P0484

Culture of Karyoplast and Cytoplast Complexes in High Osmolarity after Fusion Improve *In Vitro* Development of Porcine Nuclear Transfer Embryos

Gi-Sun Im, In-Sun Hwang, Dong-Hoon Kim, Byoung-Chul Yang, Se-Woong Kim, Hyo-Suk Park, Jin-Sung Seo, Bo-Suk Yang and Won-Kyong Chang

National Livestock Research Institute, RDA, Suwon, 441-706

Micromanipulation and fusion are essential to generate nuclear transfer embryos. In this process cytoplasmic damage is unavoidable. This study investigated the hypothesis that higher osmolarity than normal culture medium could help oocytes recover from cytoplasmic damage from micromanipulation and electric pulse. Oocytes derived from a local slaughter house were matured for 42~44 h and enucleated. Fetal fibroblast were prepared from a Day 33 porcine fetus. Fusion and activation were induced simultaneously by using two direct current (1.2Kv/cm for 30μs). Fused embryos were cultured in PZM-3 supplemented with 3 mg/ml BSA under 5% CO_2 in air at 38.5 ℃. Karyoplast and cytoplast complexes exposed to electric pulse for fusion were placed in one of 270, 320 or 360 mOsm medium for 30min. Fusion rates were from 71.8% to 73.5%. Cleavage rates were higher in 320 mOsm and 360 mOsm (61.9% and 66.9% vs. 56.4%) than in 270 mOsm. Parthenotes cultured in 320 mOsm and 360 mOsm showed higher ($P<0.05$) blastocyst rates (13.9% and 20.8% vs. 6.1%) compared to 270 mOsm. This result shows that the culture of oocytes, which are exposed to micromanipulation and electric pulse, in high osmolarity medium can improve *in vitro* development of preimplantation porcine nuclear transfer embryos.

Key words: ***Osmolarity, Activation, Culture, Nuclear transfer***