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Impact of cryopreservation method on dromedary camel ovary structure, viability, and development of antral follicular oocytes

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ABSTRACT

The objectives of this study were, a) to compare two different vitrification techniques, the solid surface vitrification (SSV) and direct vitrification (DV) method, b) to evaluate the effect of four cryoprotectant agents and their toxicity on the morphological appearance and ultrastructural of camel ovarian cortex, c) to examine the development of oocytes recovered from the vitrified ovarian cortex. Fragments of ovarian cortex were exposed to equilibration solution consisting of TCM- 199 with 10% fetal camel serum (FCS); 0.10 M sucrose and including one of the following cryoprotectants; 20% glycerol (GLY); 3.5 M ethylene glycol (EG); 3.5 M propanediol (PROH) or 3 M dimethylsulphoxide (DMSO). After vitrification of ovarian fragments, they were warmed and evaluated by histological and transmission electron microscope. The oocytes isolated from vitrified ovarian cortex were cultured in TCM-199 at 38.5 °C under 5% CO2 for 44 h. Maturation was indicated through cumulus expansion and calculated by oocytes reaching first telophase and second metaphase (TI + MII). The percentage of morphologically normal and viable follicles of SSV was significantly higher $P < 0.05$ than DV group (52.9 vs. 38.1, respectively). In conclusion, viability, histological and ultrastructural observations revealed that SSV method and ethylene glycol-based freezing solution were able to remain morphology better follicle and oocyte. Additionally, most organelles of oocytes are able to recover their normal morphology in camel ovarian cortex following cryopreservation and thawing processes, and oocytes isolated from vitrified ovarian cortex can exhibit maturation and reaches to (TI + MII).

1. Introduction

According to FAOSTAT (2006), the stock numbers of ruminants and their development from 1996 to 2005 increased substantially with a percentage increase ranging from 22% for sheep to 45% in cattle, while, camel population went down by 8% during the same period (Galal, 2007). Moreover, there is a growing need for early staged follicle and oocyte banking with regard to economically important and endangered camel species. Therefore, we utilized ovarian tissue preservation technique for the first time from camels. However, vitrification has been successfully applied for cryopreservation of ovarian tissue for a wide range of species including sheep (Demirci et al., 2001 and Salle et al., 1998), Cows (Paynter et al., 1999; Juliana et al., 2008), and humans (Mehdi et al., 2017) and proved to be an applicable technique in fertility preservation with high follicular survival rates. Although, there are no absolute rules in cryopreservation of ovarian tissue techniques, most previous studies employed two methods of cryopreservation, conventional freezing (Aubard et al., 1998; Candy et al., 1997), and developed vitrification technique (Rall and Fahy, 1985). Vitrification involves ultra cooling and warming rates in the presence of a very high concentration of cryoprotectants to avoid intercellular ice-crystal

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formation and chilling injury (Fahy et al., 1984). In turn, ultra cooling reduces the toxicity of the cryoprotectant (Parks and Ruffing, 1992). Currently, several vitrification techniques are available such as the open-pulled straws vitrification (Vajta et al., 1998), Direct Cover Vitrification (DCV), Electron Microscope (EM) grids (Park et al., 1999), and Solid Surface Vitrification (Dynnies et al., 2000). Although, studies on these methods are recorded different levels of success, there is an evidence suggesting that solid-surface vitrification is more efficient and able to retain a higher percentage of morphologically normal and functional follicles after thawing (Dynnies et al., 2000), the success of vitrification method depends on the used techniques and the proper use of a cryoprotectant agents and vitrification process. However, the most common cryoprotectant agents used are DMSO; EG: and PG/PROH (1,2-Propanediol) for ovarian cortex cryopreservation, either alone or in combination (Mazur and Schneider, 1986; Isachenko et al., 2002). In cow, Kagawa et al. (2009) vitrified ovarian slices by Cryotissue method, which is similar to the cryotop, using EG and DMSO as cryoprotectants, the authors showed that the survival rate of recovered oocytes was 89%, and concluded that the vitrification Cryotissue method could be effective method for ovarian tissue cryopreservation. In sheep, Al-aghbari and Menino (2002) demonstrated that oocytes recovered from vitrified ovarian tissues can be an exhibit in-vitro maturation rates similar to that of vitrified and non-vitrified oocytes. However, cryopreservation of camel ovarian tissues and the effect of cryoprotectants and/or freezing method on the viability of follicles within ovarian tissues has not been investigated. Therefore, the present study aimed to define the effect of the vitrification methods using different cryoprotectant agents (GLY, EG, PROH, and DMSO) on the viability, histological and ultrastructural of camel ovarian cortex. In addition, determining whether immature oocytes isolated from the follicles of frozen-thawed camel ovaries tissues can resume their activity and undergo in vitro maturation.

2. Materials and methods

This study was carried out at the Laboratory of Animal Physiology and Biotechnology, Animal Production Department, Animal Production Research Institute, during the period from April 2016 to December 2016.

2.1. Ovary collection

A total of 212 ovaries from Dromedary camels (7–10 years of age) were obtained from El-Bassatein Abattoir, Cairo, and transported immediately after slaughtering into thermos flasks at 35 °C, to the laboratory (within 2 h of slaughter) in saline solution (0.9% NaCl) supplemented with antibiotics (100 IU penicillin and 100 μg streptomycin/ml).

2.2. Ovaries washing

In the laboratory, ovaries were trimmed from adhering tissue and rinsed once in 70% alcohol and twice in phosphate-buffered saline (PBS). The ovarian cortex of each ovary was then manually dissected from medullar tissues by scalpel and cut into four fragments (about 1 mm^3). The fragments were placed into PBS supplemented with 0.1% (v/v) penicillin/streptomycin (Gibco, Paisley, UK) at room temperature (25 °C).

2.3. Experimental design and vitrification procedure

The selected ovarian fragments were randomly distributed over the experiment treatments. The first fragment was fixed in 4% paraformaldehyde for 12 h for routine histological examination where (as control), and the second fragment was fixed in 2.5% glutaraldehyde in phosphate-buffered saline (pH 7.4) for routine electron microscopy examination. The remaining fragments were submitted to the vitrification procedure (16 fragments).

2.3.1. Dehydration regimen

Dehydration prior to vitrification was performed using two steps method according to Gookv and Edgar (1999). Briefly, 16 fragments were divided into four equal groups and placed into 15 ml Falcon tubes (Becton Dickinson, Bedford, MA,USA). The fragments were submitted to solution I (SI) consisting of basic medium (TCM- 199 with 10% fetal camel serum) with one of the following cryoprotectant agents; 10% GLY; 1.5 M PROH; 1.5 M EG or 1.5 M DMSO, for 15 min followed by equilibration solution (SII) consisting of basic medium with 20% GLY; 3 M EG; 3 M PROH or 3 M DMSO, 10 ml per vial, at room temperature for 15 min.

2.3.2. Direct cover vitrification (DCV)

The ovaries were vitrified using the method described by Chen (2006). After equilibration period 8 fragments represent four treatment groups (two fragments per treatment) were placed on an aseptic absorbent gauze to remove the remaining equilibration solution, and transferred into a 3 ml plastic standard cryovial, and the vial was immediately submerged into liquid nitrogen. Then the vials were closed and transferred into canisters using nitrogen-cooled forceps for storage in a liquid nitrogen tank (196 °C).

2.3.3. Solid surface vitrification (SSV)

The remaining fragments (8 fragments) of four treatment groups were submitted to the SSV procedure according to Dynnies et al. (2000). The fragments were placed on a cold surface of steel cube covered with aluminum foil partially immersed in liquid nitrogen followed by transfer into in 5 ml cryovials in liquid nitrogen for (2–3 weeks).

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