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Effect of epidermal growth factor on *in-vitro* maturation of buffalo oocytes and subsequent development with insulin-like growth factor-1 and ß-mercaptoethanol

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The success of *in-vitro* production of bovine embryos has been improved over the time, employing a variety of embryo culture media (Bavister 1995, Anand *et al*. 2008). Culture media supplemented with serum as protein source along with somatic cell co-culture has shown to increase the percentage of embryos in caprine (Teotia *et al*. 2001). Supplementation of exogenous growth factors in culture media affected development and metabolism of embryos produced *in vitro* (Gasparini *et al*. 2006, Richter 2008). Use of ß-mercaptoethanol (ßME) (Park *et al*. 2004), growth hormone (Pozzobon *et al*. 2005) and growth factors like epidermal growth factor (EGF) (Oyamada *et al*. 2004), insulin like growth factor (IGF) (Arufe *et al*. 2009) and nerve growth factor (NGF) (Inanc *et al*. 2008) improved the quality of transferable embryos. These growth factors play an important role in both nuclear and cytoplasmic maturation of oocytes *in vitro* (Christopher *et al*. 1997). Culture of cumulus oocyte complexes (COCs) in the presence of EGF stimulates distinct cellular functions such as acceleration of the process of nuclear maturation and increase in cumulus expansion, suggests its possible effect on early development of embryos (Teruel *et al*. 2000). Addition of ßME to the maturation medium increased intracellular glutathione (GSH) synthesis (Dematos and Furnus 2000) that has many important functions in cell and embryos by improving DNA synthesis (Takahashi *et al*. 2002), transcription (Bergelson *et al*. 1994) and cytokine activity (Liang *et al*. 1989). Gasparrini *et al*. (2006) reported that the presence of thiol compound, i.e cysteamine, in the maturation medium increased the proportion of buffalo oocytes developing to compact morula and blastocyst *in vitro*. Supplementation of βME in culture medium improves the competence and viability of *in-vitro* derived bovine embryos (Lim *et al*. 1996).

The objective of this study was to workout the effect of EGF during *in-vitro* maturation of buffalo oocytes and their subsequent development post IVF with IGF-1 and ßME.

Buffalo ovaries were collected from local slaughter house. Oocytes were aspirated from follicle and the embryos were produced by the standard *in-vitro* maturation, fertilization and culture (IVMFC) protocol of our laboratory (Rajhans *et al*. 2009) with the modification of using EGF (20ng/ml) in maturation medium, IGF-1 (100 ng/ml) and ßME (100 µM) in embryo culture media. Presumptive zygotes were cultured after dividing them into 4 groups, group 1 (oocytes matured without EGF and cultured without IGF-1 and ßME), group 2 (oocytes matured in EGF and cultured without IGF-1 and ßME), group 3 (oocytes matured in EGF and cultured with IGF-1(100 ng/ml) but no ßME) and group 1V (oocytes matured in EGF and cultured with IGF-1 (100 ng/ml) and ßME (100 µM)) at 38.5 ±1°C**,** 5% CO_2 and 95% RH to produce embryos up to blastocysts stage. Presumptive zygotes of all these groups were cultured over the granulosa cell monolayer for 48 hpi (hours post insemination) followed by co-incubation with the oviductal cells to get embryos of different developmental stages.

Data were analyzed by normal deviate test described by Snedecor and Cochran (1989) wherever required.

Table 1 detailed out the impact of EGF on *in-vitro* maturation of oocytes against the control. The result shows that addition of EGF in the maturation media significantly ($P<0.01$) increases the percentage of maturated oocytes in

Table 1. Effect of EGF on maturation of buffalo oocytes

Groups	Oocytes	Maturation
Control	220	160 (72.7%)^{A}
EGF (20ng/ml)	284	236 (83.1%)^{B}

Different superscripts in the column differ significantly at $P<0.01$.

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Table 2. Effect of IGF-1 and ßME on development of buffalo embryos

Groups	Ovaries	Oocytes	Culturableoocytes	Cleaved (%)	8–16 cell (%)	Morula (%)	Blastocysts (%)
1	540	550	351	108	74 1	36	8
	77.1±3.06	78.6±2.61	50.1±1.55	15.4±1.07^{a}	0.6±0.72^{a}	5.1±0.40^{a}	1.1±0.40^{a}
				(30.76%)	(21.08%)	(10.25%)	(2.27%)
2	405	490	355	146	133	103	35
	67.5±7.50	81.7±9.89	59.2±6.25	24.3±3.98^{b}	22.2±3.31^{b}	17.2±2.04^{b}	5.8±1.47^{b}
				(41.13%)	(37.46%)	(29.01%)	(9.86%)
3	580	605	372	151	139	122	43
	64.4±4.37	67.2± 3.24	41.3±2.58	16.8±1.93^{ab}	15.4±1.99^{ab}	13.6±1.80^{b}	4.8±0.80^{b}
				(40.59%)	(37.36%)	(32.79%)	(11.56%)
4	540	560	380	162	151	139	60
	60.0±3.91	62.2± 3.83	42.2±3.24	18.0±1.24^{ab}	16.8±1.59^{ab}	15.4±1.61^{b}	6.7±0.75^{b}
				(42.63%)	(39.73%)	(35.26%)	(15.78%)

Different superscripts in the column differ significantly at P<0.05; group 1 (oocytes matured without EGF and cultured in mSOF without IGF-1 and βME); group 2 (oocytes matured in EGF and cultured in mSOF without IGF-1 and βME); group 3 (oocytes matured in EGF and cultured in mSOF with IGF-1 but no βME); group 4 (oocytes matured in EGF and cultured in mSOF with IGF-1 and βME).

comparison to the control media having no EGF. It is evident from Table 2 that group 1 which had no growth factors (EGF, IGF-I) or βME during IVM/IVC showed a significantly lower cleavage rate as compared to other groups. Study revealed that uses of EGF in maturation media (group 2) significantly (P<0.05) increases the cleavage percentage in comparison to the maturation media without EGF (group 1). During subsequent development of embryos in the culture it was observed that in group 3 (culture media containing IGF-I) the percentage of morula and blastocysts was slightly higher in comparison to group 2 (culture media without IGF-I). Addition of βME along with the IGF-I (group 4) during embryo culture further improved the cleavage percentage and blastocyst production. During the study maximum percentage of the blastocysts was observed in group 4. Though culture media containing both IGF-I and βME (group 4) increases the number of cleavage, morula and blastocyst percentage in comparison to the culture media of group 2 and group 3, but this increase was nonsignificant. It could be concluded form these results that significant increase in cleavage percentage due to EGF addition in maturation media improves blastocyst production. It supports the earlier findings (Christopher *et al*. 1997, Purohit *et al*. 2005). Better maturation rates may be due to the mitosis promoting activity of EGF that improve the preimplantation development by increasing cell metabolism and proliferation (Grupen *et al*. 1997). The influence of EGF and IGF-1 on embryo development has been evaluated during different studies (Grupen *et al*. 1997, Kurzawa *et al*. 2001).

IGF-1 in this study enhances the embryonic development with an increased percentage of blastocyst, supports the finding of earlier studies (Sirisathien and Brackett 2003, Mao *et al*. 2004). Several growth factors play important role in the control of cellular proliferation, differentiation and morphogenesis during embryogenesis and IGF-1 is the most important growth factor for cellular differentiation (Schultz and Heyner 1993). There are several effects of IGF-1, like stimulation of glucose uptake (Pantaleon and Kaye 1996), protein synthesis and increase in cell number (Doherty *et al*. 1994) during *in-vitro* development of embryo. Therefore, IGF-1 increases embryonic metabolism and cell proliferation that definitely influences embryo development, an increased percentage of blastocyst may be agreed with the suggestion that IGF-1 has an action of preventing apoptosis and increases cell proliferation (Sirisathien and Brackett 2003).

Use of ßME in culture media (group 4) after 48 hpi of IVC of presumptive zygotes showed a non-significant increase in the percentage of blastocysts when compared to groups 1,2 and 3 is in agreement of the previous findings (Gasparrini 2006, Feugang *et al*. 2004). ßME promotes the uptake of cysteine that enhance glutathione (GSH) synthesis which protects cells from oxidative damages (Takahashi *et al*. 2002) and promotes embryonic development. Therefore it could be suggested that ßME promotes embryonic development by promoting DNA synthesis. The dose of ßME (100µM) in our study was selected on the basis that 50µM ßME has no embryo protective effect as well as 100µM dose of ßME is able to reduce oxidative stress radicals in culture medium as has been suggested by Feugang *et al*. (2004).

It could be concluded from this study that addition of EGF in maturation medium has favourable effect on *in-vitro* maturation and embryo production, addition of both IGF-1 and ßME in embryo culture media improves the percentage of blastocyst.

SUMMARY

This study was conducted to find out the effect of epidermal growth factor (EGF) on *in-vitro* maturation of buffalo oocytes and their subsequent embryonic development with insulin like growth factor-1 (IGF-1) and ß-mercapto ethanol (ßME).

Cumulus oocyte complexes (COCs) were matured in the TCM-199 supplemented with EGF (20 ng/ml) or no EGF and fertilized *in vitro* followed by their culture in 4 different media as group 1 (matured without EGF and cultured without IGF-1 and ßME), group 2 (matured with EGF and cultured without IGF-1 and ßME), group 3 (matured with EGF and cultured with IGF-1 (100 ng/ml) but no ßME) and group 4 (matured with EGF and cultured with IGF-1(100 ng/ml) and ßME (100 µM). Results of this study showed that EGF increases maturation percentage significantly ($P<0.01$) in comparison to the control group where EGF was not used. There was a non-significant increase in number of cleavage, morula and blastocyst in all the groups as compared to group 1. Group 1 showed significantly ($P<0.05$) lower cleavage, morula and blastocyst number as compared to other groups is an indication that these growth factors (EGF and IGF-1) and ßME have positive effect on development of embryos.

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