CRYOPRESERVATION OF BOVINE OOCYTES BY VITRIFICATION USING MICRODROPLET METHOD

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Abstract

Recently, vitrification has been regarded as a potential alternative to traditional slowcooling method. In Vietnam, we have succeeded in freezing bovine embryo by vitrification method. The aim of this study was to apply the cryopreservation of bovine oocytes by vitrification using microdroplet method. Oocytes from slaughterhouse ovaries with multi-layers of compact cumulus cells were matured in TCM 199 medium supplemented with fetal calf serum for 20-22h at 38.5^0C under 5 % CO_2 in air. Matured oocytes were chosen and placed into pre-equilibration solutions containing 1.0 M ethyleneglycol (EG) in TCM199 medium supplemented with 20% FCS. After 15 min, oocytes were transferred into vitrification solutions (5.5M EG and 1.0M Sucrose in TCM199 supplemented with 20% FCS). Oocytes were pipetted into a glass capillary in groups of 5-8, and dropped directly into liquid nitrogen. The total exposure time of oocytes to vitrification solutions was 30 sec. The vitrified microdroplets containing 5-8 oocytes in about 6µL of vitrification solution were transferred to cryotubes to store in liquid nitrogen. After a week cryopreservation, oocytes were thawed and cultured in TCM 199 supplemented with 20% CS at 38.5^0 C under 5% CO_2 for 3h. Oocyte survival was evaluated by the color and re-expansion of cumulus cells. In total of 325 oocytes vitrified, there were 153/325 (47.08%) survival oocytes with light-color and well re-expansion of cumulus cells after thawing and culturing in

vitro for 3h. Our results indicated that we can apply vitrification using microdroplet method to store bovine oocytes in Vietnam. It suggested that micro-droplet method is very simple, effective and useful for preservation of animal genetic resources.

Keywords: bovine oocytes, thawing solution, culture solutions, vitrification solutions

Introduction

The ability of freezing sperm and embryos has been technical feasible and a widely practical procedure in the world. However, the ability of cryopreservation oocytes have been much more difficult and resulted in low survival rate. Oocytes and embryos can be cryopreserved using slow-cooling or vitrification techniques. Fuku et al. (1992)[4] and Otoi et al(1993)[9] reported the first succeeded to get calves derived from freezing and thawing IVM bovine oocytes. In 1992, Hamano et al.[5] developed a new method to preserve oocytes with higher development rate of frozen oocytes. This is vitrification method. Vitrification is defined as glass-like solidification. Vitrification achieved by using a high concentration of cryoprotectant combined with a rapid cooling rate. High concentration of cryoprotectant help oocytes avoids ice crystal formation during the cryopreservation progress (Fahy et al. [3]). In turn, rapid cooling rates reduce toxicity of the cryoprotectant and also diminish the length of time oocytes are exposed to temperature which they are sensitive (Parks et al. [10]). As the results of two advantages, vitrification method has widely used and now becomes a potential alternative to traditional slow-cooling method. There are several vitrification methods such as glass capillaries (Hochi et, al [6]), open-pulled plastic straws (Vajta et al. [12]), microdroplets (Landa [8]). Most research focused on the benefits to cryopreserve mature oocytes. Mature oocytes has stable ultrastructure against freezing damage, high membrane permeability and achieved higher survival and development after freezing-thawing than immature oocytes. In Vietnam, cryopreservation of bovine oocytes is a new method and its application for nuclear transfer and cloning technologies. Thus, we attempted to apply an easy,

simple, efficient vitrification- microdroplet method to freeze bovine oocytes in our conditions.

Materials and methods

Preparation of oocytes

Ovaries obtained from the local slaughterhouse were transported to the laboratory in saline at 30^0C within 2 to 3h. Cumulus-oocyte-complexes (COCs) were collected from follicular fluid aspirated through 18-gauge needles from follicles 2 to 6mm in diameter. COCs surrounded with multilayer compacted cumulus cells were selected for this experiment. Selected COCs were washed 3 times in maturation medium and cultured in TCM-199 medium supplemented with 5% FCS. About 20 sellected COCs were incubated for 24 h in 100µL of TCM-199 medium covered with mineral oil at 38.5^0C under 5% CO_2 in air. After maturation, oocytes were evaluated. Mature oocytes scored grade A were used for vitrification.

Vitrification of oocytes using microdroplet method

Cumulus-oocyte-complexes at metaphase II were selected and introduced into a pre-equilibration medium consisting of 1M ethylene glycol (EG) in TCM 199 medium supplemented with 20% CS for 20 min. Then 5-8 oocytes were transferred into vitrification medium consisting of 5.5M EG and 1.0 M sucrose in TCM 199 medium supplemented with 20% CS. After 30sec, 6µl of vitrification solution containing 5-8 oocytes was dropped directly in liquid nitrogen. The vitrified micro drops were transferred to a cryotube to store in liquid nitrogen.

Thawing and evaluation of vitrified oocytes

The vitrified micro drops were exposed to dilution medium containing 1.0 M Sucrose in TCM199 supplemented with 20% CS. Then they were cultured for 3h at 38.5^0C under 5% CO_2 in air. Oocyte survival was morphologically evaluated with the color and re-expansion of cumulus cell multilayers.

Results

1. The results of bovine oocytes collection and maturation

Oocytes were collected from ovaries derived from a slaughterhouse. Before maturation, they were classified.

Total of 914 oocytes collected, there were 555 (60.72%) COCs classified grade A with several well- compacted cumulus cell layers. They were used for maturation in vitro. The maturation rate in this experiment was 58.56 % (325 maturated COCs /555COCs). We chose these mature oocytes used for freezing.

2. The results of bovine oocyte survival after thawing and culturing in vitro for 3h

After thawing and culturing in vitro, bovine oocytes passed through the chilling injures. Their cumulus cells re-expanded with light-color were evaluated survival oocytes. There were 153/525(47.08%) oocytes survival after thawing and cuturing in vi tro for 3h.

Discussion

Recently, cryopreservation of oocytes from domestic species including cattle (Dinnyes et al.[2]), pig(Tamas Somfai et al. [9]), and goats (Isabella Begin et al.[7]) has been succeeded but still low survival and development to blastocyst rate. A main reason for reduced developmental competence of cryoprecerved oocytes is damage of the genetic material (Aman et al.[1]). They also reported that chilling of oocytes has been shown to cause disorganization of metaphase-II spindles, which can result in chromosomal aberrations. In order to enhance survival rate of oocyte, it is necessary to choose suitable cryoprotectants, and mature oocyte with high quality. Many researchers preferred to use mature oocytes than immature those. There are a lot of advantages of cryopreservation mature oocytes. Fuku et al.[4] reported that mature oocyte has higher membrain permeability than in oocytes. Furthermore, they also investigated that mature oocyte has higher survival and development after freezing and thawing than

immature oocytes. In experiment, we used mature oocyte with good quality. Thus, we achieved 47.08% survival oocytes after freezing and thawing.

Concentration and type of cryoprotectant have important effects on the developmental competence of vitrified oocyes. Our result indicated that cryopreservation of bovine oocytes by vitrification using microdroplet method can enhance the survival of oocytes after freezing-thawing.

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