## Semen Collection, Sperm Assessment and Cryo-Preservation in African Rhinoceroses

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The captive white rhinoceros population currently faces a demographic crisis. As a consequence substantial knowledge on reproductive biology of the female white rhinoceros has been gathered over the past years. However, little emphasis has been put on the evaluation of male fertility as a possible contributing factor to the low rate of reproduction. In the present study the reproductive fitness of ten male white and one black rhinoceros was evaluated by ultrasonography and semen assessment. Semen collection was obtained by manual stimulation (n=2) and electro-stimulation (n=9). Based on 39 semen assessment results seven males were identified as reliable semen donors. Preserved semen samples remained viable for up to four days. Cryopreserved samples showed post thaw motility suitable for assisted reproduction. Reproductive assessment provided accurate information on the breeding potential of male white rhinoceros with an implication on management decisions.

## AFLP as a Method for Genetic Wildlife Management in Rhinoceros

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Protection from habitat loss and poaching is not sufficient for rhino conservation, a specific breeding program should be applied as well. Since the migration of the remaining small populations is prevented, they can rapidly lose genetic variability and with it their capacity for genetic adaptation. To set up such a program, genetic information about the population structure is required.

The degree of inbreeding is primarily determined by the number of reproducing males in one area. Since very little is known about breeding patterns and mate choice in rhinoceros, genetic analyses can supplement behaviour observations.

Because the relationship of wild rhinoceros is not known, a molecular genetic approach was attempted to determine paternity and genetic variability. Lacking DNA sequence information, a suitable PCR method had to be found to generate genetic markers from the uncharacterised genome. We established the sequence independent DNA fingerprinting method termed "Amplified Fragment Length Polymorphism (AFLP)". To our knowledge, this is the first report of the use of AFLP markers to determine genetic relationships in wildlife.

A set of 64 AFLP primer combinations was analysed. Twelve primer combinations were selected for further investigation. They produced an average of 60-80 bands per animal per PCR reaction in a range of 50 to 510/800 basepairs. For all rhinoceros species, polymorphic bands could be detected.

The study involved 69 southern white rhinoceros (Ceratoterium simum simum) from zoological parks and a wild population of 57 animals. Additionally the study contained 5 northern white rhinoceros (Ceratoterium simum cottoni), 20 black rhinoceros (Diceros bicornis michaeli) and 6 great Indian one-horned rhinoceros (Rhinoceros unicornis).

For parentage testing a combined exclusion rate between 90 and 99% was reached. On a South African game farm with 5 white rhinoceros bulls, the most probable sire of 14 calves was determined. Simultaneously territorial status of males and behaviour of females was observed. The parentage test confirmed that females did not favour specific bulls. It showed that all males on the farm had reproduced. Courtship of a female did not guarantee fatherhood. The test reaffirmed that a female courted by a bull still mated with another male. This demonstrates that genetic analyses are important for accurate interpretations of the population structure and breeding success.

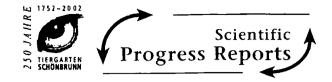
For the present, all rhino species still seem to show a high level of genetic variability, with an average heterozygosity of 0.36. This seems to be in contrast with reports for other species that have experienced near extinction.

## Digestibility Trials in the Zoo Applied to Field Studies of White Rhinoceros

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Nutrition has got a major influence on health, behavior and reproduction of animals, but only little is known about the nutrient requirement of white rhinoceros. In order to increase knowledge in this species a specific combination of zoo and field studies was conducted. The nutrient composition and the apparent digestibility of varied diets of a captive population was analyzed and compared with the feeding behavior of a free-living population.

The digestibility trials were carried out with a group of five white rhinos (1,4) in the zoo of Erfurt. Germany. The field study was conducted on free-living white rhinoceros in South Africa. Forage samples were collected from the feeding sites by following the tracks of three territorial males. Fecal samples of the same animal were collected two days later,



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## **Recent Research on Elephants and Rhinos**

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