

THE PLIGHT OF THE NORTHERN WHITE RHINOCEROS: IS THERE STILL HOPE TO PREVENT EXTINCTION?

RYDER OA¹, HERMES R², GOERITZ F², DURRANT B¹, STEJSKAL J³, HRUDY J³, VAHALA J³, LORING JF⁴, HILDEBRANDT TB²

¹San Diego Zoo Institute for Conservation Research, 15600 San Pasqual Valley Road, Escondido CA 92027-7000 USA; oryder@ucsd.edu

²Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Strasse 17, 10315 Berlin, GERMANY

³Zoo Dvur Kralove a.s., Stefanikova 1029, 544 01 Dvur Kralove nad Labem, CZECH REPUBLIC

⁴Center for Regenerative Medicine, Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037, USA

With only five surviving individuals and the prospects for reproduction compromised, the northern white rhinoceros (NWR) has been considered to be a form of rhino doomed to extinction. It may serve as a notable example in the first years of the new century of a species for which the only hope for survival is the further development and application of advanced reproductive and genetic technologies. The related southern white rhino is relatively numerous and viable hybrids have been produced with NWR. Semen has been saved from several NWR in Berlin and San Diego. Furthermore, the Frozen Zoo® of the San Diego Institute for Conservation Research has viable cell cultures from 12 NWR, arguably a sufficient gene pool to preserve a species. Fibroblast cells from Fatu, the youngest female NWR, have been transduced to become induced pluripotent stem cells (iPSCs) (Ben-Nun et al., 2011), capable of making any tissue in the body. These resources may form the basis for an ambitious effort, involving many steps, to employ advanced genetic and reproductive technologies to save the NWR. Initial efforts in San Diego are focused on comparing whole genomes of northern and southern white rhinos, further stem cell work, and the establishment of a cohort of reproductively capable female southern white rhinos to serve as ovum donors and surrogates for gestation. The genome data will shed light on the extent of genetic variation surviving in both types of white rhino, suggest their demographic histories, and allow a quantitative assessment of their genetic divergence gene by gene. These data will contribute to resolving their status as species or subspecies. The stem cell efforts will strive to produce a full representation of the surviving gene pool as represented by the living animals and the cells in the Frozen Zoo®. Further efforts will seek to produce gametes from IPS cells as has been done in the mouse. Non-surgical ovum pick-up (OPU), In vitro fertilization and intracytoplasmic sperm injection (Hermes et al., 2009) were successfully performed in southern white and black rhinoceroses. Besides the successful application of transcervical artificial insemination in white rhinoceroses (Hildebrandt et al., 2007) suitable techniques for embryo transfer have to be developed for this rhino species, as it has been done in other non-domestic species (Pitra et al., 1991). These various steps, while a speculative venture, utilize approaches that have been successfully developed and applied in other species. Furthermore, they represent the best hope for avoiding the extinction of the NWR. In the end, efforts to develop these technologies for genetic rescue will assist in efforts for other species in the future

References

Ben-Nun IF, Montague SC, Houck ML, Tran HT, Garitaonandia I, Leonardo TR, Wang YC, Charter SJ, Laurent LC, Ryder OA, Loring JF (2011): Induced pluripotent stem cells from highly endangered species. *Nature Methods* 8, 829–831

Hermes R, Göritz F, Portas TJ, Bryant BR, Kelly JM, MacMellan LJ, Keeley T, Schwarzenberger F, Walzer C, Schnorrenberg A, Spindler RE, Saragusty J, Kaandorp S, Hildebrandt TB (2009): Ovarian superstimulation, transrectal ultrasound-guided oocyte recovery, and IVF in rhinoceros. *Theriogenology* 72: 959-968.

Hildebrandt TB, Hermes R, Walzer C, Sos E, Molnar V, Mezösi L, Schnorrenberg A, Silinski S, Streich WJ, Schwarzenberger F, Göritz F (2007): Artificial insemination in anoestrous and the postpartum white rhinoceros using GnRH analogue to induce ovulation. *Theriogenology* 67: 1473-1484.

Pitra C, Hildebrandt TB, Reinsch A (1991): Embryotransfer bei Wirbeltieren in menschlicher Obhut: Die Technik und ihre Anwendungen. (Übersichtsreferat). *Mh Vet-Med* 46: 618-621.