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## **Cryopreservation of Reproductive Material and Cell Lines: Background, Benefits and Challenges**

This statement presents the challenges and uses of employing cryopreservation techniques to store samples of reproductive material and cell lines.

### **Background**

Cryopreservation, or cryobanking, seeks to preserve intact cells from reproductive material (germplasm) and cell lines, for potential future revival and use, by halting metabolic processes via specific, multi-step cooling, freezing and storage protocols which can vary between sample type and species. Samples are stored at temperatures of  $-196^{\circ}\text{C}$  and this ultra-low temperature is achieved using liquid nitrogen (usually in LN2 vapor phase).

### **Uses and Benefits**

Preservation of reproductive material such as germplasm (oocytes/ova or spermatozoa), embryos, but also ovarian or testicular tissue, can be a useful tool for population management and can be extremely valuable for protecting species from threats of extinction by maintaining genetic diversity or even reviving lost genetic lines. For population management programmes like the EAZA Ex situ Programmes (EEP), it has the potential to increase their chance of success considerably, especially when they have roles that require long-term persistence (e.g. insurance population). Furthermore, it may allow additional EEPs with important conservation roles to be established, which currently may not be feasible to manage without preservation of genetic material for future use.

Common, recognized assisted reproductive technologies that are utilizing cryopreserved reproductive material are for example artificial insemination (AI), in-vitro fertilization (IVF) and embryo transfer (ET) (Prieto et.al., 2014).

Cell lines, however, are established cultures of cells, which when provided an appropriate environment and growth medium, can proliferate indefinitely. When preserved or frozen in a way that maintains their cellular viability, they can later be thawed and used for research purposes. This eliminates the need for constant maintenance of the living, replicating cells.

### **Challenges of cryopreservation**

Cryopreservation, in contrast to biobanking, requires sample collection, transport and preservation to be done in a specific window of time (typically within 24-48 hours for spermatozoa or tissues, but less for oocytes) in order to maintain viability of the cells. Similarly, samples need to be collected from live animals, or shortly after the time of death. Prior to storage in liquid nitrogen, gametes, cell lines or embryos need to be protected by specialized media, called cryoprotectant agents (CPAs), in order to avoid structural damage such as that from ice crystals formed during the freezing process (Pegg, 2007). Therefore, protecting and freezing these samples requires specialized and often species-specific reagents, techniques and expertise. Storage requires appropriate facilities with dedicated liquid nitrogen tanks and monitoring systems, as the liquid nitrogen needs to be periodically replenished.

Most protocols associated with these techniques have been developed and tested extensively specifically for domestic or farm animal species and need to be adapted or transposed for the

purposes of use in threatened species. There are distinct challenges when attempting to apply the protocols of cryopreservation or cell-line development to wildlife, including species-specific reproductive knowledge and needs. When attempting to establish effective protocols, differences in taxon, species and tissue sample may impact variables such as CPA type and concentration as well as timing of the steps in the cooling process.

However, technology and methods are evolving rapidly, and the more species- or taxon-specific work that can be undertaken, the more information we can gain, both on the types and methods used for preservation, as well as the forms of assisted reproduction utilizing preserved materials. Any insights gained now may one day lead to benefits for threatened species. Even “temporary” or inefficient methods can be employed now in attempts to preserve these limited, finite resources, while the science and research catches up (Leibo and Songsasen, 2002).

### **Further research**

Given that questions remain regarding the effectiveness of some protocols for threatened or non-domestic species, it can be challenging for institutions who wish to use cryopreservation as a conservation tool. However, any attempts to preserve samples, when possible, should be encouraged, especially in collaboration with researchers who are working to develop or improve protocols, or other methods of biomaterial preservation. This could potentially allow for better and more comprehensive preservation of genetic material of more species in our member institutions.

In attempts to better understand the current state of wildlife cryopreservation, the EAZA Biobank is conducting a literature review of all relevant publications from the last 10 years. This will help to elucidate which samples from which taxa have already been studied, and to what end.

As genetic samples such as blood and tissue are also distinctly important for population management and conservation research needs, the EAZA Biobank will continue urging members to contribute samples of blood, serum and tissue for long-term storage, but it **cannot physically accept reproductive materials for cryopreservation at this time**. For any inquiries or more information regarding cryopreservation or cryopreservation services, please contact the EAZA Biobank Coordinator.

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