구두발표-1

The Role of MPF and MAP Kinases in Development of Embryos Produced by Somatic Cell Nuclear Transfer.

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ABSTRACT:
In embryos produced by somatic cell nuclear transfer (SCNT) the oocyte has the ability to reprogram gene expression as evidenced by the production of live offspring. However, the efficiency of successful SCNT is low and has been associated with aberrant gene expression in both the embryo and the placenta. The mechanisms involved in nuclear reprogramming are presently unknown but many factors including cell cyclestage of donor and recipient cells, methods of reconstruction, activation and culture may all impact on the successful outcome. MII oocytes contain MPF and MAP kinases which induce nuclear envelope breakdown (NEBD) and premature chromosome condensation in the donor nucleus which may be beneficial for nuclear reprogramming (Campbell KHS and Alberio R 2003 Reprod. Suppl. 61, 477-494). However, the occurrence of NEBD and PCC varies between oocytes, oocyte ages, species and donor cell type. In addition it has been shown that following enucleation, MPF activity in murine oocytes is primarily associated with the meiotic spindle reducing activity in the cytoplast, although in pig and ovine oocytes enucleation does not reduce MPF activity. The role of MPF and MAP kinases in nuclear reprogramming is controversial, studies in cattle using mitotic donors have suggested that these kinases are not directly involved in reprogramming, but may be important if intact nuclei are transferred. Recently we have demonstrated thattreatment of ovine oocytes to be used as recipient cytoplasts for

SCNT with caffeine can increase MPF and MAP kinase activities and increase the cell number of blastocyst stage embryos produced by SCNT. Although the mechanisms behind this increase in cell number are unknown the effects may be mediated through improved nuclear reprogramming. We have examined the gene expression profiles in SCNT embryos produced using caffeine treated and non-treated recipient cytoplasts and compared the expression levels with control IVF and parthenogenetic embryos. As has been previously reported in cattle aberrant expression patterns were observed for a number of genes in SCNT embryos including a down regulation of Oct-4, H2A.Z, Nanog, Hsp 27 and FGF-4 and an up regulation of INF-tau, Hsp70.1, and Sox-2.Following caffeine treatment of recipient cytoplasts the expression levels of these genes was similar to control IVF embryos. Interestingly genes whose expression levels were altered fall into two categories, stress genes (Hsp 27 and Hsp 70.1) and secondly Oct-4 and genes regulated by Oct-4 (H2A.Z, Nanog, FGF4, INF-tau and Sox-2). These two groups of genes play important roles in early development and may determine embryo quality and survival. The mechanisms by which caffeine treatment alters gene expression levels are unknown but may be indirectly or directly due to the elevated kinase activities. This paper will discuss these possibilities.