EFFECT OF SUPPLEMENTATION OF LEUKEMIA INHIBITORY FACTOR ON *IN VITRO* DEVELOPMENT OF BUFFALO EMBRYOS

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ABSTRACT

The effect of supplementation of recombinant LIF in the embryo culture medium, effect of oviductal epithelial cells and Vero cells co-culture on subsequent embryo development were studied during in vitro *culture of buffalo embryos. Oocytes collected from the slaughter house ovaries, were matured and fertilized* in vitro*. The presumptive zygotes were cultured in four different groups as control-group I, with rLIF-group II, co-culture with buffalo oviductal epithelial cells- group III and with Vero cells-group IV. There was no significant difference in the cleavage rate in all the four groups, however, development of expanded blastocysts was found to be significantly higher in all the treatment groups (13.58 to 16.57%) when compared with control (9.80%), but there was no significant difference noticed among the treatment groups. The percentage of hatched blastocysts were significantly higher in group II (10.86%) when compared with that of control (5.38%) group. From the present study it is concluded that supplementation of LIF 100ng/ml in the culture media improved the hatching rate in blastocysts.*

Key words: In vitro embryo production, Recombinant LIF, BOECs, Vero cells co-culture

INTRODUCTION

The technique of *in vitro* embryo production (IVEP) has become more popular in recent years as it provides an opportunity to generate embryos for basic research and genetic up-gradation of dairy animals. The IVEP from slaughter house derived buffaloe ovaries for both research and commercial purpose, progressed slower than in cattle. The efficiency of blastocyst production is also much poorer than that in cattle (Farin *et al.,* 2001). To enhance the IVEP, many investigators have used co-culture of the early embryos with a variety of cell types including tubal epithelial cells, uterine fibroblasts, factor (LIF) is a mediator for the beneficial effect during co-culture. LIF is a cytokine which plays an important role in implantation and enhances pre-implantation embryo development *in vitro*. Supplementation of LIF in embryo culture medium enhanced embryo development in murine (Mitchell *et al.,* 2002), human (Tsai *et al.*, 1999), ovine (Ptak *et al.*, 2006) and bovine (Yamanaka *et al.,* 2001) models. Addition of LIF to culture medium increased blastocyst hatching, decreased embryo

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degeneration and increased implantation rate after the embryos were transferred back into recipient ewes (Fry *et al.*, 1992). LIF is involved in the temporal regulation of proteinase activity in the murine blastocyst which is required for uterine invasion during establishment of pregnancy (Harvey *et al.*, 1995) and it improved the functional (hatching) and morphological (number of cells) quality of the blastocyst in bovines (Han *et al.*, 1995). Therefore this work has been carried out to study the effect of supplementation of recombinant LIF during *in vitro* buffalo embryo production.

MATERIALS AND METHODS

Oviductal epithelial cells (BOECs) were collected from buffaloes simultaneously with immature oocytes for IVM. Oviductal epithelial cells were prepared as per procedure given by Yadav *et al* (1998) and Vero cell monolayer was prepared as prescribed by Dusewska *et al*(2000). Ovaries from adult buffaloes were collected and the cumulus oocyte complexes (COCs) were aspirated from ovarian follicles of 3-8 mm diameter. The COCs containing more than three layers of compact unexpanded cumulus mass were selected for this experiment. A total of 1777 oocytes were matured in TCM199 supplemented with 20% follicular fluid, 10% FBS, 20ng/ml EGF and 50ìg/ml gentamicin in an incubator for 24h and fertilized in Fert-TALP medium at a sperm concentration of 2×10^6/ml for approximately 18h in an incubator at 38.5°C with 5% CO_2 in humidified air. The presumptive zygotes were cultured into four groups in modified synthetic oviductal fluid (mSOF); group I (control), group II (mSOF supplemented with LIF 100ng/ml), group III (mSOF co-cultured with BOECs) and group IV (mSOF and Vero cell monolayer) at 38.5°C with 5% CO_2 in air for development upto blastocyst stage. The cleavage rate was recorded on day 2 (48hpi) of culture and then the embryos were monitored daily from day 3 to day 9 at the same time in a day for embryo morphology and the percentage progression to different embryonic stages. The data were subjected to statistical analysis as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The effect of addition of LIF and LIF secreting co-culture cells on cleavage rate, blastocyst development and hatching rate are presented in Table 1. The different developmental stages of embryos produced upto hatched blastocyst are depicted in Fig.1. The cleavage rate in control, LIF, BOECs and Vero cells co-culture were 54.07%, 53.49%, 57.71% and 58.90% respectively. The cleavage percentage was slightly higher in Vero cells and BOECs co-culture groups than that of control and LIF treated groups, but not at significant level. The percentage of cleaved embryos reached morula stage were also not significantly different in all four groups. However, the development of blastocysts from morula were significantly higher in all three treatment groups when compared with control group (15.84%). It showed higher value in group II (23.13%) followed by group IV (20.95%) and group III (19.87%). But there was no significant difference amongst the treatment groups. Development of expanded blastocysts from early blastocysts were also significantly higher in all the three treatment groups when compared with that of control (9.80%). Higher number of expanding blastocysts was noticed in group II (16.57%) followed by group IV (14.68%) and group III (13.58%), but there was no significant difference noticed among the treatment groups. The percentage of hatched blastocysts were significantly higher in group II (10.86%) followed by group IV (8.61%) and group III (7.16%) when compared with that of control (5.38%) group. However, there was no significant difference among the hatched blastocysts rate in oviductal epithelial cells (group III) and Vero cell co-culture (group IV) groups.

Embryo co-culture is frequently employed to improve the rate of blastocyst production using IVM and IVF in several other species (Shamsuddin *et al.,* 1993; Yadav *et al.,* 1998). In the present experiment, culture of presumptive zygotes in BOECs significantly increased the buffalo blastocyst development and hatching rate than that of control. Similar observations were reported in buffalo by Madan *et al* (1994). This significant increase *in vitro* may be attributed to the synthesis and secretions of some of the oviductal polypeptides which might be a beneficial factor during co-culture. Vero cells have also been shown to improve the blastocyst development rate and increased the pregnancy rate after embryo transfer in humans (Menezo *et al.,* 1992). In the present experiment, though there was no significant increase in cleavage rate and morula production rate when the presumptive zygotes were cultured in Vero cells, but a significantly improved blastocyst production rate and hatching rate was observed when compared with control group. Similar results were reported by Carnegie *et al* (1999) while using high LIF secreting co-culture system for bovine embryo production, which resulted in improvement in blastocyst formation and also in a better pregnancy rate after transfer of cryo-preserved embryos into recipient cows. Kauma and Mutt (1995) reported that co-culture cells that express LIF, enhanced blastocyst development *in vitro* by decreasing embryo fragmentation and degeneration in mice. Menck *et al* (1997) also reported that Vero cells support of bovine in vitro embryo development was equal to or better than that obtained with co-culture using BOECs. Improvement in bovine *in vitro* embryo development was reported by Dusewska *et al* (2000) when cultured in Vero/BRL monolayer. Carnegie *et al* (1997) found that proliferation inactivated Vero cells supported the development of about 40% of IVM/IVF derived bovine embryos to hatched blastocysts and Pegoraro *et al* (1998) also reported a slight increase in the developmental potential of bovine embryos cultured with Vero cells. It was revealed that somatic cells might secrete embryotrophic factors, such as growth factors (positive conditioning), remove potentially harmful compounds and modify the concentration of the medium components to the levels more appropriate for embryo development (negative conditioning). Furthermore, somatic cells may lower the oxygen tension in proximity of the embryo, reducing oxidative damages caused by the oxygen species.

The advantage of rLIF over that of co-culture cells is that, it is commercially available and provides a safer and cell free approach to promote embryo development *in vitro*. Most of the studies assessing the effect of LIF in bovine embryo cultures have used recombinant human LIF (rhLIF), since there is no commercially available bovine LIF. The rationale for this is that, the human cytokine shares greater sequence homology with bovine LIF than mouse LIF. In the present study, we examined the effect of addition of 100ng/ml of rhLIF in culture media and observed a significant increase in the blastocyst development and hatching. The increase in embryonic development by the addition of rhLIF in embryo development media is in agreement with Yamanaka *et al* (2001) in bovines. Furthermore Cheung *et al* (2003) reported beneficial effect of LIF and EGF on mouse blastocyst development *in vitro* and outcome of offspring. Jeon *et al* (2001) reported increased inner cell mass with reduced cell mortality and increased Oct-4 gene expression in mouse embryos, after addition of IGF-I, LIF and TGFá in the culture media. LIF conditioned medium significantly increased the blastocyst formation rates of human cryopreseved embryos (Hsieh *et al.,* 2000). Recently, Ptak *et al* (2006) reported that LIF supplementation in sheep IVM and IVC media exerted a beneficial effect on oocytes and embryos *in vitro* at stages concomitant with the steroid hormone surge. Based on these observations and our results, we suggest that the observed improvement in

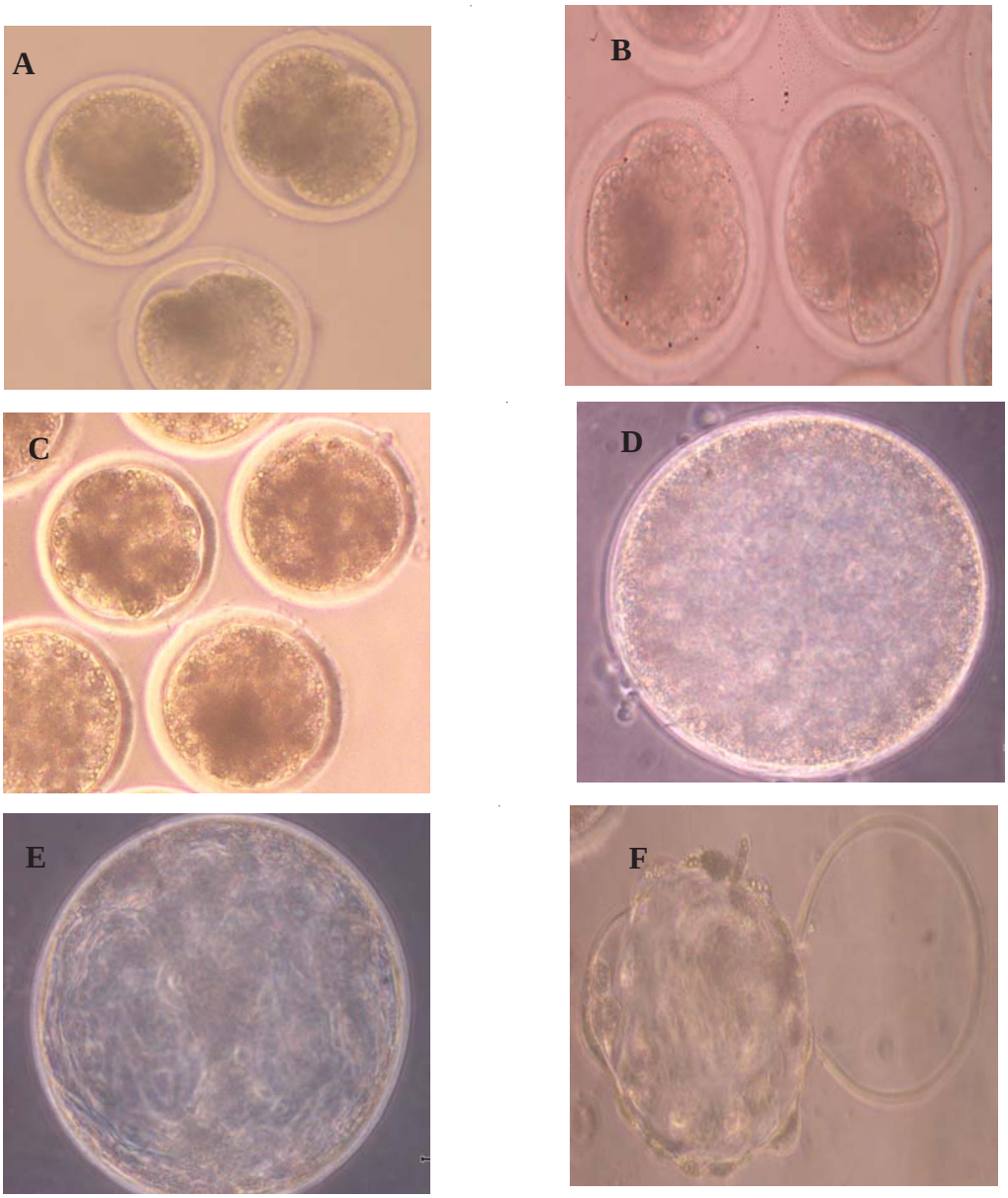
blastocyst production and hatching is probably due to effect of LIF.

Table 1.
Comparison of effect of supplementation of LIF (100ng/ml) and co-culture on development and hatching of buffalo blastocysts produced *in vitro*

Treatment	No.of oocytes cultured	Cleaved	Morula	Early blastocyst	Expanding blastocyst	Hatched blastocyst
Group I – Control	477	**258** 54.07± 1.39^{ab}	**102** 39.44 ±1.72^{a}	**42** 15.84 ±1.52^{b}	**26** 9.80 ±1.19^{b}	**14** 5.38 ±0.68^{c}
Group II – With LIF	467	**250** 53.49 ±1.18^{b}	**102** 40.67 ±1.63^{a}	**58** 23.13 ±0.85^{a}	**42** 16.57 ±1.13^{a}	**27** 10.86 ±0.55^{a}
Group III- BOEC	394	**227** 57.71 ±2.62^{ab}	**96** 42.43 ±2.42^{a}	**45** 19.87 ±1.63^{a}	**31** 13.58 ±1.48^{a}	**16** 7.16 ±0.36^{b}
Group IV- Vero cells	439	**258** 58.90 ±1.26^{a}	**116** 44.94 ±1.03^{a}	**54** 20.95 ±0.59^{a}	**38** 14.68 ±0.61^{a}	**22** 8.61 ±0.61^{b}

Fig. 1

Buffalo embryos produced *in vitro.* A: 2 cell embryos (200x). B: 4 cell embryos (200x). C: Morulae (200x). D: Early blastocyst (400x). E: Expanded blastocyst (400x).F: Hatched blastocyst (200x).



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