Is Cryopreservation the Next Noah's Ark?

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Abstract: The current change in climate, destruction of habitat and loss of organisms due to indiscriminate hunting has led to the extinction of several wild species. Reproductive technologies have long been considered as a valuable asset in the conservation of wild animals facing extinction. An important method of ex situ conservation, cryopreservation can be used in the long-term storage of genetic material. Cryopreservation also known as cryobanking can be performed either by traditional slow cooling or the newer vitrification method. Over the past few years, it has been used not only in freezing of semen samples but has also been extended to cells, tissues and organs. This is due to refinement in technique and use of better cryoprotective agents that are less toxic and more effective. While efforts are ongoing to improve existing refined techniques, cryopreservation has proven to be quite successful in species conservation.

Keywords: Cryopreservation, Ex situ, conservation, genetic resources

Introduction

In the current scenario wild animals are threatened by poaching, habitat loss, heterozygosity loss due to habitat fragmentation and inbreeding depression and climatic changes (hot and dry conditions, increasing the risk of forest fires) which could result in a significant loss of biological diversity in the coming decades. Following this, it has been postulated that in future, gene banks can become essential sources of genetic variation to ensure the long-term survival of threatened species. It has been left up to the various countries, based on the status of the species how and which species do they need to prioritise conserving. Most genetic resource bank programmes have been developed with the aim of developing effective procedures for storing gametes and embryos for future use in breeding. Despite the initial hurdles in developing a refined technique, cryopreservation can be considered as the modern-day Noah's Ark.

What is Cryopreservation?

Cryopreservation is a long-term storage technique at serial low temperatures of below -100°C to preserve the structurally intact living cells and tissues for an extended period of time at a relatively low cost. Cryopreservation is used in the preservation and storage of viable biological samples in a frozen state over an extended period of time. The traditional slow freezing method involves either freezing in a Styrofoam box in field conditions or using a programmable biological freezer. Egg yolk can be used as an extender in the cryopreservation process although the percentage used depends on the species. In this method, there is a cold chain of serial dilution temperatures starting at 5°C and shifted to -35 °C at 40 °C /min, from -35 °C to -65 °C at 17 °C /min, from -65 °C to -85 °C at 3 °C /min; and finally, the straws are plunged into liquid nitrogen at -196 °C. In the ultra-rapid freezing method diluted sperm samples are dropped from a height of 11 cm as 50 ml droplets into liquid nitrogen resulting in the formation of cryopreserved spheres or pellets which can be stored in cryogenic vials.

In the newer vitrification process cryoprotectants like acetamide and Dimethyl sulfoxide have been employed in the cryopreservation process. Vitrification can be defined as the solidification of a solution by an extreme elevation in viscosity without crystallization. In short it indicates the absence of ice formation during cryopreservation. The most recent vitrification techniques involved the use of ethylene glycol which was found to be a less toxic cryoprotectant compared to other cryoprotectants.

Use of Cryopreservation

By using cryopreserved sperm, we can improve the diversity of wildlife populations as well as work towards breed conservation. Development of gene banks should be a priority in the development of conservation programmes. Germplasm cryopreservation includes storage of the sperm, eggs and embryos and contributes directly to animal breeding programmes. Germplasm cryopreservation also assist the ex-situ conservation for preserving the genomes of threatened and endangered species. If at any given point in time, a species is facing extinction due to various non genetic and genetic factors, cryopreservation can be used to prevent the same. Further, if a population is affected by inbreeding depression or there are possible chances of mutational

meltdown cryopreservation can be used to improve the overall heterozygosity in such a population. In such cases it would be advisable to collect smaller quantities of genetic tissue material from a larger number of animals rather than collecting larger quantities from a smaller sample size.

It can also be used to introduce a new species in an environment where it was previously no present thereby resulting in an organism having to adapt to a new environment and thus ensuring greater chances of its survival. The establishment of germplasm banks using cryopreservation can contribute to conservation and extant populations in the future. Since the first successful cryopreservation of bull semen, cryopreserved bull semen has been used to propagate the rare and endangered species using assisted reproduction techniques.

In cryogene banks, the storage of frozen tissues, cultured cell lines, DNA and serum samples can be carried out. Using frozen-thawed pieces of ovary that have been replaced in a female and stimulated to ovulation (Sapundzhiev 2008), mice and sheep have also been generated. From extensive use in domestic livestock, the use of cryopreservation has now been incorporated in aquaculture for seed production, genetic management of broodstock and conservation of aquatic resources. Due to limitations with existing cryopreservation technology, so far only the eggs and larvae of eastern oyster and sea urchins have been successfully cryopreserved. Many studies on cryopreservation of fish sperm have been carried out on economically important freshwater species however it was reported that cryopreservation was more successful in marine fish than freshwater fish.

Although freshwater fish sperm are generally more difficult to cryopreserve, the fertilization rates obtained from the cryopreserved marine fish sperm are similar to those obtained with mammalian species (Tsvetkova *et al.,* 1996). The sperm of more than 30 marine fish species have been cryopreserved successfully. Higher fertility and viability rates have been obtained from frozen-thawed spermatozoa from marine species when compared against freshwater species. Successful cryopreservation of embryos is vital as for the preservation of biodiversity of both paternal and maternal genomes. While cryopreservation of mammalian embryos has been met with immense success, the same is not applicable for aquatic embryos due to their multicompartmental biological systems, high chilling sensitivity, low membrane permeability and their large size, which gives a low surface area to volume ratio (Zhang and Rawson 1995).

Conclusion

Cryopreservation as a process can be touted to be a modern-day Noah's ark. It can be used not only in the conservation of wild species but also in breed conservation of domestic livestock. However, while it may not have the space constraints Noah's ark did, it has its fair set of challenges. Chilling injury in embryos could lead to developmental difficulties, especially in fish embryos, due to inhibition of metabolic and enzymatic processes. While cryoprotectant toxicity decreases at later embryonic stages, a larger yolk reduces the chances of successful cryopreservation.

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