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## THE ROLE OF REPRODUCTIVE SCIENCE AND TECHNOLOGY IN ACHIEVING THE BIRTH OF THE FIRST SUMATRAN RHINO CALF PRODUCED IN CAPTIVITY IN 112 YEARS

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### Abstract

The critically endangered Sumatran rhinoceros (*Dicerorhinus sumatrensis*) has proven to be one of the most difficult species to breed in captivity. For the first time in 112 yr, a pregnancy established in a captive Sumatran rhinoceros was successful and produced a healthy calf. Although this calf was a product of natural mating, it is unlikely that it would have occurred if reproductive science and technology were not employed in the effort to overcome the challenges to breeding this species.

Since establishing the captive breeding program for Sumatran rhinoceros in 1984, several U.S. zoos (Bronx, San Diego, Los Angeles, Cincinnati) and our Malaysian and Indonesian counterparts have been trying unsuccessfully to breed this species.<sup>1</sup> Although aggressive behavior is not uncommon when rhinoceros are paired for breeding, it often is excessive and leads to serious physical injury if Sumatran rhinoceros are introduced when the female is not in estrus. Furthermore, Sumatran rhinoceros do not exhibit any obvious, reliable behavioral signs of estrus. Because of these characteristics and the complete lack of information available regarding the reproductive physiology of this species, breeding these rhinoceros appeared an insurmountable challenge for animal managers. By 1994, the only three Sumatran rhinoceros surviving in the United States had been moved to the Cincinnati Zoo and Botanical Garden for one last all out effort to breed the species in North America. In the fall of 1996, an intensive research effort was initiated with the primary goal to produce a Sumatran rhino calf.

The two female rhinoceros were conditioned to allow rectal ultrasound examinations. These exams were critical for evaluating the reproductive tracts and directly monitoring ovarian activity. Additionally, animals were conditioned to allow blood collection from an ear vein so that serum could be collected regularly and hormone concentrations measured. The data collected using these two technologies eventually led to the revelation and then confirmation that the younger female rhino was an induced ovulator with a 21-day reproductive cycle.<sup>2</sup> The older female appeared infertile with a large uterine mass and small, inactive ovaries. Eventually, the reproductive data acquired through this effort provided the foundation upon which a breeding program that optimized conditions conducive to safe, successful matings was developed. Animals were paired for breeding only when the female's progesterone levels were basal (< 100 pg/ml) and her ovaries contained a follicle at least 20 mm in diameter. When these criteria were met, mating typically took place on that day or, in some cases, the following day.

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A pregnancy was established after the second mating and was monitored closely by ultrasound from the day of detection (14 days after mating) until embryo loss was diagnosed on Day 42. After many subsequent infertile matings, the female became pregnant a second time but lost the pregnancy between days 79 and 90 of gestation. Three additional pregnancies were established but each lasted less than 60 days.<sup>2</sup> When the female became pregnant for the 6<sup>th</sup> time, she was administered an oral progesterone supplement (Regumate™; 16 ml; 0.044 mg/kg/day) beginning on the day of pregnancy detection (Day 16) and continuing until Day 450. She was weaned off the Regumate™ by slowly reducing the daily dosage (1.0 ml per day) until she was no longer receiving any supplement (Day 465). A healthy male calf was delivered on Day 475 after an uncomplicated labor.

Blood collection, hormone evaluations and ultrasound examinations were conducted throughout gestation for two reasons. First and foremost, the technology was employed to closely monitor the health and progression of the pregnancy. Second, it was used to generate data hormonal changes during pregnancy and fetal development. This information is the first of its kind for this species and is of interest from both applied and basic scientific perspectives.

Ultrasound examinations initially conducted every 10 days revealed that embryo development was very similar to that in horses, but after Day 83 of gestation, the fetus descended over the pelvic brim and could no longer be visualized rectally. Monitoring continued on a monthly basis via transabdominal scanning near the mammary gland with a 3.5 MHz convex probe, and the fetus could be observed until Day 223 after which it shifted cranially and no longer could be detected. From Day 223 until parturition, most information acquired through ultrasound exams was limited to allantoic fluid quality and only occasionally could a part of the fetus be seen.

The pregnant rhino's progesterone concentrations rose to luteal levels ( $1.47 \pm 0.58$  ng/ml) 10 days after breeding and remained there for the first 2 mo of gestation. Progesterone then gradually increased, rising to  $\geq 6.0$  ng/ml by 7 mo. From 7 mo to parturition at 16 mo, progesterone concentrations averaged  $9.49 \pm 2.82$  ng/ml (range, 6-16 ng/ml). By 24 h post-partum, progesterone concentrations had returned to baseline. Relaxin concentrations were basal ( $<1$  ng/ml) for the first 6 mo of gestation then rose to  $2.71 \pm 1.17$  ng/ml were they remained until spiking to  $\geq 800$  ng/ml 2 wk before parturition. Relaxin has never before been measured in a pregnant rhinoceros, and the sharp increase in relaxin 2 wk prior to parturition may prove to be a useful indicator of pending parturition.

Information generated by employing reproductive research and technology to overcome the challenges of Sumatran rhino reproduction clearly paved the way for developing a successful managed breeding program for this species at the Cincinnati Zoo. It also was critically important for detecting and overcoming the recurrent early pregnancy loss experienced by this rhino. The data will be invaluable in efforts to breed the last nine female Sumatran rhinoceros surviving in captivity. Some of the technology already has been transferred and adopted by Malaysian colleagues who are now achieving success in mating their rhinoceros, but to-date, pregnancy has alluded them.

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