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## DEVELOPMENT OF TECHNIQUES FOR SUCCESSFUL ARTIFICIAL INSEMINATION IN THE INDIAN RHINOCEROS (*Rhinoceros unicornis*)

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### **Abstract**

Assisted reproductive techniques developed in the Indian rhinoceros have resulted in the first pregnancy established from artificial insemination (AI) with frozen-thawed spermatozoa. Semen was collected by electroejaculation from anesthetized males and cryopreserved in equine extender (EQ) with glycerol or dimethyl sulfoxide (DMSO). Estrous cycles were monitored through Doppler ultrasonography, urinary estrogen conjugates (E<sub>1</sub>C), progesterone (P<sub>4</sub>) concentrations and behavioral observations. Animal conditioning permitted inseminations without anesthetics, and custom-made equipment enabled intrauterine semen deposition. Two AI techniques were developed, one involving manual insertion of the insemination rod and the other utilizing endoscopy. The method chosen depended on the tolerance of the individual females to vaginal manipulations. Both females conceived following AI, but one pregnancy ended in early embryonic loss. The established pregnancy occurred in a 15-yr-old, nulliparous cow. Insemination timing was based on ultrasound evidence of a preovulatory follicle measuring 13 cm in diameter, urinary E<sub>1</sub>C concentrations above baseline for 12 days and a pre-ovulatory spike in urinary P<sub>4</sub> concentrations. Two inseminations of 8 ml each were performed via the manual insertion method at 72 and 48 hr prior to ovulation. Ultrasonographic imaging verified semen deposition directly in the uterine body and uterine horn ipsilateral to the preovulatory follicle. Post-thaw sperm motility averaged 55% and mean total motile sperm per inseminate was  $620 \times 10^6$ . Pregnancy was diagnosed by rectal ultrasound 18 days post-ovulation. These proven techniques could facilitate gamete exchange between disparate populations, surmount behavioral incompatibility between rhinoceros pairs and effectively utilize sperm banks for enhancing genetic diversity in endangered rhinoceroses.