PA-10

Expression and Localization of Heat Shock Protein 70 in Frozen-Thawed IVF and Nuclear Transfrred Bovine Embryos

Y.J Choi, S.J Song, J.T Do, B.S Yoon, A.J Kim, K.S Chung, H.T Lee
Animal Resource Research Center, KonKuk University, Seoul

The role of heat shock proteins in shielding organism from environmental stress is illustrated by the large-scale synthesis of these protein by the organism studied to date. However, recent evidence also suggests an important role for heat shock protein in fertilization and early development of mammalian embryos. Effects of elevated in vitro temperature on *in vitro* produced bovine embryos were analysed in order to determine its impact on the expression of heat shock protein 70 (HSP70) by control and frozen-thawed after in vitro fertilization (IVF) or nuclear transfer (NT). The objective of this study was to assess the developmental potential *in vitro* produced embryos with using of the various containers and examined expression and localization of heat shock protein 70 after it's frozen -thawed. For the vitrification, in vitro produced embryos at 2 cell, 8 cell and blastocysts stage after IVF and NT were exposed the ethylene glycol 5.5 M freezing solution (EG 5.5) for 30 sec, loaded on each containers such EM grid, straw and cryo-loop and then immediately plunged into liquid nitrogen. Thawed embryos were serially diluted in sucrose solution, each for 1 min, and cultured in CRI-aa medium. Survival rates of the vitrification production were assessed by re-expanded, hatched blastocysts. There were no differences in the survival rates of IVF using EM grid, cryo-loop. However, survival rates by straw were relatively lower than other containers. Only, nuclear transferred embryos survived by using cryo-loop. After IVF or NT, in vitro matured bovine embryos 2 cell, 8 cell and blastocysts subjected to control and thawed conditions were analysed by semiquantitive reverse transcription polymerase chain reaction methods for hsp 70 mRNA expression. Results revealed the expression of hsp 70 mRNA were higher thawed embryos than control embryos. Immunocyto -chemistry used to localization the hsp70 protein in embryos. Two, 8-cell embryos derived under control condition was evenly distributed in the cytoplasm but appeared as aggregates in some embryos exposed frozen-thawed. However, under control condition, blastocysts displayed aggregate signal while Hsp70 in frozen-thawed blastocysts appeared to be more uniform in distribution.

key wards) ***Hsp70, semiquantitive RT-PCR, Immunocytochemistry***