Electroejaculation, semen characteristics and semen preservation of the brindled gnu

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Interest in the application of the techniques of artificial breeding to non-domesticated mammals has been stimulated in recent years by the failure of some species to breed in captivity and by the decreasing availability of animals from the wild. Several authors have drawn attention to the need to develop techniques suitable for exotic animals (Jones, 1971; Francoeur, 1972; Rowlands, 1965, 1974; Warner, Martin & Keeling, 1974), but as yet very few species have been studied.

A brindled gnu (*Connochaetes taurinus*) at The Zoological Society of London was sedated with Xylazine (Rompun, Bayer U.K. Ltd) and semen was collected by electroejaculation using finger ring electrodes and a sine wave stimulator (Watson & Hime, 1976). Stimulation for periods of 5 sec with 5-sec rest periods was commenced with a frequency of 10–15 Hz and a peak voltage of 7.5 V (Text-fig. 1a). After 30 sec the frequency was increased to 30 Hz and the peak voltage to 15 V (Text-fig. 1b). The penis extruded about 9–11 cm before a forceful ejaculation and semen was produced during several successive 5-sec stimulation periods.

Text-fig. 1. Oscilloscope display of the output of the stimulator used for electroejaculation of the brindled gnu. (a) Low output used to commence stimulation; (b) maximum output which evoked ejaculation.

The semen had a milky consistency and showed strong wave motion typical of bull semen. The concentration of spermatozoa in the ejaculate was calculated from haemocytometer counts of diluted semen. The percentage of motile spermatozoa was estimated immediately after dilution of semen at 30°C in buffered citrate solution. Estimates of the percentage of live spermatozoa were made using the eosin-nigrosin method of Hancock (1951). The characteristics of the ejaculates collected on 2 occasions were:

	Ejaculate 1	Ejaculate 2
Volume (ml)	10.5	12.0
Sperm concentration ($\times 10^6/\text{ml}$)	184	520
Motility (%)	50	70
% Unstained spermatozoa (eosin-nigrosin)	82	89.5

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The lower values for the first ejaculate were probably due to ageing of the spermatozoa, since the animal had not mated for several weeks before electroejaculation.

Gnu spermatozoa resemble those of domestic ungulates in having a bilaterally flattened paddle-shaped head. The midpiece inserts axially into the base of the head. Planimetry of 200 gnu spermatozoa in fixed smears lightly stained with Giemsa (Watson, 1975), at a magnification of $\times 1250$ for the sperm heads and $\times 500$ for midpieces and overall measurements, gave the following results (mean $\pm$ S.E.M.): head length 6.86 ± 0.03 μ m, breadth of head at widest point, 4.58 ± 0.02 μ m, length of midpiece 11.13 ± 0.08 μ m, overall length of spermatozoon, 66.66 ± 0.24 μ m. The head of the gnu spermatozoon is smaller than that of bull or ram spermatozoa (Bishop & Walton, 1960; Van Duijn & Van Voorst, 1971), even allowing for alterations in size caused by processing (Van Duijn, 1960; Van Duijn & Van Voorst, 1971). These measurements are consistently greater than those given in the only other report of gnu spermatozoa (Morgenthal, 1967), but Morgenthal measured epididymal spermatozoa, which in the rabbit are known to have a smaller projected head area than ejaculated spermatozoa (Pauffer & Foote, 1968), and his dimensions of spermatozoa of domestic ungulates are also smaller than those generally given.

Two experiments were performed to examine some of the diluent requirements for gnu spermatozoa and to investigate the response to freezing and thawing. Semen was diluted 5-fold at 30°C , cooled to 5°C over 3 hr and then further diluted with a glycerol-containing portion of diluent. The final dilution was 10-fold and the glycerol concentration was 7.5% (v/v). Diluted semen was sealed in 0.5 ml Cassou straws and frozen 4 cm over the surface of liquid nitrogen. Motility, percentage motile and acrosome scores were made on thawed semen as previously described (Watson, 1975; Watson & Martin, 1975). Analyses of variance were calculated for each of these parameters.

Table 1. The effects of freezing and thawing brindled gnu semen in buffered egg yolk-glucose diluent (mean scores of 3 straws/treatment)

Egg yolk conc. (%)	Equilibration time at 5°C (hr)	Motility (scale 0-4)	% motile sperm.	Acrosome damage (scale 0-3)
10	2	1.94	25.1	1.61
	18	1.79	32.9	1.15
20	2	1.53	18.3	1.15
	18	1.24	19.4	0.98

In Exp. 1 the effects of 10 or 20% egg yolk (v/v) in a glucose-phosphate diluent (Watson & Martin, 1975) were examined, and equilibration periods of 2 hr and 18 hr at 5°C after the addition of glycerol were compared. The results after thawing (Table 1) indicated that 10% egg yolk was preferable to 20% ($P < 0.05$), and equilibration for 18 hr gave a slightly, but not significantly, better revival with 10% egg yolk. Least acrosome damage occurred with the higher egg yolk level ($P < 0.01$) and the longer equilibration period ($P < 0.01$). The interaction term was also significant, indicating that the detrimental effect of a shorter equilibration was greater at the lower egg yolk level ($P < 0.05$).

In Exp. 2, a citrate diluent (Martin & Emmens, 1961) was used since, in a preliminary incubation experiment, gnu spermatozoa survived longer in an egg yolk-citrate diluent than in an egg yolk-glucose-phosphate diluent. The spermatozoa were equilibrated for 4 hr before storage for 6 months in liquid nitrogen. After thawing the spermatozoa were incubated for 0, 2 or 4 hr at 37°C (Table 2). A 4-hr incubation significantly lowered motility and the % of motile spermatozoa ($P < 0.001$), but there were no significant changes in acrosomal morphology. The lowest egg yolk concentration supported the spermatozoa better than the higher concentrations (motility score, $P < 0.05$; % motile score, $P < 0.01$). The interaction of 'egg yolk concentration $\times$ incubation time' was significant ($P < 0.05$) for motility and % motile scores, reflecting the fact that the decline in sperm viability from 2 to 4 hr of incubation in the 25% egg yolk diluent was not as great as in the lower egg yolk concentrations.

Table 2. The effects of egg yolk concentration and incubation time at 37°C after thawing on the survival and acrosomal morphology of gnu spermatozoa stored for 6 months at -196°C (mean scores of 5 straws/treatment)

Incubation time (hr)	6.25% egg yolk			12.5% egg yolk			25% egg yolk		
	Motility*	% motile	Acrosome† damage	Motility*	% motile	Acrosome† damage	Motility*	% motile	Acrosome† damage
0	2.57	60.7	1.06	2.20	48.7	1.18	1.93	43.3	1.22
2	1.87	31.3	1.35	1.70	23.3	1.32	1.20	15.3	1.26
4	1.60	23.3	1.19	0.80	10.0	1.08	1.20	12.7	1.65

* Scale 0-4.

† Scale 0-3.

The slow development of the application of artificial insemination to non-domesticated animals is partly due to the lack of information on techniques for semen collection and preservation. Although limited to a single animal, the present study shows that gnu semen can be satisfactorily preserved by freezing, as judged by laboratory tests commonly used for cattle semen.

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