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Effects of Serum in Culture Medium and Fusion/Activation Condition on Porcine Cloned Preimplantation Embryo Development and Gene Expression

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The goal of this study was to demonstrate the effects of serum in modified North Carolina State University (NCSU)-23 medium and fusion/activation condition on porcine somatic cell nuclear transfer (SCNT) embryo development and mRNA expression patterns of five selected genes [apoptosis regulator box-a: Bax, Bcl2, heat shock protein(Hsp) 70, phos-phoglycerate kinase (PGK) and integrin -1] in a single blastocyst using semi-quantitative reversetranscription-polymerase chain reaction (RT-PCR).

Supplementing 10% fetal bovine serum (FBS) in NCSU-23 medium at day 4 increased blastocsyt formation (14.3 vs. 4.7%) and inner cell masses (14.5 ± 5.0 vs. 8.3 ± 3.2) and trophectoderm (TE) cell number (46.9 ± 21.2 vs. 31.7 ± 9.5) in blastocysts. With 10% FBS supplementation *in vitro* culture condition, fusion rate was significantly increased in 1.0 mM calcium (1.0C) (81.3%) compared to control (73.4%) and 7.5 g/ml cytochalsin B (CB) (72.9%) ($P < 0.05$). The 1.0C and CB yielded significant ($P < 0.05$) higher rate in blastocyst formation (19.6 and 21.1%, respectively) than control (12.3 %). Cell numbers of blastocysts were significantly increased in 1.0C (77.4 ± 28.9) compared to control (58.5 ±

22.6) and CB (66.6 ± 26.7).

The mRNAs for five selected genes were detected in a single SCNT blastocyst. There were no significant differences in expression pattern between parthenogenetic, *in vitro* fertilized (IVF) and SCNT blastocyst. Serum in culture medium significantly increased the expression of PGK. Cytochalasin B treatment increased Bax mRNA expression compared with parthenogenetic, control SCNT and 1.0 calcium supplement groups ($p<0.05$). The Bcl-2 gene was significantly expressed in CB-treated blastocyst compared to parthenogenetic blastocyst, while PGK gene expression in CB blastocyst was higher than parthenogenetic and IVF blastocyst.

In conclusion, the present results of present study demonstrated that serum supplementation in culture medium at day 4 and higher calcium concentration in fusion/activation medium increased blastocyst formation and its quality, whereas cytochalasin B for chemical activation can increase blastocyst formation. Cytochalasin B activation and serum supplement alter the mRNA expression of genes related with apoptosis and metabolism.

This study were supported by grants from the Biogreen 21-1000520030 100000 and the Korean Ministry of Science and Technology (Biodiscovery)