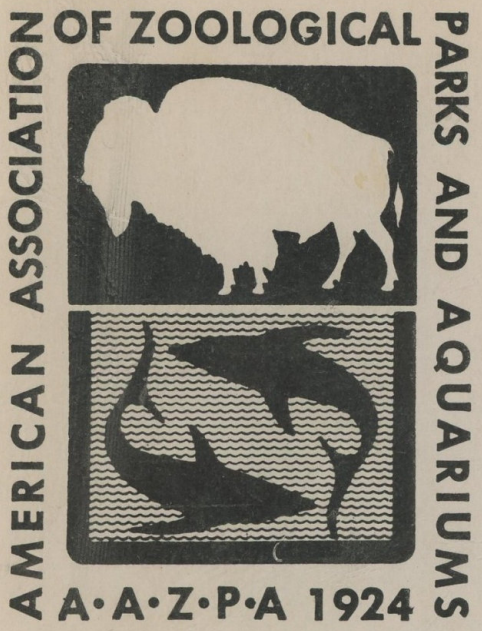

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ARTIFICIAL BREEDING PROGRAM FOR (DICEROS BICORNIS), THE BLACK RHINOCEROS

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Introduction

(Diceros bicornis), the Black rhinoceros, like all the Rhinocerotidae, is in grave danger of extinction. Although the Black rhinoceros is the most numerous of the rhinos at present, its numbers have been tragically reduced in the last ten years. Poaching for the rhino horn trade and accelerating habitat destruction have reduced some rhino populations by 90% and have scattered the remnant individuals so badly that it is unlikely that these populations are viable. The rhino horn trade is now eliminating 2,500 Black rhinos each year (Hillman, 1980). If the slaughter continues, Black rhinos will be extinct within this decade.

The position of the Black rhino in the zoo is not a particularly secure one. In 1978, 206 Black rhinos were reported in captivity. Of these, 75% were wild caught. In 1977, 11 viable Black rhinos were born in the world's zoos--a recruitment rate of 5% (International Zoo Yearbook, 1979). This recruitment rate is significantly lower than that observation in several stable wild populations in the 1960's and early 1970's (Goddard, 1967 and Mukinya, 1973). While it may be argued that mortality in zoos differs significantly from that in the wild and such recruitment comparisons are not valid, it is interesting to note that in 1968, 79% of the world's captive Black rhinos were wild caught. Recruitment in the captive population that year was slightly over 4% (International Zoo Yearbook, 1969, 1970). It is obvious that zoos are still consumers of the Black rhino and that the rhino cannot be considered self-sustaining in the zoo environment at present (Pinder and Barkham, 1978). Zoos are not making sufficient progress in the captive breeding of this endangered species and time is running out.

Initial Procedure

At the Columbus Zoo, we have kept a male Black rhino for 26 years. During that time, like many Black rhinos in captivity, he has not sired offspring. Two prospective mates were purchased over the years, but both died of systemic infections before introduction for mating occurred. The staff of the Columbus Zoo, aware that all efforts must be made to breed this endangered species, arranged a breeding loan with the Jacksonville Zoo, Jacksonville, Florida. We received their seven-year-old female Black rhino on 14 May 1978.

We anticipated a long introductory period as Black rhinos are a solitary, rather sedentary species, whose breeding behavior is sometimes marked by violent sparring and other aggressive interactions (Goddard, 1966). Accordingly, we modified our interior rhino pens by installing a system of posts and bars bewteen two transfer cages, allowing visual, olfactory, but very limited tactile contact. We began supervised, limited tactile contact on 24 July 1978. On 4 September, we permitted overnight contact through the bars of the transfer areas. Occasional sparring occurred, to which we were unable to assign any periodicity. By November, the rhinos seemed comfortable with each other, occasionally sleeping at the barrier in close contact. Winter weather prevented us from proceeding with the introduction in the outside yards.

After a winter of daily contact the pair were given access to the outside on 15 May 1979. They had individual access to the yard on alternate days for three weeks previously. The female was thought to be in cycle, based on visual and behavioral changes observed by the keepers. Violent aggressive behavior occurred on the part of both animals as soon as they entered the yard. Neither could gain a clear advantage for thirty minutes. Behavior included clubbing and goring with the anterior horn, lifting the opponent into the air by hooking the horn under a leg, and slamming the opponent into the moat wall with head and body thrusts. The only vocalizations were puffing moans and grunts. The male exhibited a bright body flush after approximately twenty-five minutes of this interaction. At thirty minutes, we began to separate the rhinos with the use of high pressure hoses, and accomplished the separation with no further injuries to the animals.

After consideration of the male rhino's age (29 at the time) the size of our yard (75' x 60') and the extreme nature of the aggression displayed, we realized that we had encountered a serious behavioral problem, of the sort that has often prevented the breeding of exotic mammals in captivity (Lang, 1972). We determined to examine all our options, but remained firmly committed to the goal of breeding this pair of Black rhinos.

Semen Collection

On 22 May 1979 we began to attempt semen collection from our male rhino. In most wild animal species semen is collected by stimulating ejaculation with an electric current. Other methods include training males to serve an artificial vagina, the use of drugs to stimulate ejaculation, massage of the intrapelvic organs, masturbation, and post mortem recovery of sperm from the reproductive tract (Goodwin, 1970 and Jones, 1971). Semen has been collected from a Black rhino under general anesthetic by electroejaculation (Platz, Seager, and Bush 1979).

We were unwilling to use drugs in semen collection with our male. The collection procedure would have to be repeated many times, since long-term semen storage techniques needed to be developed. In view of the male's age (although his overall condition is excellent) repeated administration of anesthetic agents did not seem wise.

Fortunately our male is accustomed to some degree of keeper contact, having had baths and scrubdowns for many years. We decided to attempt collection with some sort of artificial vagina (A.V.) device. We proceeded to train the male, by use of extensive food rewards, to stand steady while keepers massaged his flanks and penis. When the male rhino attained erection, a keeper washed the penis and sheath clean of dirt and sebaceous material. A second keeper stroked the rhino penis with a lubricated gloved hand. After several of these sessions, he wrapped a sheet of lubricated polyethylene around the penis and obtained a small (less than 5 ml.) semen sample after continuous gentle stroking. We collected the sample using a latex funnel mounted on a 15 ml. collecting tube enclosed in a 105° F water bath. This first sample, however, contained only a few live cells.

We experimented over a year's time with a variety of commercial and

in-house constructed A.V.'s. However, the processus glandis, or lateral lobes, of the Black rhino penis make the use of an A.V. impossible on a regular basis. We were not able to achieve consistent results and at times experienced some abrasion of the male's penis, even with a very gentle approach with a carefully designed rhino A.V.

Our complete confidence in our male rhino's conditioning encouraged us to attempt massage of the intrapelvic organs. It must be stressed that such a procedure cannot be done on an undrugged animal without a training program and a thorough knowledge of the animal's behavior. The technique consists of inserting a gloved lubricated arm into the rectum and, through the rectal wall, massaging the seminal vesicles and the ampullae of Henle. The procedure has been used in domestic stock for some years (Miller and Evans, 1943 and Goodwin, 1970). The ampullae contain accummulated sperm, being enlarged regions of the vas deferens. We concentrated our technique on these ampullae, rather than on the seminal vesicles, which secrete various seminal fluids.

Our first attempts at intrapelvic massage were not successful, but Dr. T.O. Ludwick, D.V.M., of the Ohio State University, and Robert Dahlhausen of the Ohio State University have achieved repeatable satisfactory results. (The massage technique is best learned through extensive experience with dairy cattle). We have found that the rectal wall of the Black rhino is quite thick, and if the rectum is distended with intestinal gas the wall is somewhat rigid, making determination of the intrapelvic organs difficult. Attempts to relieve the gas pressure by carrying a one meter long, 5mm. diameter semi-rigid plastic tube into the rectum were not successful. Gas is occasionally expelled during the start of the massage process, making the task somewhat easier. Of course, the rectum must be cleared of feces before the massaging process is begun.

The male Black rhino discharges semen approximately ten seconds after the ampullae are stroked through the rectal wall. Keepers collect the semen in the latex funnel and warmed collecting tube previously described. It is at times necessary to stroke the ventral surface of the penis to complete the discharge.

The semen collections range from 20ml. to 73ml. per session. It is possible to collect every week. Concentrations of sperm ranged from one million ml. to 600 million ml. Observed motility has reached 75% Cytoplasmic droplets have been observed on the tails of the sperm in some of our best samples, indicating active spermatogenesis in this aging male (droplets indicate immature sperm). Cold shock has been a factor in some of the collections, and we are further refining our techniques.

Select Sires, Inc., of Marysville, Ohio, has generously assisted us in evolving storage and processing techniques. Attempts at concentrating the samples by centrifuge have resulted in unacceptable damage to the sperm cells. We have not as of this writing discovered the ideal extender medium for Black rhino semen; but with certain samples we have achieved 40% post-thaw motility after freezing in 0.5ml. straws in liquid nitrogen. The extender used was an egg yolk-citrate/milk extender with 9% glycerol. Pre-freeze motility was 75%. This result compares favorably with reported pre- and post-freezing motility in the Black rhinoceros (Platz, Seager and Bush, 1979).

The results of the semen collection project encouraged us to proceed to an artificial insemination attempt with the Black rhinoceros.

Artificial Insemination Attempt

In December, 1978, keepers in the Pachyderm facility at the Columbus Zoo started a vaginal smear project as a practical method to increase our knowledge of the estrous cycle length and occurrence of estrus, or period of sexual receptivity, in our Black rhino (Diceros bicornis).

Starting with an intractable female Black rhino and an objective of obtaining a vaginal smear, we spent time accustoming our rhino to our presence and touch. With a scrub brush on a broom handle, a keeper would stand on a higher, adjacent level to the female and brush her back so she would stand still. In time, she became comfortable with keepers brushing and bathing her from the adjacent area. Then, while one keeper in an adjoining area would feed her produce, another keeper, talking constantly to acknowledge his presence, could enter the pen, rub the rhinoceros' back and insert a cotton swab into the vagina.

The technique for taking a smear is to spread the vulvar lips with one hand, insert the swab one to two inches into the vagina and remove it. The swab is then rolled across the microscope slide without pressure to deposit a thin layer of cells on the slide. The slide is air-dried and stored until staining. To fix the cells before staining, the slide is soaked in 100% ethanol for five minutes, then rinsed in tap water. Using Field Stain, solution A and B, the slide is dipped for six seconds in solution A, rinsed in tap water, dipped for twelve seconds in solution B, and given a final rinse in tap water. The slide is examined microscopically for the presence and percent of cornified epithelial cells, leukocytes, mucous and debris.

In the absence of any external signs of estrus, vaginal cytology proved to be a reliable monitor of the estrous cycle of our Black rhinoceros. Average cycle length was thirty-two days. During diestrus, round, non-cornified epithelial cells with distinct nuclei are seen, along with small quantities of mucous and debris. At the end of diestrus and beginning of proestrus, the epithelial cells become angular in shape, leukocytes begin to appear and debris and mucous increase. During proestrus, the epithelial cells begin to cornify. The nuclei of these cells are pyknotic, shrunken and darker, and karyolysis or breakup begins to occur, increasing the percent of debris seen. Leukocytes reach a peak during proestrus and decline towards estrus. During estrus, the epithelial cells are irregular in shape, their edges are folded over, and nuclei are absent. Debris, mucous and leukocytes are also present on the slide.

Having established regular collection of semen from our male Black rhino and the time of estrus of our female Black rhino, we decided to proceed with artificial insemination. On 20 August 1980, we determined from her vaginal cytology that our female was approaching estrus. We began to take vaginal smears twice daily. On 24 August 1980, the smears indicated that our female Black rhino was in estrus and preparations were made to artificially inseminate her. Dr. T.D. Ludwick rectally palpated the male and semen sample collected. Dr. Walter Threlfall of Ohio State University examined the semen sample and determined that there was sufficient quantity and adequate semen motility to proceed with insemination.

Dr. Harrison Gardner, Ohio State University and Columbus Zoo Staff Veterinarian, injected the female by pneumatic dart, with 3 mg. M99. With the female still standing but groggy, Dr. Threlfall entered the pen and began to cleanse the vulva. Shortly, the female laid down on her left side and remained recumbent until antidote was administered. Dr. Gardner monitored the female's heart rate while Dr. Threlfall inserted his sleeved arm into the vagina. A plastic tube attached to a syringe was inserted into the vagina and guided through the cervix. Fifty cc's of semen were injected into the uterus and the tube withdrawn. Dr. Threlfall then anally massaged the female's reproductive tract and palpated her ovaries, finding a large follicle on the right ovary. Six mg. M50-50 were injected into an ear vein of the female and within five minutes, she was standing. The entire procedure took twenty-seven minutes.

We resumed vaginal smears on 14 September 1980, to determine whether the insemination had resulted in pregnancy. At the time when the first estrus following the insemination would have been expected, a slight cornification of less than 10% was noted, increasing to 45% and 90% at the times of estrus on the second and third cycles. From the fact that our female Black rhino was cycling again, we concluded that she was not pregnant.

We remain committed to our artificial insemination program with our Black rhinos. Research on the optimum freezing and storage technique of rhino semen is proceeding. We have achieved satisfactory results with several extender mediums and freeze/thaw procedures. Our program for the detection of estrus is being expanded to include vaginal epithelium. We are also assessing environmental factors which may affect reproductive success in Black rhinos.

We have constructed a steel crate with removable bars within the rhino transfer area and have trained the female to stand in the crate for vaginal examination. With the female confined in the crate, we will be able to rectally palpate the ovaries for follicle development and ovulation. We will be able to inseminate several times over a period of days without the dangers accompanying general anesthesia. Repeated insemination with fresh or frozen semen should result in a pregnancy in 1981.

The members of the Black Rhino Project Team are (by affiliation):

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- D. Fredericks
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