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Press release

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BioRescue produces primordial germ cells from northern white rhino stem cells – a world's first for large mammals

In its race to advance assisted reproduction and stem cell associated technologies to save the northern white rhinoceros from extinction, the BioRescue consortium announces a major breakthrough: the creation of primordial germ cell-like cells (PGCLSs) from induced pluripotent stem cells of the northern white rhino Nabire. This milestone was led by specialists from Osaka University, Japan, and has never been achieved in large mammals before. Now there is one last step to master for the production of artificial rhino gametes (eggs and sperm) from preserved tissue.

When the production of artificial rhino gametes from preserved tissue is successful, this would boost the availability and genetic diversity of embryos and become a cornerstone for saving the northern white rhinoceros. The scientists describe the culture systems and processes for the induction of the PGCLCs from stem cells in a newly published paper in the journal "Science Advances".

Thirty-three-year-old Najin and her daughter Fatu are the last surviving northern white rhinos on the planet. Without a possibility for natural reproduction with only two females left of their kind, the only hope for the northern white rhinoceros lies in advanced assisted reproduction technologies. The scientists of the BioRescue consortium have already produced northern white rhino embryos by in vitro fertilisation of oocytes with sperm. Embryos are destined to be carried to term by southern white rhino surrogate mothers. Crucial to embryo production is the availability of oocytes (egg cells) and sperm. Female Fatu is the only donor of natural oocytes left and frozen sperm is available to the program from only four males – and some of these males are closely related to Fatu. With stem cell associated techniques (SCAT) the BioRescue scientists aim to overcome this bottleneck: By using stored and preserved tissue of rhino skin it should be possible in principle to create induced pluripotent stem cells (iPCSs), primordial germ cells and finally artificial gametes. This could increase the number of individuals in the founder group of a future population to 12 and permit the production of oocytes in much larger quantities. According to their most recent paper published in the journal "Science Advances", the team has now successfully cultivated primordial germ cells (PGCs) – the precursors of eggs and sperm – from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).

In sexually reproducing organisms such as humans, rhinos or mice, primordial germ In sexually reproducing organisms such as humans, rhinos or mice, primordial germ cells (PGCs) are embryonic precursors of sperm and eggs that pass on genetic and epigenetic information from one generation to the next. In order for them to be developed from stem cells, they need a very specific environment in which signals from hormones or proteins trigger the required morphological and functional transformation. This also includes a migration of the cells in the body (PGC migration). For the first time in large mammals, BioRescue scientists Masafumi Hayashi and Katsuhiko Hayashi and their team from Osaka University succeeded in creating such an environment in a culture system. They established culture systems for the southern white rhino, for which embryonic stem cells are available, and the northern white rhino, for which they used induced pluripotent stem cells derived from tissue samples. In order to succeed they needed to identify the signals which had to be introduced to the system at specific time points and the order in which they should trigger the development into PGCLCs.

The scientists relied on knowledge from the mouse model: In 2016, Katsuhiko Hayashi and his team managed to create primordial germ cell-like cells and finally germ cells from mice that were fertilised in the lab and resulted in healthy offspring being born. In the case of the white rhinoceroses, Hayashi is working in close cooperation within BioRescue with Sebastian Diecke's Pluripotent Stem Cells Platform at the Max Delbrück Center and with reproduction experts Thomas Hildebrandt from Leibniz-IZW, both of them last authors of the paper, and Cesare Galli from Avantea.

Through repeated refinement, the BioRescue team produced PGCLCs from northern white rhino Nabire under a defined condition. Because PGCs are the founder population for gametes, this accomplishment paves a way to produce functional gametes from induced pluripotent stem cells from northern white rhinos which will contribute to the effort to rewind their extinction. As soon as the creation of artificial gametes is successful, this plan merges with the procedures that BioRescue carries out with natural gametes: Just like with oocytes obtained from Fatu and sperm thawed from frozen samples, the artificially created eggs and sperm would be in vitro fertilised in the lab. The embryos created in this way would be stored safely in liquid nitrogen until a transfer to a surrogate mother is possible.
The southern white rhino embryonic stem cells which were used in Japan come from the Avantea laboratory in Cremona, Italy, where they were grown by Cesare Galli's team. The newly derived northern white rhino PGCs, meanwhile, originated from the skin cells of Fatu's aunt Nabire, who died in 2015 at Safari Park Dvůr Králové in the Czech Republic. Sebastian Diecke's team at the Max Delbrück Center was responsible for converting them into induced pluripotent stem cells.

All BioRescue procedures are accompanied by ethical risk assessments, conducted under the guidance of the Ethics Laboratory for Veterinary Medicine, Conservation and Animal Welfare at the University of Padua, Italy.

_ Statements_

Masafumi Hayashi, Osaka University:
"This is the first time that primordial germ cells of a large, endangered mammalian species have been successfully generated from stem cells. Previously, it has only been achieved in rodents and primates. Unlike in rodents, we identified the SOX17 gene as a key player in rhinoceros PGC induction. SOX17 also plays an essential role in the development of human germ cells – and thus possibly in those of many mammalian species. Additionally, we found that two specific markers, CD9 and ITGA6, were expressed on the surface of the progenitor cells of both white rhino subspecies. Going forward, these markers will help us detect and isolate PGCs that have already emerged in a group of pluripotent stem cells."

Katsuhiko Hayashi, Osaka University:
"Developing a culture system that delivers robust results has been extremely challenging since the precise orchestration of the specific signals required to induce the desired cellular differentiation is unique to every species. It was also necessary to confirm that the primordial germ cell-like cells were genetically identical to the cells from which they originated – this can be a daunting task."

Jan Stejskal, Safari Park Dvůr Králové:
"We are thrilled that this milestone was achieved by the BioRescue scientists and that Nabire, that deceased in Dvůr Králové in 2015, is still in the race for saving her kind. Unfortunately, she didn't give birth to an offspring during her life, but with the recent successes in stem cell associated techniques it seems perfectly possible that a direct descendant of Nabire will be born at some point in the future and can play an important role in repopulating central Africa with northern white rhinos."

Thomas Hildebrandt, Leibniz Institute for Zoo and Wildlife Research:
"We knew from the beginning that relying on natural gametes for the assisted reproduction technologies would not be sufficient to save the northern white rhino from the brink of extinction on the long run. So, it is crucial that we pursue a complementary strategy for making gametes available with significantly higher genetic diversity and in much larger

numbers ¬– even making it possible to create embryos from Najin through artificial gametes, which is something that was not possible with her natural gametes. It is encouraging to see that the stem cell specialists in our consortium, from the Osaka University and the Max Delbrück Center for Molecular Medicine, achieved this important step already now. It is also important to note that the plans for natural gametes and artificial gametes are not alternative paths to go, but interconnect and merge at the point where in vitro fertilization yields embryos."

Cesare Galli, Avantea
"The successful derivation of embryonic stem cells (ESCs) in our laboratory in 2018 by our colleague Dr. Giovanna Lazzari from the first SWR embryos that we were able to obtain, proved to be instrumental for the success of the work of Prof. Hayashi team because ESCs have been studied and differentiated for a long time and provided a template for the iPSCs."

Vera Zywitza, Max Delbrück Center
"After we succeeded in creating the PGCs, we must now move on to the next difficult task: maturing the PGCs in the laboratory to turn them into functional egg and sperm cells. The primordial cells are relatively small compared to matured germ cells and, most importantly, still have a double set of chromosomes. We therefore have to find suitable conditions under which the cells will grow and divide their chromosome set in half. In mice, we found that the presence of ovarian tissue was important in this crucial step. Since we cannot simply extract this tissue from the two female rhinos, we will probably have to grow this from stem cells as well."

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Northern White Rhino Nabire at Safari Park Dvur Kralove
Khalil Baalbaki
BioRescue/Khalil Baalbaki

Northern White Rhinos Najin and Fatu and Southern White Rhino Tauwo at Ol Pejeta Conservancy
Jan Zwilling
BioRescue/Jan Zwilling