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# Supplementation of Kinetin Increases Heat Shock Protein 70 and Poly[A] Polymerase Gene Expression in Porcine Nuclear Transfer Embryos and Enhances Their Development *In Vitro*

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Developmental retardation is one of distinguishing mark of the *in vitro* cultured mammalian embryos. It is known that *in vitro* culture condition affects to developmental competence. Presence of kinetin, the plant hormone known by promoting division of plant cells, synthesis of repair enzymes in DNA, superoxide dismutase activity and ribosomal RNA transcription. However, its effect on mammalian embryo development is not clear. In the present study, porcine nuclear transfer (NT) embryos were cultured with/without kinetin, and *in vitro* development and mRNA expressions of heat shock protein 70 (HSP70; stress adaptation, correct folding of newly synthesized proteins and their transcripts), glucose transporter-1 (GT-1; metabolism), and poly[A] polymerase (poly[A]; mRNA splicing) of those embryos were investigated. In the first series of experiment, porcine nuclear transfer embryos divided into four groups and cultured in (1) BSA+AAs: North Carolina State University 23 (NCSU-23) with 4 mg/mL bovine serume albumin (BSA) and amino acids (AAs: essential and non-essential amino acids) (2) PVA+AAs: NCSU-23 with 1 mg/mL polyvinyl alcohol (PVA) and AAs (3) BK200: NCSU-23 with 4 mg/mL BSA and AAs and 200 $\mu$M kinetin (4) PK200: NCSU-23 with 1mg/mL PVA and AAs and 200 $\mu$M kinetin for 7 days. In second, mRNA expression patterns of NT-derived blastocysts were analyzed by

semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). Regardless of kinetin supplementation, the cleavage rates and the numbers of cells in blastocysts were not significantly different among experimental groups. However, kinetin supplementation promoted the blastocyst formation rate from 7.5% to 15.4% in PVA supplemented medium and it was comparable to BSA supplemented counterpart (12.8 vs 13.6%, BSA+AAs vs BK200, respectively). HSP70 mRNA and Poly[A] polymerase mRNA expression level was enhanced in PK200-derived blastocysts whereas GT-1 mRNA level was not differ from PVA-AAs. These data suggest that addition of kinetin induces prevention of newly synthesized protein misfolding by heat stress control and correction of mRNA splicing, and this give rise to enhacement of porcine NT embryo development.

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