Duration of the seminiferous epithelial cycle in hybrid *Bos indicus* × *Bos taurus* bulls

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Summary. The hybrids were carrying 50% (N = 4) or 75% (N = 4) zebu (Brahman) blood. The spermatogenic cells were labelled *in vivo* with [³H]thymidine. The relative frequencies of the seminiferous epithelial cycle stages, determined from examination of 6350 tubule cross-sections, did not differ between the 2 hybrid types examined, between regions of the testis or between testes within animals. Frequencies were generally similar to those for *Bos taurus* bulls except that the frequency of stage 4 tubules was lower and that of stage 8 tubules higher. The mean duration of the seminiferous epithelial cycle was 13.41 days and was similar for both types of hybrid. The durations of meiosis and spermiogenesis were calculated to be 20.7 and 17.4 days respectively, estimates which are similar to those for *Bos taurus* bulls. From the relative frequency of cell stages and estimates of the duration of the cycle, a time divisor of 5.11 days is suggested as being appropriate to determine daily sperm production from counts of spermatid reserves in testicular homogenates of these hybrid bulls.

Introduction

In tropical zebu beef herds (*Bos indicus*), bull fertility is one of the factors that limits herd fertility (Seebeck, 1973; Seifert, Bean & Christensen, 1980). Sperm production is a function of sperm production per unit weight and testis weight, both of which are lower in zebu cattle hybrids (Entwistle, Winantea & Holroyd, 1980) than in *Bos taurus* bulls (Amann, 1970).

Techniques used for measuring daily sperm production include quantitative testicular histology, and the determination of testicular spermatid reserves (Amann, 1970). An accurate estimate of the duration of spermatogenesis is essential to derive appropriate time divisors, and such estimates have been derived for *Bos taurus* bulls (Amann, 1970; Amann, Kavanaugh, Griel & Voglmayr, 1974). While the duration of spermatogenesis within a species is nearly constant (Ortavant, Courot & Hochereau-de Reviers, 1977), strain variations do occur in the duration of the seminiferous epithelial cycle in the rat (Clermont, Leblond & Messier, 1959; Courot, Hochereau-de Reviers & Ortavant, 1970). However, no information is available for such variation in cattle. In our studies of fertility in hybrid *Bos indicus* × *Bos taurus* bulls, it was considered necessary to determine the duration of the seminiferous epithelial cycle, and hence obtain appropriate time divisors for calculation of daily sperm production in these animals.

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Materials and Methods

Animals, general management and surgical procedures

The animals used in the study were 4 F₂ generation half Brahman cross and 4 F₂ generation three-quarter Brahman cross bulls representative of the Brahman crossbred bulls widely used in tropical northern Australia. The bulls, aged 3·5 years (mean body weight 472 kg), were derived from a crossbreeding programme which started in 1968 in which purebred Brahman bulls (*B. indicus*) were mated to Shorthorn cows (*B. taurus*). Subsequent F₁ × F₁ matings led to the formation of F₂ generation half-Brahman animals. Matings of the F₁ generation animals to purebred Brahman bulls produced three-quarter Brahman animals which were also mated between themselves to produce the F₂ generation bulls used in this study.

All 8 animals were clinically normal, were of proven fertility and had satisfactory semen quality during an 8-week pre-experimental period. Throughout the experiment the bulls were maintained on native pastures supplemented with pasture hay and a concentrate ration, the amounts of which were adjusted weekly to ensure moderate (0·21 kg/bull/day) body weight gains.

Bulls were anaesthetized and surgery performed as described by Ross & Entwistle (1979). A dosage of 17·5 MBq [³H]thymidine (Radiochemical Centre, Amersham, Bucks, U.K.) was slowly injected into the surgically exposed, unbranched spermatic artery of each testis to provide an identifiable 'front' (most advanced labelled cell generation) of spermatogenic cells. The left testis was surgically removed approximately 2 h after administration of [³H]thymidine, and the right testis was surgically excised 14 days later.

Histological and autoradiography techniques

Immediately after castration, blocks of tissue 0·5–1 cm thick were cut from the dorsal, middle and ventral regions of each testis, fixed in fresh Cleland's solution (Rowley & Heller, 1966) for 24 h and embedded in paraffin wax. Six pairs of sections (6 µm) were cut from each block at approximately equidistant points through the block; 2 sections per block were stained with haematoxylin and eosin (H&E). The remaining sections were processed for autoradiography as described previously (Ross & Entwistle, 1979) and were developed and stained with H&E after exposure for 3, 4 or 6 weeks. At examination, the slides that had been exposed for 3 weeks were adequately labelled and were selected for study. Only cells showing 12 grains/nucleus were considered to have been labelled (Ross & Entwistle, 1979).

Determination of the seminiferous epithelial cycle

The frequency of the stages of the seminiferous epithelial cycle was determined by classifying seminiferous tubules in H&E stained sections into 8 stages based on the descriptions of Roosen-Runge & Giesel (1950) with the addition that stages 1, 2, 3, 4 and 8 were further subdivided into substages as proposed by Courot *et al.* (1970). Two sections from each testis block (dorsal, middle and ventral areas) were examined, giving a total of 6 sections per testis. Only tubules with regular cell layers and a circular cross-section were counted and classified.

Estimates of the duration of the seminiferous epithelial cycle were determined by the methods of Clermont *et al.* (1959) and Clermont & Harvey (1965) by determining (a) the progress of the most advanced labelled cells over a known period (Estimate A) or (b) the frequency of labelling of the oldest cell types in each stage within the testis at different intervals (Estimate B). Arrows representing the 'front' of labelled cells at 2 h and at 14 days after [³H]thymidine injection were located on a scaled diagram of the seminiferous epithelial cycle (Clermont & Harvey, 1965), in which the widths of the vertical divisions were directly proportional to the relative frequency of the cycle stages determined in this study. The duration of the seminiferous epithelial cycle was then determined by measuring the horizontal distance in time in that diagram between arrow tips.

Statistical analyses

Frequency data (expressed as a percentage) of stages of the cycle were transformed to angles and analysed by a split-plot analysis of variance model (Snedecor & Cochran, 1967). The effects of genotype and method of estimation (A or B) on the duration of the cycle were also examined by analyses of variance.

Results

The relative frequencies of the various stages are summarized in Table 1. Analysis of variance of the transformed data indicated no significant differences in the frequency of stages between genotypes, between testis regions or between testes within animals.

Table 1. Relative frequencies (%) of the stages of the seminiferous epithelial cycle in material from F₂ generation half and three-quarter Brahman cross bulls

Stage of cycle	Hybrid		Mean
	Half Brahman	Three-quarter Brahman	
1a	11.10 ± 0.57 (351)	10.97 ± 0.20 (348)	11.03 ± 0.30
1b	10.00 ± 0.38 (315)	9.95 ± 0.34 (317)	9.98 ± 0.23
1c	8.03 ± 0.30 (252)	8.80 ± 0.71 (279)	8.42 ± 0.42
2a	5.70 ± 0.15 (182)	5.83 ± 0.56 (184)	5.77 ± 0.29
2b	6.67 ± 0.37 (202)	6.23 ± 0.49 (203)	6.45 ± 0.32
3a	10.57 ± 0.38 (337)	10.70 ± 0.25 (341)	10.63 ± 0.23
3b	9.13 ± 0.24 (291)	9.83 ± 0.13 (314)	9.48 ± 0.22
4a	3.70 ± 0.10 (119)	4.13 ± 0.34 (133)	3.92 ± 0.20
4b	2.52 ± 0.12 (82)	3.17 ± 0.14 (102)	2.85 ± 0.18
5	1.77 ± 0.14 (56)	1.53 ± 0.80 (50)	1.65 ± 0.10
6	5.47 ± 0.59 (174)	5.46 ± 0.62 (177)	5.47 ± 0.42
7	8.53 ± 0.17 (271)	8.07 ± 0.43 (257)	8.30 ± 0.25
8a	7.57 ± 0.14 (240)	7.10 ± 0.17 (226)	7.33 ± 0.16
8b	9.20 ± 0.25 (291)	8.00 ± 0.15 (256)	8.60 ± 0.33

Values are mean ± s.e.m. for the no. of tubule cross-sections indicated in parentheses.

In sections taken approximately 2 h after [³H]thymidine injection, autoradiographs showed specific labelling of A type spermatogonia in all stages of the seminiferous epithelial cycle, the ratio of labelled : total A type spermatogonia being similar in all stages. Most intermediate-type spermatogonia in stages 3 and 4 were labelled although the frequency of labelling was variable. Type B spermatogonia in stages 5 to 1 were also labelled uniformly, the highest percentage of labelled cells being found in stage 1. The resting preleptotene and leptotene spermatocytes in stages 1b and 1c showed uniform labelling. However, the number of young labelled granular spermatocytes in stages 2a and 2b varied between animals. Since no older spermatocytes or spermatids were observed to be labelled, the granular spermatocytes in stages 2a and 2b were taken to be the most advanced labelled cell types at that time.

In sections from testes 14 days after thymidine injections, all young spermatocytes were uniformly labelled in all stages. Similarly, old (pachytene and diplotene) primary spermatocytes in stages 5 to 1 were uniformly labelled. The percentage of labelled pachytene spermatocytes in stages 2a and 2b varied to some extent and these cells represented the most advanced labelled cell types at that time.

Estimates of the duration of one cycle of the seminiferous epithelial cycle are shown in Table 2. There were no significant differences between the two methods of estimation, or between genotypes.

Table 2. Estimated duration of one seminiferous epithelial cycle in 4 half-Brahman cross and 4 three-quarter Brahman cross bulls

	Estimate A* (days)	Estimate B† (days)	Overall value (days)
Half Brahman cross	13.96 12.72 13.52 12.52	14.18 13.01 14.06 12.58	13.42 ± 0.30
Three-quarter Brahman cross	13.81 13.14 13.06 13.24	12.37 12.89 14.57 14.23	
Estimate mean	13.25 ± 0.18	13.59 ± 0.34	

* Determined from the frequency of labelling of the most advanced (granular leptotene spermatocytes in Stage 2a) cell types.

† Determined from frequency of labelling of the oldest cell types in each stage within the testis at different times.

Discussion

In a single testis in 2 of the 8 animals, slight leakage of the infusate was observed following withdrawal of the needle from the artery. However, in sections from these and all other testes, radioactive labelling in receptor cells was satisfactory. In contrast to tissues taken 2 h after thymidine injection, all of which were morphologically normal, there were minor histological changes such as vacuolation and exfoliation in some tubules 14 days after surgical interference. However, these were not sufficient to affect the frequency estimates or the proportions of labelled cells. Similar minor histological changes following surgery and/or intra-arterial injection were noted in previous studies (Ross & Entwistle, 1979).

Subdivision of the easily identifiable stages of the seminiferous epithelium into substages (Courot *et al.*, 1970) permitted a more accurate determination of the relative frequency of particular cell stages. The absence of any significant variation in the estimated frequency of stages between sites within the testis, between testes of the same animal, or between genotypes, confirms other reports (Amann, 1962; Hochereau, 1963), and indicates that the technique is accurate and repeatable.

Published estimates of the mean frequency of cycle stages in material from *Bos taurus* bulls and buffalo (*Bubalus bubalis*) bulls are summarized in Table 3 as a comparison with those of the present study. The values for the hybrid bulls in this study are similar to those for *Bos taurus* bulls except that the relative frequency was lower for stage 4 tubules and higher for stage 8 tubules. In general the frequency of cell stages in *Bubalus bubalis* is similar to that of *Bos taurus* (Guraya & Bilaspuri, 1976; Sharma & Gupta, 1980) (Table 3). The apparent differences between the cattle data may reflect genetic differences, but it is possible that they merely reflect a more precise estimate of the frequency of cell stages by using a further subdivision of the longer stages of the cycle, since earlier observations on the frequency of the cycle stages in cattle (Ortavant, 1959; Amann, 1962; Hochereau, 1963) utilized only 8 stages without further subdivision. Finally, the differences observed between this and other studies of cattle may merely reflect inherent biological variation between the different animals used.

Table 3. Estimates of the relative frequency and duration of the stages of the cycle of the seminiferous epithelium in *Bos taurus* bulls, *B. indicus* × *B. taurus* hybrids and in buffalo (*Bubalus bubalis*) bulls

Stage of cycle	Mean frequency (%)			Estimated duration (days)		
	<i>Bos taurus</i> *	Hybrids	<i>B. bubalis</i> †	<i>Bos taurus</i> *	Hybrids	<i>B. bubalis</i> †
1	28.6	29.4	27.5	3.9	3.9	2.4
2	12.9	12.2	14.3	1.7	1.7	1.4
3	19.6	20.1	20.1	2.6	2.7	1.7
4	10.9	6.8	10.2	1.5	0.9	0.8
5	1.7	1.6	2.1	0.2	0.2	0.2
6	5.3	5.5	7.1	0.7	0.7	0.6
7	9.1	8.3	8.3	1.2	1.1	0.7
8	11.9	15.9	10.3	1.6	2.1	0.8
			Total	13.5	13.4	8.6

* Calculated from data of Ortavant (1959); Cupps & Laben (1960); Amann (1962); Hochereau (1963).

† Calculated from data of Guraya & Bilaspuri (1976) and Sharma & Gupta (1980).

The two methods of estimation for the duration of one cycle of the seminiferous epithelium gave very similar results; there were no significant between-genotype differences and the overall mean estimate of 13.41 days was closely similar to that of 13.5 days for *Bos taurus* bulls (Amann, 1962; Hochereau, Courot & Ortavant, 1964; Amann *et al.*, 1974; Ross & Entwistle, 1979). The results of this study therefore confirm, for cattle, the suggestions by Courot *et al.* (1970) and Ortavant *et al.* (1977) that strain variations within species in the duration of spermatogenesis are not of major importance. The very short duration of spermatogenesis in buffalo bulls (8.6 days: Sharma & Gupta, 1980), which is the shortest for any domestic species except the boar (Swierstra 1968), and the low rate of daily sperm production and relatively small testes (Sharma & Gupta, 1979) are probably associated with differences in the length of the breeding season and mating behaviour of the male buffalo (Amann, 1981).

In *Bos taurus* bulls the duration of meiosis and of spermiogenesis have been estimated to be 20.5 and 17.7 days respectively (Hammerstedt, 1981). Corresponding values for the present data were 20.7 and 17.4 days, but these are only minor differences, reflecting the different stage frequencies between the two types of bulls. The values for buffalo bulls are 12.8 and 10.6 days respectively (Sharma & Gupta, 1980).

For *Bos taurus* bulls an estimated cycle duration of 13.5 days and a figure of 39.4% homogenization-resistant nuclei representing stages 4–8 of the cycle gave a time divisor of 5.32 days to calculate daily sperm production (Amann *et al.*, 1974). With this value, estimates of daily sperm production from testicular homogenization procedures more closely approximated those determined from cannulation of the vas deferens or from frequent semen collection (Amann *et al.*, 1974). From the present study, taking the mean duration of one cycle of the seminiferous epithelium as 13.4 days, and assuming that resistant spermatids represent stages 4 to 8 of the cycle (38.1%; Table 1), a time divisor of 5.11 days is suggested for calculating daily sperm production from testicular homogenates of these hybrid, *Bos indicus* type bulls.

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