EFFECT OF DIFFERENT CONCENTRATIONS OF BOVINE SERUM ALBUMIN ON *IN VITRO* MATURATION OF BUFFALO OOCYTES*

C.N. MISTRY[1], A.J. DHAMI[2#], V.S. SUTHAR[3], D.N. RANK[4] and C.G. JOSHI[5]

Department of Animal Biotechnology
College of Veterinary Science and Animal Husbandry
Anand Agricultural University, Anand

ABSTRACT

A total of 456 fresh ovaries of Surti buffaloes without CL were obtained from the local abattoir. By slicing method of 1409 oocytes were recovered with an average of 3.09 oocytes per ovary. The average recovery rate of grade A, B and C oocyte were 1.02, 1.22 and 0.85, respectively. The overall maturation rate with 0.05, 0.1, 0.3, 0.6 and 0.9 per cent concentration of BSA (Sigma) in TCM-199 medium supplemented with 10 IU/ml of eCG and 5 IU/ml of hCG was found to be 44.52, 50.99, 59.02, 84.43 and 64.29 per cent ($P<0.01$), respectively. The highest maturation rate (90.00 %) of grade A oocytes was found in BSA 0.6 % followed by BSA 0.05 % (73.91 %), while in grade B it was in BSA 0.9 % (86.09 %) followed by BSA 0.6 % (82.61 %), and for grade C oocytes the highest maturation rate was with BSA 0.6 % (80.56 %) followed by BSA 0.9 % (60.53 %). According to nuclear maturation (using Hoechst 33342 stain), the highest percentage of oocytes with germinal vesicle was found in medium containing BSA 0.3 % (15.57 %) followed closely by others, except in BSA 0.6 % (4.92 %). The highest percentage of oocytes with broken germinal vesicle (GVBD) was found in BSA 0.9 % (28.57 %), which did not differ from other concentrations. The higher percentage of oocytes with Metaphase-I and –II were in the medium containing BSA 0.6 % (22.13 and 35.25 %) followed by BSA 0.9 % (15.87 and 19.84 %), and the least in BSA 0.05 and 0.1 per cent concentrations. The percentage of degenerated oocytes decreased with increase in BSA concentration from 0.05 to 0.6 per cent (40.41 to 10.67 %), except that in BSA 0.9 % it was 23.02 per cent. The results clearly showed that BSA @ 0.6 per cent in TCM-199 medium supplemented with 10 IU/ml of eCG and 5 IU/ml of hCG is ideal over other concentrations of BSA for *in vitro* maturation of buffalo follicular oocytes.

Key words: Buffalo oocytes, Bovine serum albumin, *In vitro* maturation.

The selection of protein supplements and hormones for IVM plays an important role in the subsequent fertilization (Schellander *et al.,* 1990) and development of mammalian oocytes during *in vitro* culture (Bavister *et al.*, 1992). The culture employed in IVM not only affects the proportion of bovine oocytes that reach Metaphase-II and become capable of undergoing *in vitro* fertilization, but can also influence subsequent embryonic development (Bavister, 1992). Buffalo oocytes are generally cultured for 24 hrs in complex medium such as TCM-199 (Chauhan *et al.,* 1998[a]), Ham's F-10 (Totey *et al.*, 1993), or minimum essential medium and Waymouth medium (Ravindranath *et al.,* 2003). Kane and Headon (1980) reported that the effect of serum albumin appeared to be due to presence of a relatively high molecular weight protein. Hormones like FSH, LH, oestradiol and growth factors are reported to be beneficial for maturation of oocytes (Chauhan *et al.,* 1998[b], 1999). The present study was carried out at Department of Animal Biotechnology of the College of Veterinary Sciences, AAU, Anand from September 2008 to February 2009 with the aim to assess the best concentration of BSA

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1 Research Scholar, 2 Research Scientist & Head, Livestock Research Station, 3 Ph.D. Scholar in Veterinary Obstetrics and Gynaecology, 4 Professor & Head, Animal Genetics and Breeding, 5 Professor of Animal Biotechnology. # Corresponding author: ajdhami@aau.in.

in TCM-199 medium containing eCG and hCG for *in vitro* maturation of buffalo follicular oocytes.

Ovaries (456) of freshly slaughtered matured Surti buffaloes were collected within half an hour from the local abattoir and were transported to the laboratory in a flask containing normal saline (0.9 % NaCl) at pH 7.0 supplemented with 50 µg/ml Gentamicin (Sigma, G 3632). Extraneous tissue and fat was removed and ovaries were washed with 70 per cent alcohol to check contamination, followed by three washings with the normal saline (39°C). The washed ovaries were sliced with a BP blade and transferred into 100 mm disposable petri-dish (Tarson® INDIA) with warm normal saline. The contents of all sliced follicles were searched for cumulus oophorus complexes (COCs) in petri-dish. A stereoscopic microscope (Nikon SMZ-2B, Tokyo, Japan) was used to identify the oocytes. They were transferred to pre-warmed drops of 100 µl of gonadotropin-free hepes-buffered TCM-199 medium, which was covered with 3 ml of silicon oil (Sigma) in the 35 x 10 mm (Tarson® INDIA) plastic petri-dish. The COCs were classified according to Leibfried and First (1979) as under.

For *in vitro* maturation of COCs, TCM-199 medium supplemented with 10 IU/ml of eCG (Folligon, Intervet) and 5 IU/ml of hCG (Chorulon, Intervet) was used as the basic medium to which different concentrations of BSA (0.05, 0.1, 0.3, 0.6 and 0.9 per cent) were added to see their effect on maturation rate. The pH of all culture media was maintained at 7.4. The media were sterilized by filtration through 0.22 µ Millipore filter. Droplets of 200 µl of maturation media were prepared in a petri-dish covered with mineral oil and equilibrated for 30 minutes with 5 per cent CO_2 and 95 per cent humidity.

Selected oocytes were washed three times in 10 drops of fresh pre-warmed TCM-Air medium and subjected to final washing in 10 drops of maturation medium of TCM-Air before being transferred to the maturation droplets. About 10-20 oocytes were transferred to the droplets in TCM-199 in different batches containing different concentrations of BSA-FAF. Oocytes were cultured for 24 hrs at 5 per cent CO_2, 39°C temperature and 95 per cent humidity in an incubator.

Maturation of oocytes was assessed on the basis of the expansion (gross) and dispersion of cumulus cells surrounding the oocytes reaching at Metaphase-II with the extrusion of one polar body. To assess the nuclear maturation rate, randomly selected oocytes were stained with Hoechst 33342 stain. The maturation of oocytes was assessed under the inverted microscope with florescence unit (Leica DMIL, Germany). The percentages of oocytes showing different maturational changes were worked out and were analyzed statistically.

Different concentrations of BSA were used to determine the optimum level for maturation of buffalo follicular oocytes in TCM-199 supplemented with 10 IU/ml of eCG and 5 IU/ml of hCG as basic medium. Tissue culture medium-199 (TCM-199) with or without hormone supplementation is the most widely used complex medium for performing IVM of buffalo oocytes, except for a few studies in which Ham's F-10 has been used (Totey *et al.*, 1996).

The maturation rate achieved by BSA concentration of 0.6 per cent was significantly higher (84.43 %) than all other concentrations of BSA tried (44.52 to 64.29 %). The maturation rate with 0.9 per cent BSA concentration (64.29 %) was also significantly better, but there was no significant difference among 0.05, 0.1 and 0.3 per cent concentrations of BSA. Use of 0.05 and 0.1 per cent BSA resulted in significantly poor maturation rate (44.52 and 50.99 %, respectively). Leibfried *et al.* (1986) noted that BSA failed to support cumulus-oocyte expansion for bovine or hamster cumulus-oocyte complexes but, in our study we found graded result for different concentrations of BSA. Ocana-Quero *et al.* (1999) found similar result for BSA-FAF treatments, which yielded higher maturation rate with TCM-199, while Evecen *et al.* (2004) noted that use of 0.3 mg/ml of BSA yielded highest blastocyst rate (94.57 ± 7.43) with Whitten's medium.

The maturation rate was assessed after 24 hrs of incubation of oocytes. The oocytes which showed cytoplasmic characteristics of maturation were subjected to Hoechst 33342 stain to determine the stage of maturation. The findings of grade-wise maturation in different media are summarized in table.

The highest maturation rate of grade A oocytes was found in BSA 0.6 per cent (90.00 %), while in grade B oocytes the highest maturation rate was with BSA 0.9 per cent (86.09 %) followed by BSA 0.6 and 0.3 per cent, and for grade C oocytes, BSA 0.6 per cent was the best (80.56 %) (Table). The discrepancy in the results of present and other studies may be due to different selection criteria used for categorizing oocytes, and also the culture conditions (Motlik and Fulka, 1986), source of sera (Younis *et al.*, 1989) and the seasons during which the oocytes were collected and used for *in vitro* maturation (Selvaraj *et al.*, 1992), as these factors have been reported to influence the rate of maturation of oocytes *in vitro*.

Use of different concentrations of BSA in the basic medium TCM-199 also affected the proportion of oocytes that reached Metaphase-II. The highest percentages of Metaphase-I and II were observed in BSA 0.6 per cent (22.13 and 35.25 %), followed by BSA 0.9 per cent (15.27 and 19.84 %), and BSA 0.3 per cent (13.24 and 18.85 %) with corresponding lower percentages of degenerated oocytes, while BSA 0.05 and 0.1 per cent did not support such observations of maturation. Our findings of cytoplasmic and nuclear maturation are in agreement with the report of Bavister *et al.* (1992).

Thus overall, it is concluded that the 0.6 per cent concentration of BSA in basic TCM-199 medium supplemented with 10 IU/ml of eCG and 5 IU/ml of hCG is ideal for *in vitro* maturation of buffalo follicular oocytes.

Table : Grade-wise maturation of buffalo oocytes in TCM-199 medium with different concentration of BSA

BSA Concentration (%) in TCM	Maturation % of different grades of oocytes		
	Grade A	Grade B	Grade C
BSA 0.05	73.91	45.61	11.63
BSA 0.1	53.06	58.93	39.13
BSA 0.3	54.35	70.73	51.43
BSA 0.6	90.00	82.61	80.56
BSA 0.9	46.67	86.05	60.53

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