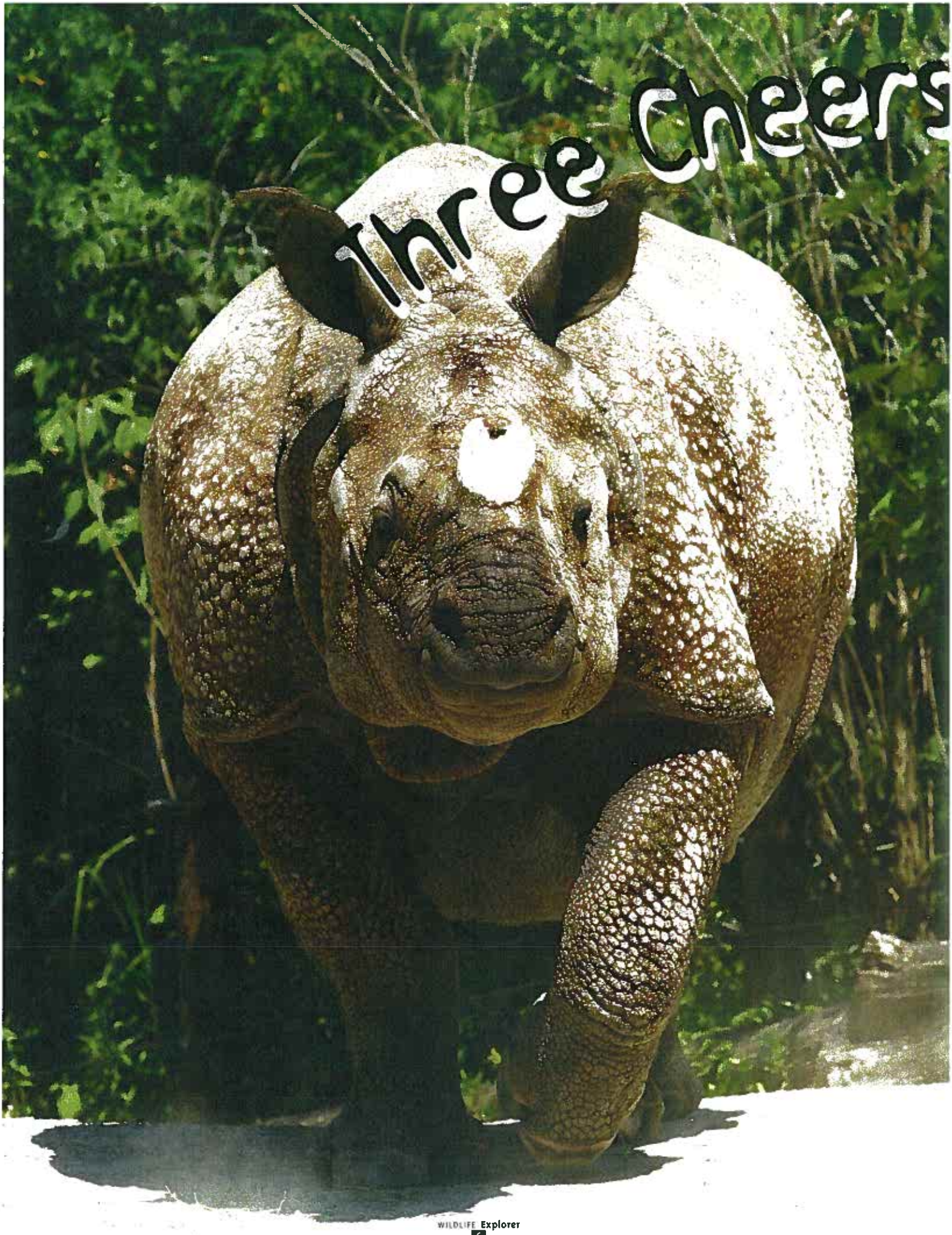
Three Cheers

for Pregnant Nikki!!

by **Dr. Monica Stoops**
CREW Reproductive Physiologist

Zoo Celebrates the Successful Artificial Insemination of the Indian Rhinoceros

Bringing a baby rhino into the world is no easy task. Just ask female Indian rhinos Nikki and Chitwan, CREW scientist Dr. Monica Stoops, and the Cincinnati Zoo rhino keeper and veterinary staff. On September 18, 2006, an ultrasound exam conducted on Indian rhino Nikki revealed she was pregnant, and at day 18 of gestation. For all of us in the rhino barn that morning, a shockwave of emotion ran through our bodies. After five years of research and dedication we were able to get the first glimpse at the result of our efforts - an Indian rhino embryo. Now, more than twelve months later we anxiously await the pitter patter of little rhino feet that are set to arrive at the end of this year. Nikki's pregnancy is important not only for the captive population of Indian rhinos, but for the monumental scientific achievement it represents as the first successful artificial insemination procedure in an endangered rhino species.

With only 60 Indian rhinos in captivity in North America and less than 2000 in the wild, successful breeding between rhino pairs is important to maintain the genetic diversity necessary to keep a population healthy and self-sustaining. Unfortunately, natural breeding attempts in captive Indian rhinos frequently result in severe aggression between the male and female. Because of this behavioral incompatibility, genetic management of the Indian rhino is a challenge.

What makes rhino breeding so complicated? Much has to do with the social behavior of rhinos. Many rhino species are solitary and only come together when it is time to breed. Additionally, timing introductions for breeding is made difficult when the female does not show good behavioral signs of estrus (receptivity to a male). Even when the female does appear to be in estrus, some male rhinos express aggression significant enough to cause serious injury and warrant separation from the female.

In order to avoid mate incompatibility while maximizing genetic management, CREW scientists took on the challenge of developing an artificial insemination technique for this species. Artificial insemination or AI is the process by which sperm is collected from a male and placed in the reproductive tract of a female. While this technique has been developed and used to produce offspring in the domestic livestock industry for decades, it had yet to be successful in an endangered rhino. As one can imagine, AI would be no small task in a 4,000 lb. animal! Before AI could even be attempted, a comprehensive understanding of the reproductive cycle of the female Indian rhino would be required to know when ovulation occurs in order to time AI at the point when the female would have the best chance for con-

Dr. Monica Stoops is joined by Head Keeper Randy Pairan and Indian rhino 'Nikki' in viewing ultrasound footage of Nikki's baby.

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Strong collaboration between Indian rhino keepers and CREW was fundamental to the success of the AI project. Here, they pose together outside Indian rhino "Chitwan's" exhibit.

ception. Sperm from male Indian rhinos would need to be collected and frozen for use in AI trials. In addition, AI tools specifically designed for the rhino's unique anatomy would need to be developed so that semen could be deposited deep in the female's reproductive tract.

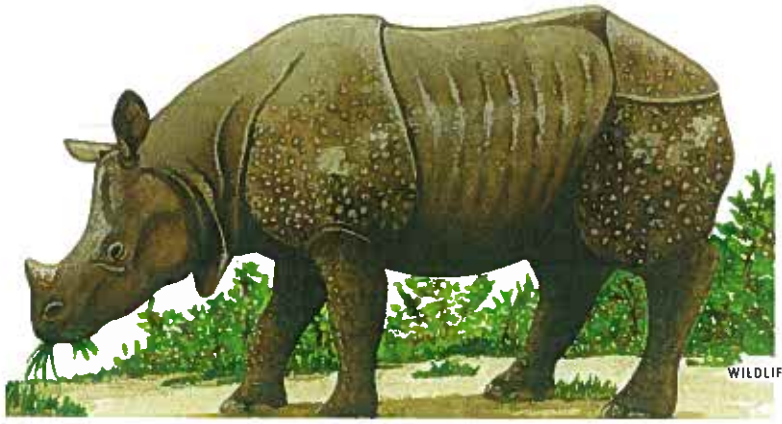
In 2001, we embarked on what turned into several years of intensive research on male and female Indian rhino reproduction. Over a two-year time frame, the estrous cycle of the Cincinnati Zoo's female Indian rhino, Chitwan, was characterized. The Zoo's rhino keepers are the best in the business and their role in the project was pivotal. The keepers worked diligently to condition the rhino to accept all the necessary scientific procedures, such as ultrasound. Typically, this was accomplished by offering her favorite food items as a positive reward in return for her cooperation. Ultrasound exams were regularly scheduled three times a week to monitor follicular growth patterns. In addition, keeper staff collected urine samples from the female that were analyzed for hormone concentrations at CREW's endocrinology lab.

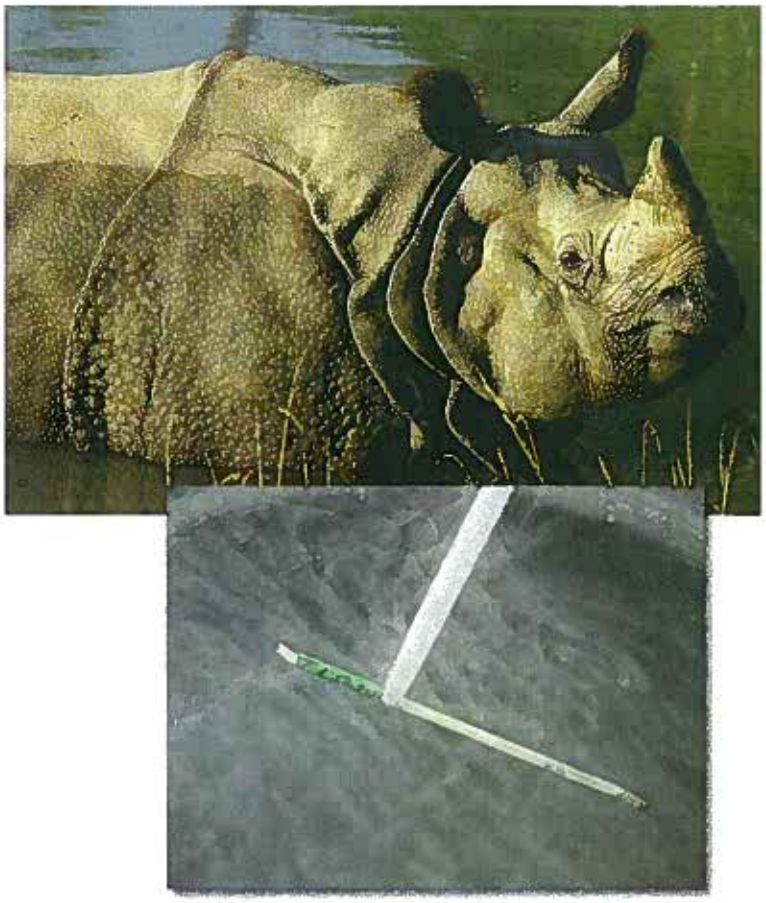
Eventually, the scientific data revealed that Indian rhino estrous cycles occur every 40-45 days. However, the unique follicular growth pattern of this species made predicting ovulation very challenging. Other rhino species grow preovulatory follicles that measure 2-3 cm in size. The Indian rhino develops a preovulatory follicle that reaches 15 cm in size! This is the largest follicle size of any mammal examined to date. While other species typically grow follicles that reach maximum size and ovulate shortly thereafter, the Indian rhino maintains maximum follicle size throughout most of the follicular phase of their cycle. In order to accurately determine when ovulation would occur so that AI procedures could be timed accordingly, we developed a multi-faceted research approach integrating ultrasound, hormone analysis and behavioral observations.

Concurrent with the work in the female Indian rhino, was ongoing research on her male counterparts. For AI to be successful, a method of collecting sperm from male rhinos was required. Additionally, by developing a technique for freezing the sperm, semen from males at other facilities could be collected and used to inseminate females at Cincinnati, thereby enhancing genetic variation. Custom made tools were mandatory for this phase of the project, in addition to our strong collaboration with other zoological institutions housing male rhinos. A method for collecting and freezing Indian rhino sperm was initially developed by working with animals at the Cincinnati Zoo and the Wilds (in Cumberland, OH). Once the procedure was optimized,

Of the five living rhino species, the Indian rhino is the second largest, weighing in at up to 6,000 pounds. It primarily inhabits the floodplain grasslands of northern India and southern Nepal. Here it grazes on tall grasses, using its prehensile upper lip to bend over stems and bite off the tops. Also on the menu are reeds, twigs, and fruits. Rhinos are important as seed dispersers. It can take up to three days to digest a meal, allowing the rhino to spread the seeds quite a distance. When not feeding, the rhino often lazes about in the water or mud to keep cool and escape biting insects.

It's the rhino's horn for which it is so revered that it is also so endangered. In traditional Asian medicine, rhino horn is believed to have medicinal properties used to treat fevers, high blood pressure, delirium, and other illnesses. In the Middle East country of Yemen, the horn is carved into ceremonial dagger handles. Laws banning the use of rhino horn have helped yet poaching and smuggling still persist. Indian rhinos, in particular, have recovered from less than 200 early in the 20th century to over 2,000 today. Continued protection and law enforcement will be necessary for their survival.

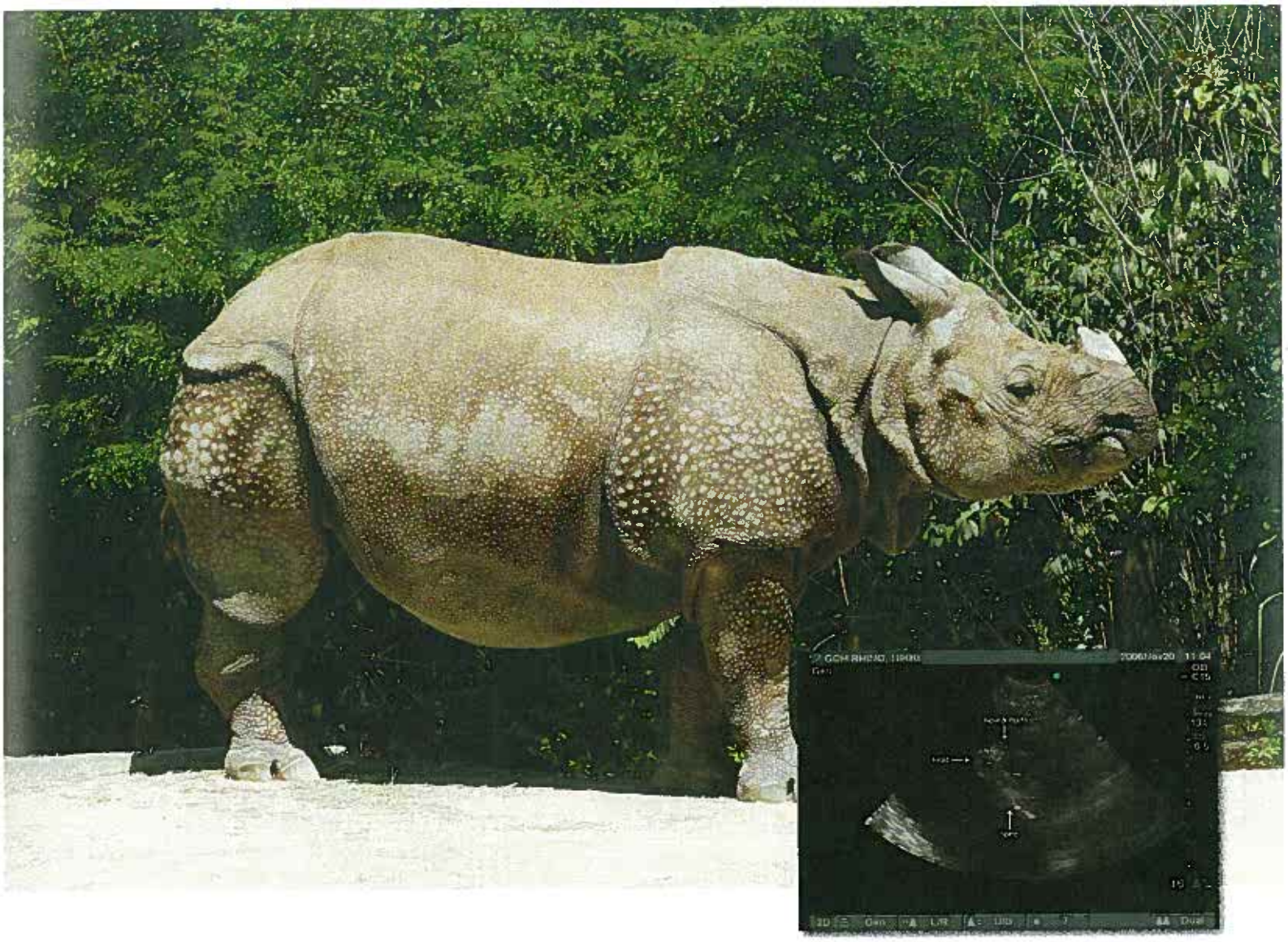




Indian rhino Nikki became pregnant from sperm that was collected in 2004 from a male Indian rhino at the Wilds named "Himal" (top). Himal's sperm was stored in straws that were frozen in liquid nitrogen for two years before they were thawed and used for Nikki's successful AI (bottom).

we embarked on collecting sperm from the most genetically valuable male Indian rhinos in North America. These males were located at the Cincinnati Zoo, the Wilds, the Bronx Zoo and White Oak Conservation Center. CREW's Frozen and Zoo and Garden now contains a male rhino genome resource bank with viable sperm from Indian rhinos that may not otherwise contribute their genetic potential to the captive population.

AI trials commenced in late 2003 on Cincinnati's single female Indian rhino, Chitwan. Substantial progress was made by using a sound scientific approach and custom made AI tools. Intrauterine inseminations were performed within 12-24 hours of ovulation. A total of six AI attempts were conducted on Chitwan, but unfortunately, she did not conceive. At this time in the project, only a limited number of AI procedures could be performed each year due to the long estrous cycle length characteristic for the species and our desire to rest the animal between procedures because of the use of anesthetics. Given the priority of the Indian



rhino Species Survival Plan (SSP) to develop AI technology in this species, a second female named Nikki joined our research program in Spring 2005. This second female provided more AI opportunities and increased our chances of establishing an AI protocol that would result in a successful pregnancy.

Because of the extensive information and experience gained through our research with Chitwan, we were able to begin working with Nikki almost immediately. Nikki's first AI procedure occurred in December 2005. While semen was successfully inseminated into her uterus, Nikki failed to ovulate. Throughout the course of our research we had wondered if anesthesia could be negatively impacting estrous cycle dynamics and/or ovulation. Therefore, in 2006, we began conditioning Nikki and Chitwan to tolerate the AI procedure without anesthesia. Nikki's mild demeanor made her a more willing subject than Chitwan. On the fourth AI attempt conducted on Nikki in August 2006, she became pregnant! The semen utilized for this successful AI was collected two years prior from male Indian rhino Himal located at the Wilds. Not only had we proven AI can be successful in the endangered Indian rhino, but that frozen-thawed rhino sperm is capable of producing pregnancies!

Several months later we were elated to discover that our second female, Chitwan, was also pregnant. Unfortunately, she underwent early pregnancy loss. However, the work continues in hopes that she too will become an expectant rhino mother soon. Meanwhile, Nikki has progressed wonderfully throughout her pregnancy and it has been exciting to see the growth (via ultrasound) of this very special rhino baby. If not for our dedicated staff and charismatic rhinos, this would not be a reality. Much time and effort has been devoted to learning as much as possible about these amazing creatures and it has been extremely rewarding. As everyone looks forward to this birth, we wonder if Nikki and her developing baby know how they have made and will continue to make history.

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