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Apoptosis and Apoptosis Related Gene Expression of Preimplantation Porcine Diploid Parthenotes Cultured in Different Protein Supplements

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This study was conducted to determine effects of polyvinyl alcohol (PVA), fetal bovine serum (FBS) and bovine serum albumin (BSA) on blastocoel formation, cell number, apoptosis and apoptosis-related gene expression of porcine diploid parthenotes developing *in vitro*. Embryos were collected from 2-cell or late 4-cell diploid parthenotes that activated with electro pulse, and *in vitro* cultured in the NCSU 23 medium supplemented without or with 0.1% PVA, 10% FBS or 0.4% BSA for day 7. The morphological analysis of apoptosis in embryos was carried out using propidium iodide staining and terminal deoxynucleotidyl transferase mediated dUTP nick end labeling. The expressions of Bcl-xL, Bak and P53 in blastocyst stage parthenotes and *in vivo*-derived blastocysts were determined using semiquantitative RT-PCR. The addition of 0.4% BSA to the culture medium enhanced the development of 2- or late 4-cell stage parthenotes to the blastocysts stage ($P < 0.01$) while FBS decreased the incidence of blastocoel formation. FBS also reduced cell numbers of blastocysts developed from both 2- ($P < 0.001$) and late 4-cell ($P < 0.05$) embryos and increased percentage of apoptosis in the blastocysts ($P < 0.001$). The relative abundance of Bcl-xL mRNA in diploid parthenotes cultured from 2-cell stage in the presence of BSA is similar with that in *in vivo* derived embryos, but is significantly higher than in parthenotes cultured with FBS, PVA or none protein supplement control. Bak mRNA showed a significant increase at the blastocyst stage in FBS supplement medium. This result suggests that apoptosis related gene expression is significantly affected by protein supplements, which may result in alteration of apoptosis and embryo viability of porcine embryos developing *in vitro*.

Key words) ***Apoptosis, dUTP, RT-PCR, mRNA, Embryo viability***