Collection and Analysis of Semen from a Black Rhinoceros

NUMEROUS FACTORS, including poaching and human encroachment, have contributed to declining numbers of the black rhinoceros *(Diceros bicornis)* in the wild. As a result, the black rhinoceros has been classed as an endangered species.

Many zoos, wild animal game parks, and preserves have attempted to establish breeding pairs, but with mixed success. One of the main problems has been incompatibility. Thus, we saw the need to develop a technique for obtaining semen from the rhinoceros. A secondary goal was to determine the ability of rhinoceros spermatozoa to withstand freeze preservation techniques, which we have applied to other mammalian species. The capability of successfully freezing rhinoceros sperm would be of obvious advantage to zoos and captive collections in this and other countries desiring to propagate the species without the risks of shipping such large and unpredictable animals. It is a simple matter to ship stored semen to those interested in artificial insemination of the species.

A black rhinoceros at the National Zoo had sired offspring in 1967, 1970, and 1978. Later, a female rhinoceros died of tuberculosis. Results of subsequent intradermal tuberculin testing included a positive response in this male. It was treated with isoniazid for 6 months, after which time it was decided to euthanatize it because of declining health and risk of spreading the disease to the rest of the herd.

The animal was given 0.6 mg carfentanil citrate (ZR 33799; Janssen Pharmaceutica, Beerse, Belgium) by pole syringe. In 11 minutes, immobilization was adequate for electroejaculation.

Electroejaculation involved administering a 110-V, 60-Hz, sine wave stimulus from a stimulator capable of an output of 0 to 60 V continuous range and 0 to 1 A.

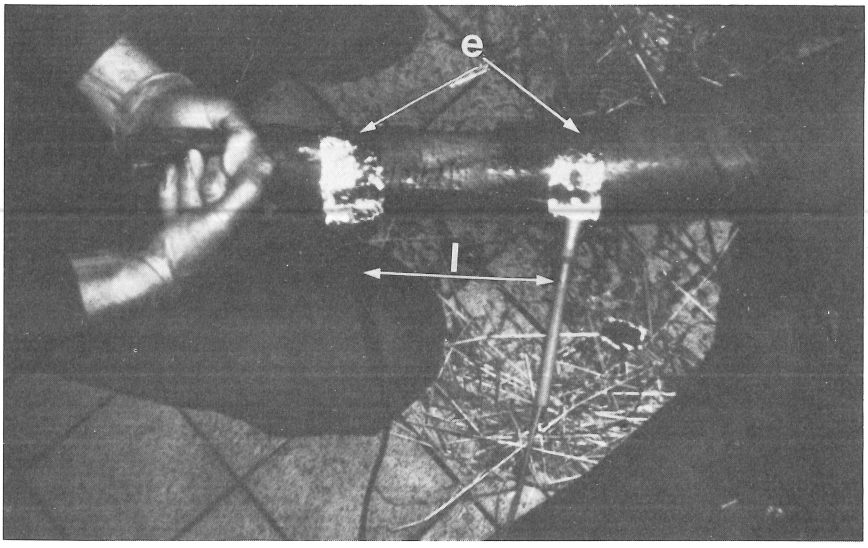

Fig 1—Aluminum foil electrodes (e) encircling black rhinoceros penis, with electric leads (l) attached.

Initially, rectal probe stimulation was combined with direct penile stimulation, by attaching one lead from the stimulator to the ventral electrode lead of the rectal probe and one lead to a 5-cm-wide aluminum foil electrode wrapped around the rhinoceros' penis. Neither erection nor ejaculation were obtained; therefore, the penis was extended manually and two aluminum foil electrodes were wrapped around the penis approximately 17 cm apart (Fig 1). Physiologic saline solution was then used to wet the area of the electrodes. The electric leads were attached to these electrode strips, and stimulus was applied. By the eighth stimulus, using 22 V and 200 mA,

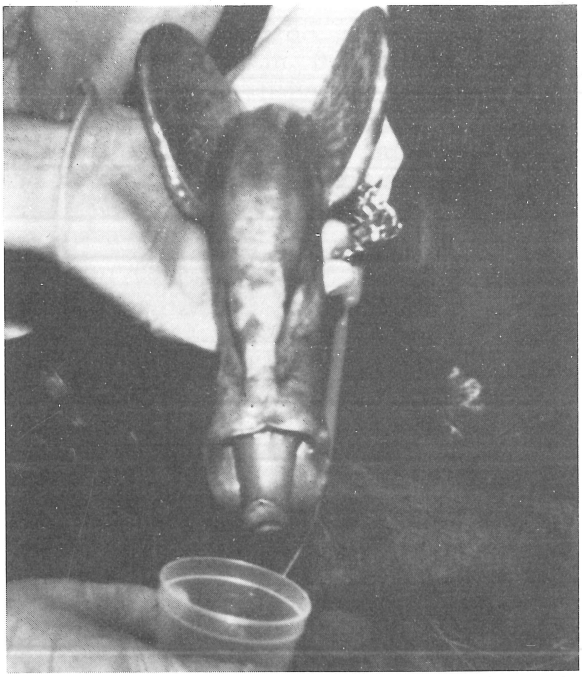

Fig 2—Penile erection and ejaculation from black rhinoceros after electrical stimulation was applied. The wing-like structures develop only on stimulation.

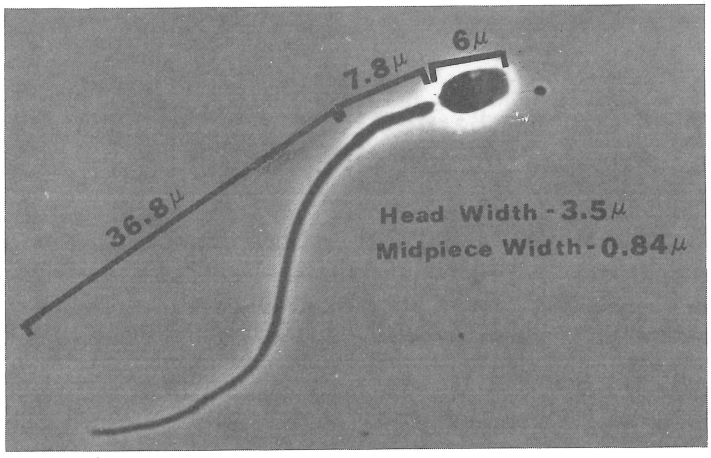

Fig 3—Black rhinoceros spermatozoa; × 2,000.

TABLE 1—Results of Semen Analysis and Freezing in the Rhinoceros

Collection	Volume (ml)	Total sperm count	Post-collection motility (%)*	Post-freeze motility (%)
1	44.6	4.4×10^8	60	20
2	18.8	1.4×10^8	40	15

* Percentage motile sperm observed at × 100.

TABLE 2—Electroejaculation Patterns Used in the Black Rhinoceros *(Diceros bicornis)*

Collection	Voltage range	Amperage range (mA)	Stimulus pattern volts (No. stimulations)
1	10–29	150–210	29(14); 10(3); 15(5); 22(20); 17(20)
2	8–25	60–200	8(4); 10(5); 15(5); 20(5); 10(3); 15(5); 20(10); 25(8); 10(4); 15(5); 10(5); 15(5); 20(6)

the penis became fully erect and an ejaculate was obtained (Fig 2). Electrical stimulation was continued, using a range of 10 to 29 V and 150 to 210 mA. The stimulus pattern was approximately 2 seconds from 0 to peak voltage, 3 seconds at peak voltage, and 2 seconds from peak to 0 voltage. After 3 seconds at 0 voltage, the pattern was repeated. Ejaculation continued through a total of 62 cycles, at which point the rhinoceros was rested for 45 minutes.

The ejaculate was analyzed, using previously reported procedures.[1,2,3] Mean (n = 10) dimensions of the spermatozoa (Fig 3) were: head length,

6.0 μm; head width, 3.5 μm; midpiece-neck length, 7.8 μm; midpiece width, 0.84 μm; tail length, 36.8 μm; and total length, 50.6 μm.

Table 1 contains the results of semen analysis and freezing. The sperm appeared undamaged after thawing from −196 C. On the basis of morphologic features and motility of the motile sperm, which we use for making an in vitro estimate of fertilizing ability, the sperm appeared capable of fertilization. Sperm motility was rapid in both ejaculates, before and after freezing. Table 2 lists the two voltage, amperage, and stimulus patterns used to obtain the ejaculates. Both patterns seemed to elicit similar ejaculatory response, although the second collection contained fewer sperm, most likely because of sperm depletion in the reproductive tract.—*Carrol C. Platz, Jr., BS, Stephen W. J. Seager, MRCVS, Institute of Comparative Medicine, Baylor College of Medicine, Texas A&M University, Houston, TX 77030; and Mitchell Bush, DVM, National Zoological Park, Smithsonian Institution, Washington, DC 20008.*

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An Anatomic Barrier to Urethral Catheterization in Male Macropodidae

A QUEENSLAND agile wallaby *(Protemnodon agilus jardini)* was anesthetized for urethral catheterization to relieve urinary retention caused by a neurologic deficit. A catheter introduced through the external urethral orifice was advanced until resistance was encountered. Because catheterization was impossible, a cystotomy was performed. During cystotomy, the catheter was introduced into the bladder lumen and directed distally through the sphincter muscle, until resistance was again encountered. Despite therapy, the animal died and was necropsied. At necropsy, the bladder and urethra were removed as a unit. An incision was made through the wall of the bladder and continued distally on the dorsal aspect of the urethra to the external urethra orifice.

A Goodfellow's tree kangaroo *(Dendrolagus goodfellowi)* was anesthetized with halothane. A male urinary catheter (Sherwood Medical Industries, St Louis, Mo) was introduced into the external urethral orifice and advanced as far as possible. Radiopaque dye (Amipaque, Winthrop Laboratories, New York, NY) was introduced into the catheter to locate its tip. Care was taken not to introduce or force contrast medium beyond where the catheter lodged, and a radiograph was made (Fig 1). A 6-ml syringe was filled with the same contrast medium and attached to the catheter. Manipulation as well as moderate digital pressure on the syringe plunger were used in an attempt to force the catheter further within the urethra, but without success. The animal was necropsied and the urinary tract was excised and incised as described for the wallaby.

Three other male Macropodidae (*Dendrolagus matschiei, Macropus robustus,* and *Protemnodon agilis jardini*), which died of unrelated causes, were necropsied. Their urinary tracts were removed and examined in a manner identical with that described for the wallaby.

The urethral lumen of all five animals was similar. Approximately 2 to 3 cm proximal to the external urethral orifice, a pair of valvelike cusps were encountered. Each cusp was approximately 5 mm long, with its free margin directed toward the external urethral orifice. Histologically, each cusp consisted of transitional epithelium overlying a submucosa of poorly vascularized fibrous connective tissue. The epithelium and submucosa were