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Surgical and Non-Surgical Gilt Transfer of *In-Vitro* Cultured Pig Embryos Cryopreserved by Vitrification

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Pig embryos are extremely sensitive to hypothermic condition. This limits their ability to withstand many conventional methods of preservation. Vitrification has shown a promise of eluding the difficulties of cryopreservation associated with cooling sensitivity and ice crystallization. This study aimed for investigating an efficiency of cryopreservation in *in vitro*-derived embryos by transfer them to recipients and resulted in pregnancy from recipients after surgical or nonsurgical transfer. *In vitro* maturation and fertilization were performed according to the procedure(Kor. J. Emb. Trans., 19:155-163, 2004). Fertilized oocytes were cultured in glucose-free NCSU23 supplemented with 5 mM sodium pyruvate, 0.5 mM sodium lactate and 4 mg/ml bovine serum albumin at 39℃ in 5% CO_2 in air for 2 days, and 10% (v/v) fetal bovine serum was added to the culture medium thereafter. Embryos developed to the morula to blastocyst stage were treated with 7.5 g/ml cytochalasin B for 30 min, centrifuged at 13,000 × g for 13 min. Freezing and thawing embryos were performed according to the procedure of Beebe *et al.* (Theriogenology, 57:2155-2165, 2002). Briefly, embryos were exposed sequentially to 2M ethylene glycol and 7% polyvinylpyrroidone solutions, aspirated into open pulled straws, and plunged into liquid nitrogen. Embryos were thawed in 39℃ medium and transferred to uterine horns of recipients by surgical or non-surgical procedures. Catheter for non-surgical embryo transfer was made by inserting a polyurethane tubing with a length of 150 cm into a conventional pig artificial insemination catheter. One hundred to 150 thawed embryos were transferred to each

recipient. Total of embryo transfers were composed of two surgically and three non-surgically transfers. Estruses were returned in 4 among 5 recipients. A pregnancy was established in one recipient gilt of surgical transfer and maintained until 45 days of gestation based on ultrasonography. Causes of the pregnancy loss were not clear. Recently vitrification of *in vivo*-derived embryos gave rise to live births (2002 Theriogenology, 57:285-302). However, to improve pregnancy rate after vitrification of *in vitro*-produced embryos, further studies would be required in many aspects of cryopreservation procedures including *in vitro* production of embryos, cropreservation vessels, and vitrification/thawing protocols.