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REPRODUCTIVE MANAGEMENT AND RESEARCH OF 1.1 EASTERN BLACK RHINOCEROS (*Diceros bicornis michaeli*) AT ZOO ATLANTA

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Very little is known about the basic reproductive biology of the black rhinoceros (*Diceros bicornis*). Through often laborious processes, researchers have made some progress uncovering the mysteries of rhino behavior, physiology, and genetics, but the questions still far outnumber the answers. There are many managers, animal caretakers, and others who have experiential knowledge of black rhino reproduction, however, this information is not common knowledge and typically not available in publications. The science of captive rhino reproduction is thus an evolving area in which resources must be tapped and integrated to maximize benefit for all of those interested in the long-term propagation of this endangered species.

Because Zoo Atlanta was recommended to breed a pair of black rhinos and has been an institution committed to research in accordance with sound management practices, a long-term project was developed that would contribute to the collective information on rhino reproduction. The three major goals of the management and research plan were 1) to introduce the rhinos as safely as possible, 2) to facilitate their breeding, and 3) to respond to the needs of the Rhino Taxon Advisory Group (TAG) for information and documentation of introductions, especially with concern for aggression levels, and reproduction.

Zoo Atlanta houses a pair of eastern black rhinoceros (*Diceros bicornis michaeli*). The female, "Rosie," was born in January 1990 at the San Francisco Zoo and brought to Atlanta in November of that year. The male, "Bo," was born in August 1986 at the Dvur Kralove Zoo in the Czech Republic and came to Zoo Atlanta in October 1989. Since their arrivals, the rhinos had daily visual, olfactory, and often tactile contact through gates and bars, but until their introduction for breeding, the rhinos were placed individually on exhibit on alternate days. The rhino off exhibit had access to the barn and corral.

The rhino exhibit is approximately 1/4-acre with grass and dirt substrate, a water moat along two sides, a dry moat along another, and walls, a gate to the service road and a gate to the corral along the fourth. A significant feature of the exhibit is a set of boulders approximately 10 feet in diameter in a central location. The original dimensions of the corral were approximately 25 ft. x 30 ft. with a primarily dirt substrate, enclosed by pressure-treated pine posts.

We began our project in the winter of 1995 and developed our management and research strategies around several specific objectives. First, acknowledging our inexperience with introducing and breeding black rhinos, we wanted foremost to seek advice from experienced zoo personnel. Second, because of Rosie's young age and a lack of observable cues to estrus, it was necessary to confirm through hormonal analysis that she had begun cycling. Third, pending hormonal confirmation, the major research objective was to collect behavioral data on both rhinos to find predictors and indicators of Rosie's estrus. This information would allow us to facilitate the breeding of this pair, as well as contribute to the general pool of information on captive rhino reproduction. To strengthen this behavioral data, our fourth objective was to correlate it to the estrous confirmation data and additional hormonal analyses on the female. The final objective was to document and disseminate the results of these introduction and breeding efforts.

As a starting point, we requested names from the Rhino TAG of animal managers who might serve as consultants, and from this list we sought advice. With consideration for curators' management styles and individual variation among black rhinos and facilities, we incorporated several suggestions

appropriate to our situation for introductions and breeding. For example, for the introduction it was suggested to wait until the female was in estrus, give the female an advantage by placing her first in the area, expect a high level of aggression, set a limit for an acceptable level of aggression, and be prepared to separate them if warranted. The first recommendation was suggested as a way to reduce potential aggression among the pair, and this idea prompted us to confirm Rosie's cycling through hormone analysis.

Of great impact on our preparations was advice given during an on-site consultation by Lonnie McCaskill, lead rhino keeper at White Oak Conservation Center. He informed us of the types of behaviors we could expect during introductions and breeding and also gave suggestions for ways to alter the corral and exhibit to accommodate such behaviors. In general the recommendations were to increase running area, remove all obstacles to running, create circular paths, and consider height and width of gated areas as possible hindrances to movement during courtship.

Deciding to give the rhinos access to both the exhibit and the corral during the introductions and future courtship periods, we designed modifications for these areas in accord with recommendations. Renovations occurred from October 1995 through February 1996. The primary task was expanding the corral to the service road, thereby including the service road gate as a second entry between the exhibit and the corral. This would create a circular path between the two areas and thus greater running opportunity. We also increased the height of the overhead roller track suspending the original gate, removed loose logs in the exhibit, and left a set of six posts remaining from the original corral perimeter to be a blind or runaround in the expanded corral.

In March 1995, when behavioral cues for Rosie's estrus were observed, the process began of gradually introducing the rhinos to one another in the corral and exhibit over the next month. The rhinos were let out together each morning and separated when aggression escalated or one of the rhinos appeared exhausted. Each introduction was recorded on videotape for documentation and later transcription. Overall levels of aggression were low with each rhino defending itself when necessary, resulting in only minor facial abrasions. The rhinos chased one another and frequently used the circular paths and structures designed as runarounds. Rosie did use the posts in the corral as an obstacle to separate herself from Bo. No breeding attempts occurred during that first estrous period together. In general, preparations for safe introductions with little aggression proved successful.

Before, during, and after the introduction process, behavioral data were collected by keepers and researchers. One beneficial data collection tool used from April 1995 to present is the daily keeper questionnaire. This is a short survey of 12 general behaviors and physical characteristics of both the male and female believed to be predictors of estrus that are marked on a 0 to 4 scale. It was designed to capture the keepers' general impressions of the rhinos without requiring time away from daily routines for formal observations. The questionnaire takes approximately five minutes to complete at the end of each day.

Analysis of a portion of the questionnaires does denote behavioral trends over Rosie's estrous cycle, which averages 23 days. These data suggest that strong pre-estrous indicators in Rosie are increased vocalizations and aggression, with more variable results in vulvar swelling, increased agitation, and increased interest in Bo. Consistent changes in Bo are increased vocalizations, aggression, hind-leg dragging, and interest in Rosie, with flehmen, agitation, and horn-digging as less dependable characteristics. By participating in behavioral data collection, the keepers are generally more aware of the rhinos' changing dispositions, allowing improved daily management of the rhinos including preparations for breeding.

Another form of behavioral research for determining pre-estrous and estrous indicators in these rhinos was a systematic study using focal animal and instantaneous scan sampling techniques. The ethogram used, created in collaboration with other institutions, included over 100 behaviors in order to identify any behavioral changes related to reproduction. Because of the general difficulty in identifying ovulation and the extreme individual behavioral variation among black rhinos, the best clues to identifying estrus may be in behaviors not typically deemed "reproductive." Focal data of single subjects were

collected for 30-minute periods, an average of four times per week, with scans of the pair taken at 30-minute intervals. This phase of research occurred from September 1995 to September 1996.

Because of the extensive nature of data collected, analyses have not yet been completed. However, initial results of the data were used to refine the keeper questionnaire and help the staff track Rosie's estrous cycle. The final results will help us reach another objective of creating a specific reproductive ethogram for our future use, with potential applications for other institutions. Because basic biological research on rhinos is in its early stages, the specific timing of ovarian activity in rhinos cannot yet be predicted by either behavioral or hormonal data. It is strongly agreed by behaviorists and physiologists alike that our best interests in understanding basic rhino reproductive biology are served by working in tandem. Towards this end, we have collaborated with two endocrinologists studying similar aspects of black rhino estrus.

Three months of daily saliva samples were collected from Rosie in the winter of 1995-96 and sent to Nancy Czekala at the Center for Reproduction of Endangered Species (CRES) in San Diego for estrous determination. Characteristic changes in estrogen and progestin levels confirmed estrus, and thus we proceeded towards the introductions. Information from behavioral data and keeper questionnaires are being correlated to this endocrine data. In addition, we collected fecal samples from Rosie over the same period to contribute to Czekala's ongoing investigations to formulate radio- immunoassay techniques for estrogen- and progesterone-metabolites for charting ovarian cycles.

Beaux Berkeley, working in affiliation with Zoo Montana, also analyzed 15 months of fecal samples from Rosie for her research to identify physiological estrus. Fecal samples were analyzed for free progesterone and conjugated estrogen concentrations by enzyme immunoassay according to a previously validated procedure (Berkeley, et al, 1997). The next step in our collaboration with Berkeley is to correlate behavioral changes in both rhinos to Rosie's hormonal changes. This information should serve as a prototype for integrated research in this area.

There is obviously much to be done with information derived from the introduction process and in the steps to identifying estrus. This information will be added to a growing database of similar behavioral and hormonal data collected at the White Oak Conservation Center, the Henry Vilas Zoo, and the Dallas Zoo. These results will be published as they are completed. Several recommendations and directions for continuing efforts arise from this program. We recommend that all institutions beginning a similar breeding program seek the expertise of colleagues in the zoo field. We also recommend designing rhino enclosures with consideration for the behavioral aspects of the rhinos, including needs for introductions, breeding, and raising infants. We believe the keeper questionnaire is a simple and worthwhile tool for tracking estrus, and it will continue to be improved. We do expect substantial behavioral criteria for predicting and indicating estrus to result from analyses of the systematic focal animal and scan data. Finally, we believe the most beneficial information for assisting black rhino breeding will come from the correlation of behavioral and hormonal data.

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