Thesis Title The Viability Testing of Frozen-Thawed Bovine Embryo Produced In Vitro

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Date of Graduation 21 May B.E. 2535 (1992)

ABSTRACT

To investigate the viability and normality after freezing and thawing of bovine embryos produced in vitro, we have accomplished the procedures to mature oocytes (in TCM-199 medium) and fertilize in vitro with heparin treated sperm (in TALP-glucose free medium).The oviductal epithelial cells were co-cultured in vitro (in TCM-199 + 10% HTFCS) and were capable of supporting normal growth of embryos to the stages at which non-surgical embryo transfer could be performed (compact morula,early blastocyst and blastocyst). Oocytes with intact cumulus cells showed higher ($P<0.01$) fertilization rate and higher ($P<0.01$) percent of embryonic development than those without the cumulus cells. Single step in freezing (1.5 M glycerol for 10 min) and thawing (0.5 M sucrose for 10 min) was employed and the survival rate of embryos tested by non-surgical transferring to recipients was highest in early blastocyst and was superior to the conventional multiple step dilution procedure (0.75 M glycerol and 0.3 M sucrose; 0.375 M glycerol and 0.3 M sucrose; and 0.3 M sucrose) reported by other investigators (50% VS 35% in compact morula; 75% VS 41% in early blastocyst; 33% VS 33% in blastocyst). In vitro testing, the competency of embryonic development to an advanced stage was higher in blastocyst than those from compact morula or early blastocyst.

The result indicated that bovine embryos produced in vitro could be frozen and thawed similar to those obtained from in vivo procedures. The survival rate tested by non-surgical transferring to recipients was better by using single step in freezing and thawing, and the early blastocysts were the best survivors.