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Effect of extract egg supplementation on expression of *mushashi* gene in caprine neural stem

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Stem cells hold a great promise for regenerative medicine but remain elusive in many tissues, including neural tissues. Stem cells are biological cells found in all multicellular organisms. For decades, it was believed that most neurons in the adult central nervous system (CNS) were terminally differentiated and usually not replaced when they died. It has been established that active neurogenesis, continues in discrete regions of the adult CNS throughout the life of all mammals, including humans (Temple 2001). NSCs are multipotent cells with the ability to self-renew and to generate mature, differentiated daughter cells of all neural lineages throughout the developing neuraxis as well as to reconstitute those cell types in ablated neural regions (Parker *et al*. 2005). Isolation and expansion of neural stem cells (NSCs) are crucial for successful development of cell therapy approaches in neurodegenerative diseases.

Musashi is an evolutionarily conserved family of RNA-binding proteins that is preferentially expressed in the nervous system. The first member of the Musashi family was identified in Drosophila. This protein plays an essential role in regulating the asymmetric cell division of ectodermal precursor cells known as sensory organ precursor cells. Level of expression of the RNA-binding protein Musashi-1 is selectively higher in NSCs than in neural precursor cells (Kaneko *et al*. 2000).

Fetal and adult neural stem cells are usually defined as undifferentiated cells (lacking markers of mature cells) that display proliferative and self-renewal capacities and multipotentiality (Gage *et al*. 1995, Gritti *et al*. 2002). Isolated and cultured stem cells not only provide an important source of cells for *in vitro* studies, but they are also an important source of CNS cells that can be used in transplantation studies (Ray *et al*. 1998). The amount of fetal bovine serum (FBS) produced for the world market is estimated to approximately 500 000 litres per year. As a consequence, more than 1 000 000 bovine fetuses have to be harvested (Jochems *et al*. 2002). In view of the animal welfare issues surrounding the production of FBS, an alternative agent allowing the replacement or reduction in the use of FBS is desirable. Growth promoting action of egg yolk extract was reported by Fuji and Gospodarowicz (1983). The goat fetal stem cells were generated using extract egg (Anees 2010, Bharadwaj 2011). However no information is available on characterization of caprine neural stem cells using NSCs specific marker mushashi using extract egg. Hence, the present study was undertaken with the object to *in vitro* characterization of caprine neural stem cells specific marker using egg extract.

The gravid uterus of goat (50–60 days) was collected from slaughter house in normal saline. Head of fetus was decapitated and incised to get brain tissue under laminar flow. The fetal brain tissue was chopped in Dulbeccos modified eagle media (DMEM) supplemented with fetal bovine serum (5%) and antibiotics. Single cells were cultured in a 6– well plate containing DMEM supplemented with fetal bovine serum (15%). Leukemia inhibitory factor (10 ng/ml) +bFGF (20 ng/ml) a control in a control media. After attaining confluency (80 –90%) cells were further passaged in control media as well as media supplemented with 1, 2 and 4% extract egg in place of FBS to study the effect of extract egg on expression of *Mushashi* gene in cells.

RNA extraction and reverse transcription: For gene expression analysis, cells were taken from culture and washed with 1X PBS. Then, cells were transferred to 2 ml centrifuge tube for RNA isolation. The total RNA was isolated by a kit. cDNA was synthesised using cDNA synthesis kit. After preparing cDNA, OD of cDNA was maintained at 600 ng/µl and used for real time PCR study.

Real time PCR: Relative quantification was performed using a real time polymerase chain reaction method. Briefly,

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a Biorad CFXManagerTM Software and EvaGreen supermix (catalog # 172–5200), as a double stranded DNA-specific fluorescent dye were used. For amplification of genes, primers were designed in beacon software. Primers for real time PCR: GAPDH F5'-GGAGAAACCTGCCAAGTATG-3' and R5'-TGAGTGTCG CTGTTGAAGTC-3'. Musashi 1 F5'- GGTGAAGGAGTGTCTGGTGATGC -3' and R5'-TCGAGTCACCATCTTAGGCTGTGC. The real time PCR thermocycling conditions were: an initial denaturation step at 95°C for 30 sec followed by 45 cycles of 95°C for 5 sec, annealing for 10 sec. Transcript level of 1 gene was quantified using the relative quantification method based on comparative threshold cycles values (Ct). The abundance of gene was determined relative to the abundance of the housekeeping gene GAPDH. The PCR products were run on 2% agarose gel. For comparative gene expression study, the relative expression of data was analysed in CFX96 Manager Software.

The present study was carried out to assess the effect of different concentrations of extract egg supplementation (1%, 2% and 4%) on expression of caprine neural stem cells specific marker mushashi. The caprine neural stem cell colonies i.e monolayer and neurospheres were developed using extract egg supplementation. Then the cells were isolated from each culture medium separately and characterized using molecular markers mushashi. GAPDH was used as a reference marker gene for molecular analysis. The normalized fold expressions of mushashi gane is presented in Fig. 1. qPCR amplified product as checked in 2% agarose, shown in Fig. 2. The caprine neural stem cell colonies and neurospheres were found positive for mushashi neural stem cells specific markers, The results indicated that the expression of mushashi gene were comparatively higher in 1% and 4% extract egg supplemented medium. In our lab goat fetal stem cells were generated using extract egg (Anees 2010). The foetal stem cell of caprine was isolated using extract egg and stem cell colony developed was larger in 4 and 6% extract egg yolk. (Bharadwaj 2011) In permanent neuronal cell line N2A, the yolk extract significantly stimulated cell proliferation as well as the growth of cell processes. Sasse *et al*. (2000) and Ying *et al*. (2011) reported cell colony formation induced by *Xenopus* egg extract as a marker for improvement of cloned blastocyst formation in the pig.

SUMMARY

Neural stem cells are multipotent cells with the ability to self-renew and to generate mature, differentiated daughter cells of all neural lineages throughout the developing neural axis as well as to reconstitute those cell types in ablated neural regions. The extract egg (1%, 2%, 4%) supplementation in DMEM showed that the normalized fold expression of the neural stem cell specific gene Mushashi was nonsignificantly higher at 1% and 4% extract egg than media containing 2% extract egg.

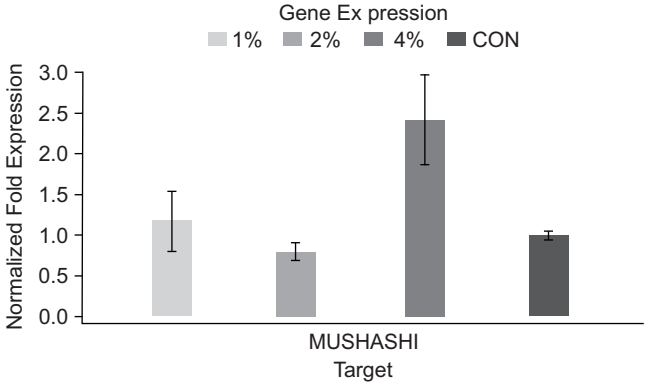

Fig. 1. Expression of *Mushashi* gene in caprine neural stem cells in different percentages of extract egg and control media.

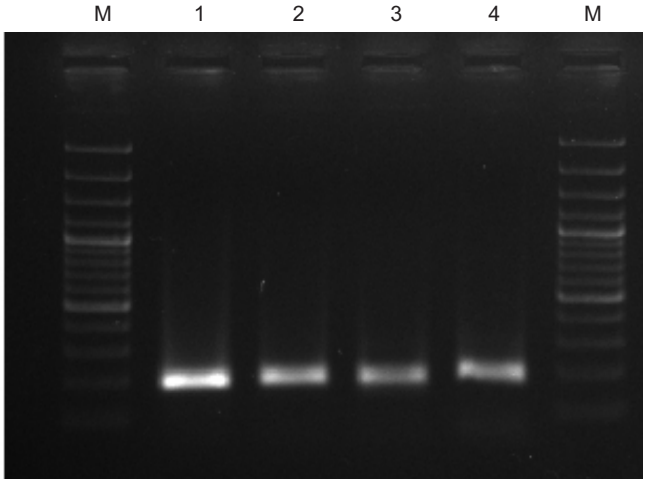

Fig. 2. Gel electrophoresis of PCR amplified product in 2% Agarose. Lane M, 100bp marker; lane 1 to 4, Mushashi (187 bp) In control, 1% EE, 2%EE, 4%EE.

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