar{x}$ 63·3
		S.E. ± 2·2

vesicles, ampullae and bulbo-urethral glands were fixed in Bouin's fluid. The Bouin-fixed material was dehydrated in alcohol, cleared in cedarwood oil and embedded in paraffin wax; sections 6 μ thick, were stained with Delafield's haematoxylin and chromotrope 2R. The Zenker-formol fixed tissue was post-chromed in potassium dichromate, washed, dehydrated, cleared, embedded in paraffin wax, sectioned and treated with Sudan Black as described by Threadgold (1957) in his method 1.

Further slices of testis were frozen on to cryostat chucks with the aid of solid CO_2 and sections cut 16 μ thick, incubated for 3 hr at 37°C to demonstrate the presence of Δ^5—3β-hydroxy-steroid dehydrogenase activity as described by Hay and Deane (1966) and Mann, Rowson, Short and Skinner (1967). Seminiferous tubule diameter was the mean calculated from 25 circular tubules measured in cross-section.

RESULTS

The reproductive tract of the impala ram is illustrated in Figure 1. The seasonal changes in the weights of the two testes, epididymides and two seminal vesicles are illustrated in Figure 2, from which it can be seen that there was a two-fold increase in testes weight during the year, the maximal value occurring two months prior to the rut and the minimal value occurring in September (spring). The decline after the rut was more abrupt than the increase preceding the mating season.

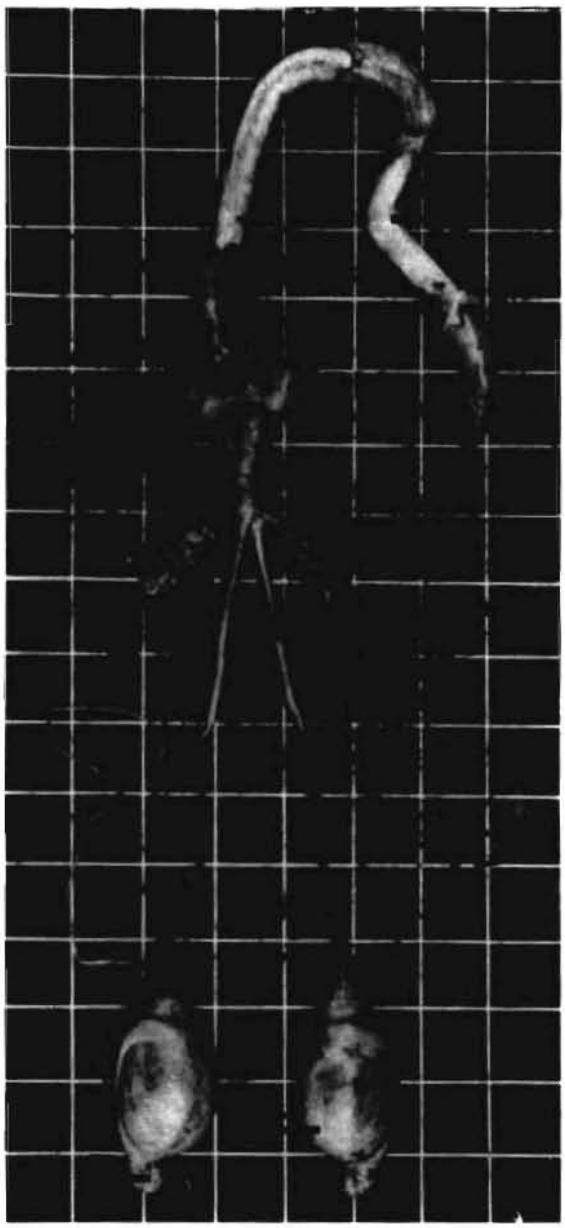

FIGURE 1
The reproductive tract of the impala ram showing testes (T), epididymides (E), pampiniform plexus (PP), vasa deferentia (VD), ampullae (A), seminal vesicles (SV), bulbo-urethral glands (BU) and the penis (P). Scale in inches.

The seasonal changes in the weight of the epididymides were equally pronounced but changes in the weight of the accessory glands were less so. The heaviest weights were found in the rut (autumn) and the lightest in early summer. Pituitary weights were relatively constant at 0·84 g throughout the year but did fall to 0·68 g two months after the rut.

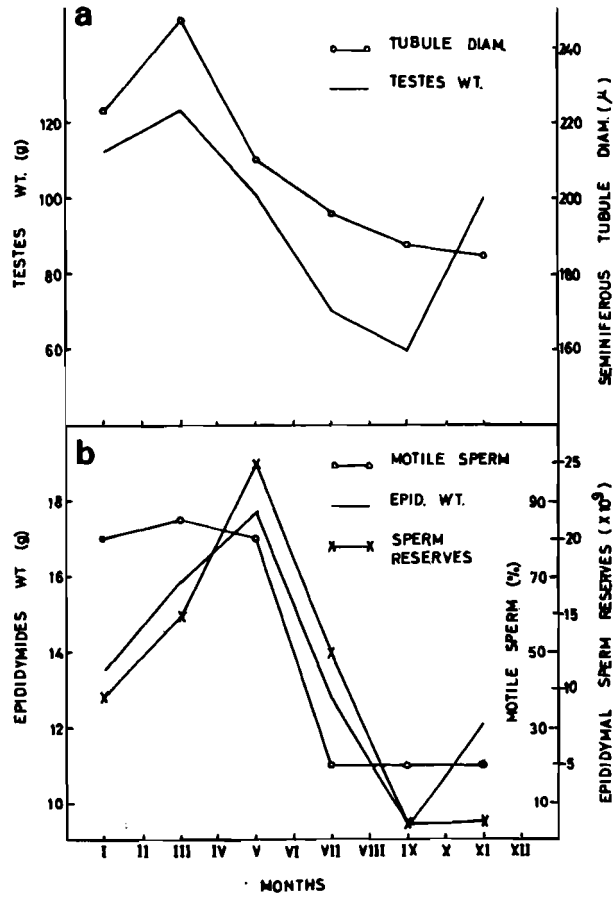

FIGURE 2
Seasonal changes of (*a*) weight of testes —— and seminiferous tubule diameter *o*——*o* and (*b*) weight of epididymides —— sperm motility *o*——*o* and sperm number *x*——*x* of the impala at the S. A. Lombard Nature Reserve.

Epididymal Sperm

Epididymal sperm reserves increased seasonally to a maximum in the rut (see Figure 2) when there were 25 times as many sperm as there were in the spring. There were always spermatozoa in the epididymis. Motility showed a similar cycle to sperm numbers, but the percentage dead and abnormal sperm did not increase with a decrease in motility. There was a considerable increase in cellular debris and other extraneous matter in the lumen of the epididymis with decline in sperm numbers.

Histology

The testes were never devoid of all signs of spermatogenic activity although tubule

diameter declined sharply after the rut and there was obviously some disruption of spermatogenesis (see Fig. 3, (A), (B) and (C)). There was a seasonal decline in spermatogenic activity matching the number of epididymal sperm but spermatogenesis never came to a complete standstill. As can be seen from Figure 2, tubule diameter increased significantly prior to the rut.

The activity of the Leydig cells showed a similar cycle. In the rut lipid was plentiful as was Δ^5—3β-hydroxysteroid dehydrogenase activity but both declined rapidly in spring (see Fig. 3, (D), (E), (F) and (G)).

Fructose and citric acid in the accessory glands

Both fructose and citric acid were identified as normal constituents of the ampullae, seminal vesicles and bulbo-urethral glands of the impala.

Vesicular fructose concentration started rising in November (462 mg/100 g) and showed a peak of 610 mg/100 g in May during the rut. Thereafter it declined rapidly to 197 mg/100 g in the spring. Citric acid showed a similar trend from a peak of 214 mg/100 g in May to 134 mg/100 g in summer. The seasonal changes in the content of vesicular fructose and citric acid (mg/total weight of seminal vesicles) in the rams are shown in Figure 4. It can be seen that fructose and citric acid reach maximum values in the seminal vesicles at the beginning of May at the onset of the rut.

The ampullae also secrete fructose and citric acid but the concentrations were lower in these glands than in the seminal vesicles, varying from 64 mg fructose per 100 g and 139 mg citric acid per 100 g just before the mating season to 23 mg fructose per 100 g and 113 mg citric acid per 100 g in summer. In the bulbo-urethral glands, concentrations varied from 5 mg fructose per 100 g and 97 mg citric acid per 100 g in autumn to 4 mg fructose per 100 g and 50 mg citric acid per 100 g in summer.

Sexual Cycle

All the gross morphological, histological and chemical observations that have been made on the reproductive organs of the impala ram demonstrate a marked sexual cycle with a peak during the rut in the autumn. This is not to say the organs were entirely inactive at other times. At no time did spermatogenesis cease altogether. The fructose and citric acid concentrations found in the accessory glands also indicated that hormone secretion was continuous and varying in quantity. The decline in sexual function coincided with a fall in body weight which itself commenced before the mating season, presumably as a result of increased fighting among the rams from March onwards. Rams which were previously grouped in large herds became scattered in ones and twos after this time. The fall in body weight after the rut was almost certainly due to a decline in the nutrient level of the veld, which may also have had an adverse effect on sexual function. However, when expressed as a percentage of carcass weight, testicular weight only showed a six per cent fall in July and September and a five per cent rise in November when compared with the values for January, March and May. Vesicular weight expressed as a percentage of carcass weight, showed a marked rise in September and November of four to five per cent. This indicated that vesicular weight did not decline as the rams lost condition.

Of particular interest is the fact that testes weight and seminiferous tubule diameter

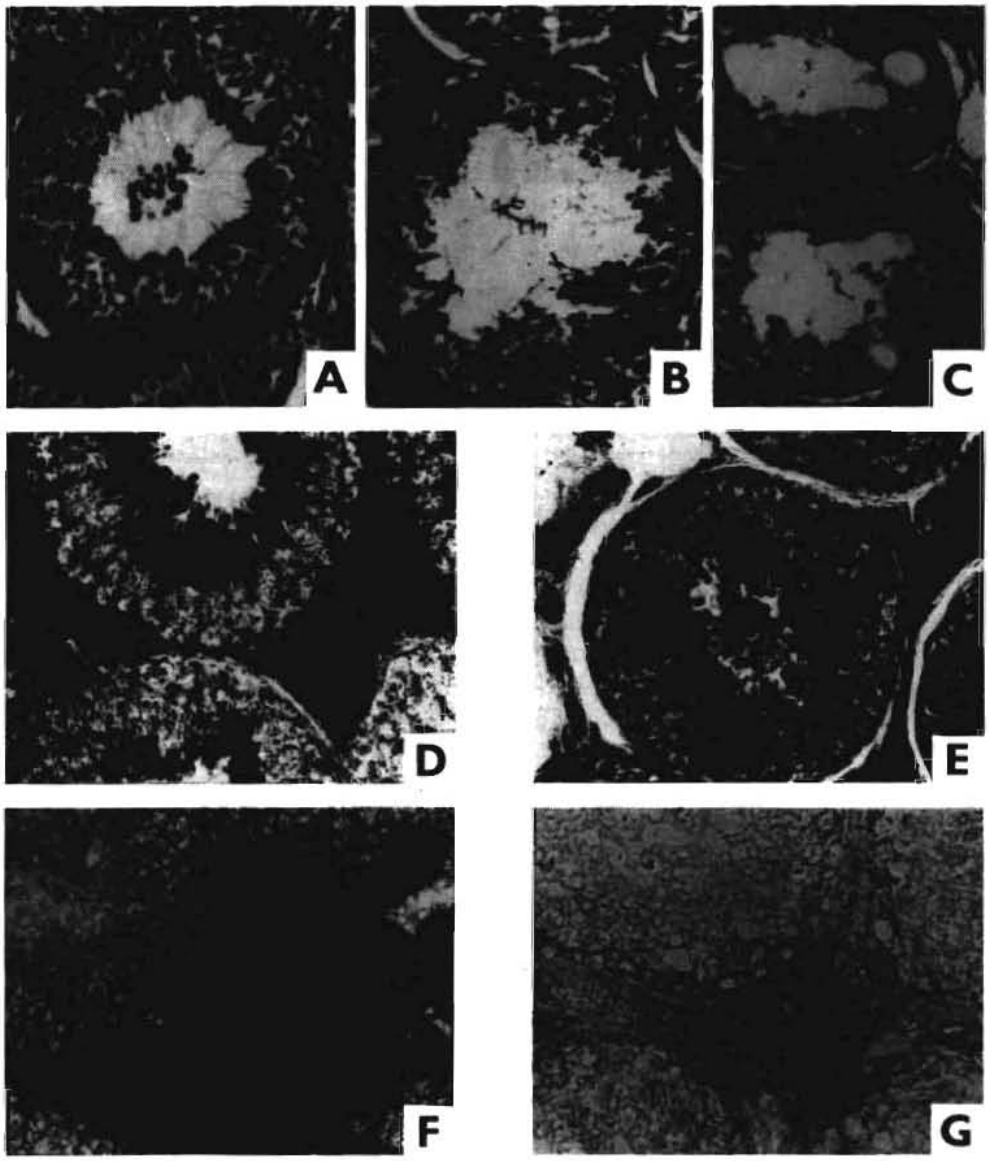

FIGURE 3
Cross-sections of the testes of impala rams (x 256). A, B and C stained with Delafield's haematoxylin and chromotrope 2R. Note optimum spermatogenesis in January/March (A) and disruption of spermatogenesis in July (B) together with reduction in tubule diameter in September (C). D and E stained with Sudan black to demonstrate lipid in the interstitium (x 256). More lipid was apparent in March (D) than September (E). F and G unfixed frozen sections from the testes of impala rams, incubated for 3 hours to demonstrate Δ^5-3β-hydroxysteroid dehydrogenase activity (x 256). There was more activity in the testicular interstitium in March (F) than in September (G).

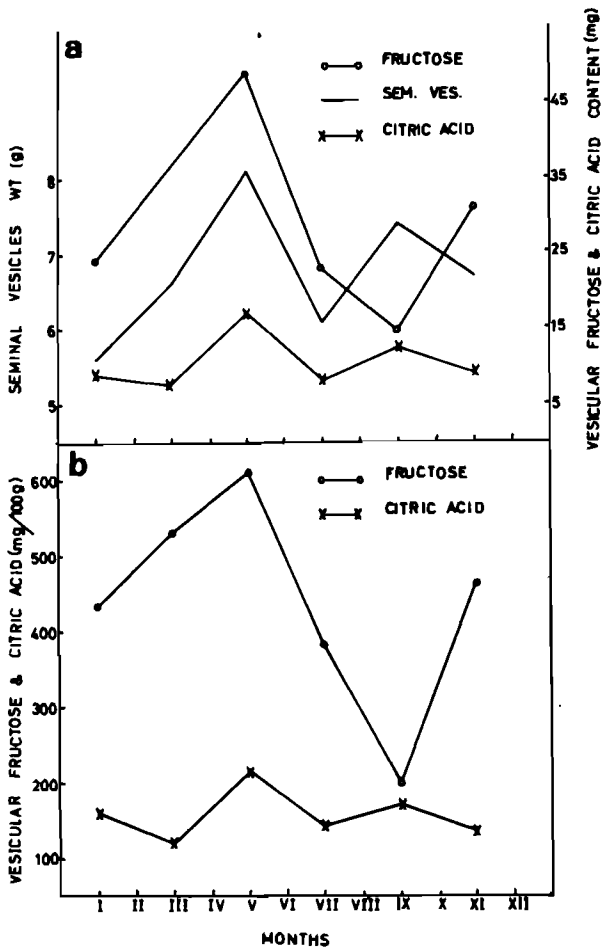

FIGURE 4

Seasonal changes in (*a*) weight of seminal vesicles —— content of fructose *o*——*o* and citric acid *x*——*x* in the seminal vesicles and (*b*) concentration of fructose *o*——*o* and citric acid *x*——*x* in the seminal vesicles of the impala at the S. A. Lombard Nature Reserve.

attain maximal values prior to the rut. At the commencement of the season, when epididymal sperm reserves are at their peak, testes weight and tubule diameter have already started to decline rapidly, a process which continues until September.

Field observations confirm that rutting is strictly seasonal. Impala ewes at the reserve have been observed to mate from 15 May onwards. Lambing usually takes place from the end of November and is practically complete within a space of 30 days. If a gestation period of 196 days is accepted (Stevenson-Hamilton 1912; Fairall 1969) and the lambing dates noted during the period 1952–1969 are converted into conception dates, we find the average date for the commencement of oestrus is May 18th. It has never commenced before

May 4th. Occasionally a few lambs are conceived as late as August. Over 90 per cent of lambs were dropped in a period of one month.

DISCUSSION

The impala rams used in this study were considerably heavier than those shot in Rhodesia (54·4 Kg, Child 1964) and the Loskop Dam Nature Reserve, Transvaal (58·1 Kg from 46 rams, range 50–66 Kg, Van Zyl, J.H.M. – Unpubl.). In fact, the animal weighing 78·6 Kg (Table 1) would appear to be a record. The rams reached maximum weight prior to the rut and then declined in condition, which confirms the results reported by Anderson (1965) in Zululand.

Anderson (1965) has demonstrated almost identical weight changes in the testes of Zululand impala. The weights of the testes were very similar to those recorded in this study, varying from a maximum of 133 g in March to 66 g in July. Weights declined to a minimum in September at the S.A. Lombard Reserve but numbers were small and there is really little difference between the two areas. Kerr (1965), collected testes from adult impala from the beginning of October to the end of March in the southern lowveld of Rhodesia and also found that a peak occurred in March. However, testes weights were considerably lower varying from 106·2 g in March to 52·6 g in November. From the results of the present study it would appear that these differences in weight are primarily due to changes in seminiferous tubule diameter.

Both Anderson (1965) and Kerr (1965) note that the rutting season in impala is of short duration. In Zululand the height of the rutting season occurs during the latter half of May and in Rhodesia it was estimated to be between mid-May and mid-June (Dasmann and Mossman 1962).

No other workers have examined impala testes histologically or studied the rest of the reproductive tract. Kerr (1965) made testis and epididymal smears, which always showed the presence of sperm. He concludes from his observations that spermatogenesis does not cease during the non-rutting season. In the present study, epididymal sperm numbers declined rapidly after the rut and it was obvious from histological sections that there was some disruption of spermatogenesis, although it was difficult to define exactly what this was. It appeared that there was an overall decline in the number of mitoses, resulting in a reduced output of spermatozoa. Sperm motility also declined but the percentage dead and abnormal sperm did not increase. It is of considerable interest to know what happened to the large number of sperm present in the epididymides at the time of the rut. If there was already a brake on spermatogenesis at this time then the numbers would decline by ejaculation but these rams were all from bachelor groups and this seems unlikely. Resorption has been accepted by one school as the principal means by which sperm are removed from the epididymis (Bishop and Walton 1960; Bedford 1965) but more recently it has been suggested that this is not a very important mechanism and that sperm pass constantly down the vas deferens and out via the urine (Lino, Braden and Turnbull 1967) presumably as a result of pressure from testicular fluid and spermatozoa. It would be interesting to see

which of these mechanisms plays a major rôle in sperm removal from the epididymis of seasonal breeders.

Under the conditions of this experiment it was not possible to measure testicular testosterone accurately. There is a close correlation between testicular testosterone and vesicular fructose and citric acid in many ruminants (Lindner and Mann 1960; Short and Mann 1966; Skinner, Booth, Rowson and Karg 1968) and it is therefore possible to conclude that androgen production shows a similar seasonal pattern to sperm production, declining rapidly after the rut. This is supported by the histological and histochemical evidence. The tremendous fall in sperm production seems unlikely to be the result of lowering in nutritional levels. It has already been shown that testicular endocrine function in the bovine is markedly affected by malnutrition whereas exocrine function in terms of sperm density, motility and morphology are not significantly changed (Mann and Walton 1957).

As in the roebuck (Short and Mann 1966) and springbok (Skinner and Van Zyl 1970), testicular endocrine and exocrine function seems to be closely related events. Tubule diameter showed a much greater out of season decline in the roebuck but in the springbok, where the sexual cycle is not as marked as either the roebuck or the impala, the decline was much less. Only in the roebuck did spermatogenesis come to a standstill. As in the roebuck, fructose and citric acid secretion preceded the onset of active spermatogenesis. The impala rams are sexually active before the occurrence of oestrus in the ewe and it would be interesting to determine whether the ewe is, in fact, responding to a change in the physiological status of the male as well as to environmental stimulus such as change in daylight length.

There were probably not enough spermatozoa present in the epididymis in September and November to ensure a fertile mating. In any event, libido would be much reduced and it would not be possible for more than one or two ewes to conceive at this time. It would appear therefore that the mating season is partly dependent on the physiological status of the male at that time.

The present hunting season in the Transvaal is from May 15 to August 15. As this coincides with the onset of the rut this is a disadvantage as far as reproduction is concerned. Moreover, males which are declining in weight are being removed. Excess males should be cropped in February–March, when maximum body weights are attained. This would also reduce the fighting prior to the rut.

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SUMMARY

A total of 12 mature impala rams was shot at two-monthly intervals at the S.A. Lombard Nature Reserve in the Western Transvaal during the course of one year. There was little change in pituitary gland weight but the reproductive organs showed a distinct cycle, reaching

a peak at the rut in May and declining to minimal values in September. Androgen secretion, as determined by fructose and citric acid concentration in the accessory glands, never ceased, nor did spermatogenesis although both declined sharply after the rut.

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