FROZEN IN TIME

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"WITH THE USE OF CRYOPRESERVATION, DE-EXTINCTION MAY BECOME A POSSIBILITY IN THE NEAR FUTURE."

Should we bring back extinct species? Many endangered species in the wild are facing the possibility of extinction. However, thanks to the developments of genetic technology, extinction does not have to be permanent. It is very possible to resurrect some of the species we once lost and even prevent endangered species from ever becoming extinct. For many years, scientists have played around with the possibility of "de-extinction"[1] and alternative methods to repopulating endangered species. With the use of cryopreservation, de-extinction may become a possibility in the near future. Cryopreservation is the the process in which certain cells such as the eggs and sperm are preserved in very low temperatures and then extracted at a later time when needed. By storing the cells in very low temperatures, it allows to extend the lifetime of cells outside their original hosts. In a way, cryopreservation allows us to freeze time and preserve the eggs and sperms of endangered species and thaw them in the future for in vitro fertilization (IVF). IVF is the process of creating embryos from eggs and sperm by fertilizing them outside of the womb and then transferring the embryo into the recipient. Thus, scientists hope that cryopreservation is the solution to saving endangered species and potentially bring back extinct species.

An endangered species is a group of organisms that are at risk of becoming extinct for one or more of the following reasons: destruction of their living environment, an increase in predators, and/or unsustainable breeding due to low member population. Over the years, scientists and researchers have explored numerous techniques in hopes of reintroducing endangered and even extinct animals back into the wild. Most endangered species are kept in captivity to breed with the remaining members of their population. Some species have even been forced to breed with closely related species, also known as inbreeding. Unfortunately, inbreeding holds potential problems such as: increased reproductive failures leading to fewer offspring, genetically undesirable individuals, and widespread damaging genes. However, this is not to say that inbreeding does not occur naturally. Certain species reproduce outside their breed on their own for their own survival. Inbreeding is a double edge sword. The results of inbreeding can produce excellent quality animals. However, excessive inbreeding can make matters worse for the species. Thus, scientists have turned to cryopreservation for solutions.

Survival of some endangered species heavily relies on "frozen zoos."[3] The Frozen Zoo at San Diego's Institute for Conservation Research was founded in 1972 as a storage for skin-cell samples from rare and endangered species. When the first samples were collected and frozen, genetic technology was still in its new stages so it was not really known how the samples would be used. "The Frozen Zoo was a

wonderful idea. They just thought: 'Well, something might happen, so we should preserve some samples for the future," says Dr Jeanne Loring, who is leading the Scripps team working with frozen samples.[3] "This is the first time that there has been something that we can do."[3] Cells preserved in frozen zoos can be added to the gene pool, increasing the chances of healthy reproduction and ultimately allowing zoos not to rely on forcing animals to breed. "If we could use animals that were already dead – even from 20 years ago – to generate sperm and eggs then we can use those individuals to create greater genetic diversity. I see it as being possible. I see no scientific barrier," Loring says.[3]

Despite some exceptions, many of the frozen cells of certain species were not suitable for the long-term preservation of undamaged DNA. Although certain species produce unsuccessful cells for cryopreservation, there are some species that produce ideal cells and work fantastically well. Scientists have successfully conducted cryopreservation on Asian elephants. The Asian elephant (Elephas maximus) worldwide population is estimated to be at around 50,000-70,000 elephants, of which approximately 15,000 are in captivity. Unfortunately, its population in the wild is not reproducing at a sufficient rate to maintain the current population. Many scientists predict that the Asian elephant will become extinct within the next few decades if their fertility rates continus to decline.

At the Hannover Zoo in Germany, thirty ejaculates were collected from six Asian elephants and one African elephant, a close living relative to the Asian elephant. Semen freezing experiments were conducted on ten ejaculate from one bull. Attempts to collect from the other Asian elephant bulls resulted in samples that were not suitable for freezing, or that could not be frozen at the time of collection[1]. The ten semen samples were evaluated and then processed for freezing with various cryoprotectant to determine the best freezing technique suitable for Asian elephant semen. The seven different concentrations of cryoprotectant that were used were: ethylene glycol, propylene glycol, trehalose, egg yolk, glycerol, and cryoprotectant-glycerol and Me2SO. In the study, the scientists concluded that glycerol was the best cryoprotectant for freezing sperm cells. According to the study, using glycerol "increases the intracellular osmolarity and by that decrease cellular dehydration and shrinkage". [1]

Still, even with the best cryopreservation techniques, the post-thaw survival rates for sperm cells are about 50%. As a consequence, fertility from artificial insemination (AI) is more worse than

Frozen DNA sample being extracted from storage

> "Extinction does not have to be permanent."

that with fresh semen in most cases. It is important to recognize the negative effects of cryopreservation as it is still quite imperfect. Several of the cellular organelles of sperm are enveloped by one or more membranes and it is known that membranes are particularly vulnerable to survival during cryopreservation. Sperm membranes affected by cryopreservation include the plasma membrane, the outer acrosomal membrane and and mitochondrial membranes.[4] There are at least two different phases the sperm cell experiences during freezing and thawing. The first relates to the effects of changing temperature and the second arises because of the formation and dissolution of ice.[4] The sperm experiences a cold shock, which traditionally refers to the extreme sensitivity to sudden cooling exhibited by spermatozoa. The cell membrane becomes fragile in the cold and the chances of survival decreases. Second, ice crystals form in the cells that can cause the membranes to rupture when thawing. The severity of the effects vary among species but are all dependent on the rate of cooling. Thus, cryopreservation is not entirely reliable for unnatural animal reproduction due to membrane vulnerability.

In addition, not only is the cell's membrane extremely vulnerable during freezing but there are also the possibility of contamination. The DNA cells are kept frozen in a liquid nitrogen storage. Liquid nitrogen can also act as a carrier for viruses, bacteria, and fungi.[2] If the liquid nitrogen is contaminated, then the cells will mostly be contaminated as well. Cryopreservation appears to be successful in some instances. However, in order to bring back extinct species, scientists need to figure out the best method that

Scientist holding a frozen DNA sample in the frozen zoo at San Diego's Institute for Consveration Research.

works for all species so that we don't waste precious DNA when sperm cells are lost. Unfortunately, cryopreservation has not been proven to have long-lasting effects on all mammals, although fish, reptiles, and many other species hatched through eggs have been farmed and have been successfully reintroduced into the wild. Nonetheless, cryopreservation appears to open many doors for the future of de-extinction and the survival of endangered species. However, there is still much to learn about cryopreservation before we can move on to the possibility of resurrecting extinct species.

REFERENCES

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IMAGE SOURCES

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