CASE REPORT: SUCCESSFUL BIRTH AFTER INTENSIVE MANAGEMENT OF AN AGED BLACK RHINOCEROS *(Diceros bicornis)* WITH A HISTORY OF ABORTIONS

Dr. Nan Schaffer
Director Rhino Reproduction Program of the Milwaukee County Zoo
and Northwestern University
(ALS, Inc., 680 N. Lake Shore Drive, Suite 1025
Chicago, IL 60611)

Dr. William Bryant, Jo Anne Berry, Ronald Powell,
Chris Barnett, and Jennifer Joyce
Sedgwick County Zoo
5555 Zoo Boulevard
Wichita, KS 67212

Elizabeth Berkeley
Deaconess Research Institute

HISTORY

Following two live births, 15 years of non-breeding, and three recent spontaneous abortions, a wild-born (1962 est.) female black rhinoceros (SB#53) at the Sedgwick County Zoo delivered a healthy male calf on August 16, 1993. According to 1992 Black Rhinoceros Studbook records, she is the oldest female to have produced an offspring in captivity, and is one of the few animals to produce after repetitive abortions. Aggressive health and dietary management, along with supplemental progesterone is believed to have assisted in maintenance of this 470-day pregnancy. Since arriving in Wichita in 1988 on breeding loan from the Detroit Zoo, she had bred regularly to a male (SB#301) but aborted fetuses at 8 months (Oct. 1989), 10 months (April 1991), and 4 months (December 1991). Trauma to the female in one case, and placentitis in the last two cases were suspected of contributing to the abortions. After each abortion, the female was allowed several months of recovery and then usually became pregnant immediately after reintroduction to the male as determined by non-return to estrous and salivary hormone assay (Czekala, pers. comm.). In March of 1992, when the female was expected to become pregnant again, close monitoring of medical and reproductive status was begun to try to elucidate the causes of reproductive failure.

DIET

The female rhinocerosceros had fluctuated in weight since arriving at the zoo. Therefore, the caloric and vitamin/mineral content of the diet was increased significantly. During the last pregnancy she was fed daily: 12 pounds ADF 16% herbivore pellets (Purina Mills, Richmond, Indiana), 3 pounds elephant supplement (Purina Mills, Richmond, Indiana), 12–16 oz of molasses, 1/2 cup of trace mineral salt (Morton Co., Hutchins, Kansas), 1 carrot, 1 onion, 1 celery stalk, 20 lbs. 1:2 prairie hay/alfalfa, 10 pounds alfalfa cubes and 30cc (vitamin E) of TPGS (Mazuri, Purina Mills, Richmond, Indiana). At mid-pregnancy, when boluses of half-chewed hay revealed she was having difficulty in chewing, the diet was divided into four feedings. Also, the prairie hay/alfalfa was shredded. In addition, 3 pounds/ daily of dairy chow (Purina Mills, Richmond, Indiana) were given during the third trimester. She experienced severe weight loss during the first half of pregnancy. During the last half of pregnancy, her weight and conditioning improved significantly.

RESTRAINT

Closer monitoring of the female's health and reproduction was possible through the use of a restraint chute. This chute is patterned after the Henry Vilas Zoo design and constructed of aluminum vertical bars (J&M Construction, Wichita, Kansas). It folds into place in the off exhibit passage between

indoor and outdoor exhibit areas. The endgates are guillotine backed by solid doors. This chute is long and flares at either end to make it flush with the exhibit gates. This flare allows the animals to swing their rumps from side-to-side. Interim guillotine gate eliminates the flare, but shortens the chute making it difficult to close the gate before the animal backs up. This female black rhinocerosceros allowed, almost immediately, cephalic vein blood collection and ultrasonography.

MEDICAL MANAGEMENT

Medical treatments were performed as indicated by weekly analysis of hematology and serum chemistries. These values remained unremarkable throughout pregnancy except for a slightly low phosphorous (3.1–4.9 mg/dl) which caused a slight imbalance in the calcium to phosphorous ratio. Also, low glucose levels (19–40 mg/dl) were treated by daily administration of molasses. The frequent appearance of mucopurulent vaginal discharges were treated by flushing the vagina with Nolvasan uterus suspension (Ft. Dodge Laboratory, Ft. Dodge, IA). An acute case of anorexia and depression in the female resolved after treating a toe abscess. Periodic skin ulcerations were controlled by a topical ointment, Nolvasan, massaged into the skin. She periodically had bouts of diarrhea. Since sand impaction had occurred in the male, Equisyl (Animal Health Care Products) or mineral oil was administered in the female's diet.

REPRODUCTIVE MANAGEMENT

Ultrasonography (3.5 MHz and 5.0 MHz linear probe and 500 Aloka scanner; Corometrics, Wallingford, Connecticut) was performed once a week for 2 weeks before conception and during the first 12 weeks of pregnancy. A dominant follicle was associated with estrous. A persistent cyst (30mm) was associated with the right ovary. Follicular development continued during the first 12 weeks of pregnancy. A 28x23mm corpus luteum was noted on Day 38 of pregnancy. On Day 16 of pregnancy, a 20x22mm vesicle was noted in the uterus. After Day 25, the vesicle was undetected until Day 50 when the membranes of the dorsal embryonic sac began to reappear.

Serum was collected at least every other week for 70 days prior to conception and throughout pregnancy from the right or left cephalic vein. Feces and urine were collected at least once or twice a week. Serum progesterone was analyzed during pregnancy by radioimmunoassay by Roche Biomedical Laboratories (Fig. 1). Steroid hormones and conjugates were also analyzed in urine, feces, and serum with enzyme immunoassay (Berkeley and Schaffer, 1992) (Fig. 2). Serum progesterone radioimmunoassay analyzed during the estrous cycle reached nadirs of <0.2 ng/ml and peaks averaging 15 ng/ml. During the first five to six months of pregnancy, values ranged from 1.2–9.8 ng/ml. After six months, the values ranged from 10.1–35.7 ng/ml with an average of 19.3 ng/ml. The patterns for serum progesterone radioimmunoassay compared closely with levels resulting from enzyme immunoassay analyses. However, the fecal progesterone levels during the last 10 months of pregnancy demonstrated a 5–10 fold increase from luteal phase levels (pregnancy: 3000–4000 ng/g dry feces; luteal phase 300–800 ng/g dry feces).

DISCUSSION

Restraint

At least nine different chutes have been built for four species of rhinoceros (Schaffer et al. 1991). The chute at Sedgwick was built according to a chute designed by the primary author at the Henry Vilas Zoo in Madison, Wisconsin. All exams or treatments were performed in this chute alleviating the need for anesthesia. Anesthesia has contributed to or been the cause of death in at least three species of rhinoceroses. Although the expense is greater and the training is more time-consuming, the use of chutes has allowed repetitive performance of a variety of procedures in four species of rhinoceros, including blood collection, ultrasonography, semen collection, bladder catheterization, and skin and foot treatments. In some instances, operators have trained rhinoceroses to tolerate ear

and cephalic vein blood collection without restraint by offering food and contact (D. Nichols, pers. comm. and M. Illig pers. comm.). While this training has provided some medical and reproductive monitoring for several months, it would have limited usefulness if the animals require intensive medical or reproductive treatment.

Monitoring and Management

Ultrasonography has been shown to be a safe method for assessment of estrous cycling, pregnancy, and pathology in the rhinoceros (Adams et al. 1992; Schaffer et al. 1992; Schaffer et al. 1991; Schaffer and Beehler 1990). Since this method has proven to be useful for identifying reproductive status in the black rhinoceros, it was used to validate circulating and excreted hormone levels. The same correlations between ultrasonographic images and hormone levels normally seen in the domestic animal were also seen in the rhinoceros. The dominant follicle was associated with decreasing progesterone levels and increasing estrogen levels. A regressing corpus luteum was associated with decreasing progesterone levels. A pregnancy vesicle was consistent with persistent progesterone levels, however, the vesicle lost shape and dropped beyond probe penetration within 25 days, making it difficult to monitor the embryo. The dorsal vesicle reappeared after 50 days. The vesicle seemed to drop rapidly which may have been due to the age and laxity of this female's reproductive tract.

Circulating or excreted hormones have been examined in many animal species including the rhinoceros (Kirkpatrick et al. 1993; Schwarzenberger et al. 1993; Hindle et al. 1992; Lasley and Kirkpatrick 1991; Ramsay et al. 1987; Loskutoff et al. 1982; Kassam and Lasley 1981). In this female, the monitoring of pregnancy by circulating (serum) hormones could be compared with excreted hormones. Both demonstrated persistent progesterone levels that were lower in the first 5–6 months than in the last 10 months. Both could be used to diagnose pregnancy. However, since the circulating peak levels of luteal progesterone (15ng/ml) compare closely with the pregnancy levels (19ng/ml), these levels can not be used to diagnosis pregnancy. Rather the persistence of increased serum progesterone levels would be indicative of pregnancy. Therefore, serial blood samples over a known estrous cycle duration would have to be analyzed. Alternatively, since the excretory hormone demonstrates a 5–10 fold increase, this high level of progesterone would be indicative of pregnancy. Thus fewer fecal samples are needed to indicate pregnancy and they are easier to acquire. These factors have resulted in the rapid, accurate diagnosis of pregnancy in several animals (Berkeley et al., 1993; Berkeley, unpublished data). The administration of the synthetic progestin may have complicated the excretory hormone analysis. However, this may be disregarded because: 1) the elevation in progesterone in the feces of this female began a month after the beginning of administration of the drug; and 2) the same peak progesterone values have occurred in other pregnant rhinoceroses.

An etiology for abortion in this female was not identified, particularly since pregnancy was successful. Abortion or re-absorption of the fetus occurs more frequently in older domestic animals, however, stress, poor nutrition, and ascending tract infection have also been implicated in other animals and could have been factors in this female.

Although this female was well over the reproductive age (25 years) of rhinoceroses in captivity, she was younger than the reported productive age (35 years) in the wild (Smith and Read 1992). Several problems were addressed that could have been sequela to her age. Her teeth were unevenly worn interfering with mastication. Shredding her food and increasing the numbers of feedings from one to four times a day probably improved her metabolism. On later examination, her cervix was found to lack normal tone and closure. This could result in ascending tract infections which were evident in her history of placentitis. In this female, a vaginal discharge was not uncommon, however, when it became copious or discolored during pregnancy it was immediately treated. In addition, the vulva would sometimes become swollen and tender.

A vaginal discharge can often occur in both cycling and pregnant rhinoceroses, particularly in the last few weeks before parturition. The discharge is usually a few drops to a few milliliters of pasty or mucoid material that can accumulate on the tail or vulva. However, one young female black rhinoceros at the Riverbanks Zoo frequently discharged large amounts, up to 250 cc, of material that sometimes formed a large mucoid plug. Investigators have tried associating this discharge in the rhinoceros with estrous, however, since estrous detection is still minimally successful this remains unclear and still needs further characterization.

During the early part of this female's pregnancy, luteal insufficiency became a concern as the cause of abortion. Luteal insufficiency can be a cause of reproductive failure in older animals and can be characterized by early abortion and low serum progesterone. This female's last abortion had occurred early in pregnancy and her serum progesterone was not increasing at the time. Since progesterone is not known to interfere with pregnancy, supplementation was instituted. Recently, pregnancy was maintained in another rhinoceros with a history of abortion when progestin was supplemented (B. Durrant pers. comm.).

Nutritional effects on pregnancy can result from over- or under-consumption or imbalances. Black rhinoceroses may have some peculiar nutritional requirements which is still being debated (E. Dierenfeld pers. comm.). Since this rhinoceros had a tendency to lose weight during pregnancy, she was offered a higher vitamin/mineral and caloric diet. Her mineral supplementation was higher than that recommended for a perissodactylid. Her weight loss may have been the greater demand by the fetus on this older female's metabolism. This was indicated since she improved after the hay was shredded and numbers of feedings increased.

Serum chemistries revealed low glucose and phosphorous levels. The low glucose could not be associated with anorexia or pancreatic insufficiencies, but increasing her feedings to four times a day resulted in maintenance of higher glucose levels. The low phosphorous may have resulted from periodic bouts of diarrhea, since the levels improved after resolution of the diarrhea. The causes of these levels remains unclear. Both can be associated with loss of pregnancy. Diabetics are known to have trouble maintaining pregnancy. Calcium-phosphorous ratios can be critical in pregnancy due to the demands of the growing fetus. The phosphorous was only minimally low and, therefore, was not treated. Phosphorous imbalances have been noted in other black rhinoceroses and is being investigated (E. Miller pers. comm.).

CONCLUSION

Intensive management and treatment improved this female's health as well as apparently resolved this animal's reproductive problem. Since 20% of the animals in the 1986 AAZPA Black Rhinoceros Survey had aborted; and as of the 1992 Black Rhinoceros Studbook, 17 females were non-producing, this problem, may need further investigation. The etiology of abortion in this female is still unknown but potential problems were identified and treated. This pregnancy can be used as a reference in investigating other aborting females.

ACKNOWLEDGMENTS

We would like to thank Mark Reed and the Sedgwick County Zoo for their many years of dedication to this project, the Milwaukee County Zoo for its continued financial support and to Julie A. Kreiner for manuscript and editorial assistance.

REFERENCES

Adams, G.P., E.D. Plotka, C.S. Asa, and O.J. Ginther. 1991. Feasibility of characterizing reproductive events in large nondomestic species by transrectal ultrasonic imaging. *Zoo Biology.* 10:247-259.

Berkeley, E.V., J.F. Kirkpatrick, and W.M. Bryant. Determination of pregnancy, ovulation, and the onset of parturition in the black rhino by using fecal steroid analysis. In: *1993 AAZPA Annual Conference Proceedings*, Omaha, Nebraska, p. 260.

Berkeley, E.V. and N.E. Schaffer. Serum, urinary, and fecal steroid correlates of reproductive behavior in the black rhinoceros (*Diceros bicornis).* In: 1992 AAZPA Annual Conference Proceedings, Toronto, Canada, p. 572.

Hindle, J.E., E. Mostl, and J.K. Hodges. 1992. Measurement of urinary oestrogens and 20-a dihydroprogesterone during ovarian cycles of black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses. *Journal of Reproductive Fertility.* 94:237-49.

Kassam, A.A.H. and B.L. Lasley. 1981. Estrogen excretory patterns in the Indian rhinoceros (*Rhinoceros unicornis*), determined by simplified urinary analysis. *American Journal of Veterinary Research.* 42(2):251-255.

Kirkpatrick, J.F., B.L. Lasley, S.E. Shideler, J.F. Roser, and J.W. Turner. 1993. Non-instrumented field tests for pregnancy detection in free-roaming feral horses. *Journal of Wildlife Management.* 57(1): 168-73.

Lasley, B.L. and J.F. Kirkpatrick. 1991. Monitoring Ovarian Function in Captive and Free-ranging Wildlife by Means of Urinary and Fecal Steroids. *Journal of Zoo Wildlife Medicine.* 22(1):23-31.

Loskutoff, N.M., J.E. Ott, and B.L. Lasley. 1982. Urinary steroid evaluations to monitor ovarian function in exotic ungulates: I. Pregnanediol-3-glucuronide immunoreactivity in the okapi (*Okapia johnstoni*). *Zoo Biology.* 1:45-53.

Schaffer, N.E., Z. Zainal-Zahari, Mohd. A. Tajuddin A., M.R. Jainudeen, and R.S. Jeyendran. 1992. Fertility analysis techniques in the Sumatran rhinoceros (Dicerorhinus sumatrensis): Semen collection and ultrasound. *AAZPA 1992 Annual Conference Proceedings* Abstract, Toronto, Canada. p. 573.

Schaffer, N.E., R.S. Jeyendran, and B. Beehler. 1991. Reproductive procedures and restraint for the rhinoceros. *Proceedings of the International Rhino Conference*, San Diego, California. pp. 153-159.

Schaffer, N. and B. Beehler. 1990. 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.

Smith, R.L. and B. Read. 1992. Management parameters affecting the reproductive potential of captive, female black rhinoceros, *Diceros bicornis. Zoo Biology.* 11:375-83.

Ramsay, E.C., L.H. Kasman, and B.L. Lasley. 1987. Urinary steroid evaluations to monitor ovarian function in exotic ungulates: V. Estrogen and pregnanediol-3-glucuronide excretion in the black rhinoceros (*Diceros bicornis*). *Zoo Biology.* 6:275-282.

Schwarzenberger, F.; R. Francke, and R. Goltenboth. 1993. Concentrations of faecal immunoreactive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (*Diceros bicornis michaeli*). *Journal of Reproductive Fertility.* 98:285-91.

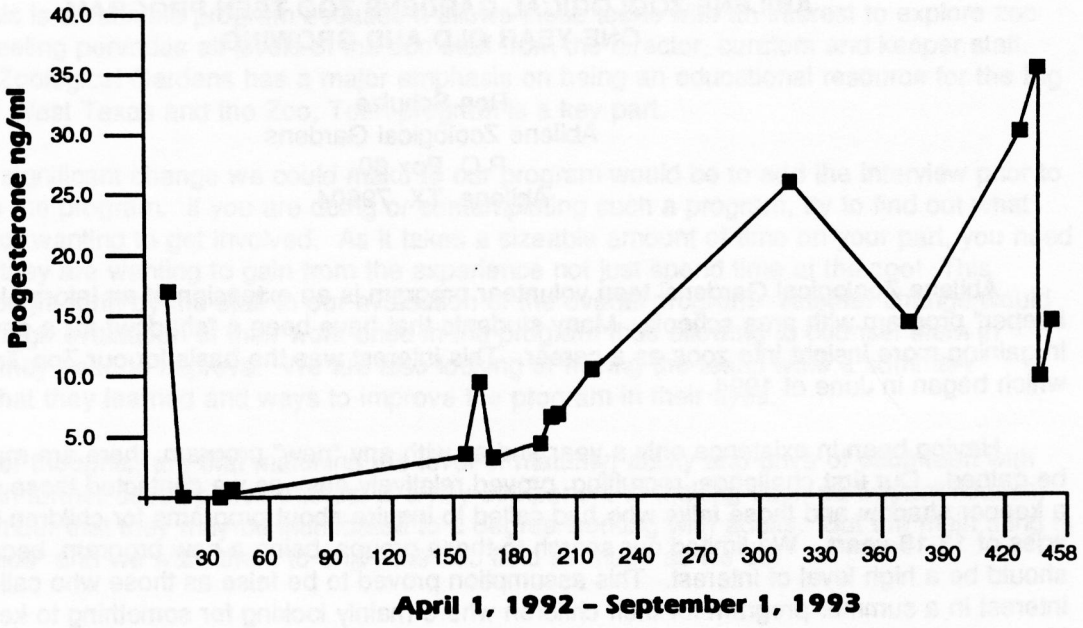

Figure 1. A Comparison of Serum Concentrations During the Estrous Cycle and Pregnancy from the Black Rhinoceros "Bibi" (SB#53). Roche Biomedical Laboratories, 1992.

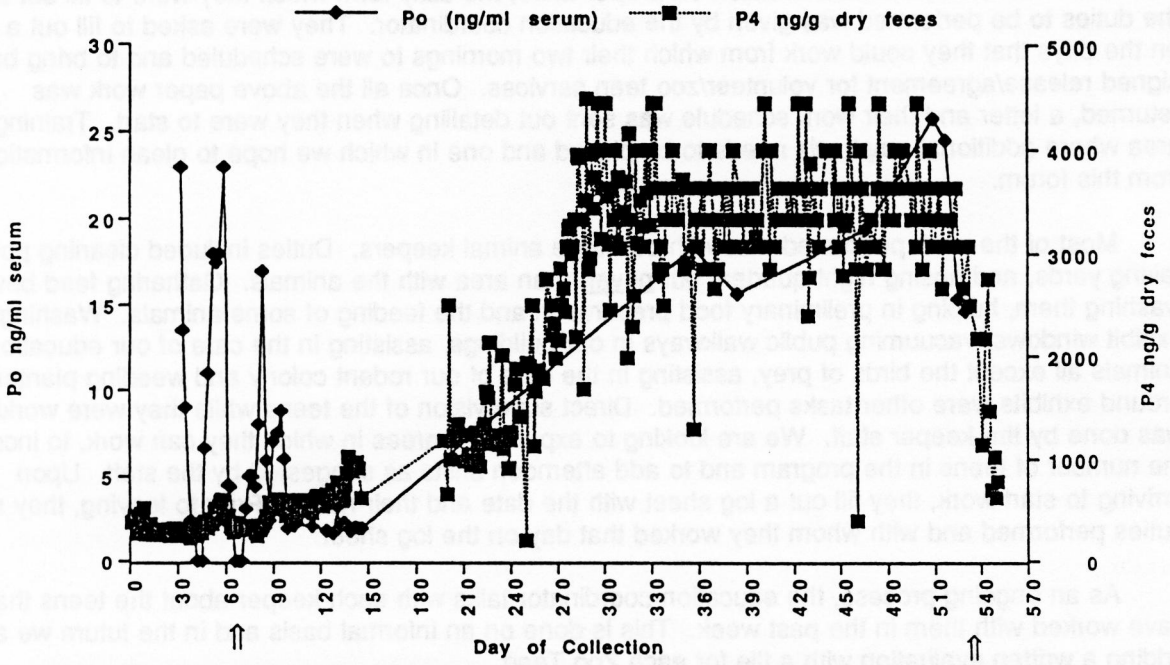

Figure 2. A Comparison of Serum and Fecal Progesterone Concentrations During the Estrous Cycle and Pregnancy from the Black Rhinoceros "Bibi" (SB#53). Reprint from: Berkeley, E.V. Fecal steroid hormone analysis of ovulation and pregnancy in the black rhinoceros (Diceros bicornis). Ohio State University, Thesis, 1994.