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Novel needle-in-straw vitrification can effectively preserve the follicle morphology, viability, and vascularization of ovarian tissue in Japanese quail (*Coturnix japonica*)

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ABSTRACT

Cryopreservation of ovarian tissue has been the only effective way of *ex situ* conservation of female germplasm in avian species. A novel needle-in-straw (NIS) vitrification method was developed to store tissue in straws instead of cryovials. Fragments of ovarian tissue from one-week old Japanese quail were transfixed on an acupuncture needle. They were immersed in equilibration and vitrification solutions containing dimethyl sulphoxide, ethylene glycol and sucrose. A layer of tin foil was rolled over the tissue fragments and the tin foil package was plunged into liquid nitrogen and inserted into a pre-cooled, 0.5-ml straw which was stored in liquid nitrogen. Tissue was also preserved using a needle immersed vitrification (NIV) method, in which tissue fragments transfixed on needles without tin foil and were stored in cryovials filled with liquid nitrogen. Cryopreserved tissue was warmed at room temperature (RT) or 37 °C and the ratio of normal follicles to total visible follicles was determined by histological methods. In addition, cryopreserved and warmed tissue was cultured on the chorioallantoic membranes of fertilized chicken eggs for 5–6 days. The viability and vascularization of the grafts were evaluated. The tissue cryopreserved by NIS and warmed at RT showed comparable follicle morphology to fresh tissue and to that preserved by NIV and warmed at RT. No significant impairment on the viability or vascularization of the grafted tissue was observed. The NIS method allows tissue to be stored and transported safely and efficiently and can be used instead of cryovials in tissue cryobanking.

1. Introduction

Avian researchers and the poultry industry have faced a drastic reduction of avian genetic resources in the past few decades, largely because of the lack of an inexpensive and reliable preservation-reconstitution regimen. Until recently, the only effective method of preserving avian genetic resources has been in living flocks but the high cost has resulted in a dramatic and continuous decline in the number of research flocks (Fulton and Delany, 2003). Meanwhile, the modern poultry breeding industry uses very few highly selected lines, leaving it vulnerable to threats such as disease or changing markets.

Cryopreservation of germplasm has been successfully used in the dairy industry, which provides an example for poultry. However, it has been extremely challenging in birds, especially for females. Avian eggs are characterized by the presence of a large amount of yolk (polylecithal), and

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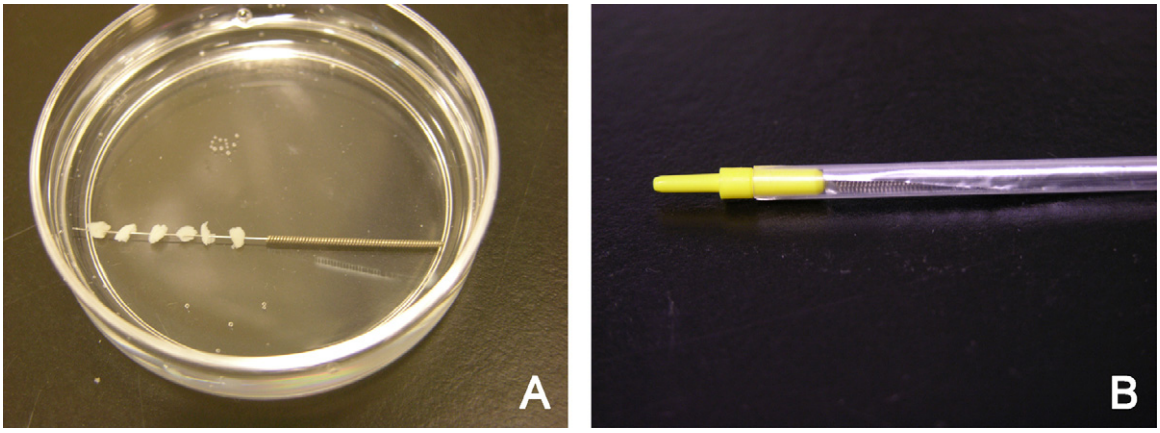

Fig. 1. Needle-in-straw (NIS) vitrification device. (A) Ovarian fragments carried by a needle; (B) a demonstration of the device: tin foil including tissue fragments on a needle was inserted into a straw, with the end of the straw sealed by an adaptor plug.

the polar position of the developing embryo (telolecithal). These characteristics prevent the application of the cryopreservation techniques that have been used for mammals. Efforts have been made to cryopreserve primordial germ cells (PGCs) to generate chimeras, but the feasibility of this strategy for genetic conservation is limited due to the low efficiency and high cost (Petitte, 2006).

Ovarian tissue contains a large pool of primordial follicles with the potential to generate mature follicles (Johnson and Woods, 2009), providing that the tissue can be recovered. This has been confirmed by recent success in ovarian transplantation in chickens (Song and Silversides, 2006, 2007) and Japanese quail (Song and Silversides, 2008). In addition, we have demonstrated that ovarian tissue of Japanese quail can be successfully cryopreserved and recovered by transplantation (Liu et al., 2010). Liu et al. (2010) showed that a vitrification method was more efficient than a conventional slow-freezing method. Vitrification likely provides better preservation of the integrity of the tissue, which is important for its functional recovery, through the process of solidification without ice crystallization (glass formation). With the conventional slow-freezing method, ice formation is induced in the extracellular space (Nawroth et al., 2007).

The vitrification method that we used previously was based on successful studies in mammals (Chen et al., 2006; Wang et al., 2008). The use of acupuncture needles as tissue carriers minimized the handling of individual samples and enhanced the efficiency of processing a large quantity of samples. However, when tissue is stored in cryovials for cryobanking, there are practical limitations such as difficulty in handling and security concerns (Kuwayama, 2007).

The objective of this study was to test the efficiency of vitrification and warming procedures using straws instead of cryovials to preserve the ovarian tissue of Japanese quail. Histology of the cryopreserved and warmed ovarian tissue and its viability and vascularization after being transplanted onto the chicken chorioallantoic membrane (CAM) were examined and analyzed.

2. Materials and methods

2.1. Birds, chemicals and tissue preparation

Quail ovarian tissue was obtained from one-week-old female chicks of White Breasted (WB) and QO lines of Japanese quail (Liu et al., 2010). Fertile chicken eggs were from the Minnesota Marker line (Pisenti et al., 2001). Both the quail and chicken lines were maintained at the Agassiz Research Centre. The research protocol was approved by the Animal Care Committee of the Agassiz Research Centre following principles described by the Canadian Council on Animal Care (2009). All chemicals were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada) unless otherwise indicated.

One-week-old quail chicks were used because a previous study (Song and Silversides, 2008) had found that recipients with ovarian transplantation at this age survived better than those at one day of age. Ovarian tissue was obtained immediately after euthanasia of the birds by cervical dislocation and immersed in Dulbecco's phosphate-buffered saline (DPBS) with 20% fetal bovine serum (FBS) on ice. The surrounding connective tissue was then gently removed and each ovary was cut to a size of approximately 2.5 mm × 2.5 mm under a dissecting microscope. All tissue fragments were kept on ice before further treatment within 2 h.

2.2. Vitrification and warming procedures

For the needle-in-straw (NIS) method, four to six ovarian tissue fragments were transfixed on an acupuncture needle (Cloud & Dragon Medical Device Co. Ltd., Jiangsu Province, China), which was modified slightly to fit the straw. Tissue fragments carried by the needle (Fig. 1A) were first submerged in DPBS with 20% FBS containing 7.5% (v/v) dimethyl sulphoxide (DMSO) and 7.5% (v/v) ethylene glycol (EG) for 10 min, then a similar solution containing 15% (v/v) DMSO, 15% (v/v) EG and 0.5 M sucrose for 2 min. This two-step vitrification protocol had been successfully used