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Review article

Cryopreservation of ovarian tissue: An emerging technology for female germline preservation of endangered species and breeds

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ABSTRACT

Many hundreds of exotic species and domestic animal breeds have been lost over the course of the last few decades. In order to avoid a similar fate to other animals threatened with extinction, it is crucial to develop and apply rescue strategies to ensure their survival for the future. One option as a safeguard measure is the cryopreservation of the main source of female gametes enclosed within the ovary: the primordial follicles. So far, there are three options to cryopreserve small ovarian follicles: whole ovary, ovarian cortical tissue or isolated follicles, with the use of slow freezing or vitrification methods. After cryopreservation, the harvested material can be transplanted or cultured, with the aim to produce mature fertilizable oocytes. The objective of this review is to summarize the current status of the cryopreservation of ovarian tissue in domestic species and non-endangered wild mammals as model for threatened and endangered species and breeds, and to provide new insights into techniques that can be applied in the future.

Contents

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1. Introduction

Over the course of the last 100 years we have been faced with a rapid and continuous decline of wild mammalian species and an indiscriminate dilution of farm animal breeds. One in every four mammalian species is under threat around the world (IUCN, 2010) and every week, an average of two domestic breeds is being lost (FAO, 2009). Despite the advances in reproductive management for breeding programs, little is known about the reproductive physiology of individual species of wild mammals, and the potential use of reproductive biotechnologies for safeguarding endangered wildlife species has had partial success. The main limiting factors are the lack of knowledge on the basic reproductive biology of these species, the absence of standard protocols for preserving female genetic material, and species-specific differences. To preserve genetic diversity, notably for the conservation of endangered species and indigenous farm animal breeds, it is essential to create genome or genetic resource banks (GRBs) of male and female gametes and embryos, made up of a very large number of individual donors in good condition (Wildt, 2000).

The ovarian cortex contains thousands to millions of primordial follicles that can remain viable even several hours after the animal's death (Silva et al., 2000). These follicles could be recovered and successfully cryopreserved for future xenotransplantation (Paris et al., 2004) or *in vitro* culture (Amorim et al., 2003a) in order to obtain mature fertilizable oocytes. Thus, cryopreservation of whole ovary, ovarian cortical tissue or isolated follicles could be used to preserve female gametes (Donnez et al., 2006), enlarging the gene pool. The preservation of ovarian tissue is cryobiologically challenging because it contains many cell types and specific extracellular matrix components (Hovatta, 2005). However, despite the fact of cryodamage, the regular reports on the development of mature oocytes and live births by means of this technology in mice (Gunasena et al., 1997; Candy et al., 2000; Liu et al., 2001; Kagawa et al., 2007), rats (Wang et al., 2002), rabbits (Almodin et al., 2004a), sheep (Gosden et al., 1994; Salle et al., 2003; Baird et al., 2004; Bordes et al., 2005; Imhof et al., 2006) and human (for a review of 6 of the 9 reported live births see von Wolff et al., 2009), clearly show the potential of this technology.

The present paper aims to review the major findings that emerged from the European Science Foundation (ESF) Exploratory Workshop on "Cryopreservation of ovarian tissue in cancer patients, farm animals and endangered species" regarding the status and achievements of ovarian tissue cryopreservation as a technology for female germline preservation of endangered species and breeds. Conclusions on human ovarian tissue cryopreservation arose from the same Workshop and can be found in the review published by von Wolff et al. (2009). The workshop had active participation of biologists, veterinarians and medical doctors, and opened new perspectives on the development of integrated strategies for the preservation of female germline.

2. Ovarian tissue cryobanking for endangered animals

2.1. Indications for ovarian tissue cryobanking

2.1.1. Threatened and endangered exotic (non-domestic) species

It is widely accepted that habitat conservation is the commonly used approach to protect and nurture species so that they can survive. However, given human activities have caused a decline in the abundance, diversity and distribution of several species, it is crucial to advocate and to create germplasm banks (Wildt, 2000), and the collaboration of specialized wildlife reproductive biologists, veterinarians and conservation managers to preserve genetically diverse sustainable populations. Apart from banking semen and embryos, ovarian tissue cryostorage has enormous potential to back up the current existing International Union for Conservation of Nature (IUCN) species action plans. The option of cryopreserving ovarian tissue avoids many of the cryobiological and practical limitations that are encountered in efforts to obtain and cryopreserve fully mature oocytes from wild mammals, such as the low number of mature oocytes that can be found and possible detrimental effects of cooling them.