# THE POST-NATAL DEVELOPMENT OF THE REPRODUCTIVE TRACT OF THE SPRINGBOK RAM LAMB *ANTIDORCAS MARSUPIALIS MARSUPIALIS* ZIMMERMANN

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A search of the literature has not revealed any reference to the development of the reproductive tract of the male springbok or any quantitative studies of the age of appearance of the various germ cells in the spermatogenic cycle. Such a study would be of importance in making recommendations for the practical management of young rams and was the object of the present work.

## MATERIAL AND METHODS

Material was provided by 73 springbok lambs allocated at random into groups for slaughter at four-weekly intervals from birth to 64 weeks of age. The lambs were marked shortly after birth being captured for the purpose at night in a large net. A system of coloured plastic marker ribbons in both ears was used so that animals of the desired age could be selected and shot from hides in the veld according to a fixed code of colours. Exact numbers in each age group were as follows: four at birth, four at four weeks, five at eight weeks, six at 12 weeks, four at 16 weeks, seven at 20 weeks, nine at 24 weeks, seven at 28 weeks, four at 36 weeks, five at 44 weeks, four at 48 weeks, five at 52 weeks, two at 56 weeks, five at 60 weeks and two at 64 weeks. Histological observations on the testes of foetuses aged 53-, 72-, 108- and 120-days have been included. The foetuses were aged according to Van Zyl and Skinner (1970).

The lambs were marked in September/October 1968 and the study was carried out over the following year at the S.A. Lombard Nature Reserve near Bloemhof in the Western Transvaal. The animals were shot in the neck with a high velocity rifle and immediately afterwards their throats were cut and the carcasses bled. The whole carcass was then taken to the laboratory, weighed and dissected. The length of the horns was measured and the number of horn rings counted. Changes in dentition were noted.

The pituitary gland, testes, epididymides, ampullae of the vasa deferentia, seminal vesicles and bulbo urethral glands were dissected out and weighed individually, sections were taken for histology and the ampullae and seminal vesicles were stored over solid $CO_2$. The amount of fructose and citric acid in these glands was estimated later (method of Linder and Mann 1960). The number of sperm in the epididymides was estimated using the method of Dott and Skinner (1967).

Pieces of testes were fixed in Bouin's fluid or Zenker-formol solution. Pieces of seminal vesicles, ampullae and bulbo-urethral glands were fixed in Bouin's fluid. The Bouin-fixed

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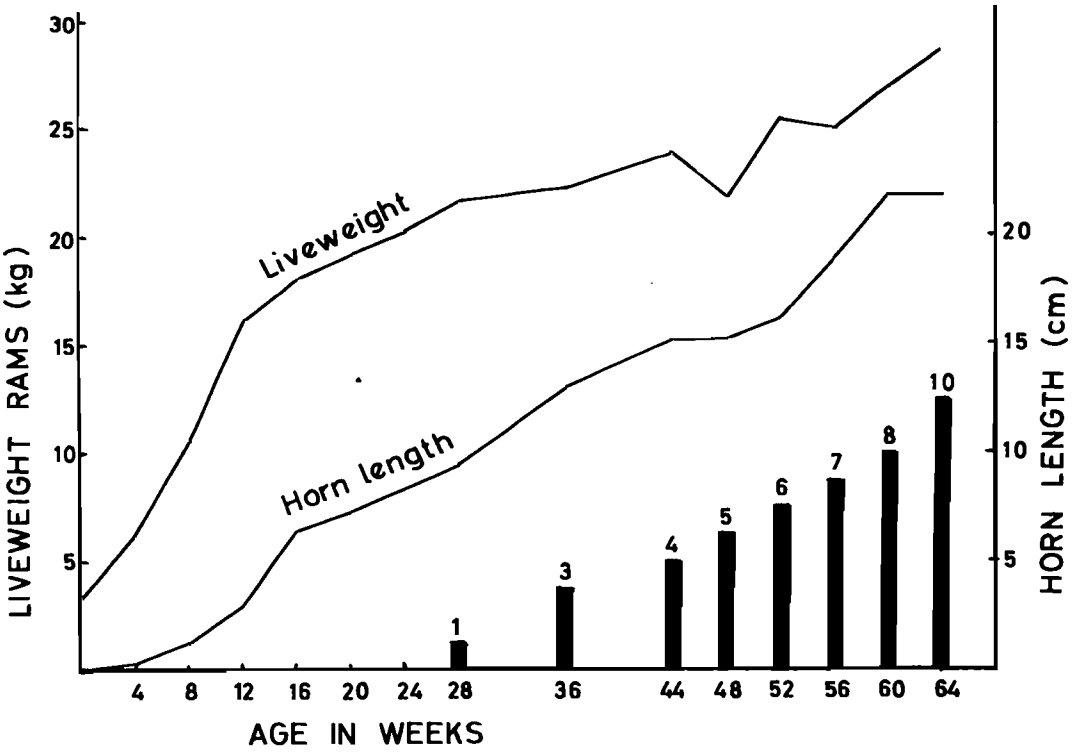



FIGURE 1
Increase in body weight and horn length of springbok ram lambs from birth to 64 weeks of age. The number of horn rings are illustrated by histograms.

material was dehydrated in alcohol, cleared in cedarwood oil and embedded in paraffin wax; sections 6 $\mu$ thick, were stained with Delafield's haematoxylin and chromotrope 2R. The Zenker-formol fixed tissue was postchromed 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 $CO_2$. Sections cut 16 $\mu$ thick were incubated for three hours at 37° C to demonstrate the presence of $\Delta 5$— 3 $\beta$-hydroxysteriod dehydrogenase activity as described by Hay and Deane (1966). The mean seminiferous tubule diameter was calculated from 25 circular tubules measured in cross-section.

## RESULTS

### *Weights*

Body weight increased sharply from birth to 28 weeks of age in May, after which there was little increase to the age of 48 weeks in November. Thereafter the weight again started

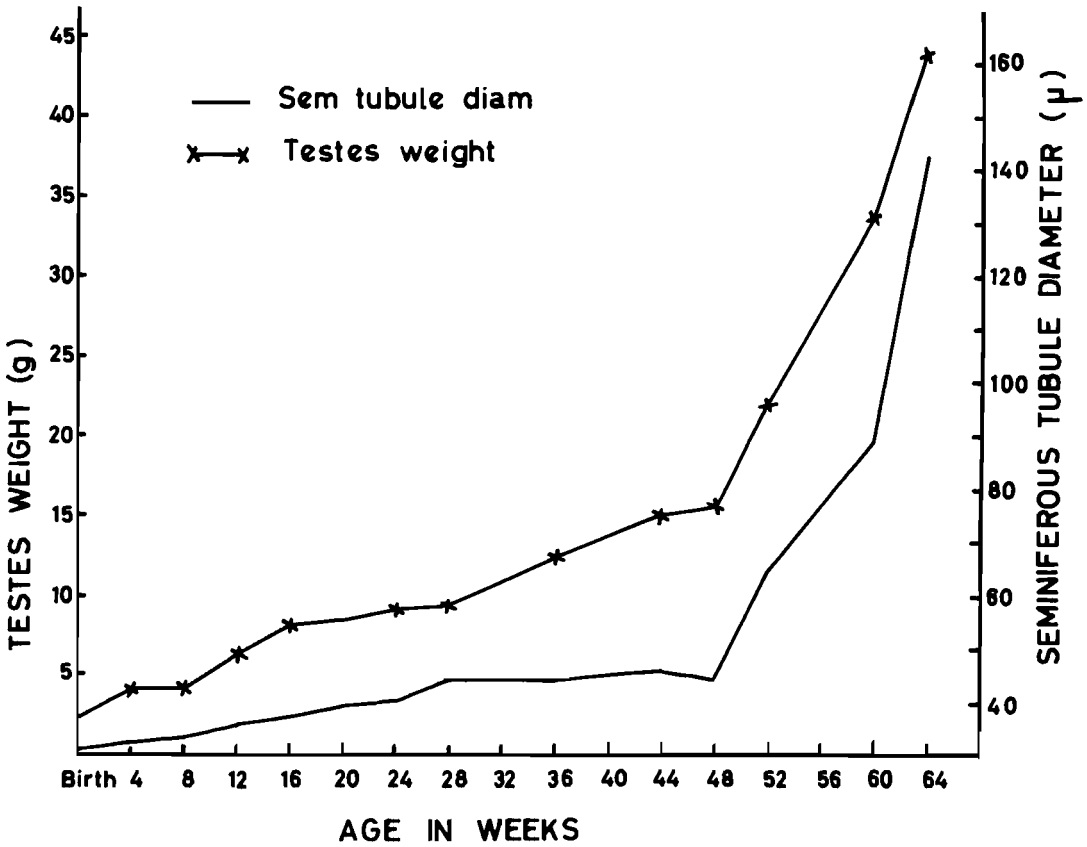



FIGURE 2
Testicular weight and seminiferous tubule diameter in springbok ram lambs.

increasing. This is graphically illustrated in Fig. 1. The pituitary gland increased rapidly in weight from birth when it weighed 0,13 g to 16 weeks when it weighed 0,25 g after which the weight remained constant until 44 weeks (0,26 g) and then increased to 0,28 g at 52 weeks and 0,33 g at 60 and 64 weeks.

As can be seen from Fig. 2 the weight of the testes increased gradually from birth to 48 weeks of age after which there was a steep rise in growth rate. Seminiferous tubule diameter showed a similar increase from 48 weeks of age. The weights of the accessory glands showed an increase with age similar to that of the testes, increasing more rapidly from 44 weeks of age. Examples are the increase in weight of the seminal vesicles and ampullae shown in Figs. 3 and 4. The weight of the epididymides increased sharply at 52 weeks when spermatozoa started passing through the vasa efferentia. The sperm in the caudae after this time were all motile.

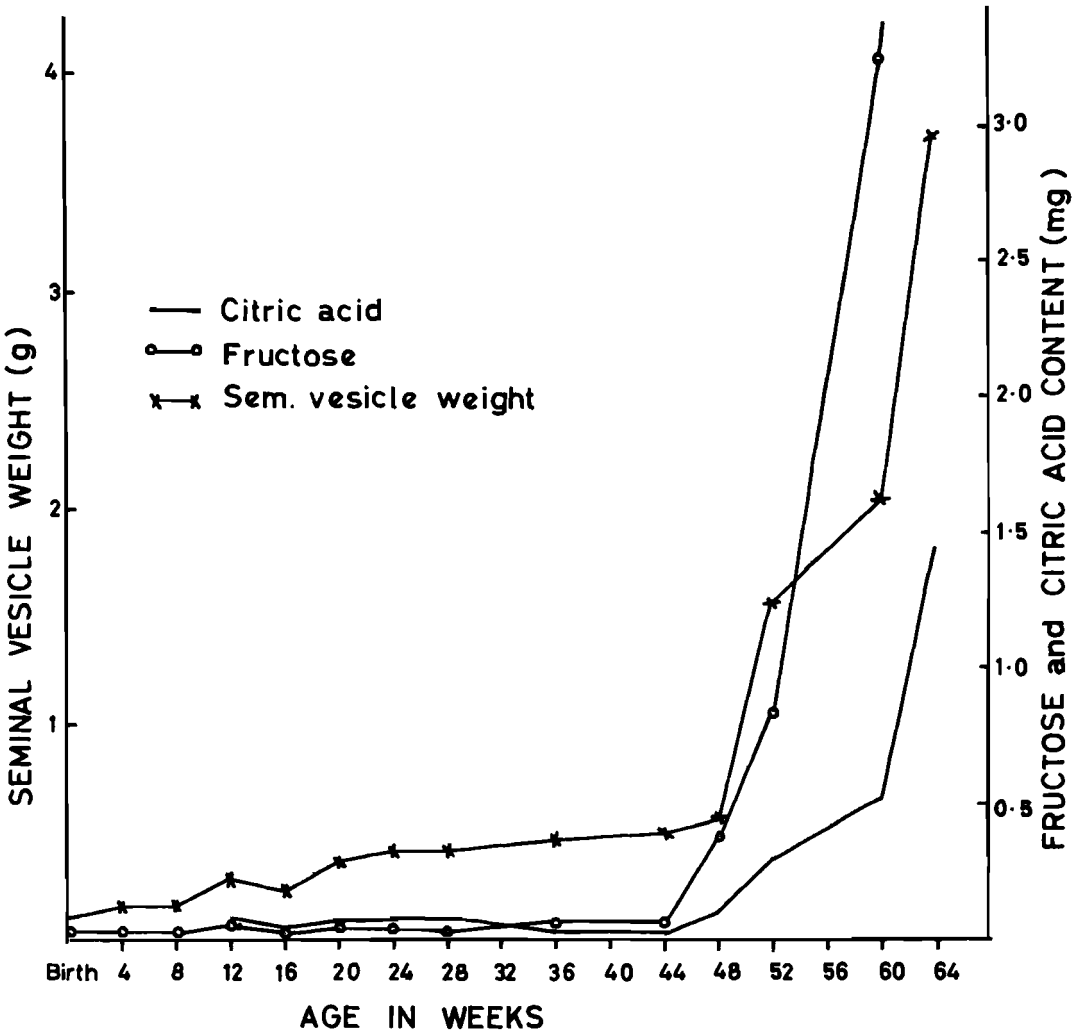



FIGURE 3
Seminal vesicle weight (paired) and fructose and citric acid content in springbok ram lambs.

*Horn length and number of rings*

Like body weight the horn length showed an increase with age as illustrated in Fig. 1 and the two parameters were in fact highly correlated (correlation coefficient 0,58, $P<O,01$). The number of horn rings increased in a step-wise fashion according to age. Both these parameters could be used to estimate ages of springbok ram lambs in the field. Replacement of the first pair of deciduous incisors started at 60 to 64 weeks of age in the animals in this study.

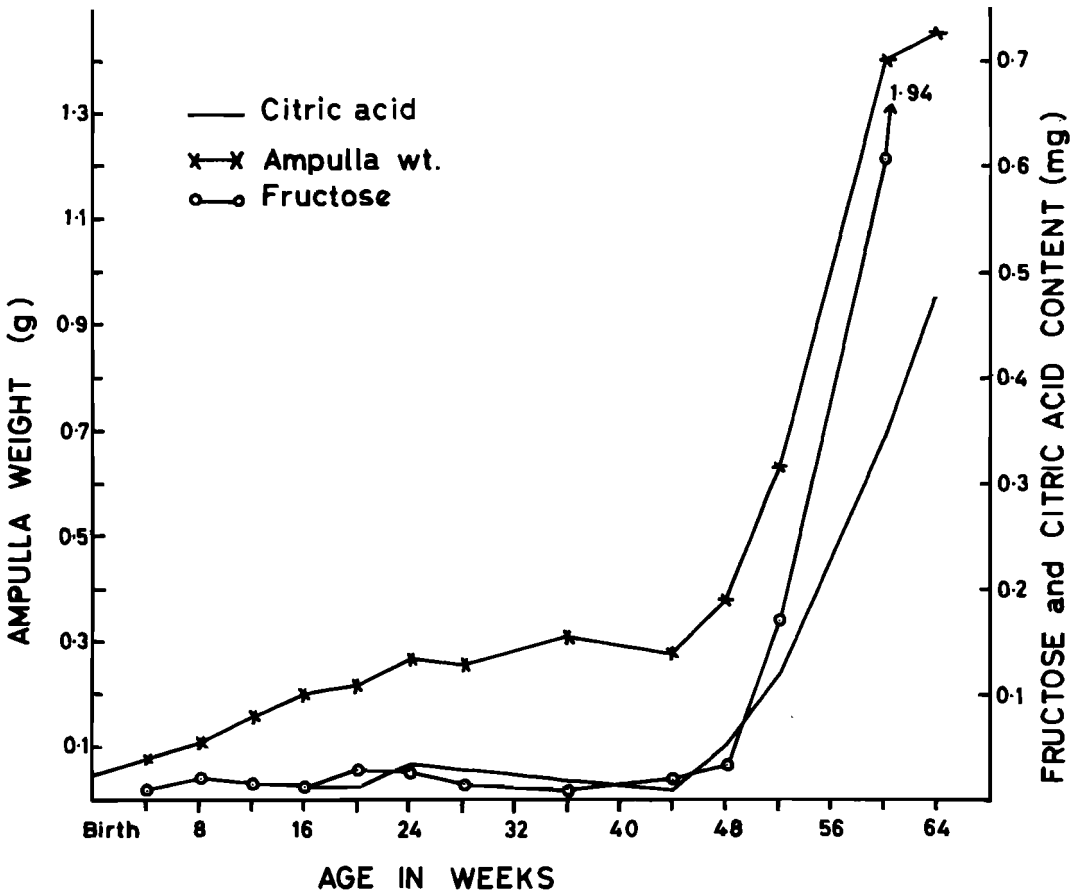



FIGURE 4
Ampulla weight (paired) and fructose and citric acid content in springbok ram lambs.

*Estimate of fructose and citric acid in the seminal vesicles and ampullae*

The mean fructose and citric acid contents of the seminal vesicles and ampullae are graphically illustrated in Figs. 3 and 4 respectively. Up to the age of 28 weeks an estimate of pooled material was obtained, after which individual estimates were determined and the mean calculated. Fructose was present in the glands from birth but there was insufficient material for citric acid determination to start with.

*Epididymal sperm*

A comparison between testicular characteristics at different ages is presented in Table 1. The number of motile epididymal sperm rose sharply from 60 weeks of age.

TABLE 1

WEIGHT OF TESTES, SEMINIFEROUS TUBULE DIAMETER, WEIGHT OF EPIDIDYMIDES AND NUMBER OF EPIDIDYMAL SPERM IN THE SPRINGBOK RAM

| *Age (weeks)* | *No.* | *Testes weight* | *Seminiferous tubule diameter* | *Epididymides weight* | *No. of sperm* |
|---|---|---|---|---|---|
| | | (g ± S.E.) | ($\mu$ ± S.E.) | (g ± S.E.) | ($x10^9$ ± S.E.) |
| 60 | 5 | 19,5 ± 3,1 | 131,9 ± 8,4 | 2,8 ± 0,5 | 0,3 ± 0,06 |
| 64 | 2 | 37,5 ± 1,3 | 162,2 ± 15,0 | 3,7 ± 0,7 | 4,4 ± 2,1 |
| Mature* | 12 | 64,1 ± 4,3 | 184,3 ± 5,3 | 8,9 ± 0,6 | 10,9 ± 1,8 |

* From Skinner and Van Zyl (1970b).

A free processus urethrae was observed at 48 weeks of age and a liveweight of 21,6 kg. Separation of the penis from the prepuce was already advanced at this stage and complete four weeks later. From these observations it would appear that springbok rams can reproduce from 52 weeks of age.

*Statistical analysis*

Seminiferous tubule diameter was significantly correlated ($P<0,001$) with the weights of testes and all the accessory glands and also with fructose concentration and content and citric acid concentration and content. Testes weight was similarly correlated with these parameters and the weights of the seminal vesicles and ampullae were significantly ($P<0,001$) correlated with their fructose and citric acid concentrations and contents.

*Histology*

*The interstitium.* Using standard methods for demonstrating $\Delta^5$–3$\beta$–hydroxysteroid dehydrogenase it was possible to demonstrate the enzyme in foetal testes and from birth onwards in the developing springbok testis. In addition, using Threadgold's (1957) method for demonstrating lipids in the interstitium, deposits could also be demonstrated in the foetal testis and from birth onwards. The enzyme and lipids are demonstrated between the tubules in Plate 1, Nos. 1 to 8.

PLATE 1

Sections of testes from springbok lambs at different ages. Nos. 1, 3, 5 and 7 unfixed frozen sections incubated for 3 hours to demonstrate $\Delta^5$-3$\beta$-hydroxysteroid dehydrogenase activity in the interstitial cells. Nos 2, 4, 6 and 8 stained with Sudan black to show lipid in the interstitial cells. Note the enzyme activity and lipid deposits between the seminiferous tubules.

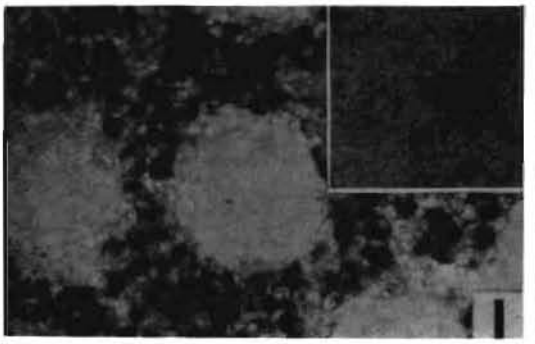
No. 1. Testis of a 108-day-old foetus, which was taken from the upper part of the scrotum just below the inguinal ring. Inset, testis of a 53-day-old foetus which was located intra-abdominally near the kidney; the enzyme is already present at this stage.

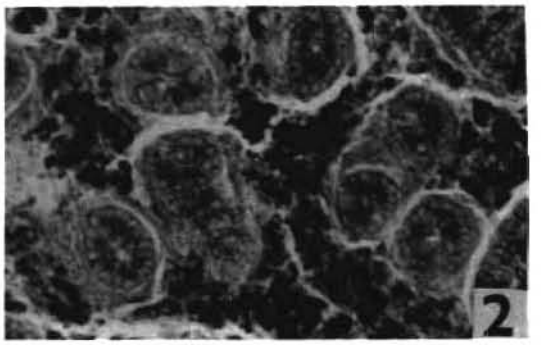
No. 2. Testis of a 108-day-old foetus.

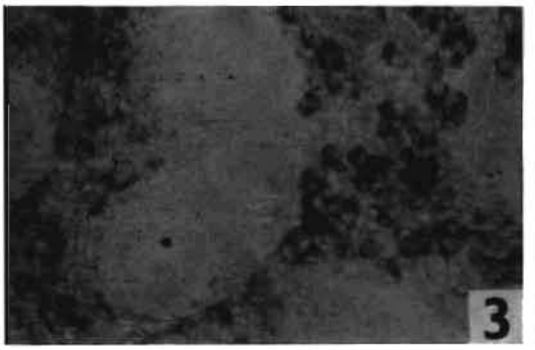
Nos. 3 and 4. Testis of a new-born lamb.

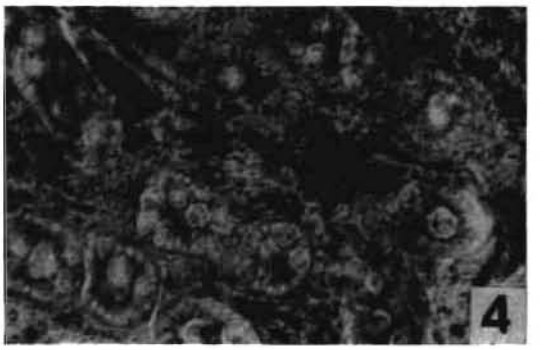
Nos. 5 and 6. Testis of a 36-week-old lamb.

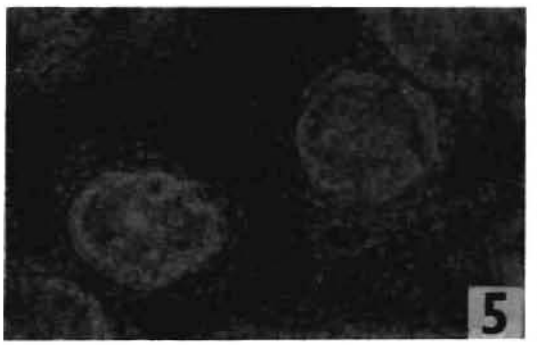
Nos. 7 and 8. Testis of a 60-week-old lamb.

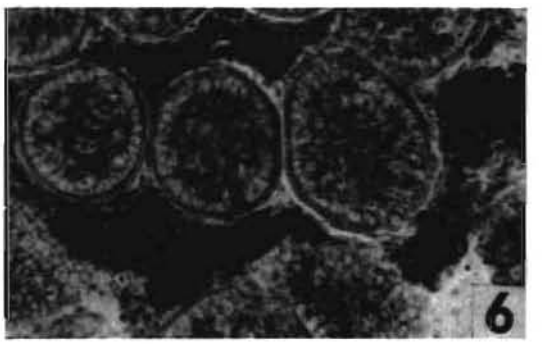


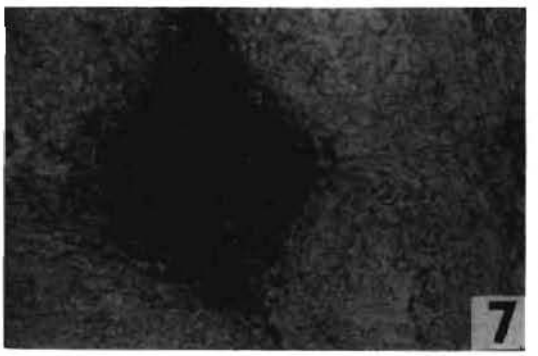



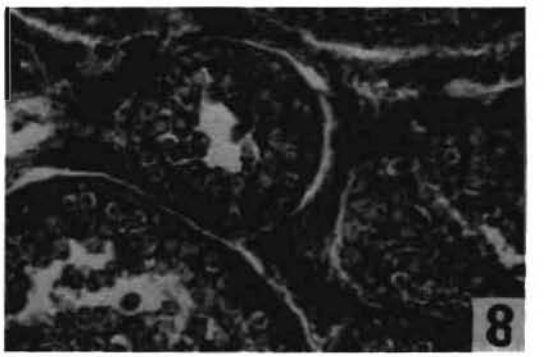



*The seminiferous tubules.* At birth, seminiferous tubules resembled those of a 108-day-old foetus (Pl. 2, No. 1). At 20 to 24 weeks of age, the seminiferous tubules still appeared quiescent with only gonocytes and supporting cells present (Pl. 2, Nos. 2 and 3). Spermatogenesis started from about 36 weeks of age (Pl. 2, No. 4), when primary spermatocytes were evident. At 48 weeks lumen formation had commenced (Pl. 2, No. 5) and spermatozoa were evident in the tubules from two animals. Supporting cells had apparently differentiated into Sertoli cells at 36 weeks of age, coinciding with the onset of spermatogenesis.

*The seminal vesicles.* The seminal vesicles of the developing lamb resembled those of the adult, being a compound tubular gland in which individual lobules are separated by trabeculae of connective tissue and smooth muscle fibres. The secretory tubules are lined by pseudo-stratified columnar epithelium of which the height varied from 13 $\mu$ at birth to 18 $\mu$ at 64 weeks and 15 to 27 $\mu$ in the adult. With increase in lumen size and "stretching" of the lobules, secretory epithelial height could even decrease (Pl. 2, Nos. 7 and 8).

## DISCUSSION

The growth of the springbok ewe and its reproductive development has been described by Skinner and Van Zyl (1970a). It is of considerable interest that unlike the sheep or goat (Skinner, Booth, Rowson and Karg 1968; Skinner 1970, 1971), reproductive development in the male springbok takes twice as long as that in the female. It nevertheless follows the same pattern as in the domestic species. Although mature springbok rams can breed throughout the year (Skinner and Van Zyl 1970b) they undergo a definite sexual cycle, and it may well be that season affects the onset of spermatogenesis in this species thus causing a delay.

Although the time intervals between sighting certain cell types have been noted, there are two major deficiencies in the present study; first, the time interval between the group autopsy was too great for this type of histological study, and second, different rams had to be killed at different stages. Nevertheless, they serve as a good indication. Seminiferous tubule diameter has already been recognised as an excellent parameter for measuring sexual function in the bull calf (Hay, Lindner and Mann 1961), ram lamb (Skinner *et al.* 1969) and in the roe deer (Short and Mann 1966), and was significantly correlated in the springbok with every parameter measured.

This study has shown that sexual development in the springbok lamb proceeds at a slow rate from birth to 44 weeks of age, after which there is a characteristic rapid increase in the parameters measured. Testicular testosterone was not measured in this experiment but there is a strong correlation between it and vesicular fructose and citric acid in many ruminants (Lindner and Mann 1960; Short and Mann 1966; Skinner *et al.* 1968). It is therefore possible to conclude that testicular testosterone is produced from birth and this is supported by the histochemical and histological observations. It is apparent therefore that androgen secretion precedes spermatogenesis by several months. This evidence that androgenesis precedes spermatogenesis has already been presented for the bull (Mann, Davies and Humphrey 1949), rabbit (Davies and Mann 1947; Skinner 1967), ram (Skinner *et al.* 1968; Skinner 1970) and goat (Skinner 1971).

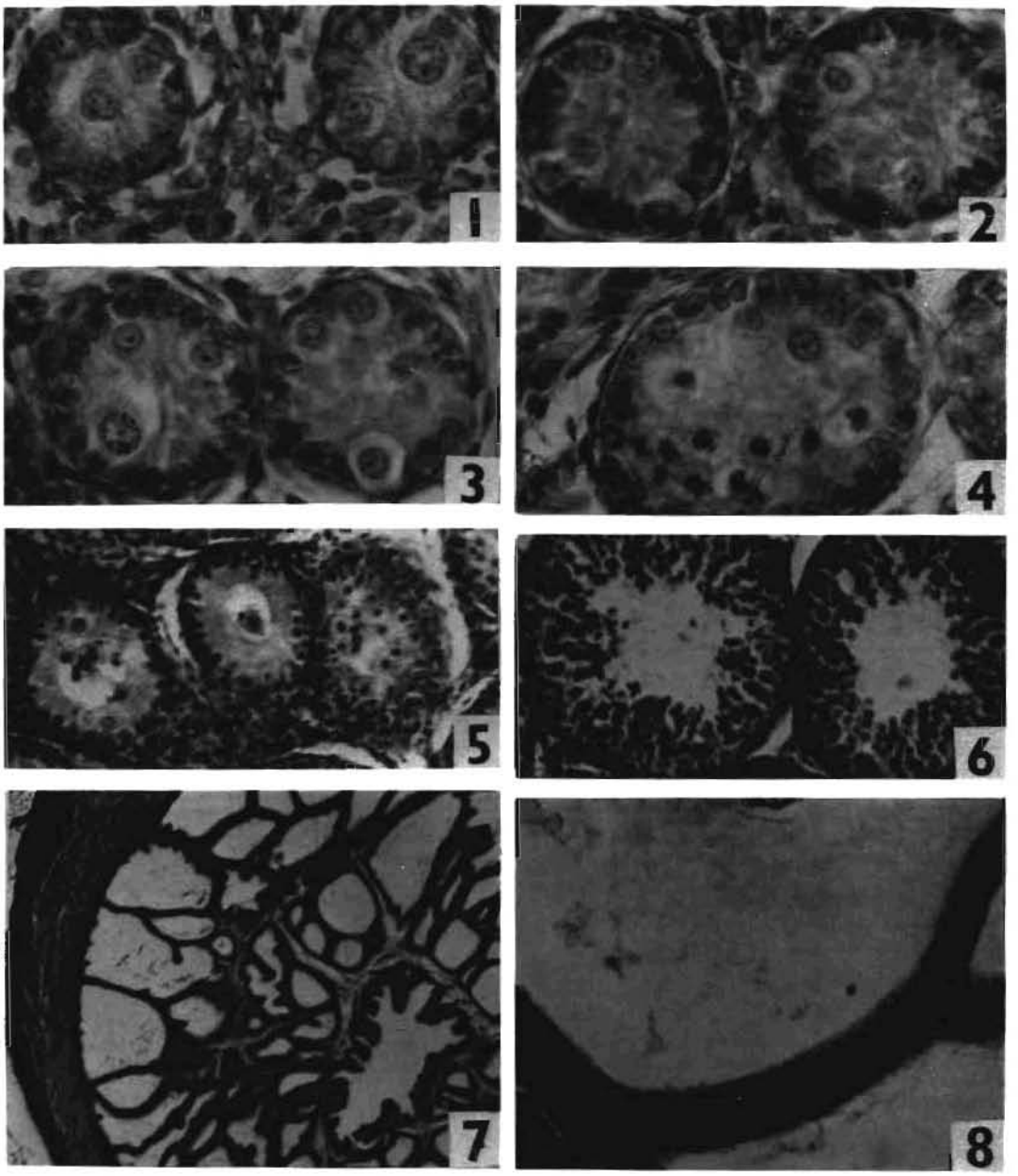



PLATE 2

Sections of testes from springbok lambs of different ages, stained with Delafield's haematoxylin and chromotrope 2R. Nos. 7 and 8 sections of a seminal vesicle.

Nos. 1, 2 and 3. Testes of a new-born lamb, a 20-week-old lamb and a 24-week-old lamb respectively. Note the gonocytes and supporting cells in quiescent seminiferous tubules.

No. 4. Testis of a 36-week-old lamb. Spermatogenesis has commenced.

No. 5. Testis of a 48-week-old lamb. Note lumen formation.

No. 6. Testis of a 60-week-old lamb showing spermatids and spermatozoa.

No. 7. Seminal vesicle of a 52-week-old lamb.

No. 8. Higher magnification of the same seminal vesicle showing columnar epithelium and secretion in the lumen.

The results of the present study favour the view that puberty occurs when the testes begin to show marked androgenic activity and the accessory glands begin to produce and secrete fructose and citric acid. This would coincide with a sharp increase in growth rate of the reproductive tract. Puberty is a phase in development in springbok lambs and spermatozoa appear towards the end of this phase. The results of this study support the definitions of puberty of Marshall (1922) and Donovan and Van der Werff ten Bosch (1965).

Springbok ram lambs are late developing but grow relatively quickly to 28 weeks of age at the beginning of winter. Growth after this time is retarded until the following spring, probably as a result of winter nutritional depressions. For meat production it would seem advisable to crop surplus male lambs just before the onset of the present hunting season. Care should be taken to ensure that the fastest growing lambs are not removed thus ensuring a measure of selection.

Reproductive development is closely related to physiological age. It would appear that horn length is a reliable indicator of age and that animals can be selected in the field on this basis.

## SUMMARY

Growth in body weight, horn length, of the pituitary and of the reproductive tract of springbok rams from birth to 64 weeks of age has been studied. Body weight increased sharply to 28 weeks of age and was significantly correlated with horn length. $\Delta^5$–3$\beta$–hydroxysteroid dehydrogenase was present in foetal testes and from birth onwards. Fructose was present in the ampullae and seminal vesicles from birth and citric acid from 12 weeks of age. There was insufficient tissue for both assays to start with. Seminiferous tubule diameter was significantly correlated with most of the parameters measured. Testicular weight increased sharply from 44 weeks of age. Spermatogenesis commenced at 36 weeks of age and spermatozoa were present in some seminiferous tubules at 44 weeks of age.

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