



CREW Conducts Research to Help Rescue Rhinos

Artificial Insemination to Improve Genetic Diversity of the Indian Rhinoceros

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Name than 12 feet long and weighing up to two tons, the greater one-horned Indian rhinoceros is the largest of the Asiatic rhino species. The critically endangered Indian rhinoceros numbers 2.400 individuals in the wild and 54 individuals in the North American captive population. Once ranging throughout much of Southeast Asia, wild populations of Indian rhinos are now found in two protected areas; Nepal and Assam. India. Being the most aquatic of the rhino species, the Indian rhinoceros spends most of its time wallowing in the water and mud of the riverine forests and tall grasslands it inhabits. With its deeply folded thick skin at the neck, shoulders, and legs, this rhinoceros appears to wear a plate of armor. Despite its fearsome appearance, the Indian rhinoceros is basically very gentle-natured...except when it comes to breeding.

Natural breeding attempts in the Indian rhinoceros frequently result in severe aggression between the male and female, and this behavioral incompatibility has made genetic management of the species a challenge. In order to guarantee



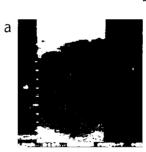
CREW scientists use ultrasonography and endoscopy to visualize the placement of semen in the reproductive tract of the female Indian rhinoceros.



In order to time AI at the appropriate phase of an estrous cycle, CREW scientists, with the assistance of rhino keepers, perform regular ultrasound exams on Chitwan to monitor follicular growth and document ovulation.

reproductive physiology, yet reproductive research on most exotic animals is still very limited. After three years of study, the follicular, hormonal and behavioral dynamics of Chitwan's estrous cycles have been comprehensively characterized. The female is a prime candidate for the procedure in that she is seven years of age, exhibits regular reproductive cyclicity and appears free of any reproductive tract pathologies. The data that has been collected from Chitwan regarding ovarian, endocrine and behavioral changes near ovulation has been used to determine the appropriate time during her cycle that AI will be performed.

Two male Indian rhinos are genetically suitable for breeding with Chitwan. One male Indian rhino, Assam, is located at our partner institution, the Wilds, while the second male, Jimmy, is at our institution. Semen samples from these males have been collected by electroejaculation and are cryopreserved in our Frozen Zoo and Garden. In July 2003,





Ultrasound images of preovulatory follicle (a) and a collapsed follicle following ovulation (b)

the highest genetic variation in subsequent generations of captive Indian rhinos, breeding and successful reproduction should occur with regularity between specifically paired males and females. As it now stands, the majority of the captive Indian rhino population is derived from a few highly prolific, non-aggressive, founder animals. With grant funding provided by the Morris Animal Foundation, CREW researchers are developing an artificial insemination. AI technique in the Indian rhinoceros to help ensure a genetically healthy and self-sustaining population of captive Indian rhinos while eliminating the risks and expenses associated with natural breeding.

Although Al has never been successful in any species of rhinoceros, CREW scientists hope to establish the procedure working with our female Indian rhinoceros. Chitwan. It is broadly known that to optimize the likelihood of conception following natural breeding or Al, ovulation must occur near the time of insemination. Therefore, the timing of breeding or Al relies heavily on an understanding of female the first AI attempt on Chitwan using frozen-thawed semen was performed less than 12 hours following a successful ovulatory estrous cycle. The procedure is technically challenging because the rhino's cervix is quite long with numerous folds of tissue making it difficult to navigate with the AI instruments. Although Chitwan did not conceive following this AI, CREW scientists made important progress in developing the technique. A second AI of Chitwan was again performed in September, 2003. This time, sperm was deposited much deeper within the cervix several hours following ovulation. Although Chitwan did not become pregnant following this second AI, the procedure itself was far more successful, and substantial progress was made towards the goal of achieving a transcervical insemination.

Even when Indian rhinos breed naturally, the chances of achieving a pregnancy can be low, but we are hopeful that the knowledge we gain from each Al procedure and use to improve our techniques in subsequent inseminations will result in a successful pregnancy in Chitwan in the near future.