B-4830
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RESEARCH ARTICLE Indian Journal of Animal Research

Effect of Cryoprotectants on Quality of Bovine Follicular Oocytes Compatible to *in-vitro* Maturation

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10.18805/IJAR.B-4830

ABSTRACT

Background: Cryopreservation of embryo is a part of most *in-vitro* fertilization programme. Various cryoprotectants can be used during embryo cryopreservation. As some cryoprotectants are very toxic to embryos therefore exposure of a suitable cryoprotectant at proper concentration and optimum equilibration time during cryopreservation of embryos is of great concern in relation to the survivability of the embryos.

Methods: All total 1224 bovine follicular cumulus-oocyte-complexes (COCs) were recovered from 900 abattoir ovaries for the present study. Total 880 numbers of COCs were used to study survivability in Propylene Glycol (PG), Glycerol (GL) and Ethylene Glycol (EG) at 5, 6 and 7 M of concentration and 5, 10 and 15 min of equilibration time.

Result: Out of the three cryoprotectants PG, GL and EG the highest per cent survivability of COCs was recorded in PG at 7 M concentration. In respect of equilibration time the per cent survivability of COCs was found better in 5 min than that of 10 and 15 min.

Key words: Bovine, Cryoprotectants, Concentration, Equilibration time, Follicular oocyte.

INTRODUCTION

Various cryoprotectants are used in vitrification process for cryopreservation of oocyte. Cryoprotectants increase the viscosity instead of crystal formation while decreasing freezing temperature during vitrification. But, due to its toxic effect to cryopreserving oocytes it is essential to select suitable cryoprotectant along with suitable concentration. Ethylene glycol, propylene glycol, glycerol and dimethyl sulfoxide are commonly used permeating cryoprotectants for oocyte vitrification as permeating cryoprotectants have least osmotic stress to the oocytes vitrified (Best, 2015). As reported by different workers 7 M and 8 M are two suitable molar concentrations of the cryoprotectants in two-step vitrification technique (Saikia *et al*., 2015). Moreover, probability of oocytes' osmotic injury can also be minimized by maintaining proper equilibration time (Vajta 2000; Prentice and Anzar, 2011). Therefore, the study is designed to study the effect of different concentrations of Propylene Glycol (PG), Ethylene Glycol (EG) and Glycerol (GL) at different equilibration periods on survivability of bovine oocytes before vitrification.

MATERIALS AND METHODS

The present study was conducted on a total of 1224 bovine follicular oocytes recovered from 900 abattoir bovine ovaries in the Department of Animal Reproduction, Gynaecology and Obstetrics and Department of Animal Biotechnology, College of Veterinary Science, Khanapara, Guwahati-781022, Assam Agricultural University from 1st August, 2019-31st July, 2020. Bovine ovaries were collected from the local abattoirs soon after the animals were slaughtered and were carried to the laboratory in a thermos flask containing warm (37°C) normal saline solution (NSS) with antibiotic Gentamicin @ 50 µg/ml (Alpagen, Alpa Laboratories Ltd.) In the laboratory, extraneous tissues were removed from the ovaries with the help of a pair of scissors. Then the ovaries were washed 3-4 times with NSS containing antibiotic prior to further processing. The oocytes were retrieved using aspiration technique and Slicing technique (Dutta *et al*., 2013).

COCs were washed 5-6 times in the washing medium and classified into four grades A,B,C and D based on their gross morphology and integrity of cumulus cells as per Jackowsha et al. (2009) and a total of 880 numbers of grade A and B (Fig 1) COCs were used in three different cryoprotectants as Propylene Glycol (PG), Glycerol (GL) and Ethylene Glycol (EG) at three different molar concentrations (5,6 and 7 M) and three different equilibration time (5, 10 and 15 min) to study survivability of the oocytes based on cohesiveness of cumulus cells layers attached surrounding the zona pellucida and homogeneity of the ooplasm.

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How to cite this article: Yasmin, K.M., Barua, P.M., Ahmed, K., Borpujari, D. and Chowdhury, S.I. (2022). Effect of Cryoprotectants on Quality of Bovine Follicular Oocytes Compatible to *in-vitro* Maturation. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4830.

Submitted: 16-11-2021 **Accepted:** 19-04-2022 **Online:** 30-04-2022

RESULTS AND DISCUSSION

Effect of cryoprotectant, molar concentration and equilibration time

The results of the present study pertaining to survivability of cumulus-oocyte-complexes (COCs) after equilibration for 5, 10 and 15 minutes in 5 M, 6 M and 7 M concentrations of Propylene Glycol (PG), Glycerol (GL) and Ethylene Glycol (EG) are presented in Table 1.

The overall per cent survivability of COCs in PG at 5 M, 6 M and 7 M concentration irrespective of equilibration time was 80.63±5.90, 86.21±1.87 and 88.51±1.77, respectively. The corresponding values in GL were 49.33±4.90, 50.13±4.68 and 62.58±4.61 and in EG were 48.44±5.64, 52.27±4.84 and 54.45±5.58. The overall mean survivability of COCs, irrespective of concentration, for 5, 10 and 15 min of equilibration time was 90.06±1.80, 86.90±2.82 and 78.58±5.19 per cent respectively in PG, 70.29±3.16, 52.78±3.59 and 38.96±3.09 per cent respectively in GL and 69.13±3.27, 53.13±3.35 and 33.07±2.94 per cent respectively in EG.

In the present study, the rate of recovery of morphologically normal oocytes was highest in PG which might be due to least osmotic stress leading to physical damage than that in GL and EG. The available literature revealed that there was quickest permeability of PG through COCs' cell membrane in comparison to GL and EG. The rate of permeability of EG was reported as almost half than that of PG. On the other hand, the oocytes in GL shrunk extensively and then it expanded marginally, indicating slow permeation (Pedro *et al*, 2005; Best, 2015). Therefore, rapid rate of permeability of the cryoprotectants favour recovery of morphologically normal oocytes.

The mean survivability of COCs in PG, GL and EG irrespective of molar concentrations and equilibration time was found to be 85.18±2.16, 54.01±2.85 and 51.78±3.07 per cent respectively. In 5, 6 and 7 M concentrations of cryoprotectant irrespective of equilibration time and cryoprotectant, the survivability of COCs was found as 59.47±3.99, 62.49±3.66 and 68.66±3.41 per cent, respectively. The survivability of COCs for 5, 10 and 15 minutes equilibration time irrespective of cryoprotectant and molar concentration, was recorded as 76.49±2.27, 64.27±3.27 and 50.21±4.05 per cent, respectively.

The findings of highest per cent survivability of COCs at 7 M concentration was comparable with that of Garg and Purohit (2007), Yadav *et al.* (2008) and Saikia (2014). It might be due to least number of COCs damage at 7 M concentration. This indicates that concentration of cryoprotectant lower than 7 M (at 6M and 5M concentration in the present study) causes physical damage to the COCs due to osmotic imbalance (Best, 2015).

In respect of equilibration time the per cent survivability of COCs was found better in 5 min than that of 10 and 15 min. This might be due to possibility of increased biochemical toxicity that occurs due to increase in exposure time (Hadi

Table 1: Per cent survivability (Mean*±S.F.) of cumulus- oocyte-complexes (COC_s) at different equilibration time in different concentration of cryoprotectants.

Concent-	Propylene glycol				Glycerol				Ethyleneglycol			
-ration	5 min	10 min	15 min	Overall	5 min	10 min	15 min	Overall	5 min	10 min	15 min	Overall
5 molar	88.7±4.65	85.72±6.51	67.41±15.17	80.63±5.90	66.43±7.84	45.91±6.13	35.66±1.36	49.33±4.90	65.98±6.99	47.16±8.28	32.18±6.42	48.44±5.64
6 molar	88.73±1.05	85.57±5.11	83.70±1.50	86.21±1.87	66.38±2.29	51.42±5.44	32.58±3.90	50.13±4.68	66.67±5.89	52.50±2.50	32.78±4.34	52.27±4.84
7 molar	92.68±3.09	89.40±4.00	84.45±1.19	88. 51±1.77	78.06±3.63	61.01±5.96	48.65±6.30	62.57±4.61	74.73±4.27	59.73±4.67	33.96±5.12	54.45±5.58
Overall	90.06±1.80	86.90±2.82	78. 58±5.19		70.29±3.16	52.78±3.59	38.96±3.09		69.13±3.27	53.13±3.35	33.07±2.94	

Table 2: Analysis of variance of survivability of cumulus-oocyte-complexes (COC_S) at different equilibration time in different concentration of cryoprotectants.

Source	DF	SS	MS	F value	P value
Cryoprotectant (C)	2	3.49108411	1.74554206	95.47	<0.0001
Molar concentration (M)	2	0.26035786	0.13017893	7.12	0.0014
Time (T)	2	1.52498458	0.76249229	41.7	<0.0001
C × M	4	0.02369695	0.00592424	0.32	NS
C × T	4	0.15769239	0.0394231	2.16	NS
M × T	4	0.01187639	0.0029691	0.16	NS
C × M × T	8	0.06012356	0.00751544	0.41	NS
Error	81	1.48097348	0.01828362		
Total	107	7.07550037			

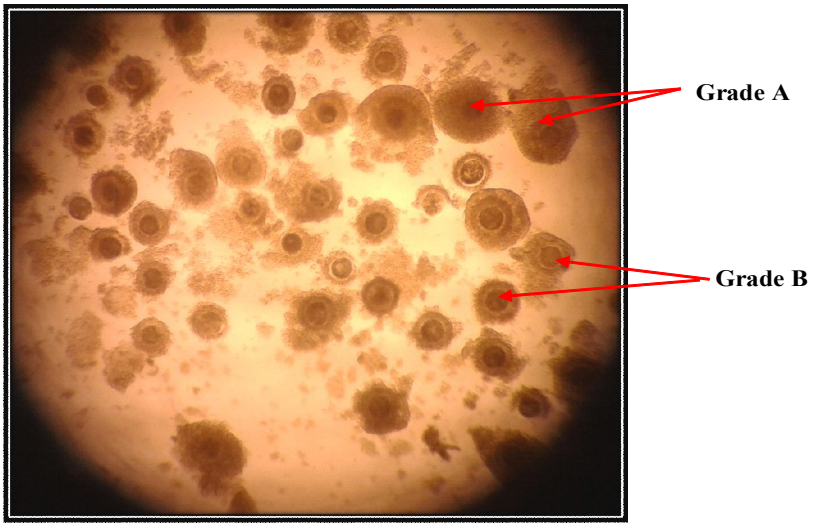

Fig 1: Grading of oocytes.

et al., 2010). The findings of the present study were comparable with that of Kuwayama *et al*. (1992) and thus it can be expressed as the survivability of oocytes increased as equilibration time is reduced.

ANOVA revealed that the percentage of survivability significantly (P<0.05) differed between treatments, between molar concentrations and between equilibration time (Table 2). Duncan´s multiple range test (DMRT) indicated that the per cent survivability of COCs was significantly (P<0.05) higher in PG than in GL and EG in 7M concentration and 5 minutes of equilibration time.

CONCLUSION

From the above discussion it can be concluded that Propylene Glycol at 7M concentration for 5 minutes equilibration time is suitable for survivability of cumulus oophorus complexes.

ACKNOWLEDGEMENT

I acknowledge Dean, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-for providing me necessary facilities to perform the research work.

Conflict of interest: None.

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