

Advanced assisted reproduction in white rhinos is safe and reliable: Evaluation of procedures

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10th oocyte collection in northern white rhino Fatu, conducted by the BioRescue Team in July 2022 Credit: Jan Zwilling

The BioRescue project develops and pioneers advanced assisted reproduction technologies (aART) for conservation in the face of the imminent extinction of most rhino species and subspecies. In a new analysis [published](#) in the journal *Reproduction*, the team evaluated 65 aART procedures conducted from 2015 to 2022.

The evaluation showed that aART is safe for the donor females with no detrimental health effects, and successful in that it yielded 51 embryos. In fact, regular OPUs benefited the reproductive health of individual female rhinos.

The procedures evaluated comprised hormonal ovarian stimulation, ovum pick-up (OPU), in-vitro oocyte maturation and in-vitro fertilization (IVF), embryo culture and cryopreservation. The evaluation showed that aART is safe for the donor females with no detrimental health effects, and successful in that it yielded 51 embryos. In fact, regular OPUs benefited the reproductive health of individual female rhinos by improving ovarian function, increasing follicle numbers and instigating the regression of pathological structures such as ovarian cysts.

As most rhino species and subspecies face impairment of natural reproduction and are threatened with extinction, new approaches to their conservation are required. A most promising new approach is the application of advanced assisted reproduction technologies (aART) such as ovum pick-up (OPU)—the retrieval of immature egg cells (oocytes) from ovaries—and in-vitro fertilization (IVF). These technologies enable the creation of embryos in the lab that can later be transferred into surrogate mothers to carry gestation to term.

The application of aART is the only option to create offspring for the northern white rhinoceros, a subspecies with only two individuals known to be alive—two females that cannot become pregnant anymore to carry their own embryos. The BioRescue project, led by the Leibniz Institute

for Zoo and Wildlife Research (Leibniz-IZW), develops and pioneers these technologies to enhance the breeding success of southern white rhinos in human care and to save the northern white rhino from extinction. The consortium takes animal safety and welfare, [quality control](#) and ethical risk assessment seriously and constantly evaluates its new scientific and veterinary procedures.

BioRescue has performed 65 aART procedures from 2015 to 2022 and now published an evaluation of these data in the journal *Reproduction*. The team of authors around Prof Thomas Hildebrandt, Dr. Frank Göritz, Dr. Susanne Holtze (from the Leibniz-IZW), Dr. Silvia Colleoni and Prof Cesare Galli (from Avantea srl.) analyzed [animal health](#) and health effects of the procedures, age and seasonality, subspecies and origins of individuals, hormonal status and cyclicity as well as the effects of the stimulation protocol in relation to OPU and IVF success rates with 20 southern and two northern white rhino females.

The set of procedures proved to be a guarantee for successful production of white rhino embryos. Altogether, 1505 [ovarian follicles](#) were counted via transrectal ultrasound. Of those, 1171 follicles were punctured, flushed and aspirated. The team retrieved 402 oocytes, 393 of which were successfully transported to the Avantea lab, and 150 of them could be matured and fertilized by employing piezo-intracytoplasmatic sperm injection (ICSI) with a single sperm cell.

Of these, 75 embryos cleaved and 51 blastocysts were finally cryopreserved—19 southern, 22 northern and 10 hybrids with southern white rhino oocytes and northern white rhino sperm. The number of oocytes collected per procedure, oocyte retrieval rate and the success rate of producing embryos considerably increased over time due to technical optimization, improved team-performance, and the accumulating beneficial effects of repeated OPUs on donor reproductive health.

Repeated OPUs yielded no indications of adverse effects on general and reproductive health, such as inflammation, pathological alterations or a declining response to ovarian stimulation. There was no evidence for detrimental effects of repeated OPU procedures with prior hormonal stimulation on reproductive health, fertility, cycling activity, ovarian morphology, follicle numbers or success across all levels of the IVF program.

Interestingly, the risk of anesthesia was assessed as negligible, as anesthesia was complication-free in all 65 reported procedures as well as in more than 500 procedures performed by the team in white rhinos for semen collection, health assessment or artificial insemination in the past. There was no sign of detrimental effects or risks even in the northern white rhino female Fatu after 10 procedures performed at three-month intervals.

In fact, there were clear indications of health benefits to individuals exposed to OPUs. A pathological cystic ovarian structure in Fatu regressed from 50 mm to 15 mm in diameter over three years and 10 OPUs. After removal of ovarian cysts in two southern white rhinos during OPU procedures, follicle numbers increased and the morphology of the ovaries improved.

Degeneration of the reproductive tract is common in female rhinos that do not regularly mate and carry a pregnancy to term. The degeneration leads to reduced fertility and premature termination of the reproductive lifespan. Repeated OPUs can prevent this from happening and help preserve or even restore reproductive [health](#), concludes the BioRescue team.

OPU procedures do not hamper natural reproduction. Two cycling females became pregnant after natural mating following four and five OPUs, respectively. One formerly non-cycling southern white rhino

female resumed cycling, became pregnant and delivered a healthy male calf after two successful OPUs. This indicates a positive influence on cycling activity. As OPU enhanced white rhino [reproductive health](#) this "mechanical ovarian cleansing" might serve as an optional fertility restoration treatment for subfertile or infertile females with minor reproductive pathologies, concludes the team.

The age of the oocyte donor is key for IVF success. In the study group, no oocytes were retrieved in approximately half of the OPUs with female white rhinos older than 24 years. In females aged 24 or younger, only 6% of OPUs yielded no oocytes. The team was not able to produce embryos at the blastocyst stage ready for cryopreservation from individuals older than 24 years.

These results imply that IVF success of the last female northern white rhino oocyte donor Fatu (23 years) may soon cease, although optimal nutrition, physical exercise, the Kenyan climate, semi-wild husbandry and possibly subspecies-related factors might prolong this period.

BioRescue emphasizes how important it is to put these newly developed technologies into practice without delay, while at the same time to learn from the results of these analyses and improve the procedures. The regular evaluation and ethical risk assessment will continue to be a pivotal element of the BioRescue mission on the edge of what is technologically possible in conservation.

The successful generation of the embryos—in particular the northern white rhino embryos—underlines the importance of the technology and its potential to address one of the most pressing global problems of our time, the dramatic loss of biodiversity. This decline causes an incalculable disturbance of crucial ecosystem services while simultaneously fostering the emergence of novel pathogens, which undermines the basis of our very existence.

More information: Thomas Bernd Hildebrandt et al, In vitro fertilization program in white rhinoceros, *Reproduction* (2023). [DOI: 10.1530/REP-23-0087](https://doi.org/10.1530/REP-23-0087)

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