Post Thaw Recovery of Buffalo Oocytes Vitrified in Propylene Glycol and Trehalose supplemented with Bovine Serum Albumin

[1]K. Loganathasamy, [2]G. Taru Sharma

[1]Assistant professor, Department of Veterinary Biochemistry, Madras Veterinary College, Chennai-600 007, Tamil Nadu
[2]Principal Scientist and Head, Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar – 243 122, Uttar Pradesh, India
Email: [1]lnsamy75@gmail.com

[Received: 24th Oct 2014; Revised: 31stOct 2014; Accepted: 31stOct 2014]

Abstract— The present study has been undertaken to assess the post thaw recovery of buffalo oocytes vitrified at different stages of in vitro maturation (IVM) by propylene glycol and trehalose supplemented with bovine serum albumin (BSA). Cumulus oocyte complexes (COCs) obtained from slaughterhouse ovaries were randomly divided into 6 different groups: control (nonvitrified oocytes were matured for 24 h in maturation medium (MM) consists of TCM-199 supplemented with 10% w/v fetal calf serum (FCS) at 38±1°C and 5% C02 in a humidified atmosphere), 0 h (vitrified before the onset of maturation), 6, 12, 18 and 24 h groups (vitrified at 6, 12, 18 and 24 h, respectively, after the onset of maturation). Oocytes were exposed to vitrification solution (VS) consists of 40% w/v propylene glycol and 0.25 M trehalose in phosphate buffered saline (PBS) supplemented with 4% w/v BSA for 3 min at 20-25°C. Oocytes in VS were loaded into 0.25 ml French mini straw with 1M sucrose solution separated by two airspace on either side of VS. The straws were sealed with hot forceps and plunged directly into liquid nitrogen (LN2; -196°C). The straws were thawed after storage period of atleast 7 days by transferring them into a water bath at 37°C for 30 sec. The cryoprotectant was removed by exposing the oocytes to 1 M sucrose solution. A sum of 495, 432, 457, 416 and 420 oocytes were vitrified in 0, 6, 12, 18 and 24 h groups, respectively. After thawing, 444 (89.70%), 384 (88.89%), 418(91.47%), 381 (91-59%) and 387 (92.14%) oocytes were recovered in 0, 6, 12, 18 and 24 h groups, respectively. It is evident that no significant difference was observed on post thaw recovery in different groups of buffalo oocytes vitrified with propylene glycol, trehalose and BSA.

Key words— Vitrification, In vitro maturation, Post thaw recovery and Buffalo oocytes.

I. INTRODUCTION

In vitro embryo production (IVEP) plays a pivotal role in propagation of superior germplasm in livestock and provides basic research tool for further investigations like embryo splitting, sexing, cloning and production of transgenic animals. IVEP uses the oocytes obtained from live animals by ultrasound guided transvaginal ovum pick-up (OPU) as well as from ovaries collected at abattoir. The continuous availability of viable, developmentally competent oocytes has been critical to recent progress in IVEP because of the relatively short fertile life span of mammalian oocytes [1]. Hence, the storage of unfertilized oocytes would generate a readily available source, which allows the experiments to be carried out at convenient time [2] and could therefore be of practical importance in the establishment of gamete bank from which particular genetic combinations could be derived [3]. Preservation of oocytes reduces the risk and expense involved in transport of live animals, hazards of disease transmission and also provides insurance against catastrophes and natural disasters. Buffalo is an important livestock, which provides milk, meat and work power. India has the largest population of buffalo in the world. It has 94.1 million heads of animals [4] which constitute more than 58% of world buffalo population. In recent years, there has been increasing interest in IVEP for faster propagation of superior germplasm in buffalo because of low efficiency of superovulation and embryo transfer in this species. This may be due to some physiological features, peculiar of this species viz., i) lower number of primordial follicles observed in buffalo ovaries, varying from 10, 000 to 19,000 (5) compared with 1, 50,000 in cattle [6], ii) lower number of antral follicles throughout the whole estrus cycle [7] and iii) high incidence of follicular atresia i.e. 82% [8] or 92% [9] as observed in slaughter house ovaries. Hence, establishment of a protocol that optimizes the survival of buffalo oocytes by vitrification is necessary to supply continuous source of oocytes during lean season.

Generally cryoprotectants are used to lower the freezing points and therefore allows the cell and freezing medium to super cooled to a specific subzero temperature before seeding. They protect the cell membrane from freeze

related injury and decrease the deleterious effects of high salt concentrations as the cells dehydrate during the freezing process [10]. We have earlier reported that propylene glycol can be used as a cryoprotectant to preserve buffalo oocytes [11]. The present study has been conducted to assess the post thaw recovery of buffalo oocytes vitrified at different stages of in vitro maturation by propylene glycol and trehalose supplemented with bovine serum albumin (BSA).

II. MATERIALS AND METHODS

A. Materials

Chemicals

Sodium chloride (NaCl) and modified Dulbecco's phosphate buffered saline (mDPBS) used for ovary collection and washing were procured from Hi-Media, India. Tissue culture medium-199 (TCM-199), antibiotics, antimycotics and other chemicals used for preparation of maturation, fertilization and embryo development media were of embryo culture or tissue culture grade, procured from Sigma Chemicals Co., USA. Propylene glycol and trehalose used for preparation of vitrification solution were also procured from Sigma Chemicals Co., USA.

Fetal calf serum was procured from Sigma Chemicals Co., USA and heat inactivated at 56°C for 30 min and stored at -20°C. Blood sample was collected from the estrus buffalo. The serum was separated, heat inactivated at 56°C for 30 min and stored at -20°C.

All the media were filtered through membrane filter (0.22 µm) and stock solutions were kept at 4°C for maximum of 1 month. The working solutions were prepared and preequilibrated 12 h before use.

Plasticware and glassware

All the plasticware used for culture viz., petriplates, culture dishes, multiwell dishes, centrifuge tubes and culture bottles etc. were purchased from Nunc: Denmark.

All the glass wares (Borosil, India) used for culture were washed, packed and sterilized at 180°Cfor 1 h. Other than glassware, rests of culture accessories were autoclaved at 120°C and 15 psi for 30 min. All the plastic wares and glass wares were UV treated for 30 min before use.

B. Methods

Collection of ovaries

Buffalo ovaries were collected from local abattoir in sterile normal saline solution (NSS-0.89% w/v) supplemented with antibiotics (Penicillin G-100 lU/ml and streptomycin-100 µg/ml) and antimycotic (Amphotericin B - 2.5 µg/ml) at 30-35°C in an isothermic container and transported to the laboratory within 2 h of slaughter.

The surrounding tissues were trimmed off and the ovaries were washed several times with sterile NSS. The ovaries were exposed to 70% ethyl alcohol for 30 sec and finally washed in mDPBS.

Oocytes collection

Oocytes from surface follicles (>3 mm) of buffalo ovaries were collected by aspirating the follicles with 18 gauge needle attached to 5 ml syringe in oocyte collection medium (OCM). Contents from syringe were poured into a 50 ml test tube having OCM. Oocytes were allowed for gravitational settlement for atleast 15-20 min. Supernatant was then discarded and the remaining fluid was poured into a petridish containing OCM. Morphologically culturable oocytes i.e. those having compact, multilayered cumulus oocyte complexes (COCs) and evenly granulated cytoplasm were selected under stereo microscope (Bausch and Lamb, USA) and transferred to another petridish containing OCM. Finally COCs were washed 5 times in oocyte washing medium and 5 times in maturation medium.

In vitro maturation (IVM)

The COCs were randomly divided into 6 different groups with approximately equal number of oocytes.

Group I (Control)

The COCs were matured in MM for 24 h at 38±1°C and 5% $C0_2$ in humidified air.

Vitrification of oocytes

Vitrification solution (VS) consisted of propylene glycol (40% w/v) and trehalose (0.25 mol l-1) in phosphate buffered saline (PBS) supplemented with BSA (0.4% w/v).

Group II (Vitrification before onset of maturation)

The COCs immediately after collection (germinal vesicle stage) were exposed to VS at 20-25°C for 3 min. Then, the COCs in VS were loaded into 0.25 ml French mini straw (15-20 COCs in each straw) with sucrose (1 mol l -1) solution separated by two air space on either side of VS. The straws were sealed with hot forceps and plunged directly into liquid nitrogen (LN2).The straws were thawed after storage period of 7 days by transferring them into a water bath at 37°C for 30 sec. The cryoprotectant was removed by exposing the COCs to sucrose (1 mol ¬- l Finally, the COCs were matured in MM for 24 h at 38±1 °C and 5% CO, in humidified air.

Group in (Vitrification at 6h maturation)

The COCs were matured for 6 h and were vitrified, stored and thawed as in Group II. They were further matured for 18 h to complete 24 h maturation.

Group IV (Vitrification at 12 h maturation)

The COCs were matured for 12 h and were vitrified, stored and thawed as in group II. They were further matured for 12 h to complete 24 h maturation.

Group V (Vitrification at 18 h maturation)

The COCs were matured for 18 h and were vitrified, stored and thawed as in group II. They were further matured for 6 h to complete 24 h maturation.

Group VI (Vitrification at 24 h maturation)

The COCs were matured for 24 h and were vitrified, stored and thawed as in group II.

The straws were thawed after 7 days of storage in LN2 by transferring them into a water bath at 37°C for 30 sec. After thawing, oocytes from 0, 6, 12, 18 and 24 h were counted for post thaw recovery under stereo-microscope.

Statistical analysis

Statistical analysis was carried out by standard method described by Snedecor and Cochran (10).

III. RESULTS AND DISCUSSION

A sum of 3285 buffalo ovaries was collected from local slaughterhouse and used for this study. From 3285 ovaries, 2991 oocytes were harvested by follicle aspiration method. Out of 2991 oocytes, 2542 culturable quality oocytes were selected under stereomicroscope. An average of 0.91 oocyte per ovary was recovered and out of which, 0.77 culturable oocytes per ovary was selected. Fully/partially denuded oocytes as well as oocytes with unevenly granulated cytoplasm comprised of around 15% of total population. These oocytes were not included for this study.

A total of 2542 oocytes were used in this experiment, out of which 2220 oocytes were allotted into five different vitrification groups (495, 432, 457, 416 and 420 oocytes for 0,6,12, 18 and 24 h, respectively; Table 2). The oocytes were stored in LN2 for at least 7 days and then oocytes were thawed by transfer to a water bath at 37°C for 30 sec.

After thawing, 444 (89.70%), 384(88.89%), 418 (91.47%), 381 (91.59%) and 387 (92.14%) oocytes were recovered, whereas 51 (10.30%), 48 (1111%), 39 (8 53%), 35 (8.41%) and 33 (7.86%) oocytes were lost during vitrification and thawing processes in 0, 6, 12, 18 and 24 h groups, respectively.

In this study, buffalo oocytes were vitrified at different stages of maturation in 40% w/v PROH and 0.25 M trehalose supplemented with 0.4% w/v BSA in PBS to assess the post thaw morphology, survivability, nuclear status and developmental competence. PROH (low MW permeating cryoprotectant) has high glass forming tendency and wholly amorphous state of its aqueous solution has great stability at subzero temperature which limits the formation of ice crystals [12]. Trehalose (low MW nonpermeating cryoprotectant) induces cellular dehydration through changes in osmotic pressure without toxic effect on cells [13]. BSA (high MW nonpermeating cryoprotectant) suppresses the formation of small ice crystal [14] and protects cell membrane [15] during freezing.

The average number of harvested and culturable quality oocytes obtained for this study is 0.91 and 0.77, respectively. A major limiting factor in the IVEP in buffalo species is very poor recovery rate of immature oocytes from slaughterhouse ovaries. When, the method of oocyte retrieval employed is aspiration of 2-8 mm follicles, the average recovery of total oocyte per ovary is 0.7 [16], 1.7 [17] and 2.4 [7]. Because of high incidence of atresia, mean recovery of good quality oocytes per ovary is further reduced i.e. 0.4 [16], 0.9 [17], 1.76 [18] and 2.4 [19]. Variations in the oocyte yield among different studies are due to differences in geographical location in relation to status of animals slaughtered, season of ovary collection, number of ovaries processed and techniques employed by different technical personnel, Furthermore, criteria for selecting ovaries at slaughterhouse might have influenced the oocyte yield in different studies [16,20].The oocyte recovery rate in buffalo is therefore much lower compared with cattle, from which 8-12 good quality oocytes are obtained on an average per ovary [21].

The post thaw recovery rate in different vitrification groups varied from 89 to 92% in this study. The recovery rate of vitrified thawed oocytes has been reported to vary from 80 to 100% in different species [22, 23] which is in agreement with the present study. Loss of oocytes during the process of freezing and thawing is well documented in several studies [24, 25, 26, 27, 28]. Such loss of oocytes occur due to sticking of oocytes on inner wall of straw adhering to crack or rough surface (developed sometimes during thawing) or oocyte disintegration due to improper vitrification. Also, oocytes may be removed with sealed end when it is cutoff after thawing [29].

Generally cryoprotectants are used to lower the freezing points and therefore allows the cell and freezing medium to super cooled to a specific subzero temperature before seeding. They protect the cell membrane from freeze related injury and decrease the deleterious effects of high

salt concentrations as the cells dehydrate during the freezing process [30].

IV. CONCLUSIONS

The present study indicates that post thaw recovery of buffalo oocytes vitrified at different stages of in vitro maturation were not altered by vitrificatiion solution consisting of different types of cryoprotectant like propylene glycol, trehalose and bovine serum albumin (BSA).

V. ACKNOWLEDGEMENT

We are grateful to acknowledge IVRI, Izatnagar and ICAR, New Delhi for rendering technical and financial aids for the conduct of this study.

REFERENCES

[1] J. E. Parks, and N. A. Ruffling, "Factors affecting low temperature survival of mammalian oocytes," Theriogenology, vol. 37, pp. 59-73, 1992.

[2] R. M. Moor, and I. M. Crosby, "Temperature induced abnormalities in sheep oocytes during maturation," J. Reprod. Fertil.,vol. 75, pp. 467-473, 1985.

[3] D. G. Whittingham, "Fertilization in vitro and development to term of unfertilized mouse oocytes stored at -196°C," J. Reprod. Fetil., vol. 49, pp. 89-94, 1977.

[4] FAO, Production Year Book, FAO, Rome, 2001.

[5] H. A. Samad, and A. A. Nasseri, "Aquantitative study of primordial follicles in buffalo heifer ovaries.Compendium 13 FAO/SIDA," Int. Course Anim. Reprod., 1979.

[6] B. H. Erickson, "Development and sensescene of post-natal bovine ovary,"J. Anim. Sci., vol. 25, pp. 800-805, 1996.

[7] A. Kumar, V. S. Solanki, S. K. Jindal, V. N. Tripathi, and G. C. Jain, "Oocyte retrieval and histological studies of follicular population in buffalo ovaries," Anim. Reprod. Sci., vol. 47: pp. 189-195, 1997.

[8] M. B. Ocampo, A. T. de Asis, L. Ocampo, and H. Kanagawa, "Histological observation of follicular atresia in swamp buffalo," Buffalo Bull., vol. 13, pp. 51-55, 1994.

[9] P. Palta, N. Banzai, P. S. Prakash, and M. L. Madan, "Endocrinological observation of atresia in individual buffalo ovarian follicles," Ind J. Anim. Sci., vol. 68, pp. 444-447, 1998.

[10] W. F. Rall, "Factors affecting the survival of mouse embryos cryopreserved by vitrification," Cryobiology, vol. 24, pp. 387-402, 1985.

[11] K. Loganathasamy and G. Taru Sharma, "Influence of Propylene Glycol on the Post Thaw Morphology of Buffalo Oocytes Vitrified dt different Stages in Vitro Maturation," International Journal of Advanced Chemical Science and Applications, vol. 2, no. 1, pp. 41-45, 2014.

[12] G. W. Snedecor, and W. G. Cochran, "Statistical methods," The Iowa State University Press USA, 1987.

[13] P. Boutron, and A. Kaufmann, "Stability of amorphous state in the system water 1, 2 propanediol. Cryobiology," vol. 16, pp. 557-565, 1979.

[14] J. Crowe, L. Crowe, and R. Mouradian, "Stabilization of biological membranes at low water activities," Cryobiology, vol. 20, pp. 346-356, 1983.

[15] F. Franks, M. H. Asquith, C. C. Hammond, L. B. Skaer, and P. Echiin, "Polymeric cryo protectants in the preservation of biological structure," J. Microsc, vol. 110, pp. 223-238, 1977.

[16] S. P. Leibo, "Cryopreservation of embryos," XI International Congress on Animal Reproduction and Artificial Insemination, 1988.

[17] S. M. Totey, G. Singh, M. Taneja, C. H. Pawshe, and G. P. Talwar, "In vitro maturation, fertilization and development of follicular oocytes from buffalo (BubalusBubalis)," J. Reprod. Fertil., vol. 95, pp. 597-607, 1992.

[18] G. K. Das, G. C. Jain, V. S. Solanki, and V. N. Tripathi, "Efficacy of various collection methods for oocyte retrieval in buffalo," Theriogenoiogy, vol. 46, pp. 1403-1411, 1996.

[19] H. A. Samad, I. Q. Khan, N. U. Rehman, and N. Ahmed, "The recovery, in vitro maturation and fertilization of Nili-Ravi buffalo follicular oocytes," Asian Aust. J. Anim. Sci., vol. 11, pp. 491-497, 1998.

[20] B. Gasparrini, G. Neglia, V. Caracciolodi Brienza, G. Canpanile, and L. Zicarelli, "Effect of cysteamine during in vitro maturation on buffalo

embryo development," Theriogenoiogy, vol. 54, pp. 1537-1542, 2000.

[21] P. Palta, and M. S. Chauhan, "Laboratory production of buffalo (Bubalusbubalis) embryos," Reprod. Fertil. Dev., vol. 10, pp. 379-391, 1998.

[22] I. Gordon, "Aspiration technique: old and new. In laboratory production of cattle embryos," Wallingford, UK, CAB International, pp. 71-72, 1994.

[23] E. Fuku, T. Kojima, Y. Shioya, G. J. Marcus, and B. R. Downey, "In vitro fertilization and development of frozen-thawed bovine oocytes," Cryobiology, vol. 29, pp. 485-492, 1992.

[24] M. A. Nowshari, R. L. Nayudu, and J. K. Hodges, "Effect of cryoprotectant concentration, equilibration time and thawing procedure on survival and development of rapid frozen-thawed mouse oocytes," Theriogenoiogy, vol. 42, pp. 1193-1204, 1994.

[25] S. Hamano, A. Koikeda, M. Kuwayama, and T. Nagai, "Full-term development of in vitro matured, vitrified and fertilized bovine oocytes," Theriogenoiogy, vol. 38, pp. 1085-1090, 1992.

[26] T. Miyake, M. Kasai, S. E. Zhu, T. Sakurai, and T. Machida, "Vitrification of mouse oocytes and embryos at various stages of development in an ethylene glycol based solution by a simple method," Theriogenology, vol. 40, pp. 121-134, 1993.

[27] J. M. Lim, J. J. Ko, W. S. Hwang, H. M. Chung, and K. Niwa, "Development of in vitro matured bovine oocytes after cryopreservation with different cryoprotectants," Theriogenology, vol. 51, pp. 1303-1310, 1999.

[28] A. Dhali, R. S. Manik, S. K. Das, S. K. Singla, and P. Palta, "Post-vitrification survival and in vitro maturation rate of buffalo (Bubalusbubalis) oocytes effect of ethylene glycol concentration and exposure time," Anim. Reprod. Sci., vol. 63, pp. 159-165, 2000.

[29] H. S. Luna, I. Ferrari, and R. Rompf, "Influence of maturation of bovine oocytes at time of vitrification on the incidence of diploid metaphase II at completion of maturation," Anim. Reprod. Sci., vol. 68, pp. 23-28, 2001.

[30] J. M. Wright, "Commercial freezing of bovine embryos in straws," Theriogenology, vol. 23, pp. 17-29, 1985.