Recent Research on Elephants and Rhinos

### Utero – Ovarian Pathological Complex of the Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

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Sumatran rhinoceros complicated reproductive biology has contributed to the failure of this species to flourish in captive breeding facilities. Physiological failures have included absence of intromission, intromission lacking conception and reabsorption. Twenty-two females were in captivity since 1984. Of the seventeen examined at least 50% exhibited reproductive tract pathology. Uterine tumors and cysts were also noted with ultrasonography. Leiomyoma and cystic endometrial hyperplasia was reported postmortem. One female exhibited 8-year-long galactorrhea and another an unusual occurrence of multiple corpora lutea on the ovaries and an enlarged uterus. Uterine pathology appears to begin after 10 years of age, assuming animals were mature when captured. The pathology becomes prevalent in animals over 15 years old. Of the remaining ten females in captivity, eight are copulating of these three have no pathology, and the other five are copulating with mild or significant pathology. Though these animals are reproductively active this pathology may interfere with conception. The etiology of these pathologies needs further investigation.

# Influence of Alpha-2-Agonists on Manual Semen Collection in a Standing White Rhinoceros (*Ceratotherium simum*)

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#### Abstract

Reproductive technologies, such as artificial insemination, require routine methods of semen collection. The common technique to obtain semen samples from rhinoceros is electroejaculation. The objective of this study was to develop a less invasive procedure without the necessity of anaesthesia in order to perform repeated deseminations. Based on successful pharmacologically induced *ex copula* ejaculations in stallions using alpha-2-agonists (MCDONNELL and ODIAN, 1994; TURNER et al., 1995) a study with two white rhino bulls (age 20 and 30 years) was carried out at Salzburg Zoo Hellbrunn. We used Detomidine-Hydrochloride (*Domosedan*, Pfizer Corporation Austria Ges.m.b.H., A-1071 Wien) and a combination of Detomidine-Hydrochloride and Butorphanol (*Butomidor*, Richter Pharma AG, A-4600 Wels) applied IM. The preliminary results presented here are based on our experiences with the older male white rhino.

The study was undertaken in the new rhino housing facility at Salzburg Zoo Hellbrunn with a thirty years old male white rhinoceros. The rhino was conditioned to enter a restraint chute and to tolerate manual stimulation of penis and preputium. The indoor restraint chute enables easy access to the genital region of the white rhino while providing the highest standard of safety for the operating person. Various dosage-related protocols using Detomidine-Hydrochloride and a combination of Detomidine-Hydrochloride and Butorphanol applied I.M., as well as non-medicated trials, were performed at an average of three times per week over a period of three months. Prior to I.M. injection, dermal anaesthesia was achieved by applying an eutectic mixture of local anaesthetics (*Emla*, 5%- Creme, Astra Ges.m.b.H., A-4020 Linz). In order to maintain a strong erection and to induce the ejaculatory reflex, manual stimulation of the preputium and penis was performed similar to descriptions by SCHAFFER et al. (1990) and WALZER et al. (2000). Immediately after each session, a questionnaire scaling several objective and subjective parameters was answered. The scaling of the subjective parameters occured separately by the keeper and by the first author.

Analysis of the questionnaire allowed a comparison of non medicated manual stimulation and medicated penile stimulation using Detomidine-Hydrochloride and Detomidine-Hydrochloride combined with Butorphanol.

Referring to the scaled subjective parameters, neither aggression, fear, avoidence behaviour nor heightened nervousness were noted at any time during the study. A superior

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degree of erection was achieved using medication. A positive trend could be demonstrated when applying Detomidine-Hydrochloride. The combined medication lead to a significant increase in the erection strength (p<0,001; CHI<sup>2</sup>-Test), figure 1.



#### Figure 1:

Frequency of the different erection categories\* achieved by non medicated manual stimulation in comparison to manual stimulation with combined medication.

\*(The parameter "erection strength" was classified into 5 categories: Category 1: No erection induced by manual stimulation. Categories 2 to 5: Induction of different degrees of erection strength, 5 as the maximum degree possible.)

Analysis of the objective parameters demonstrated that the white rhino entered the chute immediately in most cases. The duration of manual stimulation, the number of pulsatile contractions of penile muscles and the volume of obtained fluids showed a broad variation. The onset of the penile prolapse and subsequent erection was significantly shorter (p<0.001, Mann-Whitney-U-Test) when applying Detomidine-Hydrochloride and Butorphanol, (see figure 2), and was induced by the medication without the need of additional manual stimulation. The duration of crection was significantly longer when Detomidine-Hydrochloride and Butorphanol were used in combination as compared to the non medicated trials (p<0.05, Mann-Whitney-U-Test), figure 3.

Seminal fluids (mean: 4,36; ranging from 0,2ml to 40ml) could be collected in 15 out of 60 attemps. Semen was only obtained in six of sixty trials using alpha-2-agonists and revealed poor concentration (< 10000/mm3). However only few malformations (5%) and a high motility and vitalitiy (75% to 80%) were noted.



#### Figure 2:

Onset of penile prolapse and subsequent erection after beginning with manual stimulation in non medicated and medicated trials using the combined medication





#### Figure 3:

Duration of penile erection induced by non medicated manual stimulation and by manual stimulation with application of combined medication.

To date, a routine collection of semen samples for cryopreservation trials and artificial insemination in white rhinos using manual penile stimulation could not be established with this study.

*Ex copula* collection of fluids in a standing white rhinoceros containing semen could be induced by application of Detomidine-Hydrochloride, Butorphanol and additional manual stimulation, but not regularly and predictably. This is similar to other researchers (HERMES at al., 2001).

The positive influence of Detomidine-Hydrochloride applied in combination with Butorphanol on the onset, strength and duration of penile erection could serve as a basis for further investigations including different temperature and pressure stimuli on the penis in order to induce the complete ejaculatory reflex.

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## Genetic Kinship and Social Structure in White Rhinos at the Zoological Center Tel Aviv Ramat Gan (Safari)

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The decline in reproductive rate of rhinos in zoos around the world, including in the F1 generation has been identified as a major concern affecting the future wellbeing of these herds. The major factors, which may contribute to reproductive decline, include ageing, behavioural-incompatibility of individual males and females, behavioural incompetence, genetic- inbreeding depression, hormonal or reproductive tract dysfunction or dietary aspects.(Patton et al 1998).

The Zoological Center Tel Aviv Ramat Gan (Safari) is now grappling with this problem in its herd of white rhinos.

The white rhino herd at the Safari in Ramat Gan originated as a group of 4.4 imported from South Africa to Israel in 1973 at the estimated age of two -three years. Between 1978-1996, 20 rhinos were born in the park. Births ceased in 1996, although copulations continue. The herd structure currently consists of 5 males and 6 females of which four males and 3 females were born in the park. The rhinos in the Safari are managed as a free-ranging multi-male herd on an area of about 0.7 km2. The animals are not separated at any time; therefore paternity cannot be known with certainty from behavioural observations.

This study sets out to determine the genetic parentage and establish the current social behaviour of the population. It is part of a larger study to analyze the associated with reproductive decline to help make intelligent management decisions for white rhinos in the safari park.

#### Methods

#### Molecular analysis

Genomic DNA was extracted from hair follicles as described by Ausabel (1987) with some modifications. Primers were obtained from UBC (University of British Columbia Biotechnology Lab) Primer Synthesis Project, set 100/4 and 100/2 for PCR amplification.

#### **RAPD** procedure

The accepted criterion for ascribing paternity to a male candidate is a t least 3 bands of the RAPD pattern (using three different primers) are found in the pattern of the male but not in the mother's (Neveau et al 1996).



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