BIORESCUE

Northern White Rhino -Extinction or Survival?

The aim is to breed and rear Northern White Rhino calves through creating pure NWR embryos that can be implanted into Southern White Rhino surrogate mothers.

BY KES HILLMAN SMITH

orthern White Rhinos (NWR) (*Ceratotherium simum cottoni*) are the second largest land mammals in Africa, yet until recently they were facing possible extinction in our lifetimes. Can they be saved? In a world where the news seems to be dominated by wars, resource conflicts, climate change and iconic losses, it is good to be able to answer: "Yes! On the basis of results from the exciting advanced assisted breeding programme known as BioRescue, it looks very possible".

The fact that we have even come to very near extinction of an iconic sub-species is not a great reflection on humanity, but we have personally fought for their survival in the wild for over four decades, most of which were in the wide expanses of Garamba National Park, Zaire/Democratic Republic of Congo (DRC). That challenging story will be told in the sequel article to this one. For now, it is summarised by the graph which shows that the main factors leading to huge losses of both rhinos and elephants in Central Africa have been wars and armed conflict combined with periods of little or no support. Increases have been when there was external support to complement the national conservation agency, which demonstrates the value of donor contributions and dedicated efforts.

The rhinos themselves, in a natural environment, were breeding at 9.5 per cent annually, producing 50 calves during the 22 years of the Garamba National Park Project (GNPP), from 1983 to 2005. (Hillman Smith et al 2014). During the same period, captive Northern Whites in Dvur Kralove Zoo had four offspring. Even considering individual wild rhinos: F3 Kunalina during that period had nine calves, three grand-calves, and eight great grand-calves, ie 20 offspring. F4 Boletina had six calves, 10 grand-calves and two great grand-calves, a total of 18. They breed well when they can establish their normal social structure, but the big challenge in the wild is security. Protection is hard to achieve when surrounded by

BELOW

Right to Left: Female NWR *F4 Boletina*, calf *4f Nauoloko*, with Sub-adults *5cM Molende* & *4cF Noel*, Garamba National Park (1995)







PHOTO BY KES HILLMAN SMITH

political instability, armed conflict, weapons, ammunition and human power play. Rhinos in captive institutions tend not to breed well, but they can be protected. Without them, this programme would not be possible. Subspecies survival now is dependent on crucial international collaboration between wild and captive institutions, science and technology. In addition, the whole ecosystem of their wild habitat in Garamba is now being protected by African Parks with far more resources than our GNPP had.

Following many years of interactions and discussions between those of us involved in both wild and captive rhino conservation, the apparent loss of most wild Northern Whites in Garamba and South Sudan, and the simultaneous development of reproductive technologies, it was finally agreed to bring some captive rhinos to Africa to improve chances of breeding. Four captive Northern White rhinos from Dvur Kralove Zoo, two males *Sudan* and *Suni* and mother and daughter females, *Najin* and *Fatu* were transported in 2009, from the snows of Dvur Kralove Zoo in the Czech Republic to Ol Pejeta Conservancy in Kenya. Despite matings, no natural pregnancies resulted and both males have now died, although their semen was harvested. But the females are the main subjects of the BioRescue Programme.

BioRescue is based on years of research and development, led by Professor Thomas Hildebrandt and his team. It is a collaboration between the Leibniz Institute for Zoo and Wildlife Research, Berlin (IZW), Avantea Institute, Italy, the Dvur Kralove Zoo in the Czech Republic, Kenya Wildlife Service, Ol Pejeta Conservancy Padua University, Italy (ethical assessments of our work), Max Delbrück Center for Molecular Medicine, Germany, and Kyushu University, Japan. The rhinos were transported by the Back to Africa programme of wildlife vet Pete Morkel and Hamish Currie.

The aim is to breed and rear Northern White Rhino calves by creating pure BELOW

Millenium baby in Garamba NP: 4dF Minzoto & 4da Millenium, 2000.

BELOW

Dead young female NW Rhino, Garamba 2004.







NWR embryos that can be implanted into Southern White rhino surrogate mothers since the uterus of *Fatu* has a degenerated endometrium and could not carry a foetus and *Najin* has weak hind limbs. (*Hildebrandt pers comm., Saragusty et al 2016*). Oocytes have been harvested from the females, with 10 immobilisations of *Fatu* so far, for ovum pick up and a record number of 23 oocytes collected in July 2022. In July 2021, oocyte harvesting from then 32-year-old Najin was stoppped after an ethical risk assessment because of her age. The IZW team are extremely experienced and the health of the rhinos comes first. *Najin* will continue as an ambassador of the programme and with her social contributions as a grandmother.

Each procedure involves the team travelling from Europe with complex logistical arrangements coordinated by Jan Stejskal of Dvur Kralove, KWS and Ol Pejeta. Approval has to be confirmed from CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) for the international movement of the rhino material. Working with the KWS vets, the Kenva Wildlife Research and Training Institute and Ol Pejeta senior staff and rangers, the team is very efficient. Frank Göritz, OPC and KWS vets manage the anaesthesia at the rhino's head end. Thomas, Suzanne and Robert manage the probe at the rear watching on a computer screen and flushing out the oocytes. Robert left the team at the end of 2021. Later the fluid is laboriously checked through a microscope to select the oocytes. When all the procedures are finished and the rhino is safely revived, the team flies out and the oocytes have to be transported straight to the Avantea laboratory

PHOTO BY OL PEJETA/BIORESCUE

ТОР

Leibniz Institute for Zoo and Wildlife Research (Leibniz-IZW) team flushing oocytes from Fatu.

BELOW LEFT Oocyte selection by Thomas Hildebrandt.



HOTO BY OL PEJETA/BIORESCUE

in Italy where they are combined with stored semen to produce embryos. So far there are 22 quality embryos created by the group in Italy, led by Cesare Galli. The female ova are from *Fatu*, the male spermatozoa from *Suni* and stored semen from another bull, *Angalifu*.

The next stage is to implant embryos into surrogate SWR females. First the method has to be thoroughly tested on Southern White Rhino embryos implanted in Southern White females in Ol Pejeta before applying it to the precious Northern embryos. The female has first to be made receptive with a sterile bull, and timing of the implantation is crucial. Photography and filming are also key for reporting and publishing the work since money has to be raised to continue it. Ami Vitale of National Geographic produces the photos and films. Conservation and study of the Northern White rhinos in the wild have been most of my life's work, and I feel privileged to be a small part of the team and to take morphometric measurements to compare with those we made in the wild, which are part of the differences between sub-species.

Other methods for possible countering extinction have also been under development. Under the guidance of Oliver Ryder from the United States, an international "Frozen



Since stem cells are the un-programmed building blocks of organisms, this gives the possibility of re-creating even extinct species.

zoo" has been established to cryo-preserve tissue from endangered species. It includes ear notch tissue from wild NW Rhinos. It is now possible to create stem cells (Induced Pluripotent Stem Cells or IPSCs), from such stored body tissue (*Korody et al. 2021*). Since stem cells are the un-programmed building blocks of organisms, this gives the possibility of re-creating even extinct species.

So all is not lost for saving the unique genotype of the Northern White Rhinos and I believe it is part of human responsibility to prevent extinction if at all possible, now that the means exist. The ideal would have been to have had enough support both financial and political when we were conserving the rhinos in Garamba. It would have been easier to protect them if the Sudanese had not been fighting a war next door and for the Congolese and neighbours not to have been fighting within the country, leading to a plethora of arms and ammunition, lawlessness and **TOP LEFT** Managing anaesthesia of the rhino.

CONSERVATION



power play. But conservation is rarely in ideal situations. At least, at the 11th hour, technology and a unique international collaboration of top scientists is providing the possibility of countering extinction. The techniques have wider applications and the money involved would not have been available for field conservation. There are also still possibilities that some rhinos may exist in the wild in Sudan and DRC in the more forested cover where they hide. There have been some very credible signs found from ongoing surveys. (P.Winter pers.comm)

At the same time the rhinos' key ecosystem, Garamba National Park in DRC, which is a World Heritage Site is being effectively protected through the support of African Parks, (with some 40 times the financial budget that we had). It is now considered safe enough to try introducing Southern White Rhinos into. Having rhinos in Garamba again contributes considerably to its value for conservation, but they will have some adapting to do and may also have disease factors to cope with. The principles of the IUCN African Rhino Specialist group are to keep sub-species pure and in indigenous habitat when possible. This begs the question of the ethics of re-introducing artificially produced pure Northern whites at a later stage if Southern whites are there. But, although they diverged 0.46 to 0.97 million years ago, they are defined as sub-species and can inter-breed and the northern genes will be there to enhance survival in the habitat.

Basic needs of the White rhino are short grass, water and cover. The cover was particularly important in Garamba for them to hide from danger, but was generally provided by long grass rather than thick bush. The morphological differences between the subspecies appear to be selected for survival in their indigenous habitat, dominated by seasonal long grass. Their heads are shorter and smaller than those of Southern whites and can be held higher. Legs are proportionally longer and bodies shorter. These characteristics make it easier to run through two-metre high grass. Their hairy ears could possibly have been against biting insects, or perhaps to increase sound capture or simply less rough thorny bush. These are inherited characteristics, but learning from their mothers how to use the environment is another stage of growing up as a Northern White Rhino.

TOP LEFT

Mama Giningamba and new baby 5aM Giningamba, illustrating the higher head carriage and smaller head of the Northern White Rhinos, Garamba NP (1985).

BELOW

Fatu, Tauwo and Najin after procedure.

PHOTO BY OL PEJETA/BIORESCUE/JAN ZWILLINGS





It is sad that it has come to this. When I think back to how much went into their conservation in Garamba and how exciting it always was when we found a new baby rhino with its mother. But it is also fantastic that methods have been developed to make it possible, which have wider applications, and that the Northern white rhinos have been selected to be saved from extinction. It will be wonderful when a Northern white baby rhino is born again. It still has to grow up and learn the environment, but at least it is a means of conserving Northern white genes to enhance survival chances and to save the second largest land mammal from extinction.

Thanks to the collaboration between wild and captive conservation, science, research, technology, vets and filmakers, extinction of the Northern white rhino may be reversed.

In recognition of the importance of the BioRescue programme, Kenya is even honouring the work with paintings of *Najin* and *Fatu* adorning a Kenya Airways DreamLiner aircraft.



KES HILLMAN SMITH has a BSc and PhD in Zoology. She did the first pan-African Rhino survey, founding the IUCN African Rhino Specialist Group, and spent 22 years in Garamba conserving the Northern White Rhinos and ecosystem.

References

Harley E.H. M.de Waal, S.Murray & C.O'Ryan (2016) Comparison of whole mitichondrial genome sequences of northern and southern white rhinoceroses (Ceratotherium simum): the consequences of species definitions.

Hillman Smith, K et al. (2014) Garamba, Conservation in Peace & War;

Holeckova D. (2009) Breeding Endangered Species at Dvur Kralove Zoo Vol3

Korody M.L., S.M.Ford, T.D.Nguyen, Cullen G. Pivaroff, I Valiente-Alandi, S.E.Peterson, O.A.Ryder, J.F Loring (2021), Rewinding Extinction in the Northern White Rhinoceros: Genetically Diverse Induced Pluripotent Stem Cell Bank for Genetic Rescue; Stem Cells and Development Vol 30 : 4, 177-189

O Connor M.R 1982 Resurrection Science; St Martins Press;

Saragusty et al. (2016) Rewinding the Process of Mammalian Extinction; Zoo Biology 9999:1-13

PHOTO BY OL PEJETA/BIORESCUE/AMI VITALES

тор

Dream-liner with Najin & Fatu. Kenya is the home to the only surviving northern white Rhino's and to celebrate these wild animals and market tourism in the country, Kenya Airways has decorated two of its planes with the photos of these two animals.