EFFECT OF CHEMICAL ACTIVATION TREATMENTS ON PARTHENOGENETIC DEVELOPMENT OF *IN VITRO* MATURED BUFFALO OOCYTES

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ABSTRACT

This study was designed to investigate the effect of chemical activation treatments on parthenogenetic development of *in vitro* matured fresh (24 h) and aged (30 h) buffalo oocytes. Six trials were conducted each with control, ethanol and ionomycin activation treatments. In control group, none of the oocytes cleaved. In the ethanol activation (EA) group, the cleavage rates of fresh and aged oocytes were 10.75 ± 3.02 and 21.19 ± 2.24, respectively and there was significant difference (P<0.01) among them. In the ionomycin activation (IA) group, the cleavage rates of fresh and aged oocytes were 18.33 ± 1.89 and 26.13 ± 5.32 per cent, respectively and there was significant difference (P<0.01) among them. No significant difference (P>0.05) between the cleavage rates of fresh and aged activated oocytes with ethanol and ionomycin. It can be concluded that both ethanol and ionomycin can parthenogenetically activate buffalo oocytes and a minimum duration of oocyte maturation interval of 30 h was required for the efficient activation.

Key words: Buffalo, chemical activation, oocyte aging, parthenogenesis.

INTRODUCTION

Understanding the critical molecular components as well as the morphological changes during the initial stage of oocyte activation would be of significance for effective use of activated cytoplasts for cloning by nuclear transfer. Different artificial protocols have been developed to activate mammalian oocytes by simulating the biochemical and physiological events that normally occurred during sperm-oocyte interaction. Parthenogenetic activation of mammalian oocytes could be induced by exposure to ethanol (Rho *et al.,*1998) and ionomycin (Kitiyanant *et al.,* 2002). Ethanol induced a single Ca^{2+} rise that resulted both from extracellular entry and from some mobilization of intracellular store (Shiina *et al.,* 1993). Ionomycin on the other hand was a potent Ca^{2+} ionophore, which enhanced Ca^{2+} influx, but instead of directly facilitating the transport of Ca^{2+} across the plasma membrane, it mobilized intracellular Ca^{2+} which in turn triggered capacitative Ca^{2+} entry (Morgan and Jacob, 1994). Hence, the present study was designed with the objective of, compare the effect of chemical activation treatments on parthenogenetic development of *in vitro* matured buffalo oocytes.

MATERIALS AND METHODS

All chemicals were purchased from Sigma Chemicals Company (St.Louis,Mo,USA) and disposable plastics wares from Nunc (Denmark), unless otherwise stated.

Cumulus oocytes complexes (COCs) retrieved from buffalo ovaries by slicing method were morphologically graded based on the cumulus cells investments and homogeneity of ooplasm (Nandi *et al.,* 1998). Only COCs of A, B, and C grades were chosen for *in vitro* pathenogentic activation. Oocytes were matured in TCM 199 medium with 10 per cent fetal bovine serum (FBS) 40 IU/ml pregnant mare serum gonadotrophin (PMSG) and 10 ng/ml epidermal growth factor for 24h (fresh oocytes) and 30h (aged oocytes). *In vitro* maturation was assessed based on cumulus cell expansion after 24h of incubation

(Kobayashi *et al.*, 1994). Oocytes with degree 1 and 2 expansion were considered as mature. Further after denuding ,oocytes were evaluated for the presence of first polar body.

Matured buffalo oocytes both fresh and aged were randomly allocated to the following treatments viz. Ethanol (7 per cent) TCM 199 for 5 min (Nagai, 1992). Ionomycin (5 μM) in TCM 199 (Ware *et al.*, 1989) and in TCM 199 for 5 min (Control).

Following activation, oocytes were washed thrice in *in vitro* culture (IVC) medium before transferring to 2 h pre-equilibrated 50 μl IVC droplets (10-15 / droplets) containing TCM 199 supplemented with 10 per cent FBS, streptomycin (100 μg/ml) and penicillin (100 U/ml). Embryos were co-cultured with Buffalo Oviductal Epithelial Cell (BOEC) explants at 38.5°C in a humidified atmosphere of 5 per cent CO_2 in air and maintained for 7 days. Approximately at every 48 h, embryos was fed with fresh IVC medium. Cleavage was assessed at 48 h post activation and monitored every 24 h, upto 5 days for subsequent developmental stages. The statistical analysis of the data was done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

In the control group, 111 fresh oocytes and 115 aged oocytes were used for activation and none of the oocytes cleaved. In EA group, of 104 fresh and 103 aged oocytes, the cleavage rates were 10.75 ± 3.02 per cent and 21.19 ± 2.24 per cent, respectively and there was significant difference ($P<0.01$) among them. The percentages of embryos which developed to the 4 cell stage from fresh and aged activated oocytes were 2.89 ± 1.34 per cent and 6.09 ± 0.52 per cent, respectively and the difference was not statistically significant ($P>0.05$). None of the embryos developed further from fresh activated oocytes where in aged activated oocytes, 1.95 ± 1.32 per cent developed to the 8-16 cell stage. However, no significant difference ($P>0.05$) was observed in the development to 8-16 cell stage due to ageing of oocytes.

In IA group, of 121 fresh and 110 aged oocytes the cleavage rates were 18.33 ± 1.89 per cent and 26.13 ± 5.32 per cent, respectively and there was significant difference ($P<0.01$) between them. In the fresh oocytes 7.58 ± 1.15 per cent and in the aged oocytes 10.63 ± 2.99 per cent developed to 4-8 cell stage embryos. None of the embryos from the fresh activated oocytes developed to 8-16 cell stage whereas 3.64 ± 2.00 per cent from the aged activated oocytes developed to the 8-16 cell stage embryos.

In the present study, there was no significant difference ($P>0.05$) between the cleavage rates of fresh and aged activated oocytes with ethanol and ionomycin treatments. Furthermore, ionomycin showed a higher percentage of cleavage and development to 4-8 cell stage embryos than ethanol treated counterparts. The explanation probably resides, atleast in part due to the different methods of calcium mobilization induced by the two chemical activators. In addition, to its intracellular stores, ionomycin was likely to have induced a smaller and therefore less cytotoxic, rise in intracellular calcium levels than ethanol (Loi *et al.*, 1998). The possible reason that could be advanced for this suggestion is that ethanol induced an extracellular influx as well as intracellular release of calcium.

The results suggested that both ethanol and ionomycin were able to parthenogenetically activate buffalo oocytes. It has been demonstrated that maturation age of the oocytes was an important factor for parthenogenetic activation (Kaufman, 1981). Nagai (1987) reported that bovine oocytes cultured for 27-33 h before ethanol treatment resulted in the activation of 60-68 per cent of the oocytes, whereas maturation for 24-26 h resulted in a low activation rate of 25-38 per cent. Ware *et al.* (1989) also described that both calcium ionophore and electric stimulation in bovine oocytes cultured for 26 and 30 h resulted in an activation response similar to the ethanol activation reported by Nagai (1987).

In the present study, the cleavage rate of oocytes activated with ethanol and ionomycin were

significantly higher ($P<0.01$) in aged oocytes. This was in agreement with the observations of Nagai (1987) and Ware *et al.* (1989). In conclusion, both ethanol and ionomycin could parthenogenetically activate buffalo oocytes and a minimum duration of oocytes maturation interval of 30 h was required for the efficient activation.

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