New strategies in Sumatran and northern white rhinoceros conservation based on advanced assisted reproduction techniques combined with cellular technologies

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The Sumatran rhinoceros (SR, *Dicerorhinus sumatrensis*) and the northern white rhinoceros (NWR, *Ceratotherium simum cottoni*) are the two megaherbivores which suffer from the most accelerated population decline on our planet over the last decade. The NWR rhino is reduced to a none self-sustaining group of three individuals which are kept under protected condition in Ol Pejeta Conservancy, Kenya. The SR, divided into two subspecies, is dwindled down to fewer than 100 individuals, largely scattered over the islands of Sumatra (under 90 SR) and Borneo (approx. 10 SR). The NWR is already declared as extinct in the wild and the SR has a high risk to follow the same destiny. The current approach of habitat protection will not save the NWR and most likely the SR either. For an effective conservation program, it is critical to increase their reproductive rate substantially. With the application of advanced assisted reproduction techniques such as in-vivo gamete collection and in-vitro embryo production, followed by embryo transfer into surrogate females, combined with emerging cellular technologies based on induced pluripotent stem cells (iPSCs), it might be possible to maintain the genetic variability necessary for self-sustaining populations and rewind the extinction process of these two rhinoceroses. The authors will present the various options embedded in a strategic road map developed at a multidisciplinary meeting under the name “Conservation by Cellular Technologies” and published online in Zoo Biology on May, 3rd 2016 (doi: 10.1002/zoo.21284).

Etorphine free anesthesia protocols optimized for frequent reproductive interventions ranging from semen collection, artificial insemination to Ovum-Pick-Up (OPU) in four rhino species

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In order to elucidate the problems of poor reproductive performance in captive rhinos the EEP and IZW have encouraged intensive and serial reproductive monitoring. A multi-disciplinary and multi-institutional research proposal was initiated. The use of restraint chutes and recent implementation of medical training offered new possibilities of safe handling and management of rhinoceros. However, serial ultrasonographical reproductive assessments, artificial inseminations, semen collections and OPU, as well

as bronchial lavages for reliable tuberculosis diagnostic require safe immobilization and anesthesia protocols in rhinos. In the past we mainly used etorphine in combination with detomedine and butorphanol (Walzer at al. 2002). Due to negative side effect known for etorphine (e.g. V/Q mismatch with the result of hypoxemia, we especially noticed in Sumatran rhinos) and because of safety and legal aspects (e.g. prohibition to import opioids into Asian countries) we developed a safe and effective etorphine free anesthesia protocol for different rhino species and optimized dosages regarding species-specific potency (e.g. Indian rhino > White rhino > Black rhino) and regarding procedure-specific level of anesthesia needed (e.g. transrectal ultrasound < artificial insemination < OPU). The author will describe the specific anesthesia protocol (I. premedication/standing sedation: injection via dart or by hand i.m. of combination of detomidine, butorphanol, midazolam and ketamine; II. drop dose/bolus: ketamine i.v.; III. maintenance: quad-drip i.v., CRI) and will demonstrate its use by means of elected procedures.

First results of oocyte maturation and In-Vitro-Fertilisation (IVF) in Sumatran and northern white rhinoceroses

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Over the last two years a total of seven ovum-pick-up (OPU) procedures were performed in two Sumatran rhinoceroses (SR*, Dicerorhinus sumatrensis*) housed at the field research station in Tabin, Sabah, Malaysia. Parallel, eight OPUs were performed in six southern white rhinoceroses (SWR, *Ceratotherium simum simum*) housed in different European zoos. In SR a total of 12 oocytes were harvested and three developed into metaphase 2 stage during incubation in a modified horse media. None of these three oocytes developed into an embryo after In-Vitro-Fertilisation by intracytoplasmatic sperm injection (ICSI). In SWR a total of 17 oocytes were harvested, three entered the metaphase 2 stage and ICSI was performed. Two of the 17 harvest SWR oocytes developed under similar In-Vitro conditions used in SR into an early embryo. Both stopped at 5-cell-stage. Based on these first results there is a clear indication that both maturation and fertilization protocols need to be improved for SR and SWR. New In-Vitro approaches will be discussed.