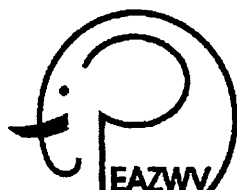


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## DEVELOPMENT OF AN ARTIFICIAL INSEMINATION TECHNIQUE IN THE AFRICAN RHINOCEROS

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### Summary

The status of the wild white and black rhinoceros populations and poor reproductive success in captivity emphasizes the critical need for advanced knowledge of these species' reproductive biology and the development of assisted reproduction techniques. Anatomical (n=2) and ultrasonographical (n=15) studies of the reproductive tract in vivo in the black and white rhinoceros led to the development and application of an artificial insemination (AI) technique adapted to the rhinoceros' anatomy. The Application of this innovative catheter system resulted for the first time in the intra-cervical and intra-uterine insemination during so far five AI attempts in 0.3 white and 0.1 black rhinoceros. The accurate positioning of the designed catheter system during the procedure was guided by endoscopy and ultrasonography. The development of an efficient AI technique for the African rhinoceros seems imperative to enhance genetic diversity and limited captive breeding success. Successful AI in these species may serve as model to other endangered rhinoceros species.

### Zusammenfassung

Der unsichere Status der Wildbestände sowie der eingeschränkte Zuchterfolg von in Menschenhand gehaltenen Breit- und Spitzmaulnashörnern begründet die Notwendigkeit, das noch immer limitiertes Wissen über die Fortpflanzungsbiologie zu erweitern sowie Massnahmen der assistierten Reproduktion bei diesen Spezies zu entwickeln. Post mortale (n=2) und sonographische Studien des Geschlechtsapparates in vivo (n=15) beim Breit- und Spitzmaulnashorn bildeten die Grundlage für die Entwicklung und Anwendung eines speziell an die Anatomie des Nashorns angepassten Besamungsbestecks. Durch den Einsatz dieses innovativen Kathetersystems gelang erstmals bei bisher fünf Besamungsversuchen bei 0.3 Breit- und 0.1 Spitzmaulnashörnern eine intra-zervikale bzw. intra-uterine Insemination. Die Positionierung des Besamungsbestecks wurde dabei unter endoskopischer und ultrasonographischer Darstellung vorgenommen. Die Entwicklung einer effizienten künstlichen Besamungsmethodik bei Afrikanischen Nashörnern trägt zu einer verbesserten Reproduktion und genetischen Diversität in Menschenhand gehaltener Populationen bei. Eine erfolgreiche künstliche Besamung kann auf andere ebenfalls stark vom Aussterben bedrohte Nashornarten modellhaft übertragen werden.

### Résumé

Le statut des populations de rhinocéros blanc et noir dans la nature et le faible taux de reproduction en captivité, soulignent la nécessité absolue d'une meilleure connaissance

de la biologie de reproduction de cette espèce et du développement des techniques de reproduction assistée. Des études in vivo de l'anatomie (n=2) et l'ultrasonographie (n=15) du système reproducteur des rhinocéros noir et blanc, ont conduit au développement et à l'emploi d'une insémination artificielle (IA) adaptée à l'anatomie des rhinocéros. L'utilisation d'un cathéter développé spécialement dans ce but, a permis pour la première fois une insémination intra-cervicale et intra-utérine lors d'essais d'IA chez 0.3 rhinocéros blanc et 0.1 -noir. Le bon positionnement du cathéter pendant la procédure était contrôlé par endoscopie et ultrasonographie. Le développement d'une technique efficace d'IA pour le rhinocéros africain semble indispensable pour augmenter la diversité génétique et améliorer la reproduction en captivité. Le succès de l'IA chez cette espèce pourrait être un modèle pour les autres espèces menacées de rhinocéros.

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## Keywords

African rhinoceroses, ultrasonography, endoscopy, artificial insemination

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## Introduction

Extensive population declines in the wild (black (*Diceros bicornis*), greater one-horned (*Rhinoceros unicornis*), Sumatran (*Dicerorhinus sumatrensis*), and Javan (*R. sondaicus*) rhinoceros) has brought most rhinoceros species on the verge of extinction (6). The southern white rhinoceros (*C. s. simum*), recently downlisted to threatened, still suffers from limited distribution and increasing threats from poachers. The northern white rhinoceros subspecies (*C. s. cottoni*), is considered the rarest rhinoceros taxon in the world. The status of the wild white and black rhinoceros populations and poor reproductive success in captivity emphasizes the critical need for advanced knowledge of these species' reproductive biology and intensified reproductive assessment of captive populations. The development of assisted reproduction techniques such as artificial insemination (AI) and safe, reliable semen collection procedures (3, 5, 7, 8) might improve opportunities to overcome long-present difficulties in breeding rhinoceros in captivity (4).

## Methods

Anatomical features (1) relevant for the development of an artificial insemination technique were evaluated in two post mortem preparations of a black and a white rhinoceros (nullipara, 4 / 9 years) and in vivo during 15 transrectal ultrasound examinations (Hitachi, EUB 405, 3.5 MHz, / 5.0 MHz; ATL, HDI 1000, 2 - 4 MHz, 4 -7 MHz), which has been accustomed to the rhinoceros anatomy with as-shaped steel extension. Five artificial breeding attempts (0.3 white and 0.1 black rhinoceros) have been performed so far. AI attempts were performed on immobilised animals in lateral recumbency. Videochip-endoscopy was used to visualise the vestibule, the vagina and the portio cervicalis under sterile conditions (Pentax, EPM-300, EL 380 IM, Sony, monitor, PVM-2130 QM). Based on the anatomical studies a special insemination catheter was designed and patented (Fa. Schnorrenberg, Chirurgiemechnik, Woltersdorf, Germany) to achieve a semen deposition deep into the genital tract.

### Female reproductive anatomy:

#### Relevant aspects for the development of assisted reproduction techniques (ART)

Detailed studies on the reproductive tract anatomy and identification of anatomical structures relevant for intra-cervical or intra-uterine semen deposition were necessary for the development of an AI technique specifically adapted to the rhinoceros. The two main anatomical obstacles for an assisted reproduction in the female rhinoceroses were the hymenal structure in nulliparous animals and the very tortuous cervix. Clinical palpation of the caudal urogenital canal revealed that four out five nulliparous females examined still had an intact hymenal structure, thus all females were in breeding situations for a long time. Post mortem preparations and in situ endoscopy illustrated the hymenal structure 10 -18 cm from the urogenital tract opening dorsocranial to the urethral orifice. Three openings were identified in this hymenal membrane. Two of these openings with a diameter of 0.2 - 0.3 mm ended as blind pouches presumably representing rudimentary Wolf' ducts. Only the third opening represented the vestibular - vaginal orifice leading to the vagina. With a diameter of 0.3 -0.4 mm this opening was slightly larger than the blind ducts and after a distance of approx. 2 -3 mm divided by a septum. A passage into

the vagina was therefore only possible through one of the three documented hymenal openings. In uniparous (n=2) and one nulliparous female examined only remnants of the hymen were palpated and access to the cervical Os was given at a distance of 30 - 45 cm from the urogenital opening. The cervix, the second obstacle for AI was characterised by highly convoluted folds of dense fibrous connective tissue. The external cervical Os at the cranial aspect of the vagina had a rosette folded appearance with a prominent small uvula covering the opening of the cervical canal. The length of the cervix ranging from 12 - 20 cm and 90° turns of the cervical canal dictated the material, the length, the diameter and the tip-angle of the specially designed insemination catheter.

## AI procedure

Transrectal ultrasound evaluated the in situ position, dimensions and morphology of the of the vagina, cervix and uterus prior to the insertion of the catheter system. Under sterile conditions a semi-rigid speculum dilated caudal urogenital canal. The previously palpated hymenal structure in nulliparous insemination candidates (n=3) was visualised 10 -18 cm from the external opening by video-chip-endoscopy. In these nulliparous females a mandrin as well as flexible and rigid insemination cathetersystem was used for insemination. The three fold structure of the vestibular-vaginal orifice was identified in a cranio-dorsal orientation. Under endoscopic guidance the rigid catheter was inserted through the hymenal opening. Ultrasound evaluated the correct intra-cervical or intra-uterine positioning of the rigid catheter, before a flexible insemination catheter was advanced and monitored the semen deposition deep into the genital tract.

## Discussion

To date, AI attempts in the rhinoceros have been very limited due to the complexity of such procedures. Thanks to a collaborative effort of many individuals and institutions combined expertise on endocrine activity, reliable anaesthesia protocols, the evaluation of the reproductive soundness of AI candidates and prediction of ovulation have been prerequisites to so far five AI attempts (2, 9, Walzer, unpublished data). Problems associated with the low rate of reproduction especially among captive-born female Southern white rhinoceros, world wide only 8% of the F1 population has reproduced successfully, are of great concern (4). The successful development of an AI technique in one rhinoceros species might be a future key to enhance conservation efforts in other African or Asian rhinoceros species, amplifying genetic diversity and breeding success in captive and wild populations.

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