Short communication

Effect of EGF on development of bovine embryo cultured in G1/G2 sequential media

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Abstract The nutritional requirements of mammalian embryos changes throughout pre-implantation period, coincident with changes in the secretion of the female reproductive tract. Therefore, it has been suggested that sequential culture media may support nutritional requirements for optimal growth of the mammalian embryos. In this study, we investigated the effect of two different concentrations (10 or 100 ng/ml) of epidermal growth factor (EGF) in sequential media on bovine embryo cleavage rate and blastocyst formation. Oocytes were aspirated from 2- to 6- mm follicles and transferred to maturation media. After 24 h incubation in a CO_2 incubator, matured oocytes were inseminated. Presumptive fertilized oocytes after 22 h incubation were cultured in human sequential G1/G2 media containing 0, 10 or 100 ng/ml EGF. The experiment was performed in three replicates and the data were recorded as percentage of cleaved embryo and blastocysts formed. EGF did not significantly affect the cleavage rate but more blastocysts were formed in media containing 100 ng/ml EGF ($p<0.05$). But the results also showed that human sequential media G1/G2 containing EGF can be used in bovine embryo culture.

Keywords: growth factors, preimplantation, sequential media, bovine

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Introduction

Improving the quality of in *vitro* produced embryos is important in embryos transfer in cattle. Studies have shown that the rate of blastocyst formation is higher in the media supplemented with fetal bovine serum (FBS) or bovine serum albumin (BSA) (Pinyopummintr and Bavister, 1991; Krisher et al., 1999), and this has been attributed to the presence of factors in the serum, such as growth factors (Lonergan et al., 1996). After EGF binding to the cell surface, a series of postreceptor processes occur, resulting in increased DNA synthesis and cell proliferation (Carpenter and Cohen, 1990). Use of EGF increased blastocyst formation rate in bovine embryo *in vitro* culture (Palma and Brem, 1995). It has been demonstrated that *PDGF-b* gene is expressed in the bovine oviduct; therefore, these growth factors may have a role in normal growth of embryos (Viuff et al., 1995). Presence of EGF receptor mRNA from two-cell stage to blastocyst was demonstrated in bovine embryo, indicating its positive role in embryonic development (Yoshida et al., 1998).

A synergic effect of EGF and FGF on embryo growth was proporsed by Lee and Fukui (1995). A synergic effect on rate of blastocyst formation was also observed between EGF and IGF-I when added in synthetic oviduct fluid with amino acids (SOFaa) medium (Sakagami et al., 2012). In an experiment analyzing the effects of growth factors, it was shown that these factors had a significant effect on growth during early embryonic stages in serum-free media (Palma and Brem, 1995). In mouse and cattle, the blastocyst rate was improved and the number of cells increased when a high dose (100 ng/ml) of EGF was added to the culture medium (Glabowski et al., 2005; Sakagami et al., 2012). Nutritional requirements of mammalian embryos changes during the preimplantation period, co-incident with changes in the secretions of the oviduct and uterus. Therefore, use of sequential media, properly supplying growth-propmoting substances, was proposed for optimal embryonic growth (Gardner, 1999). A recent study showed that use of EGF in SOFaa medium increased the quality of blastocysts

due to increased inner cell mass (ICM) (Sakagami et al., 2012).

Regarding these findings, Gardner and Lane (1998) made a sequential medium for human embryo culture. The blastocysts produced by this medium were superior in quality compared with the co-culture containing somatic cells (Lane et al., 2003). Composition of this sequential medium is according to changes in carbohydrate and amino acid requirements of embryos and increases the blastocyst rate (Gardner, 1994). The present study aimed at determining the effect of two doses of EGF in the sequential medium on the cleavage rate and blastocyst formation in cattle.

Material and methods

All chemicals and reagents were purchased from Sigma Aldrich Company, unless specified.

Sample collection and in vitro maturation

Ovaries were collected from a slaughterhouse and transferred to the laboratory within 2 h in physiological saline at 30 to 35°C. Cumulus-oocyte complexes (COCs) were aspirated from 2- to 6-mm follicles. Oocytes with homogenous cytoplasm and at least three layers of cumulus cells were isolated and washed two times in aspiration medium (TCM-199 supplemented with 3 mg/ml BSA, 20 mM sodium pyruvate and 50 g/ml gentamicin sulfate). After being washed three times in the maturation medium, COCs were cultured in 50 µl droplets of maturation medium (TCM199) containing 10% FBS, 20 mM sodium pyruvate, 0.5 mg/ml FSH, 5 mg/ml LH (porcine), and 50 g/ml gentamicin sulfate and 100 ng/ml EGF). Culture plates were incubated at 38.5°C with 5% CO and maximum relative humidity for 24 h.

In vitro fertilization

Fresh epididymal motile sperm cells were washed twice in HEPES TALP medium and separated by swim up method. Matured oocytes with expanded cumulus cells were washed three times in TCM199 containing 3 mg/ml BSA and transferred in groups of 10 to 15 oocytes into 50 µl droplets of IVF medium (IVF-TALP with 25 µg/ml heparin and 6 mg/ml fatty acid free BSA). Oocytes were fertilized with 10µl of 2×10^6/ml sperm concentration. Plates were incubated at 38.5°C and 5% CO2 for 22 h.

In vitro culture

Presumptive zygotes were denuded from cumulus cells by mechanical treatment, washed and cultured in human embryo sequential media G1+ 5% FBS in two groups of 10 and 100 ng/ml EGF and a control group without EGF. Undivided cells were removed and after 72 h. Embryos were incubated in G2 medium for nine days. The experiment was replicated three times under similar conditions. The results were recorded as rate of cleavage and rate of blastocyst per cleavage.

Statistical analysis

Data were analyzed, as a completely randomized design, using the GLM procedure of the SAS software, and the mean separation was performed with LSMEANS and Tukey test at α=0.05.

Results and discussion

A total of 382 oocytes were used in this study. Supplementation with EGF (either 10 or 100 ng/ml) had no effect on the cleavage rate ($p > 0.05$), whereas supplementation at 100 ng/ml EGF significantly increased the blastocyst rate ($p < 0.05$) in comparison with control group (Table 1). Developmental stages of the embryos are shown in Fig. 1.

Bastan et al. (2010), comparing the effect of different doses of EGF on bovine oocyte maturation and embryo development, did not find any significant differences between the two media in blastocyst formation rate, although the use of EGF in the maturation period increased embryo development to blastocyst stage in both media. In the porcine species, supplementation of the maturation medium with EGF and/or amphiregulin increased maturation rate and total cell number (Jeong et al., 2012). Supplementation of EGF (100 ng/ml) in the maturation medium positively affected the meiotic maturation, cumulus expansion and cleavage rate (Lonergan et al., 1996).

Table1. Effect of EGF in G1/G2 media on cleavage and blastocyst rates (mean% ± SE)

Treatment	Total oocytes	Percentage of cleavage	Total blastocysts	Percentage of blastocysts per cleavage
No EGF	119	81.62 ± 1.48 [a]	32	32.93 ± 1.21 [a]
10 ng/ml EGF	126	82.10 ± 5.85 [a]	36	35.42 ± 1.45 [ab]
100 ng/ml EGF	137	79.73 ± 7.52 [a]	44	40.39 ± 1.33 [b]

Values in the same column with different superscripts differ significantly ($p < 0.05$)

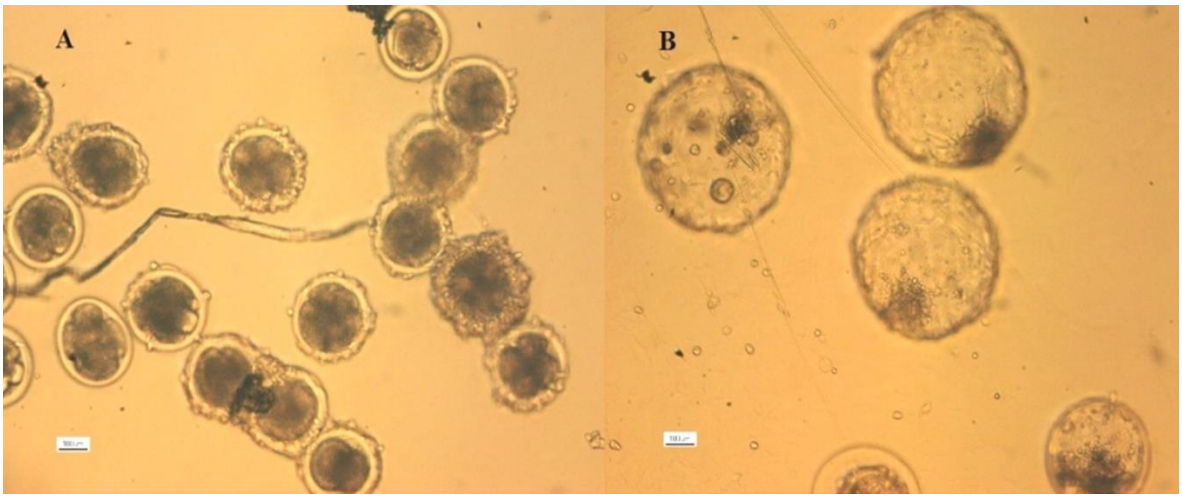

Figure1. A: embryos at 8-16 cell stage, B: hatched and blastocyst stage embryos.

Sakagami and colleagues (Sakagami et al., 2012) examined the effect of different doses of EGF in SOFaa on bovine embryo development and showed that EGF at a concentration of 100 ng/ml significantly increased the rate of blastocyst formation, whereas EGF at concentration of 200 ng/ml had no effect on cleavage rate (Bastan et al., 2010). Similarly, Yang et al. (1993) reported that the addition of 10 or 100 ng/ml EGF to SOF + 10% FBS increased the rate of embryo development. High concentration of EGF was accompanied with increased protein synthesis in the mouse (Wood and Kaye, 1989). Zahmatkesh et al. (2013) showed that EGF at a rate of 100 ng/ml in monoculture and sequential media had no effect on cleavage but increased the bovine blastocyst rate. Considering the importance of the concentration of growth factors, we used a low and a high concentration of EGF (10 and 100 ng/ml) in sequential culture media, but surprisingly did not find any significant difference in the cleavage rate. Our finding was concordant with the studies of Lonergan et al. (1996) and Sakagami et al. (2012) who showed that addition of EGF in SOF medium did not affects the cleavage rate. However, concentration of EGF at 100 ng/ml in sequential culture media significantly increased the rate of blastocyst development ($p<0.05$). Concordant with the study of Lonergan et al., this result confirms the role of this factor on embryonic development. Presence of EGF receptors has been demonstrated in the bovine embryos (Gardner and Lane, 1997). The positive effect of EGF can be because of its role in protein synthesis (Wood and Kaye, 1989) or its mitogenic effects on embryo (Lonergan et al., 1996). This study seem be the first investigation on the effect of different levels of EGF in the human sequential culture media (G1/G2) on bovine cleavage and blastocyst rates.

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اثر EGF برروی نمو رویان گاو کشت داده شده در محیط دو مرحله‌ای G1/G2

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چکیده نیازهای طبیعی رویان‌های پستانداران طی مرحله پیش از لانه گزینی تغییر کرده، که با تغییر در ترشحات لوله تناسلی ماده همزمان می‌باشد. بنابراین، پیشنهاد شده است که محیط کشت دو مرحله‌ای می‌تواند نیازهای طبیعی برای رشد بهینه رویان‌های پستانداران را فراهم نماید. در این مطالعه، اثر دو غلظت متفاوت (۱۰ یا ۱۰۰ ng/ml) فاکتور رشد اپیدرمال (EGF) در محیط کشت دو مرحله‌ای برروی نرخ تقسیم رویان گاوی و تشکیل بلاستوسیست بررسی گردید. اووسیت‌ها از فولیکول‌هایی به قطر ۲- تا ۶- mm آسپیره شده و به محیط بلوغ منتقل گردیدند. بعد از ۲۴ ساعت کشت در انکوباتور CO_2، اووسیت‌های بالغ به محیط لقاح انتقال یافتند. بعد از ۲۲ ساعت کشت در محیط لقاح، اووسیت‌های بارور شده احتمالی به محیط دو مرحله‌ای G1/G2 دارای صفر، ۱۰ یا ۱۰۰ ng/ml EGF منتقل گردیدند. آزمایش دارای سه تکرار بوده و نتایج به صورت درصد رویان‌های تقسیم شده و درصد تشکیل بلاستوسیست گزارش گردید. EGF تاثیر معنی داری برروی نرخ تقسیم رویان نداشته اما بیشترین درصد بلاستوسیت ($p<0.05$) در محیط دارای ۱۰۰ ng/ml EGF حاصل گردید. همچنین نتایج نشان دادند که محیط کشت دو مرحله‌ای انسانی G1/G2 غنی شده با EGF می‌تواند برای کشت رویان گاوی مورد استفاده قرار گیرد.