P-14

Survival and Development of Porcine Embryos Produced *In Vitro* using Open Pulled Straw Methods

S. Y. Lee, J. S. Yu and C. K. Park[1]

Gyeongsangnam Province Advanced Swine Research Institute
[1]College of Animal Life Sciences, Kangwon National University

The purpose of this study is to investigate the effects of vitrification in open pulled straws (OPS) methods on *in vitro* survival ability of porcine embryos. For *in vitro* maturation of immature oocytes, the porcine ovaries were collected from local slaughter-house. The cumulus- oocytes complexes were aspirated from 2 to 6 mm follicles. The collected oocytes were cultured for *in vitro* maturation in NCSU-23 medium with 5 mM hypotaurine, 0.57 mM cysteine, 10% porcine follicle fluid, 10 IU/mL PMSG and 10 IU/mL hCG for 21~22 hrs. Then, the oocytes were more cultured 21~22 hrs *in vitro* maturation in medium removed hormones. The frozen- thawed spermatozoa were washed by centrifugation 2 times for 10 min at 1,500 rpm in D-PBS with 5.56 mM glucose, 0.33 mM Na-pyruvate, 100 IU/mL penicillin, 100 μg/mL streptomycin and 4 mg/mL BSA. The fertilization medium used mTBM with 2 mM caffeine and 2 mg/mL BSA and adjusted to a pH of 7.2 to 7.4. The final concentration of spermatozoa was adjusted to 2.5×10^6 cells/mL motile sperm during fertilization *in vitro*. At 8 hrs after inse-mination, the oocytes were transferred into NCSU-23 medium with 5.0 mM hypotaurine, 4 mg/mL BSA and 10 ng/mL EGF and cultured for 7 days. When the blastocysts of different stages were frozen- thawed by OPS methods, the proportions of embryos with normal morphology were significantly ($p<0.05$) higher in embryos frozen-thawed at expanded blastocyst stage (38.9%) than in early blastocyst stage (28.3%). On the other hand, the proportions of embryos damaged after frozen- thawing were significantly ($p<0.05$) higher in embryos frozen at early blastocyst

stages than in expanded blastocyst stage. In another experiment, the normal embryos morphology after frozen- thawing were further cultured for 48 hrs. After culture, the proportions of embryos hatched were 6.7, 20.0 and 33.3% for embryos frozen-thawed at early blastocyst, mid-blastocyst and expanded blastocyst stages. These finding indicate the possible broader application for OPS methods, as frozen-thawed embryos may be accompanied by developmental stages according to requirements of the survival ability after freezing of blastocyst stage in the pig.

Key words) *Frozen-thawed embryos, In vitro development, OPS methods, Pig, Survival ability*