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OVERVIEW OF PROCEDURES AND RESULTS OF SEMEN COLLECTION FROM AMBULATORY RHINOCEROSES

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INTRODUCTION

Artificial manipulation of the reproduction in rhinoceroses is essential to accelerate the propagation of these animals in captivity. Semen collection is a prerequisite of these manipulative procedures. Although many collection methods are available for use in domestic animals, they have not been applied extensively to the rhinoceros. Rhinoceroses have been anesthetized for electroejaculation but only limited attempts can be made without risk to the animal. Multiple samples can be obtained with minimum or no risk to the animal by semen collection from unanesthetized rhinoceroses. This summary reports on the procedures for semen collection from ambulatory rhinoceroses and the processing of this semen.

COLLECTION PROCEDURES

RESTRAINT

Semen can be collected successfully from unanesthetized rhinoceroses. These rhinoceroses have been unrestrained or restrained during collection. All of the collection methods described in this report were developed and successfully used on unrestrained animals.

Several years ago, development of techniques to collect semen from unrestrained rhinoceroses was undertaken (Young, 1976; Peachey, 1980). Initial work was carried out on a black rhinoceros (*Diceros bicornis*) at the Columbus Zoo and, more recently, on an Indian rhinoceros (*Rhinoceros unicornis*) at the Milwaukee County Zoo. Although unrestrained, these animals were amiable and tolerant. Both of these rhinoceroses were receptive to procedures done within their exhibit. Over one hundred seminal fluid samples have been collected and analyzed on each of these rhinoceroses. The semen collection methods reported in this paper were initially developed on the Milwaukee County Zoo Indian rhinoceros. This author's experience with this animal has lead to successful application of techniques to other animals. Some of these rhinoceroses, however, have required restraint measures.

Equipment manipulations and personnel protection is facilitated by restraining rhinoceroses in chutes. A white rhinoceros (*Ceratotherium simum*) at the Houston Zoo, a black rhinoceros at the Henry Vilas Park Zoo in Wisconsin and an Indian rhinoceros at the Oklahoma City Zoo, have all been restrained in chutes, but these restraint systems are not optimum. During procedures at these zoos, observations were made on the effectiveness of current chute systems in restraining rhinoceroses. Some assessments were determined. Careful consideration of the following factors will improve the function of future systems.

Chutes should be strong enough to bear the weight plus the force of the rhinoceros. Six- to eight-inch pipes embedded in the ground have restricted these animals. Four-inch pipe has been used but it must be reenforced and firmly anchored.

Some of these structures were built with dead-ends, which some rhinoceroses have been reluctant to enter. Rhinoceroses become more rapidly accustomed to chutes that they pass through every day. These chutes are more convenient for personnel. Rhinoceroses loaded in chutes have become accustomed to the close quarters. However, aprehension in some individuals has caused them to either mount the chute or lie down. These movements can cause injury to the rhinoceros or personnel. To prevent mounting, the chute should be higher than the length of the animal and lack places to leverage the front foot or head. High vertical bars have best resolved this problem. Vertical bars also prevent personnel from becoming wedged if the animal sits down. However both vertical bars and horizontal bars will trap personnel, if movement of the animal is extensive within the chute. Tight-fitting chutes have stabilized rhinoceroses and allowed more technical manipulations with the animal. Procedures are also facilitated if the lower part of the chute on either side of the rear of the animal can be opened. This allows better maneuverability of different equipment. Different equipment and a greater variety of procedures are applicable on rhinoceroses restrained in chutes.

COLLECTION METHODS

The various methods that have been developed for semen collection from domestic animals are applicable to the rhinoceros (Pickett et al., 1987; Ball et al., 1983; Kenny et al., 1983; Hurtgen, 1984). These methods were developed in domestic animals for acquisition of semen from non-breeding animals. In addition, semen is obtained by these techniques for fertility analysis and preservation. These methods have been successful in the collection of semen for analysis in nine out of the ten unanesthetized rhinoceroses (Table 1). In one of these Indian rhinoceroses, two previous attempts to collect semen by electroejaculation were unsuccessful. These attempts were made while the animal was under anesthesia. These semen collection procedures include penile massage, artificial vaginas, rectal massage and electroejaculation. All of these methods were performed without sedating the animal.

Penile massage is a method not generally used in domestic animals, except in boars (Basurto-Kuba and Evans, 1981) and dogs. It has been successfully applied to the rhinoceros. Direct massage of the penis begins with slow stroking that proceedes to vigorous massaging by the operator until the rhinoceros ejaculates. Rhinoceroses are the most receptive to this procedure and therefore it has been the method most frequently used. It is also the easiest to apply. This method was immediately successful in two black rhinoceroses, however, in two white and two Indian rhinoceroses, protracted periods of training were required. For instance, ten months of training were required to obtain seminal fluids with significant sperm counts in one Indian rhinoceros (Table 3). However, with further conditioning with penile massage, this animal began to ejaculate fluids that contained sperm concentrations in the billions (Table 4). This method can be successful within a short period in high libido and cooperative rhinoceroses. In less receptive animals, the procedure will take more training, but the results can be highly productive.

Further stimulation of the penis can also be induced by artificial vaginas (AV). These are usually latex coverings filled with warm water. They provide warmth and pressure stimulation to the penis. AVs have been minimally successful in collection of semen from rhinoceroses. Although routinely used with domestic animals, their use is usually preceeded by teasing the animal to cause an erection (Asbury and

Hughes, 1964; Zemjanis, 1962). Teasing measures have not been attempted with the rhinoceros. Therefore, lack of sustained erections in the rhinoceros has made placement of AVs difficult. Furthermore, use of AVs caused ejaculation of only sperm-free fluids. AVs may prove to be useful with further training of rhinoceroses, in high libido animals or olifactory stimulated animals.

Rectal massage in the rhinoceros involves massaging the accessory glands just inside the anus. This same procedure is used in domestic animals (Salisbury et al., 1961). In domestic animals it has been ineffective when used alone, but the method is recommended in preparation for other procedures (Ball, 1976). Rectal massage alone was also ineffective in producing seminal fluids from the rhinoceroses of this report. However, when rectal massage was followed with penile massage, seminal fluids were acquired from one white and one Indian rhinoceros (Table 2). In addition, with this combination of methods, ejaculates with higher sperm concentrations were obtained from one Indian rhinoceros. These concentrations were significantly higher than penile massage alone (Table 3). Rectal massage may be useful in rhinoceroses before performing any semen collection procedure.

Electroejaculation is extensively used in beef bulls (Ball et al., 1983), but not in boars and horses unless they are under anesthesia (Clark, 1976; Stover et al., 1981). Rhinoceroses have been anesthesized for electroejaculation (Howard et al., 1983; Platz et al., 1979), but unanesthetized rhinoceroses have been successfully electroejaculated with both rectal probe and hand-held ring electrodes (Schaffer, 1988). As with rectal massage, this stimulation produced seminal emission into the posterior urethra. Semen was then retrieved only after penile stimulation. These fluids also had higher sperm counts than penile stimulation alone (Table 3). Electroejaculation has been minimally used in the rhinoceros and with further development and improvement, it may become an effective method in the unanesthetized rhinoceros.

Other methods that have been used to acquire semen from rhinoceroses are post-coitus collection from the female (Bryant, 1988) and first of stream urinary collection. Both of these methods have indicated the presence of sperm, but determinations about other semen parameters are limited.

COLLECTION RESULTS

SEMINAL ANALYSIS

Analysis of seminal fluids from rhinoceroses is given in Table 4. The broad ranges in this table reflect variation between individuals as well as between collection methods. Ejaculate volumes are high in rhinoceroses. The low volumes in white rhinoceroses have resulted from incomplete ejaculation in these animals. The highest sperm counts were from an Indian rhinoceros. However, these values resulted from sperm-rich samples resulting from the ejaculation of a highly conditioned animal. Sperm motilities were high in these rhinoceroses. These samples had very little accessory gland fluids in them and therefore were probably not representative of a normal ejaculate. Sperm abnormalities were high in these species. White and Indian rhinoceroses exhibited primarily sperm tail abnormalities while the black rhinoceros had a high percentage of more serious head abnormalities (Saacke and White, 1972; Dott, 1975). Many more semen samples need to be acquired and viability tested on some of these individuals before assessments about

the fertility of rhinoceroses can be determined.

CRYOPRESERVATION OF SEMEN

Semen has been cryopreserved from three species of rhinoceroses (Table 5). Both pellet and straw cryopreservation methods have been used in the rhinoceros.

Poor recovery of rhinoceros sperm from freezing is indicated by low post-thaw motilities. Some improvement in recovery is noted in the Indian rhinoceros, however only the sperm-rich fraction of this rhino's ejaculate was frozen. In the sperm-poor fraction, sperm were not successfully cryopreserved. This sperm-poor fraction had higher concentrations of accessory gland fluids. In the horse (Pickett et al., 1987) and boar (Zavos and Liptrap, 1987), separation of ejaculate fractions is recommended before semen is processed. Seminal fractions have not been identified in other rhinoceroses. Accessory gland fluid is indicated as one of the factors contributing to the difficulty in freezing rhinoceros semen.

EXTENDED LIFE MEDIUM

Due to the problems with cryopreservation, an extended life medium is being developed for use in rhinoceroses in cooperation with Dr. Barry Bavister at the University of Wisconsin in Madison. Samples retrieved from rhinoceroses are initially examined for viability and then mixed with the extended life medium. This medium holds the sperm at room temperature for overnight express shipping back to Madison. At the Madison labs, highly technical analyses of sperm are performed. Preliminary trials with transport of semen have been successful in the rhinoceros. Detailed analysis of rhinoceros semen will lead to significant assessments about fertility and lead to development of semen handling techniques for artificial insemination. Zoos that have access to rhinoceros semen are encouraged to participate in this program. A video of procedures and requirements is available from the Milwaukee County Zoo.

VIDEO

A video of semen collection and semen processing procedures is available from: Dr. Nan Schaffer, Milwaukee County Zoo, 10001 W. Bluemound Road, Milwaukee, Wisconsin 53226.

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Table 4. Ranges of Seminal Parameters of Ambulatory Rhinoceroses Collected by Various Methods

Species	Volume (ml)	Sperm		
		Concentration ($x10^6$/ml)	Motility (%)	Abnormality (%)
Black (n=3)	0.2-60	0.1-80	0-90	40-90
White (n=6)	0.2-8	2.0-300	0-80	20-86
Indian (n=2)	0.1-500	0.0-20,000	0-95	5-92

Table 5. Post-Thaw Motility of Cryopreserved Sperm from the Rhinoceros

Species	Method Cryopreservation	Post-Thaw Motility (%)
Black	Pellet[1]	20
Black	Straw[2]	20
White	Pellet	30
Indian	Pellet	70
	Straw	60

[1]Platz et al., 1979 [2]Peachey, 1980

Table 1. Number of Successful Semen Collections from Unanesthetized Rhinoceroses

Species	Number Attempted	Number Collected
Black	4	4
White	4	3
Indian	2	2

Table 2. Number of Ambulatory Rhinoceroses Successfully Collected by Different Methods*

Species	Penile Massage	Artificial Vagina	Rectal Massage	Electroejaculation
Black	3	1	0	1
White	6	1	1	0
Indian	2	1	1	2

*Animals were repeated for different procedures.

Table 3. Comparison of Semen Parameters Between Various Semen Collection Methods Used in an Indian Rhinoceros

Collection Methods	Semen Parameters	
	Volume (ml) mean range	SC ($x10^6$/ml) mean range
Penile Stimulation		
Penile massage (PM, n=4)	2.3 (0.2-3.0)	89.5 (2-208)
Rectal Stimulation		
Rectal massage (RM)	0 0	0 0
Electroejaculation (EE)	0 0	0 0
Combination of Methods		
RM + PM (n=4)	12.4 (5.0-20.0)	138.6 (18-352)
EE + PM (n=4)	0.7 (0.1-2.0)	186.4 (5-500)