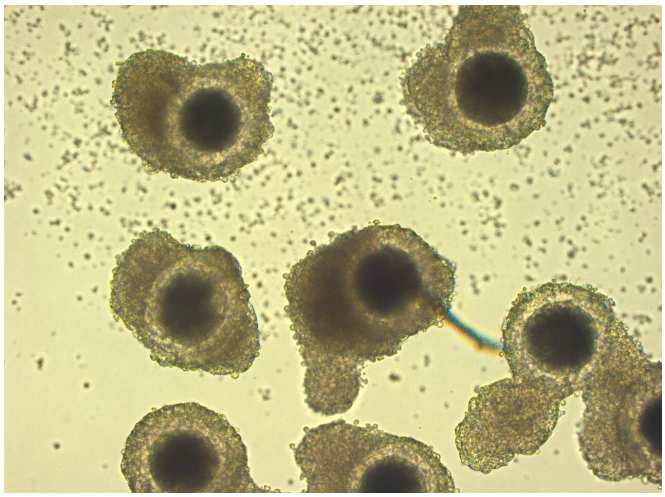
Scientists produce the first in-vitro embryos from vitrified African lion oocytes

18 January 2021



Freshly isolated African lion oocytes. Credit: Jennifer Zahmel/Leibniz-IZW

A team of scientists from the Leibniz Institute for Zoo and Wildlife Research (Leibniz-IZW) inGermany, Givskud Zoo–Zootopia in Denmark and the University of Milan in Italy succeeded in producing the very first African lionin-vitroembryos after the vitrification of immature oocytes. For this specific method of cryopreservation, oocytes are collected directly after an animal is castrated or deceased and immediately frozen at -196°C in liquid nitrogen. This technique allows the storage of oocytes of valuable animals for an unlimited time, so that they can be used to produce offspring with the help of assisted reproduction techniques. The aim is to further improve and apply these methods to save highly endangered species such as the Asiatic lion from extinction. The current research on African lions as a model species is an important step in this direction. The results are reported in the scientific journal *Cryobiology*.

Lion oocytes are presumed to be very sensitive to chilling due to their high lipid content, resulting in poor revival following slow cooling. Vitrification can circumvent this problem, as the cells are frozen at ultra-fast speeds in solutions with a very high concentration of cryoprotective agents. This method prevents the formation of ice crystals in the cells, which could destroy them, and enables them to remain intact for an unlimited time to allow their use later on.

For the present research, the scientists collected oocytes from four African lionesses from Givskud Zoo—Zootopia after the animals had been euthanised for the purpose of population management. Half of the oocytes (60) were vitrified instantly. After six days of storage in liquid nitrogen, the vitrified oocytes were thawed and subjected toin-vitromaturation in an incubator at 39°C for a total of 32-34 hours. The other half (59) were used as control group and directly subjected toin-vitromaturation without a step of vitrification. Mature oocytes of both groups were then fertilized with frozen-thawed sperm from African lion males. "We could demonstrate a high proportion of surviving and matured oocytes in the group of vitrified oocytes. Almost 50 % of them had matured, a proportion similar to that in the control group," says Jennifer Zahmel, scientist at the Department of Reproduction Biology at the Leibniz-IZW. Of the vitrified group, seven early embryos developed, whereas in the control group only three embryos developed. "To our knowledge, this is the first time thatin-vitroembryos were produced following vitrification of oocytes from African lion or any other wild cat species," says Martina Colombo from the University of Milan and guest scientist at the Leibniz-IZW.

In a recent scientific study on the domestic cat, conducted in cooperation with colleagues from the University of Milan and the University of Veterinary Medicine Vienna, the reproduction biology team of the Leibniz-IZW demonstrated that an immediate on-site vitrification of felid oocytes is the best option to obtain a high number of good quality gametes

and consequently a higher number of developing embryos. This study was also published in *Cryobiology*. On-site vitrification is especially useful if samples are collected from wildlife kept in zoos and then need to be transported to a suitable lab. "Transportation of fresh oocytes and ovarian tissue across international borders is often complex and critical in terms of time, whereas on-site vitrification of oocytes allows a safe time period for transportation. The oocytes can be fertilized at a later point, once they have been transported to a suitable lab and sperm of a male is available," Zahmel explains.

The present research demonstrates successful vitrification,in-vitromaturation and fertilization of African lion oocytes for genetic resource banking. Yet, none of the early embryos from vitrified oocytes developed beyond the 4-cell stage. Clarifying the pathways that are affected by vitrification would be important to get a better understanding of the specific needs vitrified oocytes may have after thawing. "Though embryo development was still impaired, our results give hope that felid oocytes can be cryopreserved and stored in biobanks in future," says Katarina Jewgenow, Head of the Department Reproduction Biology at the Leibniz-IZW. "Our aim is to further improve these methods in model species such as domestic cats and African lions in order to use them one day for the assisted reproduction of endangered felids such as the Asiatic lion," Jewgenow adds.

More information: Jennifer Zahmel et al. Maturation and fertilization of African lion (Panthera leo) oocytes after vitrification, *Cryobiology* (2020). DOI: 10.1016/j.cryobiol.2020.11.011

Martina Colombo et al. Ovary cold storage and shipment affect oocyte yield and cleavage rate of cat immature vitrified oocytes, *Cryobiology* (2020). DOI: 10.1016/j.cryobiol.2020.11.003

Provided by Forschungsverbund Berlin e.V. (FVB)