Reproductive management strategies for an aging female Black rhinoceros *(Diceros bicornis micheali*) at the Denver Zoological Foundation

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Overall, the recruitment rate of the captive black rhino population has remained stable. Unfortunately, only a few facilities have shown consistent success in reproduction of this species. Currently, the total number of both species of black rhino held in North America is 119 individuals (2005 Studbook). The sex ratio of both populations is slightly biased towards males at 65:54 (2005 Studbook). According to the most recent information, the Eastern black rhino (*Diceros bicornis michaeli)* population has shown slight growth after remaining level over the last decade. Even with growth, both subspecies continue to be plagued with numerous disease syndromes, and lack of breeding space due to a skewed ratio of males.

A successful breeding program for any species must consider the following factors that will help managers in their efforts to increase the reproductive success of this species. This includes husbandry and behavior, the reproductive histories of individuals, and the ability to detect and monitor ovarian activity. Historically, the Denver Zoological Foundation has produced twelve calves with three different pair of rhino. Our last successful birth of a black rhino occurred over a decade ago in 1993. In 2000, we acquired an 18 year old female Eastern black rhino in hopes of re-establishing our breeding program. Since her arrival at the zoo, this female has become pregnant at least two times but unfortunately both pregnancies resulted in losing the fetus. This paper will discuss the reproductive history of this female, the recurrent abortions, and the future Denver Zoo management strategy for this species.

Reproductive History

The 18 year old female rhino "Shy-anne" came to us from Cheyenne Mountain Zoo (CMZ) in Colorado Springs, CO. Her only successful pregnancy occurred when she was 13 year old, delivering a healthy male calf. Following the death of the breeding male at (CMZ), the recommendation by SSP was to move her to our facility which housed two males. The two males in our program were a 19 year old proven breeder, and an inexperienced 7 year old. Upon her arrival, she was slowly introduced to the 19 year old male via visual access with a stall between the two animals, and then eventually allowed access to one another between the bars of the stalls. The introduction of the rhinos to the yard was hampered due to muddy, slick conditions, so an estrus cycle was missed at this time. Even though the cycle was missed, it provided information on what behavioral and physical signs this female presented when coming into an estrus cycle. The physical signs included frequent strong bursts of urine, pacing and presenting her rear towards the male, and then pressing it up against the bars. During the next cycle

(December 2000), the pair was introduced and several breedings occurred over a two day period. In January 2001, the female became increasingly aggressive towards the male, suggesting that she might be pregnant. At this time, operant conditioning training was instituted to assist in blood collection. Samples were not obtained until April 2001 when a pregnancy was confirmed. In May 2001, this pregnancy ended in an abortion of a 12 inch long fully formed male fetus. After the abortion, a uterine infection developed resulting in an immobilization to flush the uterus, and the administration of Lutalase® (Pharmacio & UpJohn Co., Kalamazoo, Michigan 49001) injection to help induce her to cycle. After she began cycling normally again, this pair copulated at least 38 times, but none resulted in a pregnancy.

Recently, there has been an increase in male captive black rhinos over the age of 10 developing testicular calcification (Hermes, 2004). It was decided that we would perform reproductive assessments on both of our males to determine if this syndrome was occurring in our animals. Free standing rectal ultrasounds were performed, and findings indicated that indeed our 19 year old male had developed severe calcification. The 7 year old male showed no abnormalities, and therefore became the primary breeding male at our facility. Since this male had no breeding experience, and was slightly smaller than the female, the introduction of this pair occurred over several weeks. Once the pair had been introduced, it was clear that this male had strong instinctual drive, but was locationally challenged. After two days of mounting her head, the female being more experienced, presented her rear towards him each time he would start to move towards her head. Eventually, with her help, the correct location was found and he appeared to breed successfully. Once again, pregnancy was confirmed in May 2003, and sadly terminated in another abortion sometime after October of 2003.

Recurrence of Abortions

One factor may have influenced the first abortion in our female. The necropsy performed on the recovered fetus showed an absence of a spinal cord. The genetic line of the 19 year old male included a female calf that died at 15 months due to leukoencephalomalacia (Kenny, 1996). This particular disease syndrome affects the development of the white matter leading to severe neurological conditions. This calf presented with lip smacking, circling to the left, and showed fine motor control deficits (Kenny, 1996). The prior history of this disease may be possible a factor in the first spontaneous abortion. The detection of the second pregnancy occurred fortuitously during a reproductive assessment of this female performed by Dr. Robert Hermes (IZW) while he attended the 3rd Rhino Keeper Workshop held in May 2003. During the assessment, the ultrasound demonstrated a 1.74 cm embryonic disc. Based on the last breeding date and appearance of the disc, the embryo's gestational age was estimated to be 17 days. Upon discovery of this pregnancy, a supplemental program was implemented. This protocol entailed giving three oral supplements on a daily basis; 20 ml altrenogest (Regu-mate ®; Intervet Inc., Millsboro, DE 19966), 1500 mg aspirin (Monument Pharmacy, Monument, Colorado 80132), and 40 gm Beta-Carotene.

Low serum progesterone levels have been associated in early pregnancy loss in mares (Bugfeldt, et al, 1992). The age of our female, and the prior loss of a fetus caused us to consider using alternogest which is commonly prescribed in such cases, and appears to be the only form of progesterone known to assist in pregnancy (Roth, et al, 2003). It has been used successfully in maintaining the pregnancies of a black rhinoceros (Berkeley, 1997), and Sumatran rhino (Roth, 2001) that had experienced early fetal loss. A dosage was prescribed using the calculation of (0.0444 mg/kg) used in horses (Roth, 2003). The supplement was offered on alternogest soaked bread.

Forty grams of Betacarotine, a dry powder vitamin had to be mixed with applesauce, and then spooned into cored out apples which were hand-fed to the rhino. It was found that feeding her this supplement in the afternoon before her main diet resulted in her ingesting most of the supplement. Betacarotine was recommended for maximizing the uterine environment for developing fetus (Hermes, personal comm.).

Antiphospholipid syndrome (APS) is coagulation defect causing the formation of clots in blood vessels obstructing the flow of blood throughout the circulatory system, as well as causing recurrent abortions (Ball and Marrow 2003). This disease syndrome in humans has been compared to several similar clinical conditions observed in the black rhino (Ball and Marrow, 2003). In the hopes of preventing another fetal abortion, 1500 mg of aspirin therapy was given daily.

Future Management Strategies

After repeated suspected fetal losses it became evident that an immobilization was required to ascertain the reproductive viability of our female. In December 2004, a reproductive assessment was conducted by our veterinary staff, and with the assistance of equine surgeon, Dr. Dean Hendrickson from Colorado State University, Fort Collins, Colorado 80523. The ultrasound evaluation concluded that the uterine body and both horns appeared to be normal. The ovaries were quiescent, and we hypothesized that the estrus cycles might be behavioral and not hormonally driven. Blood was collected for a hormonal panel consisting of progesterone, estrogen, testosterone (UC Davis Laboratory, One Shields Ave. Davis, CA 95616). Additional samples were obtained when this female developed an eosinophilic mouth ulcer that required another immobilization in January. This individual's cycle duration lasts between 25-28 days in length, and is compatible to findings in other captive females (Radcliffe, 2001). Recent studies have suggested that wild black rhino females may show reproductive seasonality with most reproductive activity occurring during the summer and rainy months (Garnier, 2002). This reproductive strategy ensures that conditions are optimal for the survival of the calf in the wild. In horses, a mare will produce anovulatory follicles during the period of transition between regular cycles (Stabenfeldt and Edquist 1997). Radcliffe et al(2001) documented through ultrasound the formation of similar follicles in females at Fossil Rim suggesting that seasonality may also be occurring in captive animals.

Findings from the blood panels are indicative of hormonal changes occurring in this female, and may potentially support this theory of seasonality in black rhino females.

Black Rhino "Shy-Anne" Hormone Analysis determined at UC Davis Lab

(Prolactin level from University of OHIO below measured range)

Sample/date	Progesterone	Testosterone	Estrone Sulfate	Inhibin	Estradiol
Reference range (equine)	Estrus or anoestrus: **0.1-0.5** ng/mL Diestrus/pregnant: **1.0-15.0+**	Non-pregnant: **20-45** pg/mL pregnant: **50-250** pg/mL	Non-pregnant: **0.1-6.0** ng/mL Pregnant: **6.0-2000**	Non-pregnant: **0.1-0.7** ng/mL	Cycling: **20-45** pg/mL
12/1/04	0.6	103.1	9.5	0.42	22.9
1/6/05	1.4	83.4	12.6		11.8
3/14/05	0.6	27.8	1.7	0.57	41.6

During this same period, both males showed behavioral interest in breeding this female perhaps supporting a hormonal estrus. Physical cues included frequent defecation, urination, feet scraping and flehmen response. When the female appears to be in estrus our 22 year old male develops laminitis producing inflammation of the cornary band and lameness that lasts two or three days in which the animal becomes lethargic and remains recumbent. In horses, it has been observed that a mare will show behavioral estrus even after the ovaries have been removed , and the male continues to behave as if she is actively cycling indicating that it may be behaviorally driven and not a hormonal function (Hendrickson, personal comm.) We had to re-evaluate our reproductive protocol making collection of blood through operant conditioning the number one priority for this female. Hopefully, we will be able to interpret the hormonal panels which will help us determine estrus cycles and if she is pregnant. This female had been a reluctant participant in training in the past, and blood could not be obtained on a consistent basis. Fecal progesterone has been proven to be a reliable method of reproductive monitoring, but the cost was prohibitive for the use in our program. In retrospect, valuable information about the progression and termination of pregnancies could have been determined through regular blood collection. With the success of the training program we are now collecting blood for monthly progesterone and estradiol levels. The information obtained will be charted and may further support the suggestion that black rhinos show seasonality in the captive environment, therefore help in the management of this endangered species.

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