

BRIEF COMMUNICATION

PUBERTY IN THE MALE RABBIT

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Marshall (1922) stated that: "in those animals in which during immaturity the testicles remain in the body cavity, it is at puberty that these organs first descend into the scrotal sacs". Subsequent work has shown that in the rabbit the growth of testes follows a sigmoid curve, increasing rapidly during puberty (Lipschütz, 1924; Kuboshima, 1951; Gaddum, 1964). Brief reports have been published by Lipschütz (1924) and Davies & Mann (1947) on the endocrine function of the pubescent rabbit testis. The object of the present study was to investigate the time-relationship between the androgenic and gametogenic function of the rabbit testis during puberty.

The rabbits came from the Unit's own closed colony which originated from cross-bred rabbits, and in which males reach a mature weight of 3 to 4 kg at 7 months of age. Weaned rabbits were transferred in groups to colony pens. It was observed that fighting began amongst these animals at 60 days of age and 'bucking' (attempts to mount) about 10 days later. At this stage seven bucks were separated and trained to ejaculate into an artificial vagina, and thereafter, ejaculates were collected at weekly intervals until autopsy at 126 days. The following were determined: Volume of semen (seminal gel, if present, was included); sperm density, in a haemocytometer; the percentage of live and abnormal spermatozoa, as described by Glover (1960); and fructose and citric acid (Mann, 1964). The main results of semen analyses appear in Text-fig. 1 and Table 1, which also includes some data on six older (1-year-old) bucks. At 84 days of age the concentration of fructose and citric acid was fairly high and at 126 days some values were within the range found in the ejaculates of adult bucks. The low citric acid estimates in the mature bucks may be due to the fact that the gel was removed before analysis; citric acid is associated with the gel fraction (Mann & Parsons, 1950).

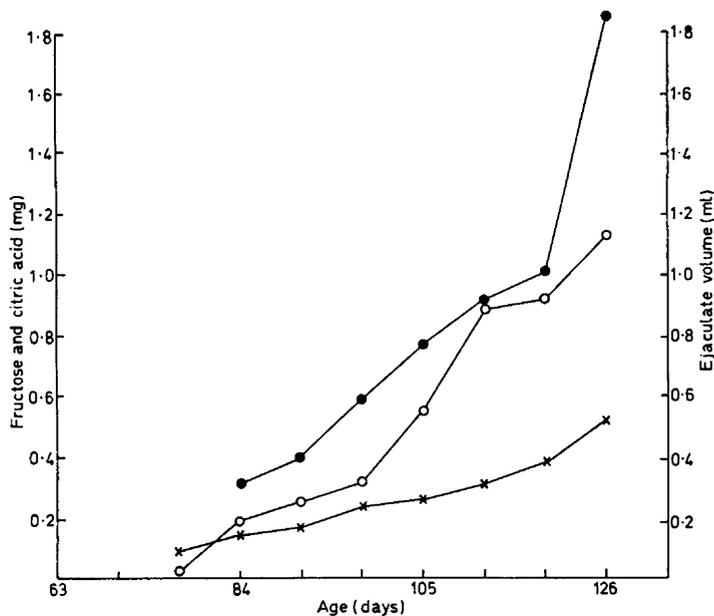
Spermatozoa, many of them motile, first appeared in ejaculates at 119 days. A large proportion of these spermatozoa bore proximal droplets (Table 1) but the abnormalities soon decreased, concurrent with the increase in the proportion of live spermatozoa. At 126 days sperm motility was as high as in adult bucks.

Both testes were removed from sixteen animals, including three littermates at birth, three at 42 days, three at 63 days and seven at 126 days. After weighing,

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slices of testis were frozen on to cryostat chucks, sectioned at  $16\ \mu$ , and examined for the presence of  $\Delta^5\text{-}3\beta$ -hydroxysteroid dehydrogenase by Maier's (1965) method. Additional testis slices were fixed in Bouin's fluid, embedded, sectioned at  $6\ \mu$ , and stained with Delafield's haematoxylin and chromotrope 2R.

In the 42-day old animals the accessory organs (combined prostate, ampullae, glandula vesicularis and glandula seminalis) contained  $6.25\ \text{mg}/100\ \text{g}$  of fructose, which indicates that testosterone secretion had begun. The testes of these animals were small and subinguinal. Hydroxysteroid dehydrogenase activity was present in the interstitium although the seminiferous tubules were still in a quiescent state and the testes small and subinguinal. Scrotal pouching and hair shedding had also started.



TEXT-FIG. 1. The volume ( $\times$ ), total fructose ( $\circ$ ) and citric acid ( $\bullet$ ) in the ejaculate of pubescent buck rabbits (mean from seven animals).

In the 63-day old animals, the enzyme activity in the interstitium was distinctly higher, spermatogonia were present in the tubules and the testes had descended into the scrotum. From then onwards, testicular growth accelerated, spermatogenic activity increased and the aggressive behaviour became more pronounced.

Spermatogenesis started between 42 and 63 days of age but spermatozoa did not appear in the ejaculate until 119 days, conforming with the estimate of 70 days for the duration of spermatogenesis in the adult rabbit (Fox, Jackson, Craig & Glover, 1963).

In the past, two main definitions were used to describe puberty in the male. Some authors, notably Asdell (1946), have identified puberty with a definite time when the male first becomes capable of reproducing due to the appearance of spermatozoa, others, including Marshall (1922) and, more recently, Donovan & van der Werff ten Bosch (1965), regard puberty as the stage when the endo-

crine function of the testes first becomes clearly evident. The results of the present study favour the view that the onset of puberty coincides with the time when the testes become androgenically active, the accessory glands begin to secrete fructose and citric acid, (Mann & Parsons, 1947) and the animal

TABLE 1  
COMPARISON OF SEMEN CHARACTERISTICS IN PUBESCENT AND MATURE BUCKS

	Pubescent bucks			Mature bucks
	84 days	119 days	126 days	About 1 year
Number	7	7	7	6
Weight (g)	2088 ± 221	—	2800 ± 101	3778 ± 179
Semen (ml/ejaculate)	0.150 ± 0.027	—	0.520 ± 0.069	0.730 ± 0.064
Spermatozoa/ml semen (10 <sup>6</sup> )	—	—	13.41 ± 9.2	46.0 ± 16.1
Spermatozoa/ejaculate (10 <sup>6</sup> )	—	—	7.41 ± 4.6	35.4 ± 15.0
Live sperm (%)	—	64	77	80
Abnormal sperm (%)	—	—	—	—
Deformed heads	—	50	35	27
Abaxial attachment	—	17	20	29
Coiled tails	—	7	5	1
Tailless	—	8	7	3
Proximal droplet	—	12	2	3
Fructose (mg/100 ml)	111.1 ± 38.4	—	230.0 ± 35.0	292.0 ± 48.7
Fructose (mg/ejaculate)	0.189 ± 0.069	—	1.128 ± 0.156	2.264 ± 0.555
Citric acid (mg/100 ml)	180.0 ± 30.0	—	381.0 ± 67.9	178.0 ± 27.5
Citric acid (mg/ejaculate)	0.317 ± 0.098	—	1.861 ± 0.387	1.270 ± 0.219

assumes the characteristically male behaviour. The findings are also in accord with the observation of Davies & Mann (1947) that androgenic activity precedes the histological evidence of onset of spermatogenesis. In this context, the appearance of spermatozoa occurs towards the end rather than the onset of male puberty.

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