MANAGEMENT TOOLS FOR THE IMPROVEMENT OF REPRODUCTION IN RHINOCEROSES AT BERLIN ZOO

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Summary

This paper gives a general view of examinations and measures taken at Berlin Zoo to enhance natural and artificial breeding efforts in different rhino species. Altogether 8 anaesthesias are described which were executed in a White-, an Indian, and 3 Black rhinoceroses.
Semen was collected by means of electroejaculation from an aged Black and White rhinoceros. One female Black rhino was artificially inseminated. In a female Indian and Black rhinoceros pathological changes in the genital tract were observed by means of ultrasonographic examination and therapeutic measures taken.

Zusammenfassung

Die Arbeit gibt einen Überblick über die in den letzten Jahren im Zoo Berlin durchgeführten Untersuchungen und Maßnahmen zur Unterstützung der natürlichen bzw. künstlichen Zucht der verschiedenen Rhinozerosarten. Insgesamt wurden acht Anästhesien bei einem Breitmaulnashorn, einem Panzernashorn und drei Spitzmaulnashörnern im Zoo Berlin durchgeführt, die in dieser Arbeit protokolliert sind.
Bei einem älteren Spitzmaul- und einem Breitmaulnashorn-Bullen wurde durch Elektroejakulation erfolgreich Samen gewonnen. Ein weibliches Spitzmaulnashorn wurde künstlich besamt. Bei einem weiblichen Panzer- und einem Spitzmaulnashorn wurden durch Ultraschalluntersuchung pathologische Veränderungen am Geschlechtsapparat festgestellt und eine entsprechende Therapie durchgeführt.

Resumé

Ce document presente l'ensemble des examens effectués et des mesures prises au Zoo de Berlin pour contribuer à la reproduction naturelle et artificielle des différentes espèces de rhinocéros. Un total de 8 anesthésies, pratiquées chez, un rhinocéros blanc, un rhinocéros indien, et 3 rhinocéros noirs sont décrites.
Du sperme d'un rhinocéros noir et d'un blanc fut recolté par électro-éjaculation. Une femelle rhinocéros noire fut inséminée. Chez deux femelles de rhinocéros indien et d'une rhinoceros noir, des altérations pathologiques du tractus génital ont pu être observées par ultrasonographie et des mesures thérapeutiques prises en consequence.

Key words
Black rhinoceros, White rhinoceros, Indian rhinoceros, anaesthesia, electroejaculation, ultrasonography, artificial insemination.

Introduction

In-situ and ex-situ situation of the rhinoceros

All 5 rhinoceros species for decades are listed to be highly endangered. Particularly threatened from extinction is the Javan rhinoceros (*Rhinoceros sundaicus*) with an estimated 50-60 individuals, the Sumatran rhinoceros (*Diceros sumatrensis*) with ~300 individuals, and the Northern White rhinoceros (*Ceratotherium simum cottoni*) with 25 individuals left in the wild (table 1):

Species	Subspecies	Wild	Population Captive source: *
		Global Studbook at July 1999	
AFRICAN rhinoceros population			
B L A C K rhino	Southwestern	740	0
	Northwestern	10 ~	0
	Eastern	485	65
	Southern	1.365	170
	total Black rhinos	2.600	235
W H I T E rhino	Southern	8.440	704
	Northern	25	9
	total White rhinos	8.465	713
TOTAL African population		**11.065**	**948**
** The Global Studbook for African Rhinoceroses is kept at Berlin Zoo since 1966*			
ASIAN rhinoceros population			
	Indian/Nepalese	2.520	136
	Indonesian Javan	50-60	0
	Vietnamese Javan	5-8	0
	Eastern Sumatran < Borneo >	50 ~	3
	Western Sumatran < Sumatra/Malaya >	250 ~	14
TOTAL Asian population		**2.880 ~**	**150**

Table 1:
Wild and captive living populations of rhinoceros at July 1999. Source: IUCN/SSC African & Asian Specialist Groups

Other rhino species and subspecies with self-sustaining wild populations in some countries have successfully been protected, however, poaching and political instability still represents a potential threat to their future survival. Reproduction in most captive rhino species is low or has as yet never occurred such as in the Javan rhinoceros (8). Reproduction management and today's assisted reproduction techniques will greatly enhance efforts to establish self-sustaining captive populations. The cause for this stagnation or insufficient reproduction of captive populations varies from species to species. In general the percentage of losses caused by accidental death, traumata, infectious diseases in subadult individuals, or false nutrition has been relatively high. The current captive White rhinos' age structure reveals that the majority is rather advanced in years. All the same, a large number is kept in non-reproductive pair situations. Breeding is mainly seen in lager groups, although exceptions are recorded (pair keeping in Poland).
The age distribution in the captive Black rhinoceros seems more satisfactory, however, it is to be noted that the adult male population is too low in number and the mortality in young individuals rather high.
In the captive Indian rhino a main issue is the high percentage of problems of the plantar surface of the foot, endocrine disorders in females or suspected development of leiomyomas in the female genital tract (2).

One aspect of research on the reproduction biology in rhinoceroses has been the development of techniques for semen collection and endocrine monitoring. Electroejaculation in immobilised male individuals, subsequent semen evaluation, processing, and preservation has been reported (6, 7, 9). It has been reported that also manual penis massage to stimulate ejaculation in the Black rhinoceros is a successful method to collect semen (10, 11). As this requires a close animal/keeper relationship and sufficient facility space this technique will remain limited to only few institutions.
Since 1990 faecal progesterone analysis in females allows the determination and monitoring of the oestrus cycle (1, 5, 13, 14).
Recent progress in the research on the reproduction biology and mainly the evaluation of the soundness of breeding as well as artificial insemination in elephants has been tremendous (3, 4). As a consequence management decisions are now based on hard physiological data of the individual. Through regular collection of semen in a number of African elephants sperm preservation techniques were established. The most recent progress is the successful artificial insemination of 4 individuals (12).

Material and Methods
Over the past 10 years Berlin Zoo has increasingly applied modern reproduction management techniques to meet the afore mentioned reproduction problems in rhinos and to actively improve the management of captive breeding of the different rhino species.

This paper summarises the efforts made to enhance natural and artificial breeding in 3 rhino species at Berlin Zoo:
1) Development of protocols for safe standard anaesthesia.
2) Ultrasonography, electroejaculation, collection, processing and cryopreservation of semen from male rhinos.
3) Assessment of females by means of ultrasonography.
4) Attempt of artificial insemination.

1) Development of protocols for safe standard anaesthesia.

For reproductive management procedures 8 anaesthesias have been performed in Black, White, and Indian rhinoceroses at Berlin Zoo between 1998 and 1999. Standard protocols were established and are summarised in table 2.

Species/ Gender/ House Name	Age in.ys	Initial drug / dosage Immobilon Revivon ml i.m.	Yohimbin mg i.m.	Xylazin mg i.m.	Extension Ketamin	Antidote ml i.v	ml i.v.
BLACK rhino							
Male Kibo	34	1,3	40	40	2 x 0,5 ml Immobilon i.m.	2,6	4,0
Male Kibo	35	1,3	40	40	1 x 0,5 ml Immobilon i.m	2,6	4,0
Female Sita	9	1,3	40	40		2,6	3,0
BLACK rhino							
Female Ine	13	1,3	30	30	after 40 minutes: 0,5 mlImmobilon i.m.	3,0	4,0
WHITE rhino							
Male Hlambamans	40	1,3	30	30	after 15 minutes:	4,6	5,0
			0,4 ml	Immobilon plus			
			250 mg	Ketamin i.v. (ear) while still on its feet 1 h later: 0,6 ml Immobilon i.m.			
Male Hlambamans	40	1,5	40	40	after 40 minutes: 0,4 ml Immobilon i.m.	4,0	4,0

Table 2:
ANAESTHESIA protocol for rhinoceroses at Berlin Zoo between 1998 and 1999

Table 2: (continued)

Species/ Gender/ House Name	Age in.ys	Initialdrug/ dosage Immobilon ml i.m.	Xylazin mg i.m.	Extension Ketamin mg i.m.	Antidote Revivon ml i.v	Yohimbin ml i.v.
I N D I A N rhino						
Female Narayani	14	1,5	50	50	3,0	6,0
Female Narayani	14	1,5	50	50	3,0	5,0

Table 2: (continued)
ANAESTHESIA protocol for rhinoceroses at Berlin Zoo between 1998 and 1999

2) Ultrasonography, electroejaculation, collection, processing and cryopreservation of semen from male rhinos.

At Berlin Zoo indication for the employment of assisted reproduction techniques such as ultrasonography and electroejaculation was given in 2 rather aged male rhinoceroses. The Black (wild born) rhinoceros (Kibo) for 3 years had shown chronic hind leg lameness with progressive inability to mate. Whereas the (wild born) male White rhino (Hlambamans) for approximately 29 years had lived in a nonreproductive pair situation when in 1992, after the death of the (wild caught) female, this male began showing a rather remarkable increase of activity. The medical and historic indicators of the 2 individuals were motive to execute ultrasonographic examinations of the genital tract, electroejaculation, and semen analysis for evaluation of their reproductive state. The ultrasonographic visualisation characterised the in-situ topography and showed a fully developed breeding state of their urogenital organs. Since the evaluation of the ultrasonic examination of the genital anatomy proved fundamental in the White rhino, globally the first successful electroejaculation was performed in this species. At a total of 4 operations viable semen was collected from both animals. The freshly collected semen from the White rhinoceros was used in 2 artificial insemination attempts in Salzburg (Austria) and Schwerin (Germany). As the Black rhino had shown a rapid decline in general health, for the preservation of his genetic material the semen was cryopreserved over a period of 12 respectively 6 months for future artificial insemination attempts.

3) Assessment of females by means of ultrasonography.

The reproductive soundness of 1 female Indian and 2 female Black rhinos was evaluated through transrectal ultrasonography.

In the Indian rhino individual regular oestrus and copulation in 1990 was seen and became subject to suspect pregnancy. With the missing of oestrus since then, a number of possibilities presented themselves regarding the failure of reproduction. For this reason transrectal ultrasonic examination was executed to detect the cause for the absence of oestrus. In the uterus was found a fluid filled cavity (21 cm in diameter) with high echogenic reflections which suggested a retained pregnancy. In addition, follicular cysts were found on both ovaries. The treatment with Prostaglandin F_{2a} (Iliren®, Hoechst Roussel) resulted in extreme nervousness and excitement for 2 hours. The therapeutic result of this treatment was evaluated through a second ultrasonic examination 17 days later when the uterus proved to be free from the previously imaged echogenic fluid cavity. No discharge was to be observed since treatment. On one ovary merely one cyst of 6 cm in diameter remained. However, due

to the reduced size and thus improvement for imaging the uterus, an additional leiomyoma was diagnosed in the uterine horn.

The aim of the assessment of the 2 Black rhinoceroses was to determine viable candidates for artificial insemination. Ultimate goal was the utilisation of the genetic material of the previously mentioned male (proven breeder) which by that time had become incapable of mounting.
In one of the females (Sita) of prime breeding age with longterm aggressive behaviour and suspected silent heat vaginoscopy and ultrasonic examination was executed. Monitoring revealed multiple ovarian cystic structures on both ovaries, multiple endometrial cysts, and a necrotic vestibulo-vaginitis. Douche was done of the vagina and vestibule with 500 ml Lotagen solution (4%) under endoscopic guidance after a diagnostic biopsy had been taken. Treatment with the antibiotic Verazin compositum® (Albrecht), a longterm Penicillin/Streptomycin-combination over a period of 6 days was based on the identification of *Staphylococcus* and *Micrococcus* in the cultured material.

4) Attempt of artificial insemination.

The second candidate for artificial insemination was a 13 year old cow (Ine) (proven breeder). The accurate timing of a forthcoming ovulation was based on reproductive-cycle-related changes in behaviour (15) and on the observation of morphological changes of the external genital tract. Measurement of the vulva and clitoris showed a minor increase in size over a period of 72 hours after vaginal discharge had been observed. The ultrasonographic viewing of one dominant follicle (2,7 cm) on one ovary validated behavioural and morphological indicators for a forthcoming ovulation. Intrauterine insemination with preserved (thawed) semen was performed under endoscopic and ultrasonographic guidance. 20 ml Receptal® (GnRH Analogue, Hoechst Roussel) was administered to support maturation and ovulation.

Discussion

In view of the persisting political instability and the problem of poaching in the countries of origin as well as the low breeding rate in the captive population, the survival of the species by no means may be called safe. Modern technology for assisted reproduction seems a reasonable tool to further illuminate reproductive problems in the captive population and to enhance the chance for natural breeding and artificial insemination.
The threatened situation of the 5 remaining rhino species in-situ and ex-situ practically demands the employment of modern technologies of assisted reproduction. A major requirement for the application of methods such as electroejaculation, ultrasonic examination, and artificial insemination is to establish standard anaesthesia protocols.

The 8 anaesthesias executed at Berlin Zoo in the last 2 years in Black, White, and Indian rhinos proved to pose no problem with the drugs and dosages applied (table 2), although some of the individuals were of rather old age. However, it became apparent that with the application of almost the same dosage reactions varied perceptibly in the different animals. Relatively often, 15 or 20 minutes after the initial dosage an extension had to be applied to the still standing or sitting animal either intramuscular or intravenously in the ear. The initial dose was applied intramuscular between the creases of the skin on the side of the neck by means of injection pistol with a 6 to 8 cm long hypodermic needle. The male White rhino after the second anaesthesia had to be assisted in getting back on his feet by rolling him on his chest. For the first time the forthcoming of an ovulation and its symptoms such as changes in behaviour, swelling of the vulva, and vaginal discharge - which was only seen in the female Black rhino Ine - was confirmed through ultrasonographic observation of the ovaries. Whether or not the therapeutic measures taken on the ground of the sonographic examination in the female Black rhino Sita and the Indian rhino Narayani may be called successful remains to be seen.

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