REPRODUCTIVE PROCEDURES and RESTRAINT for RHINOCEROSES

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Understanding rhinoceros fertility can improve management of the species, particularly of critically limited populations. For example, if fertility of the animal is analyzed first, then effort and expense can be properly spent on treating and moving only fertile animals. Rhinoceros fertility can be determined by assessing the quality of semen of males and the cycling or pregnancy in females. For making these assessments in the rhino, new procedures for semen collection and ultrasound were developed and used to establish normal reproductive parameters. With these procedures and an understanding in reproductive physiology, assisted reproductive techniques such as artificial insemination can be performed to help maintain the genetic balance of small populations.

The increased inter-cooperation of zoos and the recent development of chutes for restraining rhinos for examination has significantly increased sample sizes and the relevance of studies. However, more extensive studies are needed to understand the basic reproductive physiology of the species. This summary proceeds from a new basic chute design for restraint to brief descriptions of fertility assessment techniques such as ultrasound and semen analysis and concludes with the preliminary use of assisted reproductive techniques such as semen cryopreservation. The information summarized in this paper was compiled from 10 years of personal observation and the efforts of many individuals and cooperating institutions. The rhino species examined were the black (*Diceros bicornis*), white (*Ceratotherium simum*) and greater one-horned Asian (*Rhinoceros unicornis*).

RESTRAINT CHUTES

Intensive medical and reproductive examinations of the rhino requires repetitive restraint. Since anesthesia is risky and of limited use for repetitive procedures, chutes were built to restrain these animals. Chute designs at different zoos demonstrate a variety of limitations. Following personal experience with eight chutes of various design, we suggest a design for efficient handling of animals. Although the following description covers major considerations, such variables as available space, animal size, and animal disposition must be individually addressed. Often, many variables are not apparent until the chute is in place. Therefore, management must be prepared to make modifications.

Since rhinos are similar to elephants in their response to a regular handling regime, daily examination of the rhino is recommended. Therefore, permanent pass-through, indoor chutes are the most convenient. Indoor chutes prevent inclement weather from interfering with studies. The chute should allow restraint of the animal when it is passing through the chute in either direction so the shifting routine of the animal is not interrupted (Fig. 1). Two vertical bars that push in from the sides of the chute to the shoulder of the rhino (Fig. 2) alleviate the excessive forward movement of the rhino when it lowers its head. Quick release of these shoulder bars often relieves agitated animals without having to release them completely. The width of the chute should limit side-to-side movement while still allowing the animal to comfortably lie down. Animals will be come wedged in tight fitting chutes if the sides can not be released.

High-walled chutes or bars over the top keep the animals from climbing or rearing up. Horizontal bars, in the chute's entry gates and sides, are hazardous for examiners when the animals lie down. Vertical bars on the sides can trap the operator's arms if the animal can move forward. If the animals forward and side-to-side mobility can be limited, vertical bars

on all sides are recommended. The distance between these bars along the sides of the chute should be great enough to prevent the animal's foot from becoming wedged if it rolls on its side in the chute. For examiner safety, this distance should be divided with removable vertical bars.

Since rhinos slam swinging doors, sliding or guillotine gates are the most effective (Fig. 3). A rectangular opening in these gates for performing palpation should not pin the arm of an examiner when the animal is shifting or becoming recumbent. The distance between the vertical sides of this rectangular opening must be wide enough for examiner safety while still limiting the space in which a rhino could squeeze through. Also, the horizontal bottom bar of this rectangle should be only a few inches from the ground since animals frequently become recumbent. Solid doors on the outside of these gates should be used to stop rhinos, since they will attempt to charge even small openings. Good lighting and electrical sources are useful.

This chute design successfully restrains the male and female white rhino for routine examination, even when they become highly agitated and fractious. Many of the specifications of this improved design were instituted at the Henry Vilas Zoo in Madison, Wisconsin. Ultrasound examination of the female revealed that she was non-cycling; etiology and treatment are being pursued. Semen collection techniques are being developed on the male.

FERTILITY

With a basic understanding of the physiology, anatomy and histology of the rhino, comparisons can be made with domestic animals which allows researchers to borrow standard techniques for fertility assessment. These techniques are used to establish normal parameters for rhinos from which abnormalities or pathologies can be determined. Techniques presented here include ultrasound examination of the female reproductive tract and semen collection and analysis from the male.

Ultrasound

Only recently has the normal structure of reproductive organs of the rhinoceros been described. Schaffer and Beehler (1990) provided gross postmortem descriptions and diagrams of both the male and female reproductive tracts in three species of rhinoceros. These reproductive structures were compared with in vivo ultrasound images. Godfrey, *et al.*, (1991) give descriptions of postmortem tracts in female African rhinos.

The male reproductive tract is characteristic of both the stallion and the bull. The accessory glands lie within the pelvic canal and can be imaged with a 5.0 MHz probe. The accessory glands include paired vesicular and bulbourethral glands and a prostate. The testicles in the rhinoceros are extra-abdominal and lie dorsolaterally to the penis in the same skin-fold.

The female anatomical structures compare to both the mare and cow. Distinguishing characteristics include a convoluted cervix with interdigitating folds that appear on ultrasound as dark and light swirls above the dark image of the bladder. A short bifurcated uterine body leads to a bicornate uterus. The uterus lies loosely on top of the intestines and courses cranially toward the kidneys. The females ovaries lie 70–100 cm from the vulva, therefore, ultrasound probes (principally 5.0 MHz) need to be attached to extensions so that operators can reach the ovaries to image them through the rectal wall.

The ovaries are covered with a thick *tunica albuginea* and consist of an outer cortex (*zona parenchymatosa*) and a central medulla (*zona vasculosa*) similar to those seen in ruminants (Ken Ilio, personal communication). The ovaries are ovals that are flat if quiescent or round during active folliculogenesis. Appearance of *corpus luteum* on surface of ovaries suggests ovulation occurs from the surface, rather than into a fossa as in the mare. A broad infindibulum with extensive fimbria that covers the entire ovary supports this supposition.

Ultrasound examination has allowed the determination of early pregnancy (Greg Adams, personal communication) and late pregnancy (Schaffer, personal observation) in a black rhino. Follicles and pregnancy corpus luteum have been identified on the ovary. Hormonal assays to monitor cycling of the female could be validated using ultrasound.

Pathological conditions that have been identified in rhinos with ultrasound include ovarian and endometrial tumors and cysts in non-cycling white and black rhinos. Ovarian bursal cysts also occur and must be differentiated from ovarian cysts and follicles. Leiomyofibromas (Montali, this volume), (Griner, 1983) and ovarian fibromas and cysts (George Foley, personal communication) have been identified in older animals at necropsy. Diagnoses and treatment resolutions may be determined with ultrasound examination which would expedite breeding success.

Semen Collection

Semen evaluation provides insight on the fertility status of the male. Ejaculates have been acquired from anesthetized animals with electroejaculation (Platz, *et al.*, 1979; Howard, *et al.*, 1983) and unanesthetized animals (Young, 1967; Spellmire and Booth, 1981; Schaffer and Beehler, 1988). The first fraction of the first urine voided in the morning by the rhino has often included sperm (Schaffer, personal observation). This provides evidence of sperm production, but not its viability since urine is detrimental to sperm quality. Various techniques of semen collection from a greater one-horned rhinoceros (such as penile massage, rectal massage, artificial vagina, and rectal probe electroejaculation) were detailed previously (Schaffer, *et al.*, 1990).

Most animals required protracted periods of training (1-3 years) before semen samples could be regularly collected. In a few animals semen was collected during initial attempts using penile massage. Collection of semen by penile massage was successful in three black, five white and one greater one-horned rhino. Rectal massage was successful once in this same greater one-horned rhino. One white, one black and two greater one-horned rhinos ejaculated into artificial vaginas but samples recovered had few to no sperm. Low voltage electroejaculation applied to two black, one white and two greater one-horned rhinos did not induce ejaculation. In one greater one-horned rhino, sperm concentrations increased in fluids from penile massage when this method was preceded by electroejaculation or rectal massage (Schaffer, *et al.*, 1990).

Penile massage is the easiest to apply and most widely used method, however, further modification and development of other methods will provide options for collecting semen and may improve the quality of semen samples.

Epididymal sperm is a viable source of gametes for assisted reproductive technologies. Epididymal sperm was obtained from five rhinoceroses (two greater one-horned, two white and one black species) at postmortem.

To obtain epididymal sperm an incision should be made along the lateral side of the base of the penile sheath to allow removal of the testicle surrounded by its parietal vaginal tunic. To remove semen from the epididymis different techniques were used for the African and Asian species, since the morphology of the epididymis of each was significantly different. The tubules in the African species were so small and densely packed that the tail had to be chopped and the tissue rinsed to recover semen. Only a few (1–2 ml) were recovered. In the greater one-horned rhino, the tail of the epididymis was round, firm and bulbous, protruding from the distal pole of the testicle. The lumen of the tubules were large, allowing semen (total 18.7 ml and 25.0 ml) to be easily removed by squeezing the tissue after puncturing the tail with a 16 gauge needle several times or slicing through it once. Sperm counts from the epididymis were: black rhino, 8.9×10^8/ml; white rhino, 1.3×10^7/ml and 12.3×10^8/ml; greater one-horned rhino, 6.5×10^{10}/ml and 85.3×10^8/ml.

Semen Analysis

Normal seminal parameters of rhinos were presented by Schaffer and Beehler (1988). These parameters have been updated in Table 1. Since many zoos are in the process of handling rhinos for semen collection, knowledge about the quality of initial semen samples is useful to evaluate the progress of training.

For comparison, initial semen samples were collected by manual massage of the penis from ambulatory rhinos, including three black, six white and two greater one-horned rhinos. Ejaculates were examined for color, consistency, volume, sperm count, sperm motility, pH, sperm morphology, cytology and sediment. Most of the first 1–30 samples in all three species were of poor quality and had common characteristics. These samples were primarily dribbles of thin, white, brown or yellow translucent to opaque fluid. The pH was 7.5–9.0; volumes were 0.1–5.0 ml. Although in a few of these individuals, the first attempt at collecting semen resulted in a sample with several million sperm, often, subsequent samples had few to no sperm. Several million per ml epithelial and white blood cells could occur particularly in smaller sample volumes. Sediment was extensive, consisting of calcium carbonate crystals and cells. The sediment was white and sometimes flocculent due to strands of mucus with trapped cells and crystals.

Later samples from individuals varied between species. In three of the white rhinos collected approximately every 1–2 weeks for 1–2 years, samples have not changed remarkably from initial quality mentioned above. Sperm count ranged from 0.01×10^6–32.0×10^6. After the first few (1–5) semen samples in one 39-year-old black rhino (Spellmire and Booth, 1981) and one greater one-horned rhino (Schaffer, *et al.*, 1989), the samples became clear to cloudy white and thicker in consistency, with total volumes of 0.5–150.0 ml. Sperm concentrations were 0.2×10^6/ml–5.0×10^9/ml, and motility 0-90%. These samples had a pH of 7.0–8.5, and contained few to no cells or crystals. These ejaculates were produced in drips or squirts of fluid. In the black rhino, sampling continued several times a year for 10 years. Samples remained consistent except for a recent lowering of sperm count.

In the greater one-horned rhino, after four years of semen collection approximately twice a month, samples (30th to 68th) in the fourth year became primarily white in color with volumes of 0.2–15.0 ml. Sperm concentrations were 1.8–20.2×10^9/ml, motilities were 0–50%, pH 6.5–8.0, and they contained very little sediment.

Sperm abnormalities in all the samples were similar within all three species. The abnormalities were, primarily: neck and mid-piece cytoplasmic droplets; bent, kinked or folded mid-pieces; coiled tails; and detached heads. Some variation in head size and abaxial tail attachment to the head was also seen.

Table 1. Ranges of Seminal Parameters of Ambulatory Rhinoceroses Collected by Penile Massage

SPECIES	VOLUME (ml)	Concentration (x 10^6/ml)	SPERM Motility (%)	Abnormality (%)
Black (n=3)	0.2–164.5	0.0–600.0	0–90	40.0–90.0
White (n=6)	0.2–8.0	0.0–32.0	0–20	20.0–86.0
Greater one-horned (n=1)	0.1–500.0	0.0–20,000.0	0–95	5.0–92.0

Although fertility/infertility cannot be differentiated among these samples, the above characteristics (volume, sperm concentration and motility and sediment), may be used for comparison as semen collection progresses.

ASSISTED REPRODUCTION

Although artificial insemination and embryo transfer are desirable for reproductive management of rhinos, further development of techniques is necessary. Equipment needs development for dealing with the complicated cervix and lengthy uterus of the female. Detailed identification of events in the female reproductive cycle will aid in the development of ovarian stimulation and estrus synchronization regimens, as well as timing for artificial insemination. In addition, effective collection and preservation of gametes needs to be developed. These obstacles can be overcome with dedicated commitment by zoological institutions. The resulting protocols would then be readily applicable to rhinos already acclimated to routine handling.

Cryopreservation

Cryopreservation of semen of the black rhinoceros using one type of extender resulted in successful recovery of post-thaw sperm motility (Platz, *et al.*, 1979; Spellmire and Booth, 1981). Only one type of extender was used in these studies and motility recovery was less than 50%. Different extenders may improve recovery of sperm viability.

Ten different milk and egg yolk extenders with 4% and 7% glycerol were applied to the split ejaculates of a 30-year-old greater one-horned rhinoceros. Only similar ejaculates "sperm-rich fractions" (1.5–13.8 x 10^9; 30–60% motility) which were low in accessory gland fluid were used to cryopreserve with the "pellet" method. Extenders were ranked according to the results of sperm motility, exclusion of eosin dye staining, and sperm responds to hypoosmotic swelling. Significant differences were not demonstrated between milk and egg extenders, however, viability improved in all extenders containing less glycerol (4%). This study suggested that this rhino's sperm was sensitive to glycerol. This may have also been a factor in low recovery of sperm of a black rhino which was frozen in 9% glycerol (Spellmire and Booth, 1981).

CONCLUSION

These procedures are applicable to zoological institutions and can be used to establish parameters that will help to qualify the fertility of the population. Regular monitoring of the health and reproductive status of the rhinoceros would significantly improve their management. This insight can be gained with minimum expense in cage modification and efforts by personnel. Once the fertility of the animal has been determined, reproductive management strategies can be developed to help preserve genetic variability in the rhino population.

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Videos on semen collection techniques and chutes or ultrasound techniques are available from Dr. Nan Schaffer, Northwestern University Medical School, Dept. of Obstetrics and Gynecology, Prentice 1504, 333 E. Superior Street, Chicago, IL 60611-3095, U.S.A., (312) 908-0902/FAX: (312) 908-6643.

REFERENCES

Godfrey R.W.; Pope C.E., Dresser B.L., Olsen J.H. Gross anatomy of the reproductive tract of female black (Diceros bicornis michali) and white rhinoceros (Ceratotherium simum simum). Zoo Biology 10:165-175, 1991

Griner, L.A. Pp 487 in Pathology of Zoo Animals. Zoological Society of San Diego, San Diego, California, 1983.

Howard J.G.; Bush M.; Collby L.; de Vos V.; Wildt D.E. Electroejaculation techniques and semen evaluation in rhinoceroses. American Association of Zoo Veterinarians Annual Conference Proceedings, Abstracts, Tampa, Florida. pp 74-75, 1983.

Platz C., Seager S.W.J.; Bush M. Collection and analysis of semen from a black rhinoceros. Journal of the American Veterinarian Medical Association 175:9, 1002-4, 1979.

Schaffer N.E.; Beehler B. Overview of procedures and results of semen collection from ambulatory rhinoceroses. American Association of Zoological Parks and Aquariums Annual Conference Proceedings, Milwaukee, Wisconsin. pp 273-279, 1988.

Schaffer N.; Beehler B. Preliminary studies on the anatomy and ultrasonic images of the reproductive structures of three species of rhinoceroses (*Rhinoceros unicornis, Diceros bicornis, Ceratotherium simum*), American Association of Zoo Veterinarians Proceedings, pp 215-220, 1990.

Schaffer N.E.; Beehler B.; Jeyendran R.S., Balke B. Methods of semen collection in an ambulatory Greater One-Horned rhinoceros (Rhinoceros unicornis). Zoo Biology 9:211-221, 1990.

Spellmire T.J., Booth B.H. Artificial breeding program for Diceros bicornis, the black rhinoceros. American Association of Zoological Parks and Aquariums Regional Conference Proceedings, Peoria, Illinois, pp 296-301, 1981.

Young E. Semen extraction by manipulation technique in the black rhinoceros. International Zoo Yearbook 7:166-167, 1967.

Fig. 1 RHINO CHUTE (side)

Rhinos foot should easily slip through 1 to 2 space, but not through space 3 to 4.

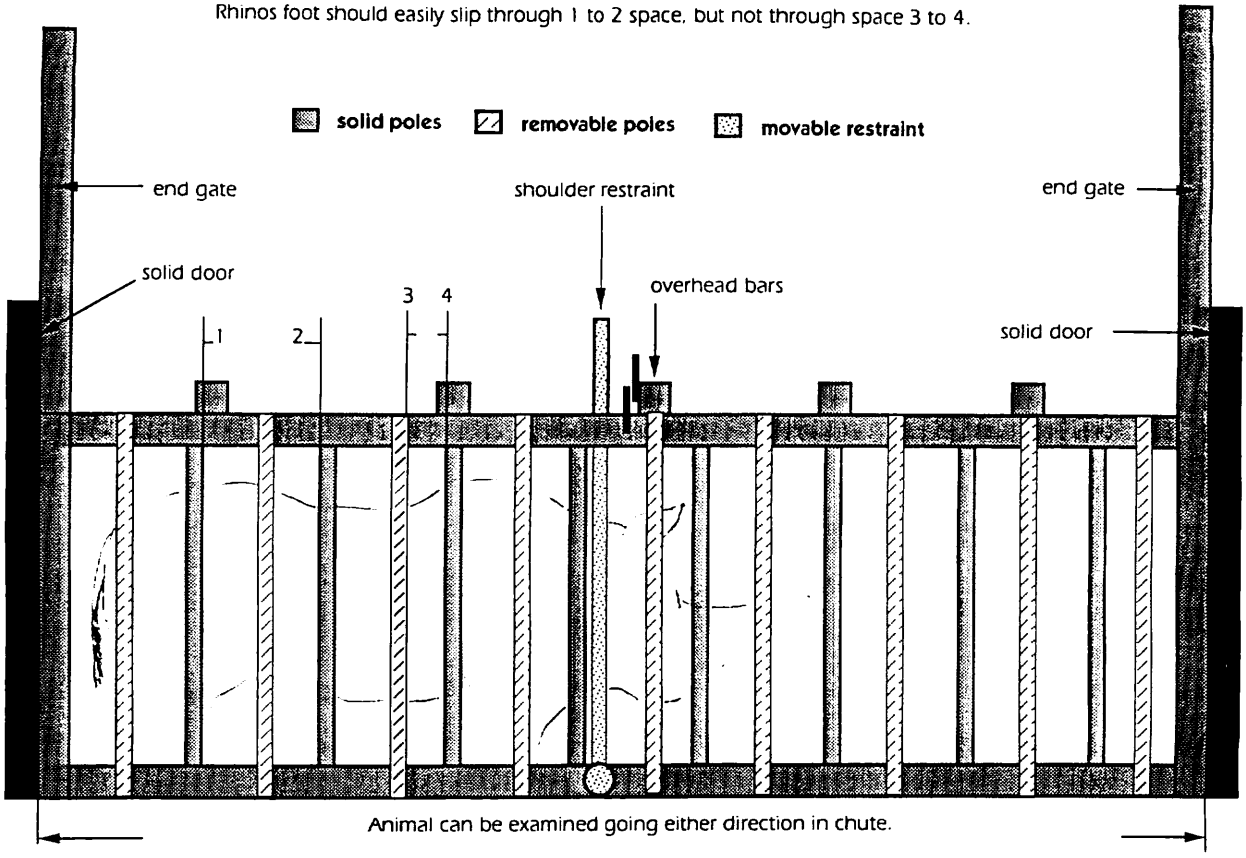

Fig. 2

SHOULDER RESTRAINT

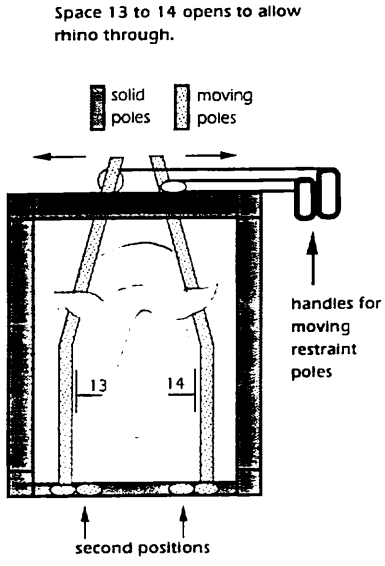

Fig. 3

END GATE

(front and back guillotine gate)