P-66

Effect of *In Vitro* Matured Oocyte and Parthenote Selection with Polar Body Extrusion and Early Cleavage on Development of Porcine Follicular Oocytes

Hyun-Jong Kim, Sang-Rae Cho, Sun-Ho Choi, Chang-Yong Choe, Dong-Soo Son, Byung-Don Sang, Man-Hye Han, Il-Sun Ryu, In-Cheul Kim, Il-Hwa Kim and Kyung-Soon Im[1]

Animal Genetic Resources Station, NLRI, RDA, Namwon, 590-832, Korea
[1]Department of Animal Biotechnology, Seoul National University, Seoul, 151-742, Korea

The aim of present experiment was to improve the developmental rate of micro-manipulated oocytes up to hatching stage. In this experiment, oocyte selection with polar body extrusion and embryo selection with early cleavage were carried out. Ovaries were obtained from prepubertal gilts at a local abattoir and brought to the laboratory in physiological saline with antibiotics at 30~33℃. The ovaries were washed and wiped, and then cumulus-oocytes complexes in the follicular fluid were aspirated form surface-visible follicles (2~6 mm in diameter) with 10 mL syringe fitted with an 18-gauge needle. After being washed three times with modified phosphate- buffered saline (DPBS; Gibco, USA) containing 0.3% BSA, the COCs were suspended in maturation medium, NCSU-23 containing 10% (v/v) porcine follicular fluid, 10ng/mL epidermal growth factor (EFG; Sigma, USA), 10 ug/mL follicular stimulating hormone (FSH; Sigma, USA) and 35 ug/mL luteinizing hormone (LH; Sigma, USA), cysteine 1 mg/mL (Sigma, USA), 100 IU/mL penicillin G, and 100 ug/mL streptomycin sulfate (Gibco, USA). After 24 hours, the COCs were transferred to the same medium without hormones. The matured oocytes were denuded in DPBS containing 300 IU/mL (w/v) hyaluronidase (Sigma, USA) by vortexing for 3 minutes to eliminate cumulus cells. After treatment with hyaluronidase, the denuded oocytes

were selected with polar body and then the polar body extruded oocytes and non-extruded oocytes returned to the maturation medium without hormones. After 65h of maturation, oocytes were exposed to PBS with 7% ethanol (v/v) for 7 minutes, and then the oocytes were washed and cultured in TCM199 containing 5 ug/mL cytochalasin B (Sigma, USA) for 5h at 38.5℃ in an atmosphere of 5% CO_2 and 95% air with high humidity. After cytochalasin B treatment, the presumptive parthenotes were cultured in PZM-5 medium (IFP, Japan) and cleavage of the parthenotes was assessed at 48h of activation, Normally cleaved parthenotes, hyper-cleaved parthenotes and non-cleaved parthenotes were cultured for 8 days to evaluate their ability to develop to blastocyst and hatching stages. The FBS were added at Day 4 with concentrations of 5% for improving hatching ability. The parthenotes that did not examined with polar body and early cleavage were shown 16.7% BL rate and 5.0 % hatching rate and the parthenotes that did not examined with polar body but examined with early cleavage were shown 31.7% BL rate and 20.0% hatching rate. The parthenotes that examined with polar body but did not examined early cleavage were shown 39.0% BL rate and 30.0% hatching rate and the parthenotes that examined with polar body and early cleavage were shown 49.0% BL rate and 38.0% hatching rate. This results mean polar body examination was important for high developmental rate and early cleavage examination also improved the developmental rate.

Key words) *Porcine follicular oocyte, Polar body extrusion, Cleavage, Hatching, Parthenote*