Indian Journal of Animal Sciences **81** (1): 12–14, January 2011

Expression of messenger RNA encoding LIF receptor beta in buffalo pre-implantation embryos produced *in vitro*

S ESWARI[1], G SAIKUMAR[2] and G TARU SHARMA[3]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 23 December 2009; Accepted: 11 November 2010

ABSTRACT

Leukaemia inhibitory factor (LIF) is a pleotrophic cytokine from interleukin–6 family and plays an essential role during embryonic development and implantation. LIF exerts its biological effects via binding to a specific receptor system; LIF receptor beta (LIFRß) and glycoprotein 130 (gp130). While LIFRß serves as the specific binding unit for the LIF, the gp130 subunit is similarly used by other cytokines. The temporal expression pattern of genes for several growth factors and their receptors have been examined in pre-implantation buffalo embryos using RT- PCR. This study investigated the expression of buffalo LIFRß in immature, *in vitro* matured oocytes and pre-implantation embryos. A pool of immature oocytes, matured oocytes, 2–4 cells, 8–16 cells, morulae and blastocyst were collected and used for total RNA isolation. The transcripts encoding LIFR were detected by employing RT-PCR. LIFRß mRNA were present during the pre-implantation stages of development from immature oocytes to the hatched blastocyst. The presence of functional LIFR in the preimplantation embryos indicated the important role of cytokine LIF in embryo development.

Key words: Buffalo, Gene expression, *In vitro* embryo development, LIFR

Successful implantation of embryo requires presence of several factors. These include an embryo with qualities favorable to growing to the blastocyst stage and hatching, adequate endometrial receptivity, and successful interaction between the embryo and the endometrium. Leukemia inhibitory factor (LIF) is a secreted glycoprotein that was initially identified through its ability to induce macrophage differentiation in murine myeloid leukaemic cell line H1**.** LIF also plays an essential role during early differentiation and implantation in embryos (Fry 1992). Of the many observed actions of LIF, its outstanding ability to preserve the totipotentiality of murine embryonic stem cells has assured its continuing role in experimental biology (Williams and Hilton 1988). LIF exerts its biological effects via binding to a specific LIF receptor beta (LIFRß) (Nichols *et al.* 1996). Information on gene expression profiling in the course of *in vitro* embryo production in buffalo is an unexplored area, which promises to provide valuable leads for not only optimizing the IVF procedure in buffalo but will also help in answering some of the vexed physiological questions to explain reproduction potential in this species. The present study was undertaken to examine whether the LIFR is transcribed in buffalo oocytes and pre-implantation embryos and also to find out whether they have a capacity to synthesize a functional receptor.

Present address: [1]Assistant Professor (e mail drseswari @gmail.com), Department of Veterinary Physiology, Madras Veterinary College, Chennai 600 007.

[2] Senior Scientist, Division of Pathology; [3]Head, Division of Physiology and Climatology (e mail: gts@ivri.up.nic.in).

MATERIALS AND METHODS

In vitro *embryo production:* Ovaries from adult buffaloes were collected from a local slaughterhouse at Bareilly, Uttar Pradesh, and transported to the laboratory in an insulated container in 0.9% saline solution (37°C) fortified with antibiotic-antimycotic solution (penicillin 100 IU/ml and streptomycin 100 mg/ml). Ovarian follicles of 3–8 mm diameter were aspirated and the cumulus oocyte complexes (COCs) containing more than 3 layers of compact unexpanded cumulus mass and evenly granular cytoplasm were selected and matured in TCM199 supplemented with 20% follicular fluid, 10% FBS, 20ng/ml EGF and 50µg/ml gentamicin at 38.5°C for 24 h with 5% CO_2 in humidified air and fertilized in Fert-TALP (tyrode - albumin - lactate - pyruvate supplemented with pyruvate (0.2 mM), fatty-acid-free BSA (6 mg/ml), heparin (10 mg/ml) penicillin (100 IU/ml) and streptomycin (100 mg/ml) medium at a sperm concentration of 2×10^6/ml at 38.5°C for approximately 18 h with 5% CO_2 in humidified air and the presumptive zygotes were cultured in modified synthetic oviductal fluid medium (mSOF) supplemented with BSA (3 mg/ml), 10% FBS, 1%

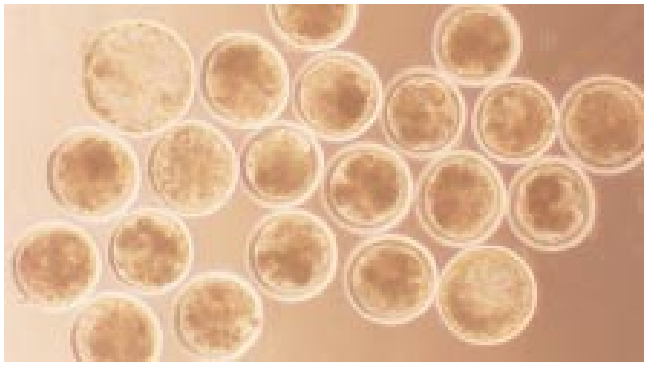

Fig. 1. Different stages of *in vitro* produced buffalo pre-implantation embryos.

essential and non essential amino acids up to 9–10 days or blastocyst development.

RNA extraction and reverse transcription: Samples of immature, matured oocytes and embryos at different developmental stages (Fig. 1) were collected at 48, 96, 120 and 192 h post insemination (hpi) for 2–4 cells, 8–16 cells, morulae and blastocyst stage respectively and the total RNA were isolated from immature oocytes (20), matured oocytes (20), 2–4 cells stage (20), 8–16 cell stage (20), morulae (10) and blastocysts (4) by using RNeasy micro kit. The reverse transcription reaction was carried out using a mix of random hexamer primer and moloney murine leukemia virus reverse transcriptase (MMLV-RT) enzyme to prime the RT reaction and to produce cDNA. Tubes were heated to 70°C for 5 min to denature the primer and template mix, and then the RT reaction was completed with the addition of 50 units of superscript RT-PCR enzyme. They were then incubated at room temperature for 10 min and then at 42°C for 60 min to allow the reverse transcription of RNA, followed by 99°C for 5 min to denature the RT enzyme.

Polymerase chain reaction: Primer pairs were designed from human LIFR*ß* sequence (Accession Number ×61615) using Beacon designer and the designed primers were obtained from integrated DNA technologies. The sequences of primers used and the size of expected PCR fragments are shown in Table 1. RT negative and PCR negative controls were also set for all PCR reactions to negate the DNA contamination in cDNA templates and for ascertaining the authenticity of the PCR. The PCR products were visualized on 2% agarose gel in 1X TAE (Tris 10 mM and EDTA 1 mM) buffer containing 0.5µg/ml of ethidium bromide.

RESULTS AND DISCUSSION

The sensitive technique of mRNA detection by RT-PCR was applied to total RNA samples isolated from pools of immature and matured oocytes and *in vitro* produced embryos at 2–4 cells, 8–16 cells, morula and blastocyst stages. The assays were repeated 3 times with each sample pool. Fragments with the expected size were amplified, and no PCR products for LIFRß genomic DNA were detected in any of the RT-PCR assays (Fig. 2b). The identities of PCR products were further confirmed by sequencing and the homology was tested with the sequences of other species (DNA STAR). A house keeping gene ß actin was also tested with the samples (Fig. 2a).

Supplementation of LIF in embryo culture medium enhanced embryo development in murine (Mitchell *et al*. 2002), ovine (Ptak *et al*. 2006) and bovine (Yamanaka *et al*. 2001, Rodriquez *et al*. 2007) models. Reinhart *et al.* (1998) reported that oviductal cells synthesize LIF to promote and condition the embryo for implantation. In rabbits, the transfer of embryos to LIF-treated recipients significantly increased pregnancy and implantation rate when compared with controls (Liu *et al.* 1999). The similar effects were also found by Vogiagis *et al.* (1997) in ewes. Furthermore Cheung *et al*. (2003) reported beneficial effect of LIF and EGF on mouse blastocyst development *in vitro* and outcome of offspring. In mice, the potential of LIF to keep embryonic stem cells in

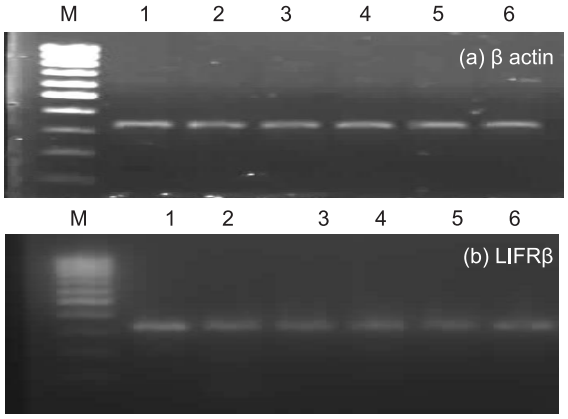

Fig. 2. Detection of mRNAs encoding LIFRß in buffalo oocytes and pre-implantation embryos by RT-PCR. (a) A single fragment of 327 bp encoding actin. (b) A single fragment of 329 bp encoding LIFRß. M, 100 bp DNA ladder; lane 1, immature oocytes; lane 2, matured oocytes; lane 3, 2–4 cells; lane 4, 8–16 cells; lane 5, morulae; lane 6, blastocyst.

Table 1. PCR primers for detection of LIFR ß and ß actin gene expression

Gene	Primers direction	Primer sequence	Fragment size	Annealing temperature
LIFR	Forward	5-ATGTGGTTGTGTCCTATTGTC-3	329 bp	52°C
	Reverse	5-CTCACTGTTGCTGTCTATGG-3		
actin	Forward	5-GGAGGCCCCTCTGAACCCCAA-3	327 bp	60°C
	Reverse	5-CTCTTTGATGTCACGCACGAT TTC-3		

the undifferentiated state suggested that LIF was important for maintaining the pleuripotency of the inner cell mass. Additionally, Cai *et al.* (2000) reported that LIF enhanced the murine blastocyst formation and outgrowth in culture.

Expression of LIF and its receptor have been studied in several species. However, there were no reports in the buffalo. The data presented here document the first detection of LIFR expression in buffalo oocytes and pre-implantation embryos. In this study we collected evidence that LIFR is transcribed in buffalo oocytes and throughout pre-implantation development. These observations confirm those made by Eckert and Niemann (1998) in bovine. Nichols *et al.* (1996) demonstrated that LIF is exclusively expressed in the trophectoderm, whereas the mRNA of LIFR is primarily localized in the inner cell mass in mice. Yang *et al.* (1995) identified LIFR in rabbit uterus during early pregnancy. Similarly Ding *et al.* (2008) detected LIFR and gp130 during implantation process in golden hamsters. Rizos *et al.* (2003) detected LIF and LIFR mRNAs at higher levels in *in vitro* produced bovine blastocysts, irrespective of culture system, than in *in vivo* derived blastocysts. In cattle, Eckert and Niemann (1998) reported differences in the expression of LIF and LIFR between *in vitro* and *in vivo* derived embryos. They also reported that some perturbations of the mRNA expression patterns of the specific LIF/LIF-receptor system occur during the *in vitro* development of embryos leading to abnormal development of the inner cell mass and trophectoderm in the blastocyst. Niemann and Wrenzycki (2000) detected LIFR mRNA from immature oocytes upto blastocyst stage, whereas the LIF transcripts in morula not only in from matured oocytes up to 16 cell stage but as well as in blastocyst. The conditions of culture *in vitro* alter gene expression in the embryo. The analysis of such differences in mRNA expression may explain the differences between *in vivo* and *in vitro* produced embryos and allow the opportunity to modify gene expression through modification of culture systems. In this study, the expression of LIFR gene indicated the potential autocrine and/or paracrine role of LIF in the regulation of pre-implantation embryo development, possibly through binding to specific LIFR on the embryos.

In conclusion, buffalo oocytes and preimplantation embryos express LIFR mRNA. The expression of this transcript indicated that preimplantation embryos may be responsive to LIF originating either from the surrounding environment or from the embryos themselves and exerting its function in a paracrine and/or autocrine manner.

REFERENCES

Cai L Q, Cao Y J and Duan E K. 2000. Effects of leukaemia inhibitory factor on embryo implantation in the mouse. *Cytokine* **12**: 1676–82.

Cheung L P, Leung H Y and Bongso A. 2003. Effect of supplementation of leukemia inhibitory factor and epidermal growth factor on murine embryonic development *in vitro*, implantation and outcome of offspring. *Fertility and Sterility* **80**: 727–35.

Ding T, Song H, Wang X, Khatua A and Paria B C. 2008. Leukemia inhibitory factor ligand- receptor signaling is important for uterine receptivity and implantation in golden hamsters. *Reproduction* **135**: 41–53.

Eckert J and Niemann H. 1998. mRNA expression of leukemia inhibitory factor (LIF) and its receptor subunits glycoprotein 130 and LIFreceptor ß in bovine embryos derived *in vitro* or *in vivo*. *Molecular Human Reproduction* **4**: 957–65.

Fry R C. 1992. The effect of leukemia inhibitory factor (LIF) on embryogenesis. *Reproduction Fertility and Development* **4**: 449–58.

Liu C Q, Yuan Y, Wang Z X and Lu S H. 1999. Mifepristone regulation of leukemia inhibitory factor and uterine receptivity in rabbits. *Contraception* **60**: 309–12.

Mitchell M H, Swanson R J and Oehninger S. 2002. *In vivo* effect of leukemia inhibitory factor (LIF) and an anti-LIF polyclonal antibody on murine embryo and fetal development following exposure at the time of transcervical blastocyst transfer. *Biology of Reproduction* **67**: 460–64.

Nichols J, Davidson D, Taga T, Yoshida K, Chambers I and Smith A. 1996. Complementary tissue specific expression of LIF and LIF-receptor mRNAs in early mouse embryogenesis. *Mechanisms of Development* **57**: 123–31.

Niemann H and Wrenzycki C. 2000. Alteration of expression of developmentally important genes in preimplantation bovine embryos by *in vitro* culture conditions: implications for subsequent development. *Theriogenology* **53**: 21–34.

Ptak G, Lopes F, Matsukawa K, Tishner M and Loi P. 2006. Leukemia inhibitory factor enhances sheep fertilization *in vitro* via an influence on the oocyte. *Theriogenology* **65**: 1891–99.

Reinhart K C, Dubey R K, Mummery C L, van Rooijen M, Keller P J and Marinella R. 1998. Synthesis and regulation of leukaemia inhibitory factor in cultured bovine oviduct cells by hormones. *Molecular Human Reproduction* **4**: 301–08.

Rizos D, Gutierrez-Adan A, Perez-Garnelo J, De La Fuente J, Boland M P and Lonergan P. 2003. Bovine embryo culture in the presence or absence of serum: implications for blastocyst development, cryotolerance and messenger RNA expression. *Biology of Reproduction* **68**: 236–43.

Rodriquez A, DeFrutos C, Diez C, Caamano J N, Facal N, Duque P, Garcia-Ochoa C and Gomez E. 2007. Effects of human versus mouse leukemia inhibitory factor on the *in vitro* development of bovine embryos. *Theriogenology* **67**: 1092–95.

Vogiagis D, Fry R C, Sandeman R M and Salamonsen L A. 1997. Leukemia inhibitory factor in endometrium during the oestrus cycle, early pregnancy and in ovariectomized steroid-treated ewes. *Journal of Reproduction and Fertility* **109**: 279–88.

Williams R L and Hilton D J. 1988. Myeloid leukemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature* **36**: 684–87.

Yamanaka M, Amano T and Kudo T. 2001. Effect of the presence period of bovine leukemia inhibitory factor in culture medium on the development of *in vitro* fertilized bovine embryos. *Animal Science Journal* **72**: 285–90.

Yang Z M, Le S P, Chen D B, Yasukawa K and Harper M J. 1995. Expression patterns of leukemia inhibitory factor receptor (LIFR) and the gp130 receptor component in rabbit uterus during early pregnancy. *Journal of Reproduction and Fertility* **103**: 249–55.