

Successful Electroejaculation and Other Semen Procurement Methods from four Species of Rhinoceros (*Ceratotherium simum*, *Diceros bicornis*, *Rhinoceros unicornis*, *Dicerorhinus sumatrensis*)

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Several semen procurement techniques, including penile massage, rectal massage, artificial vagina, and rectal probe electroejaculation were applied to four rhinoceros species. Penile massage, rectal massage, and artificial vaginas were applied to cognizant animals; rectal probe electroejaculation was applied to anesthetized animals. In some instances, multiple procedures were applied to individual animals. Success of these procedures was based on whether or not sperm was acquired. Penile massage was the direct manual massage of the penis. Rectal massage was manual manipulation of the accessory glands through the rectal wall. Artificial vaginas were applied to the erect penis during penile massage. Rectal probe electroejaculation utilized an ultrasound probe incorporated into a custom engineered electrical probe (Schaffer et al. 1998). These procurement techniques were applied to a total of 21 animals. Penile massage was successful for all 5 black, all 5 white, 2 of 4 Greater One-horned Asian and 3 of 5 Sumatran rhinoceroses. However, only two animals, one black and one Indian, became trained to reliably produce samples with sperm. Rectal massage facilitated other methods, but was unsuccessful when used alone on 2 black, 1 white, 2 Greater One-horned Asian and 2 Sumatran rhinoceroses. An artificial vagina was applied to 2 black, 1 white, 2 Greater One-horned Asian and 1 Sumatran rhinoceroses, but was effective in only the black rhino that had been successfully trained for penile massage. Rectal probe electroejaculation was applied successfully to 2 black, 2 white and 1 Greater One-horned Asian. Seminal fluid was successfully collected in all 12 attempts to electroejaculate these 5 animals. Sperm was not present, however, in the fluids of one attempt in the Greater One-horned Asian. Rectal probe electroejaculation proved the most repeatable and reliable technique in producing seminal fluid with sperm.

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An IRF/SOS Rhino Sponsored Integrated Approach for the Enhancement of Reproductive Performance of White Rhinoceroses in the EEP

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The goal of this IRF-SOS Rhino funded multi-disciplinary, multi-institutional research is to work on possible solutions to overcome the reproductive problems occurring in the captive white rhinoceros population. Until recently these problems were summarized as 1) acyclicity and variable estrous cycle lengths of 35 or 70 days, 2) mating failure due to acyclicity, or silent estrus due to sibling relationship/mate choice problems and 3) conception - pregnancy failure due to presumed uterine pathology. Reasons for these problems are not definitively identified, but our results suggest that in addition to the problems summarized above, male infertility and ovarian pathology are severe additional problems and causes for the low reproductive rate of captive white rhinoceroses.

Our combined approach works to overcome these problems; the techniques used include: 1) endocrine monitoring; 2) transfer of animals to enhance natural breeding; 3) clinical examinations of reproductive soundness in male and female white rhinos and 4) development of artificial insemination (AI). In addition, work on conditioning 2 males for manual semen collection is currently underway.

The goals of this project are to provide management recommendations, which will help to increase the reproductive rate of the captive population. In order to achieve this goal research into the causes for the low reproductive rate is necessary and thus currently the major focus is to increase our reference data basis of male and female reproductive biology and pathology. Particular focus is being placed on the old non-reproducing animals in order to conserve their genetic potential and to be able to better manage white rhinos in the future. Generation of the presently available data was only possible by combining the techniques of endocrine monitoring with ultrasonographic examinations and the development of a reliable anesthesia protocol. Although work is still in progress, currently available results make the white rhino EEP one of the most progressive due to direct inclusion of research results into management decisions.

Females with missing ovarian cycles are a major cause for the low reproductive rate of the captive white rhinoceros population. Ultrasound examinations in combination with endocrine monitoring revealed several reasons for the state of acyclicity. The two major groups are 1) females with no luteal activity (true flatliners) and 2) females with persistent luteal activity. In adult animals the reasons for acyclicity are either small cysts, 'micro

corpora lutea' or inactive, small ovaries. Females which had persistent luteal activity over more than 6 month had cysts of up to 10 cm in diameter and severe uterine pathologies, causing infertility. In addition to female related infertility, infertile or sub-fertile males with inadequate ejaculate quality are a major cause of the low reproductive performance in captive white rhinoceroses.

Besides the research already carried out in this project, our future efforts to overcome reproductive problems will include behavioral research and research project in *in situ* populations (i.e. on game ranches in Africa). However, it is strongly emphasized that it is essential that the future research is carried out in combination with endocrine and ultrasonographic examinations.

Artificial inseminations (AI) were performed in reproductively sound animals, which are in a non-breeding situation due to missing ovarian cyclicity and/or diminished male fertility. Different protocols for ovulation induction were used and seven AI attempts have been performed. AIs were performed in three animals after ovulation induction and in two other animals during natural estrus. Results demonstrate that a reliable ovulation protocol and an AI technique suitable to overcome difficult anatomical structures of the female genital tract is available, however, attempts have not resulted in pregnancy so far (results still pending in one animal).

Conclusions

1. The combination of endocrine monitoring with ultrasonographic examinations of the entire female reproductive tracts under anesthesia provided information which neither of these techniques on its own would have been able to supply.
2. The combination of Detomidine, Buthorphanol, followed by Etorphine and i.v. Ketamine proved to be a reliable anesthesia protocol for white rhinoceroses.
3. Semen collection by electroejaculation proved to be a reliable technique; ejaculates gathered by this method were used for cryopreservation and results suggest that post thaw quality is sufficient for artificial insemination.
- 4) In addition to female related infertility, infertile or subfertile males with inadequate ejaculate quality are a major cause of the low reproductive performance in captive white rhinoceroses.
- 5) Acyclicity is a major cause of infertility in female white rhinoceroses. Acyclic females consist of two groups, a) females with no luteal activity (flatliners) and b) females with persistent luteal activity. Ultrasound examinations revealed several causes for flatliners; in adult females the causes are either small ovarian cysts, 'micro corpora lutea' or inactive and small ovaries. In contrast to the small ovarian cysts in 'flatliners', females with persistent luteal activity had cysts of up to 10 cm in diameter; the permanent estrogenic state of these large cysts cause severe uterine pathologies resulting in infertility.
- 6) Uterine pathologies were identified in about 55% of the females investigated; about half of these animals (27.5 %) were classified post reproductive due to severity of pathologies, whereas the other 27.5 % were still considered to be fertile beside mild pathologies were present. Females which do not bred before the age of 12 - 15 y, are likely to develop uterine pathologies. In contrast, these pathologies were not seen in old (>25 y) proven breeders.
- 7) A reliable technique for artificial insemination and for the intra-uterine placement of sperm is available.

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