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Methods of Semen Collection in an Ambulatory Greater One-Horned Rhinoceros (*Rhinoceros unicornis*)

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Illegal poaching and reduced habitats have led to the endangered status of rhinoceroses. Collection of semen for preservation and future artificial insemination would therefore increase the reproductive potential of the rhinoceros. Although various collection methods have been attemped with individual rhinos, comparison between methods on a single animal have not been reported. This report details the application of six semen collection techniques on an unsedated and unrestrained greater one-horned rhino. The methods included different types of penile and/or rectal stimulation. Artificial vaginas and an inflatible probe for electroejaculation were specially constructed for semen collection. Of the various methods employed, penile massage consistently resulted in sperm-poor seminal fluid, but when preceded by either rectal massage or electroejaculation, seminal fluid with high sperm concentration was obtained.

Key words: rhinoceros, rectal massage, penile massage, artificial vagina, electroejaculation

INTRODUCTION

Various semen collection techniques have been developed for domestic animals, but not for rhinoceroses. Although electroejaculation on anesthetized rhinos [Platz et al., 1979; Howard et al., 1983] and penile massage and/or artificial vaginas on unanesthetized rhinos for semen collection have been reported [Young, 1967; Peachy, 1979], there have been no detailed investigations on comparing these collection methods on an individual animal. This report details and compares six semen collection techniques over a 12-month test period on an unanesthetized and unrestrained greater one-horned rhinoceros (*Rhinoceros unicornis*) housed at the Milwaukee County Zoo.

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MATERIALS AND METHODS

This 30+-year-old animal had sired one offspring in 1967. After the death of his mate in 1975 he remained unpaired. He was healthy except for penetrating cracks in his rear foot pads that periodically inflamed into supportive erosive pododermatitis causing him to limp. His exhibit area included one outside and two inside (6.1m $\times$7.6m) areas separated by a door and bars. Entry or escape from the rhino was possible from rear doors left partially open or along the front moat of each of the inside enclosures. Relative to other rhinos, this rhino was very tolerant of new objects and personnel in his cage. Therefore, collection procedures proceeded without physical restraint or sedation. The study period was from Fall 1983 to Fall 1984 and occurred in the inside exhibit areas.

This animal could usually be controlled for these procedures by offering him food or by scratching his head.

Penile Stimulation

Penile stimulation consisted of two methods: penile massage and artificial vagina.

Penile massage. A penile massage collection proceeded with two operators kneeling at the rear of the rhino to massage the penis. Rubbing the medial aspects of the rear legs and the stomach always induced let-down of the penis from the sheath so it could be massaged directly. When released from the sheath, the semi-erect penis curved caudally. Proximal to the curve were lateral, longitudinal folds. After the penis was released from the sheath, it was washed with warm water. Silicone gel (K-Y, Johnson & Johnson, New Brunswick, NJ) was then smeared on the distal 15–20 cm of the penis (end of penis to beginning of lateral folds), but the penile tip was kept free of lubricant. Complete erection was induced by rubbing back and forth on the top and bottom of this area with one hand. Gentle rubbing would become more vigorous as the penis became erect. With erection, the curve of the penis straightened, swinging the tip of the penis forward. The lateral folds (20–25 cm in length) expanded into slightly cupped projections extending from the middle of the penis. A 60 cc syringe (Monojet, Sherwood Medical, St. Louis, MO) case was held over the tip of the penis to collect the ejaculate (Fig. 1). Forceful ejaculations of seminal fluid occurred as the penis became fully erect. Occasionally, shaking of the back legs and thrusting of the penis would occur. Another operator would help support the penis as it became fully erect. The entire length of the fully erect penis was 60–70 cm. The tip of the penis needed to be covered at all times during stimulation since semen could squirt or dripple out at any time. After a forceful ejaculation, the penis would relax and the animal would not usually remain still for further manipulation. Periods to induce erection and ejaculation lasted from 10–20 minutes. If ejaculation did not occur at full erection, the penis was allowed to relax. The process was repeated until the rhino would no longer stand still, which usually occurred in 40–60 minutes.

Artificial vagina. Two different water-filled artificial vaginas (AV) were constructed.

A water-filled AV similar to a standard domestic animal AV which provides the warmth and pressure needed to induce ejaculation was developed (Fig. 2). Briefly, an 18 cm $\times$ 50 cm PVC pipe was used as support for two 15 cm latex liners: one inside the other (Edwards Agricultural, Baraboo, WI). The outside liner was stretched over

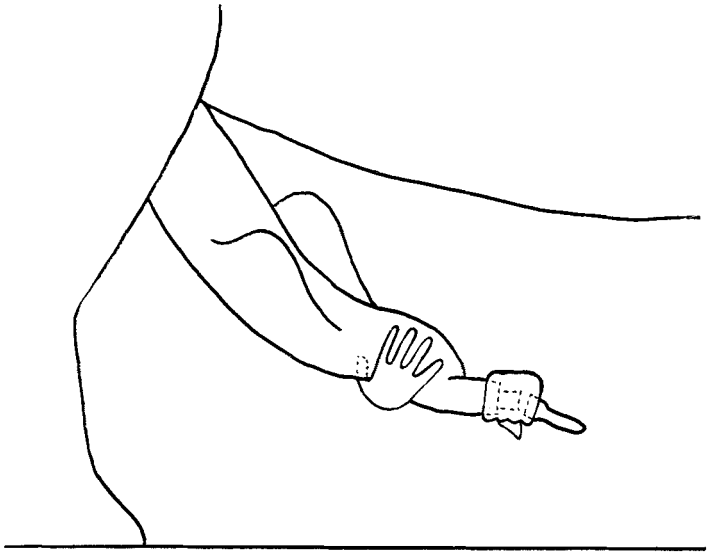

Fig. 1. Placement of hands for penile massage and semen collection from rhinoceros.

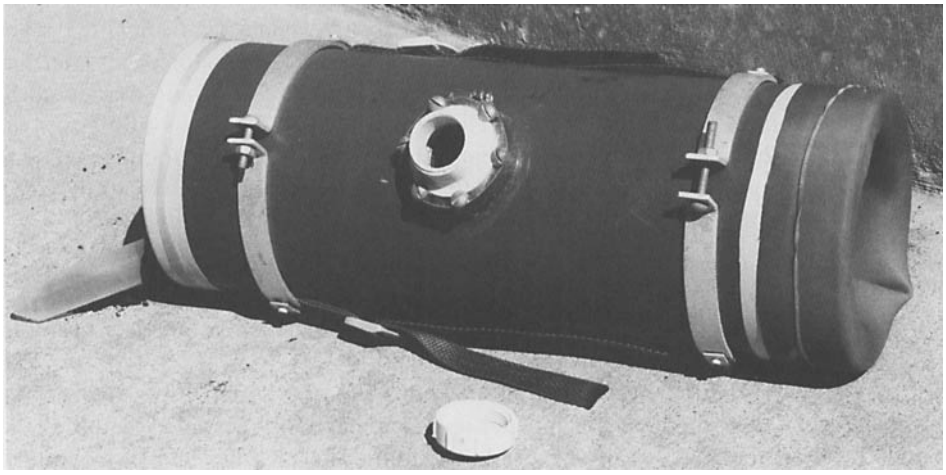

Fig. 2. Water-filled AV of hard construction for penile stimulation.

each end of the pipe. The cavity between the latex and pipe was then approximately half-filled with warm water (~35°C–45°C) through a sealable hole in the pipe. The other latex insert was stretched over one end of the pipe, with the other end sealed (vulcanized) except for a 3 cm hole that could be stretched over a 50 cc centrifuge tube (polystyrene, American Scientific, McGraw-Park, IL). Because the AV weighed approximately 25–30 lb when filled, handles were fitted on both sides so it could be held by more than one operator. Lubricant was then applied to the interior of the AV. This AV covered the end and lateral folds of the erect penis.

A modified AV was developed based on the experience of the previous AV. A

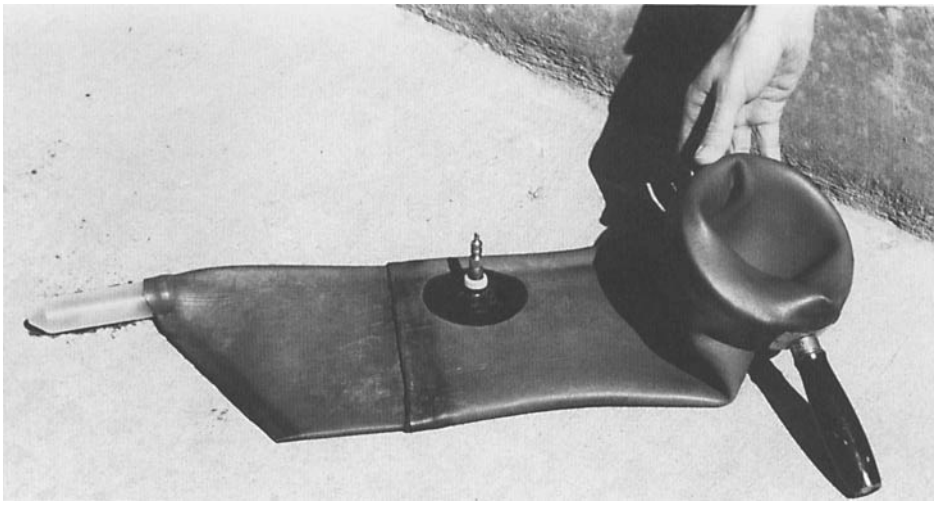

Fig. 3. Water-filled latex AV for stimulation of distal tip of rhinoceros penis.

15 cm diameter latex liner was doubled over and vulcanized together with a 15 cm diameter metal ring (within the latex) attached to a wooden handle (Fig. 3). The handle allowed this 50 cm long AV to be held by only one operator. The other end of the folded liner was also vulcanized closed, except for an opening that could be stretched onto a 50 cc centrifuge tube. This AV was constructed to only cover the distal part (15–20 cm) of the penis. To determine the optimum temperature needed to induce ejaculation, two different temperature ranges (35°C–40°C and 40°C–45°C) were employed in the AV.

Rectal Stimulation

Stimulation inside the rectum was induced by either manual massage or by an electrical probe.

Rectal massage. For rectal massage, an operator inserted a plastic-sleeved, lubricated hand through the anal sphincter and then manually massaged the accessory glands through the mucosa of the caudal rectum. The prostate and bulbourethral gland, which were about 10–15 cm proximally to the anal sphincter, were massaged with rhythmic side-to-side downward pressure by the operator's fist. Massage proceeded in 5 minute intervals with resting 1–2 minutes between intervals. Total time for massage never exceeded 15–20 minutes.

Electroejaculation. Standard electrical probes used for domestic animals could not be utilized since they usually rolled in the gas-expanded rectum of the rhino. Because of this, the electrodes would intermittently lose contact with the rectal mucosa and therefore current could not be effectively controlled. The development of an inflatible rectal probe allowed complete and secure contact of electrodes to the mucosa (Figs. 4,5). After placing the probe in the rectum, a sturdy 28 cm diameter balloon (punch ball, National Latex, Ashland, OH) taped (electrical tape, 3M, St. Paul, MN) securely to the body of the probe (2.5 cm O.D. × 60 cm PVC pipe) was inflated with an air-mattress hand pump (Tex-Sport, Houston, TX). The balloon

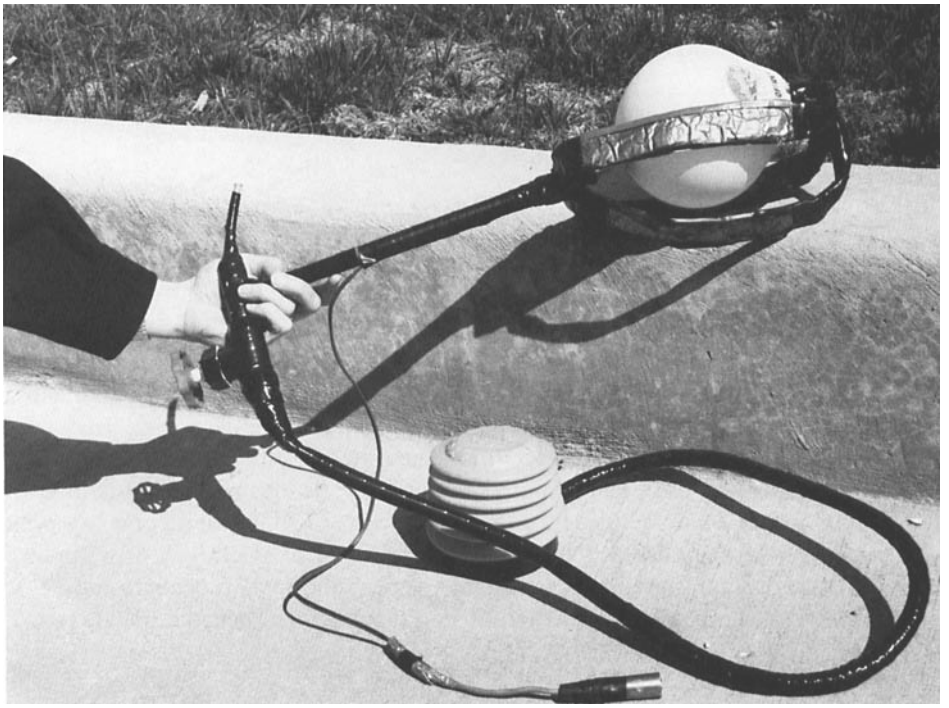

Fig. 4. Inflatable, electrical probe developed for rectal stimulation in rhinoceros.

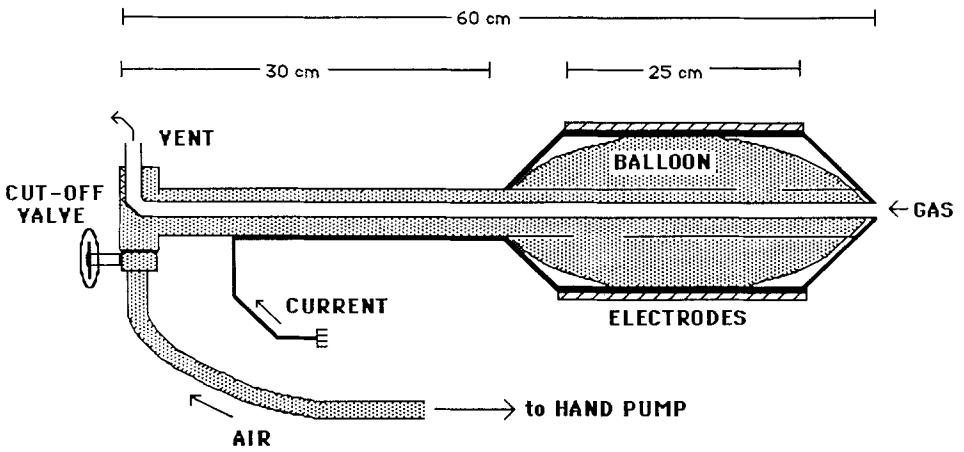

Fig. 5. Cross-sectional diagram of inflatable electrical probe.

pressed three loosely held (by wire), encircling copper electrode strips (25 cm × 4 cm) into contact with the mucosa. Air in the balloon was retained by a PVC hydrate-type cutoff valve. A 0.8 cm tube through the entire body of the probe allowed intestinal gas to exit without filling the rectum. Thus, the probe remained in contact with the mucosa.

TABLE 1. Seminal parameters (means and ranges) for various collection methods from a greater one-horned rhinoceros

Method	Volume	Sperm count	Total sperm count	Motility
Penile massage (n = 12)	16.9(0.2–50)	7.1(0.0–208)	55.2(624)	15.8(0.0–80)
Artificial vagina (n = 3)	46.7(30–60)	—	—	—
Rectal massage (n = 1)	3.0	55	190	30
Electroejaculation	—	—	—	—
Rectal & penile massage (n = 6)	10.6(2–20)	142.5(0.1–352)	1364.4(0.2–2,700)	50(30–80)
Electroejaculation & penile massage (n = 4)	0.7(0.1–2)	186.4(5–500)	77.4(0.5–250)	73(70–80)

After inflating the balloon until the probe was in contact with the rectal mucosa, the cutoff valve was closed. The hand pump was removed and electrode leads were connected to wires hanging from the ceiling. The rhino was then free to move around the exhibit area during electrical stimulation by a 20 cycle electroejaculation machine (Lane P-IIIZ, Lane Manufacturing Company, Denver, CO). Three second sine wave pattern stimuli were applied at the 2V, 40 mA level for two series of 60–80 pulses each. With a 5 minute rest interval between series, the total procedure took from 10–15 minutes. The procedure was terminated if the animal became irritated.

RESULTS

Six months of training was required to develop the various procedures and for acclimation of the rhino. Over the next 12 month test period, penile and rectal stimulation methods were attempted separately or in combination, at an average of once a week at different times of the year. Any of these methods were unsuccessful if the rhino was very hungry or thirsty. Activity around his cage would distract the rhino's attention from collection procedures, so collections were generally performed in the morning before the zoo opened. While the keepers fed or scratched the rhino's head, procedures could be applied. If he was not receptive to a procedure, he would walk or back away from operators. If this persisted, application of procedures ended. He was never physically or chemically restrained from movement.

Penile Massage

The rhino was the most receptive to penile massage. It was the most frequently successful of collection methods (Table 1). Fluids from this method were characteristically clear and viscous with poor sperm concentration. Ejaculate volumes obtained from penile massage were consistently higher than the other methods investigated.

Artificial Vagina

Inducing a fully erect penis by penile massage was required before an AV could be fitted. AVs intended to cover the penis had to have diameters large enough for the width of the penis when the lateral folds expanded into projections. The initial AV's construction with PVC pipe and its snug fit often resulted in abrasion to these lateral projections. This AV was abandoned for the modified lighter and all-latex AV that could be handled by one operator.

Although trauma to the penis was relieved by the modified AV, maintaining the

erection after fitting the AV was still a problem. Only three of ten attempts with the AVs resulted in ejaculates. These samples had high volumes but poor sperm concentrations. These ejaculates occurred when the AV temperature was between 40°C–45°C.

Rectal Massage

The rhino was initially reluctant (flicking his tail and walking around) to have a plastic-sleeved, lubricated hand pass through the anus, but after four to five sessions, he became receptive to palpation. The rhino's penis would drop from the sheath during rectal massage, but never became fully erect. Fluid drippled from the penis only once, after it had dropped from the sheath during rectal massage.

Rectal and Penile

Although the total stimulation period was not shortened by combining these two procedures, the seminal fluids obtained by rectal massage followed with penile massage had higher sperm concentrations than penile massage alone (Table 1).

Electroejaculation

The inflatable probe was easily inserted and removed when deflated. Electrical pulses at the 2V level were kept consistent as monitored by the strip chart patterns (Miniservo® Esterline Angus, Indianapolis, IN), but amperages varied between 40–50 mA during stimulation. Electrical stimulation by itself did not produce erection or ejaculation. The animal would stand or walk around his cage and occasional muscle contractions were seen in his rear legs. He did not become agitated (flicking his ears, rapid movement) unless amperages rose above 50 mA, and at that point the procedure was then terminated.

Electrical and Penile

Electrical stimulation followed by penile massage produced sperm counts comparable to the combination of rectal and penile massage. Total sperm counts from this combination were lower than rectal and penile massage probably due to low sample volumes obtained. Following electroejaculation, longer massage periods (20–30 minutes) were necessary to induce let-down of the penis from the sheath, in comparison to when only penile massage was performed. Ejaculates were not forceful and the volumes were small but the penis became erect. The animal was always restless following electroejaculation, so penile massage trials were not repeated.

DISCUSSION

Although penile massage routinely induces ejaculation in humans and carnivores, it is usually ineffective in other large mammals. In the elephant, penile massage was successful only after the bull was prevented from mounting the female [Jainudeen et al., 1971], but it appears to be an effective method for semen collection in the stallion [Crump and Crump, 1989].

In this study, low sperm concentrations of observed ejaculates from both penile massage and AV collections are probably due to insufficient stimulation of the animal. Artificial vaginas have been used effectively in many domestic species [Mattner and Voglmayr, 1962; Asbury and Hughes, 1964; Ball et al., 1983] and in the elephant

[Heath et al., 1983]. To be effective, the AV must be properly constructed and the animals preconditioned by teasing the male [Salisbury et al., 1961; Kenney and Cooper, 1974; Pickett et al., 1987] before semen collection. The teasing technique, such as allowing the animal to mount the female or an inanimate object, improves sperm concentration in the ejaculate by priming emission of semen into the posterior urethrea [Hale and Almquist, 1960; Seidel and Foote, 1969; Newman et al., 1982]. Lack of teasing is probably the cause for poor semen quality observed in this study. Attempts to heighten libido in the rhino by exposing feces or urine from an estrus female (Terry Blasdel, personal communication) or placing inanimate objects in cages for mounting [Sedgwick, 1988] have been generally ineffective. The stallion [Schumacher and Riddell, 1986] and the elephant [Jainudeen et al., 1971] were collected without a mount but the males were still teased with a female. Intensive training may alleviate the need for preconditioning the rhino. Semen has been collected from highly trained elephants without teasing [Heath et al., 1983]. With time and repeated application of procedures, a noticeable improvement in sperm concentration occurred in this Greater one-horned rhino [Schaffer and Beehler, 1988]. Peachy [1979] also reported an increase in sperm concentration with training of the rhino. Similar to our initial observation, Young's [1967] semen collection trials by penile massage from an unconditioned black rhino also resulted in ejaculates with poor sperm concentration.

Domestic animals are trained to respond to handlers and also some form of restraint when collecting semen [Salisbury et al., 1961; Kenney et al., 1983; Hurtgen, 1984; Bowen, 1986]. This rhino might have been trained sooner if he had not been allowed to walk away from procedures. With restraint caging, application of collection procedures has been possible in less amiable rhinos [Schaffer and Beehler, 1988].

In addition to teasing, several other variables such as size, shape, pressure, and temperature of the AVs had a direct effect. Standard domestic AVs for bulls and stallions had to be widened to accommodate the lateral folds of the rhino's penis. This adaptation made the AV extremely heavy and difficult to maneuver. In addition, the hard PVC tube construction often resulted in trauma to the lateral folds on the penis. Peachy [1979] also found that AVs resulted in trauma to the penis and inhibited performance of the rhino. The smaller, modified AV that covered only the distal end of the penis was less traumatic to the rhino, but was only marginally effective. Ejaculates can be collected from other animals such as boars by exerting pressure on the distal end of the penis by hand [Zavos and Liptrap, 1987]. The end of the penis was also sensitive to stimulation in the rhino [Young, 1967]. It appears that the AVs for rhinos need to be light-weight and large enough to cover the entire penis and to apply pressure to the distal end.

It has been reported that the temperatures of AVs should be higher than the animal's body temperature. For example, AV temperatures (38C°–55°C) are reported for stallions and bulls [Salisbury et al., 1961; Seidel and Foote, 1969; Hillman et al., 1980; Ball et al., 1983; Pickett et al., 1987]. Similarly, our data suggest that the rhinos may ejaculate with higher (40C°–45°C) than normal rhino body temperatures (37C°–39°C). However, due to the many variables involved, further trials are needed to determine the optimum temperature.

Stimulation of perineal nerves can result in seminal emission, erection, and ejaculation [Ball, 1976]. Rectal massage may have caused seminal emission in this rhino, but the stimulation was not sufficient to cause ejaculation. Although it is not

an effective method for semen collection in most species, it is used as a priming technique for other collection methods [Carpenter et al., 1982]. Rectal massage has been combined with penile massage [Jainudeen et al., 1971] (John Bradford, personal communication) or with an AV [Heath et al., 1983] for semen collection in elephants. Ball [1978] recommended massage in bulls before electroejaculation to improve collection results; however, it may also increase the cellular debris and accessory gland contributions [Austin et al., 1961; Salisbury et al., 1961]. In our investigation, using rectal massage prior to penile massage, higher sperm concentrations were obtained than when penile massage was used alone. Unlike in the bull, increases in volume and debris were not noted.

Although parameters for electrical stimulation by rectal probe are not available for the rhinoceros, parameters for electrical stimulation of the penis were reported to be 22 V and 200 mA for one black rhino [Platz et al., 1979]. However, this animal was euthantized after electroejaculation. No report of tissue damage was available. Parameters for rectal stimulation of bulls ranged from 4–6 V and 150–200 mA [Salisbury et al., 1961] and 10–30 V for elephants [Wildt et al., 1983]. Higher stimulation levels have been reported in rectal probe electroejaculation of anesthetized rhinos and unanesthetized bulls than were used in our investigation. Application of optimum voltages on stationary animals usually requires that animals be restrained for electroejaculation. Boars and stallions have been anesthetized for electroejaculation [Clark, 1976; Basurto-Kuba and Evans, 1981; Stover et al., 1981]. Elephants have also been anesthetized for electroejaculation [Rueedi and Kuepfer, 1981; Wildt et al., 1983]. Elephants have not been physically restrained for electroejaculation. Trained domestic bulls can be successfully electroejaculated while restrained in stanchions. Higher voltages successfully produced semen in later studies on the restrained Greater one-horned rhino; however, stimulation was specifically applied to rectal areas by hand-held ring electrodes [Schaffer and Beehler, 1988]. This rhino had previously been anesthetized and unsuccessfully collected by rectal probe electroejaculation. Higher voltages could not be used on this study's rhino because he could not be restrained.

Electrical stimulation followed subsequently by penile massage yielded seminal parameters that were comparable to ejaculates collected by electroejaculation from sedated rhinoceroses. The ejaculate volumes (0.1–2.0 ml) obtained by combining electroejaculation with penile massage were similar to earlier reports of 0.2–8.5 ml [Howard et al., 1983], but much lower than 18–48 ml for penile stimulation [Platz et al., 1979]. However, sperm concentrations of electroejaculation combined with penile massage were comparable to the sperm concentrations of electroejaculation in both of these studies. Low-voltage stimulation was apparently sufficient for seminal emission. The brief periods of penile massage were effective in obtaining some fluid. Longer electrical stimulation periods and/or longer periods of penile massage may result in larger volumes of ejaculate.

CONCLUSIONS

Various semen collection methods were compared in an unrestrained, ambulatory Greater one-horned rhinoceros. Of the various types of stimulatory techniques, rectal stimulation followed by penile massage yielded ejaculates of improved semen quality. It is concluded that the combination of stimulatory methods such as the

above, with proper training of the animal, may result in ejaculates of acceptable semen quality for insemination or preservation procedures.

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