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ULTRASONOGRAPHIC MONITORING OF ARTIFICIALLY STIMULATED EJACULATION IN THREE RHINOCEROS SPECIES (*CERATOTHERIUM SIMUM, DICEROS BICORNIS, RHINOCEROS UNICORNUS*)

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Abstract: Manual massage of the penis and rectal electroejaculation methods have been minimally effective for collecting semen from the rhinoceros. These two methods for stimulating ejaculation were evaluated by rectal ultrasonography. One individual each of three rhinoceros species (*Ceratotherium simum simum, Rhinoceros unicornis* and *Diceros bicornis*) was stimulated by manual massage of the penis and two black rhinoceroses (*Diceros bicornis*) were electroejaculated. On ultrasonography, neither manual massage nor rectal electroejaculation affected the accessory glands. The pelvic urethra remained empty during manual massage of unconditioned animals; however, this area was filled before commencement of massage of conditioned animals. The pelvic urethra also filled during electroejaculation. Semen accumulated in the pelvic urethra during pauses between electrical stimulations and was moved distally into the penile urethra by rectal and penile massage. The volumes of seminal fluid recovered from electroejaculation in this study exceeded previously reported attempts. This study demonstrated the potential of transrectal imaging for improving the recovery of semen by these methods.

Key words: Rhinoceros, ultrasonography, electroejaculation, seminal emission, *Ceratotherium simum simum, Rhinoceros unicornis, Diceros bicornis.*

INTRODUCTION

Ejaculation by the rhinoceros differs from that by other species. Rhinoceros intromission is prolonged compared to that of other mammals. In addition, procedures used to collect semen from other species often fail in the rhinoceros. Manual massage is successfully used for collecting semen in the dog, stallion, human, and a few wild animals, but requires extensive training of the rhinoceros.[21] Rectal ejaculation has been associated with adverse reactions in anesthetized rhinoceroses (M. Briggs, pers. comm.).

Various techniques have been used to monitor both normal and artificially stimulated ejaculation in other species. Rectal ultrasonography during ejaculation by the stallion demonstrated the emptying of accessory glands.[24] Human ultrasonography has revealed the alternating association of seminal emission and ejaculation.[6] Radiography of electroejaculated sheep suggested that some semen may be retroejaculated into the bladder.[7] We have described the ultrasonography of rhinoceros reproductive structures[16,22] and used these procedures in the present study to investigate the stimulatory effects of two semen collection techniques on the urogenital structures of the rhinoceros.

MATERIALS AND METHODS

Rectal ultrasonography was performed with a handheld 5.0-MHz linear probe attached to an Aloka 500 scanner (Corometrics, Wallingford, Connecticut 06492, USA), and recorded (Hi-8 video recorder, Sony EV-C100, Sony Corp., Tokyo, Japan). The accessory glands in each animal were examined for size and echogenicity. The pelvis was examined from the bladder neck to the external pelvic brim. The structure of the pelvic urethra was identified postmortem by filling unfixed reproductive tracts with water. The effects of stimulation on the urethras of live animals were examined before and during procedures.

Two reproductive tracts from postmortem animals were analyzed: a white rhinoceros (*Ceratotherium simum simum,* studbook no. 40, 28 yr old, had sired offspring), and a greater one-horned Asian rhinoceros (*Rhinoceros unicornis*, studbook no. 14, 31 yr old, had sired one aborted fetus). Five healthy, adult male rhinoceroses were stimulated to produce ejaculation: three black rhinoceroses (*Diceros bicornis*, rhino 1, studbook no. 301, 14 yr old, had sired 1 offspring; and rhinos 2, studbook no. 409, 42 yr old, and 3, studbook no. 68, 40 yr old, neither of which had sired any offspring), one white rhino (rhino 4, studbook no. 697, 27 yr old, had

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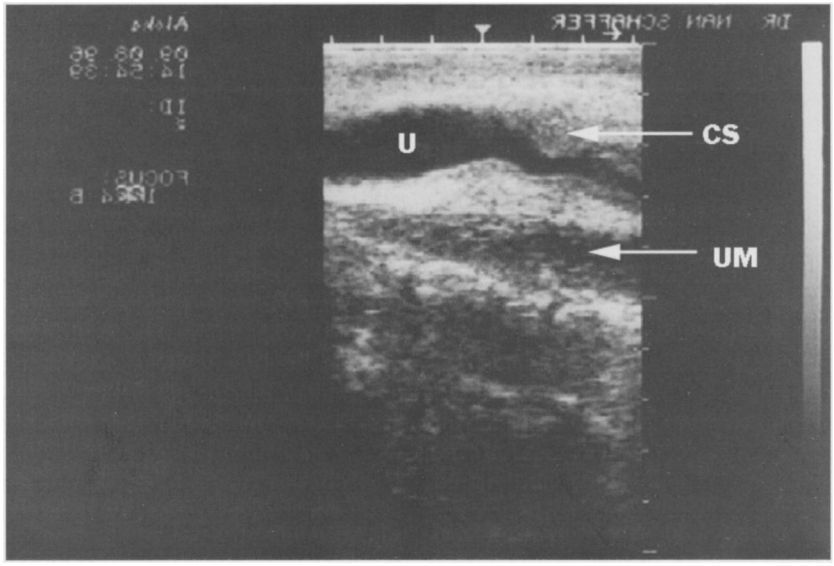

Figure 1. Postmortem sagittal longitudinal ultrasonographic image of water in pelvic urethra (U) of a greater one-horned Asian rhinoceros; the colliculus seminalis (CS) is the dorsal bulge into the lumen. Cranial is to the right. UM = urethral muscle.

sired no offspring), and one greater one-horned Asian rhino (rhino 5, studbook no. 14, 31 yr old, had sired one aborted fetus).

Rhinoceroses 1 and 2 were anesthetized with 1 mg i.m. of carfentanil (Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado 80524, USA) for rectal electroejaculation. A 10-cm-diameter by 21-cm-long rectal probe plated with two thin, 6 × 12-cm flat electrodes was placed just inside the anus. Electrical stimulations (Lane P-IIIZ, Lane Manufacturing Co., Denver, Colorado 80222, USA) were performed in 2-sec pulses repeated 10 times each at 4, 5, 6, 7, and 8 V successively and, after a 1- to 2-min rest, at 6, 7, 8, and 9 V. The rectum and penis of rhinoceros 1 was massaged at the termination of electrical stimulation. Rhinoceros 2 was massaged throughout stimulation.

Rhinoceroses 3, 4, and 5 were trained to stand quietly with food enticement for rectal ultrasonography with or without restraint in a chute. Rhino 3 was also conditioned to penile massage and regularly produced semen samples. Rhino 4 had no prior penile massage conditioning, and rhino 5 was initially examined before a semen sample had been collected and again after the animal was producing semen samples approximately every other week (unconditioned = 5a, conditioned = 5b). The back legs of rhinos 3, 4, and 5 were stroked until the penis was let down from the sheath, and the penis was then massaged directly.[17]

Volume and sperm concentration of ejaculates from the stimulatory procedures were analyzed.[26]

RESULTS

Ultrasonography of the postmortem water-filled reproductive tracts demonstrated a flattened bulbous expansion of the pelvic urethra between the bulbourethral glands and the prostate in both the white (8 × 5 × 2 cm) and greater one-horned Asian rhinoceroses (5 × 5 × 2 cm) (Fig. 1). Constriction of the bladder neck and prostatic urethra was the cranial border of the expansion, and constriction of the urethra at the bulbourethral glands was the caudal border. A dorsal–cranial bulge into the pelvic urethra just caudal to the cranial constriction was the colliculus seminalis (Fig. 2). Ultrasonography of postmortem morphology was similar to the images from the living animals (Figs. 3–5) except for the occurrence, in the live animals, of several small hypoechogenic lines in the colliculus seminalis. These lines were apparent in both resting (Fig. 3) and stimulated animals (Fig. 4). Neither these ducts nor accessory glands grossly changed in their size or echogenicity during stimulatory methods.

Ultrasonography demonstrated that no fluid occurred in the pelvic urethra before electroejaculation began. Electrical stimulation caused rear leg extension and penile retraction. In one animal (rhino 1), the pelvic urethra filled in approximately 4 min after electrical stimulation was terminated (Fig.

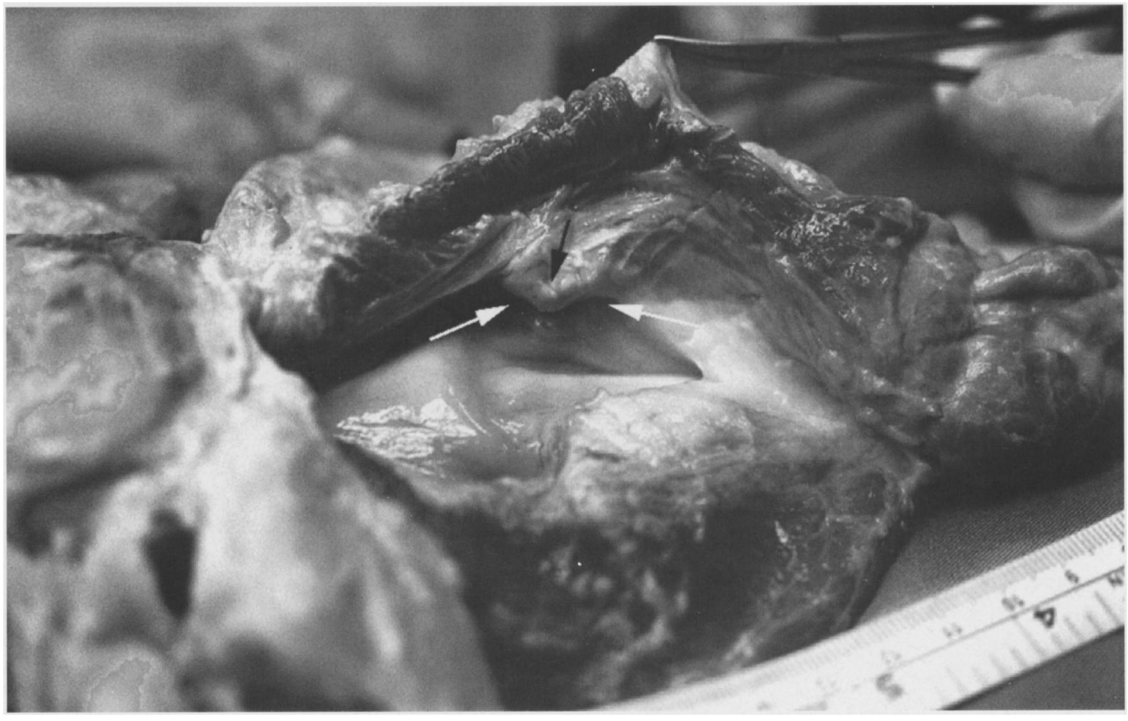

Figure 2. Sagittal longitudinal dissection of the pelvic urethra of a greater one-horned Asian rhinoceros; the colliculus seminalis (arrow) bulges from the dorsal urethral surface. Cranial is to the right.

4). In animal 2, some filling occurred during the 1-min rest period between stimulation series, but maximal filling occurred at termination. In both animals, the urethra filled to a maximum depth of 2 cm, with lateral extensions of the fill area (Fig. 4). This expansion was similar to the postmortem images.

Massage during electroejaculation kept the penis out of its sheath and appeared ultrasonographically to cause clonic contractions of pelvic musculature

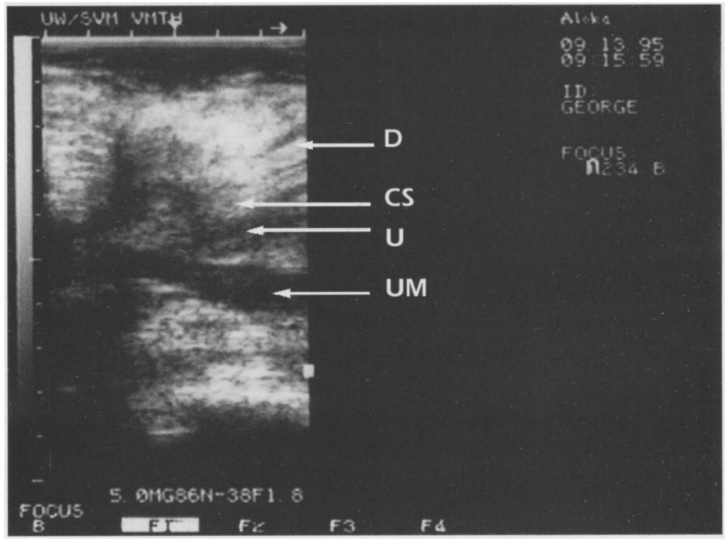

Figure 3. Sagittal longitudinal ultrasonographic image of empty pelvic urethra (U) of a black rhinoceros. Cranial is to the right. CS = colliculus seminalis, D = ducts, UM = urethral muscle.

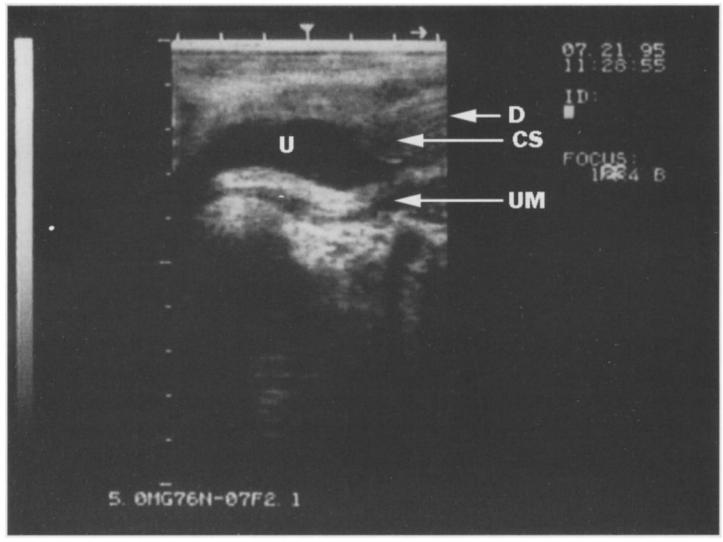

Figure 4. Sagittal longitudinal ultrasonographic image of semen in the pelvic urethra (U) during electroejaculation of a black rhinoceros. Cranial is to the right. CS = colliculus seminalis, D = ducts, UM = urethral muscle.

and peristalsis of the pelvic urethra. The caudal urethral constriction opened allowing semen to flow into the penile urethra while the cranial constriction remained closed. Initially, when the semen moved into the penile urethra a slight delay occurred before expulsion. Downward pressure through the rectum onto the pelvic urethra and movement of the hand from the prostate caudally appeared to mechanically move the remaining fluid into the penile urethra. Once the urethra emptied, the muscle contractions stopped quickly and seminal emission did not appear to continue even with further stimulation of the penis. Volume and sperm concentration of the ejaculates are presented in Table 1.

For penile massage collections, stroking of the back legs stimulated the penis to drop from the sheath. Ultrasonography prior to massage revealed fluid in the pelvic urethra in the two conditioned rhinoceroses (rhino 3 and sample 5b) (Fig. 5), but not in the pelvic urethras of the unconditioned animals (rhino 4 and sample 5a). In the conditioned rhinoceroses, the filled areas of the urethra resem-

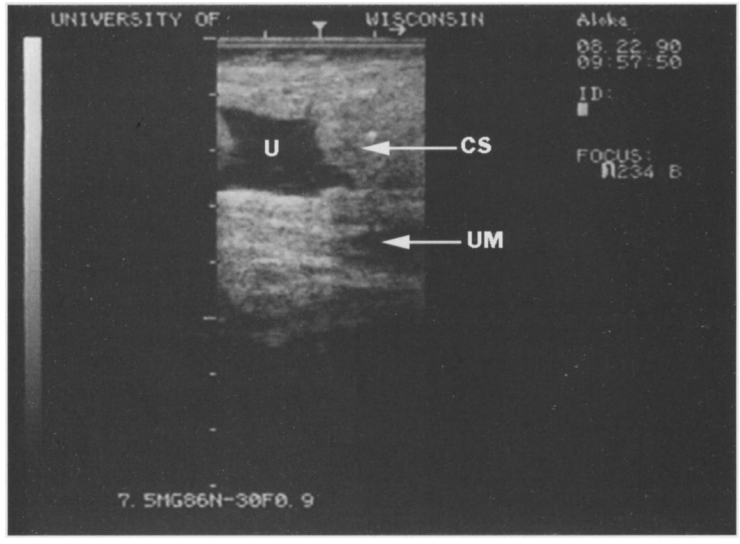

Figure 5. Sagittal longitudinal ultrasonographic image of semen in the pelvic urethra (U) during penile massage of a black rhinoceros. Cranial is to the right. CS = colliculus seminalis, UM = urethral muscle.

Table 1. Seminal parameters of electroejaculated black rhinoceroses.

Sample	Studbook no.	Age (yr)	Volume (ml)	Sperm concentration (cells/ml)
1	301	14	15	200×10^6
2	409	42	20	1.0×10^6

bled and responded similarly to those in the electroejaculated animals. In the two conditioned rhinoceroses, muscle contractions continued until the urethra emptied. In the two unconditioned rhinoceroses, only a few contractions occurred with initial contact with the penis. Fluid with sperm was collected from three of the animals (Table 2). In rhino 4, fluid was not evident in the urethra, but a small volume was collected during massage. In rhino 5 (conditioned sample 5b), the urethra was filled, but emptied quickly with contact with the penis. The urethra did not refill upon further massage. In rhino 3, the urethra remained filled and the flow of seminal fluid continued (Fig. 5). Massage of this animal was terminated when the cloudy fluid being collected began to clear, as this change had previously indicated a drop in sperm count. When massage was terminated in this animal, semen slowly drained from the pelvic urethra. Whether this semen flowed into the penile urethra or bladder was not apparent on ultrasonography. Semen did not subsequently flow from the penis. Rectal massage was not pursued with any of the animals given penile massage.

DISCUSSION

Ultrasonography was useful for monitoring the effects of stimulation procedures on pelvic urethral morphology and seminal flow in the rhinoceros. These rhinoceroses exhibited the stages of ejaculation common to most mammals: seminal emission and ejaculation. For most species, seminal emission is the issuance of fluids from the vas deferens and accessory glands into the urethra, and ejaculation is the propulsion of fluid from the pelvic urethra by contraction of perineal musculature.

Ejaculation of seminal fluid in other species resulted in changes in structure and echogenicity of the vesicular and ampulla glands.[11,24] However, no such changes occurred in these rhinoceroses. The accessory glands of these rhinoceroses appeared to be normal.[16] Ampullae have not been evident anatomically or histologically in the white, black, or greater one-horned Asian rhinoceros (G. Foley, pers. comm.). The vesicular gland in the human and stallion reduces in size after ejaculation. In the stallion, the saclike morphologic shape of the vesicular gland demonstrated an obvious change in shape after ejaculation. However, the rhinoceros vesicular gland compares more closely to the multisacculated structure of that in the bull, and changes in it may not be obvious.

Table 2. Seminal parameters of manually stimulated rhinoceroses.

Species[a]	Sample reference[b]	Studbook no.	Age (yr)	Volume (ml)	Sperm concentration (cells/ml)
B	3	68	40	62.2	15.0×10^6
W	4	697	27	0.7	10.0×10^6
GOHA	5a	14	31	0	0
	5b	14	42	8.0	$13{,}900 \times 10^6$

[a] B = black rhinoceroses (*Diceros bicornis*); W = white rhinoceros (*Ceratotherium simum simum*); GOHA = greater one-horned Asian rhinoceros (*Rhinoceros unicornis*).

[b] 5a = unconditioned; 5b = conditioned.

Significant changes in size of the glands might be evident if measurements are made. Measurements were difficult with the linear 5.0-MHz probe because the lengths of glands were not represented on the scanner monitor. Measurements of these large glands would be facilitated by the larger image field capacity of convex probes. Also, the artificial stimulations of this study probably did not simulate normal ejaculations in these rhinoceroses. These rhinoceroses could be under- or overstimulated with subsequent effects on the accessory glands. Changes in accessory gland size might be evident before and after breeding.

On ultrasonography, seminal emission filled a flattened bulbous expansion of the pelvic urethra. This pouch was similar to the area in the water-filled postmortem specimens. It was shorter and closer to the ischial arch in the greater one-horned Asian rhinoceros as compared to the African species. This was similar to previous examinations of postmortem reproductive tracts.[16] This pouch expanded laterally as well as longitudinally and was bordered by cranial and caudal constrictions. In the human and dog, this area was referred to as a "pressure chamber."[10–12] Filling this chamber optimized contraction efficiency of muscle at the time of ejaculation. This chamber in the rhinoceros may provide volume and force to the propulsion of semen through the extensive length of the penis and through the complicated cervix of the female. This expanded area occurred in these rhinoceroses caudal to the colliculus seminalis. On postmortem examination, the colliculus encompassed the openings

into the urethra of the ejaculatory ducts of the prostate, vesicular gland, and vas deferens. These ducts appeared as hypoechogenic lines in all of the animals except the postmortem specimens. These lines helped to locate the area of the urethra that would be subsequently filled by emission.

Semen emitted into the pelvic urethra of the rhinoceroses in this study appeared to persist in the urethra for a longer period of time than reported in other species.[6,24] In both electroejaculated and manually massaged rhinoceroses, filling and emptying of the urethra took several minutes. In normal ejaculation by humans, the period is measured in seconds.[6] No accumulation was reported in the urethra of the stallion, but a brief accumulation during ejaculation may have been missed.[24] In the rhinoceros, insufficient stimulation by these methods may lengthen the process. Collection of semen in stallions was prolonged by the manual massage method.[2] However, in the ram, this area filled and emptied quickly during electroejaculation.[7] Prolonged filling may be normal for the rhinoceros. Sheep breed very quickly, and consequently semen has a brief period in the pelvic urethra[23]; in contrast, rhinoceroses have a very protracted intromission and semen may stay longer in the urethra.

As long as semen was in the urethra of both massaged and electroejaculated rhinoceroses, penile massage stimulated clonic contractions of perineal musculature. This resulted in expulsion of fluid from the urethra, but filling did not reoccur. This suggested that penile massage may not be effective unless it is preceded by a technique that produces seminal emission. In this study, the electroejaculation and the conditioning of animals produced seminal emission before contact with the penis. Arousal may also produce seminal emission in the rhinoceros and thus be useful before penile massage. Arousal has improved recovery of semen by manual massage from other species and has been recommended for the rhinoceros.[2,25] The ability of techniques to produce seminal emission into the pelvic urethra could be investigated with ultrasonography.

A few drops of fluid were collected from unconditioned rhino 4 in this study, although fluid was not evident in the pelvic urethra. Other unconditioned rhinoceroses have produced small volumes from first attempts to collect semen from penile massage.[19] The occurrence of sperm in these fluids may be the result of passive emission. In sheep, small accumulations of sperm in the urethra and urine resulted from passive emission.[9] Passive elimination may occur in the rhinoceros, as sperm has been collected in their urine.[21] Also, nocturnal accumulation of semen was indicated when higher quality samples were collected in the morning before urination from manually massaged animals (N. Schaffer, pers. observ.).

Ultrasonography indicated that manipulation of the reproductive tract may have improved the recovery of semen from electroejaculation. Samples collected by electroejaculation from these rhinoceroses were significantly greater in volume than previously published reports (less than 9 ml)[8] or unpublished reports (S. Seager, T. Gross, E. Miller, pers. comm.). Previous electroejaculation in rhino 2 resulted in only a few drops of semen with a few sperm (J. Gunther, pers. comm.). None of these researchers reported monitoring ejaculation or manipulation of the penis or rectum.

Monitoring of semen in the pelvic urethra during electroejaculation may have resulted in the retrieval of semen from these rhinoceroses at the appropriate time. Similar to the ram, the pelvic urethra of these rhinoceroses filled during pauses between electrical stimulations.[7] In these rhinoceroses, several minutes were required to fill the urethra. The constraints of anesthesia of the rhinoceros typically result in rapid electroejaculation attempts with short rest periods and abrupt termination. Semen samples may be missed if they appear after stimulation and collection efforts have ceased. Also, longer periods of rest may be necessary to induce seminal emission into the pelvic urethra. However, a prolonged rest period may result in loss of semen, as semen remaining passively in the pelvic urethra may flow into the bladder. Immediate removal of semen from the urethra may be critical in preventing such loss. Retroejaculation occurred in both normal and artificially stimulated ejaculation in other species. Concentrations of sperm have increased in the bladder after normal ejaculation in humans and dogs[3,6] and after artificial simulation in the cat, bull, ram, macaque, and gorilla.[4,5,7,13,18,20]

Massage of both the rectum and the penis improved recovery of semen during electroejaculation. Rectal massage of reproductive organs has been suggested before stimulation by other methods in the bull and rhinoceros.[1,17] In this study, semen was mechanically removed from the pelvic urethra after electroejaculation. Such stripping of the pelvic urethra has improved the recovery of semen following electroejaculation in primates.[15] Penile stimulation was employed in one study of electroejaculation that demonstrated similar seminal quality to this study.[14] The rhinoceros penis can be tightly retracted during electroejaculation, and the sigmoid curve may inhibit the release of semen. Encouraging the penis to straighten through massage may

improve the release of semen from the pelvic urethra. Further studies utilizing ultrasonography for monitoring the effects of these techniques on ejaculation may produce more effective semen collection methodology.

CONCLUSION

Ultrasonography was useful for monitoring the effects of various stimulation techniques on the process of ejaculation in the rhinoceros. In this study, ultrasonography demonstrated that different techniques had varying effects on the occurrence of seminal emission and the fate of semen in the posterior urethra. This information was used to recover larger volumes of semen from these rhinoceroses than reported previously. Ultrasonography may be useful for monitoring semen collection techniques in other species.

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