

Effect of Superoxide Dismutase on Development and Survival Ability *In Vitro* of Frozen-Thawed Porcine Embryos by Ultra-Rapid Cooling Method

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This study was performed to investigate cryopreservation by ultra-rapid cooling methods in embryos of pigs. Pig's ovaries were collected from slaughterhouse and transported to laboratory at 35~37°C in 0.9% saline. Cumulus-oocyte complexes were cultured for *in vitro* maturation in NCSU-23 medium and fertilized *in vitro* in mTBM with 2 mM caffeine and 2 mg/ml BSA. Early development of oocytes fertilized *in vitro* were cultured NCSU-23 medium containing hypotaurine and BSA. After 7 days of culture, embryos were cryopreserved by vitrification methods, and were thawed and cultured in NCSU-23 medium for examination of survival ability. The blastocysts of different stages were frozen-thawed by ultra-rapid cooling methods, the proportions of embryos with normal morphology were 30.8, 38.6 and 35.5% in embryos cryopreserved at early, blastocyst and expanded stages. There are no significant differences in the proportions of normal morphology among different stages of blastocysts cryopreserved. In another experiment, the embryos with normal morphology after frozen-thawing were further cultured. After 48 hrs of culture, the developmental rates of embryos frozen-thawed at expanded blastocyst stage (38.7%) were significantly ($P < 0.05$) higher than in early (30.0%) and blastocyst (32.5%) stages. The proportions of embryos expanded and hatched were higher in medium with 1 unit/ml than SOD of 0 and 10 units/ml. These findings indicate the possible broader application for ultra-rapid cooling methods, as frozen-thawed embryos may be accompanied by developmental stages according to requirements of the survival ability after freezing of different blastocyst stages in the Pigs.

Key words) *embryos, in vitro development, survival ability, Pigs*