

## **Survival after Frozen-thawed of Bovine IVF Blastocysts on Sucrose Concentration in Ethylene Glycol Based Freezing Medium for Slow-cooling**

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The present study was undertaken to investigate post-thawed survivability of bovine embryo according to different ethylene glycol and sucrose concentration bovine ovaries were collected at local slaughterhouse and the cumulus-oocyte-complexes aspirated from ovaries were *in vitro* matured, fertilized and cultured at 39° C in an atmosphere of 5% CO<sub>2</sub> incubator. for conventional slow-freezing, embryos at expanded blastocyst stage were collected from day 7 or 8 post insemination in serum free medium and equilibrated in 1.5 M and 1.8 M EG with 0.1 M and 0.3 M sucrose in Dulbecco's phosphate-buffered saline(DPBS) supplemented with 0.5% bovine serum albumin. embryos were then loaded individually into 0.25mL-straw and placed directly into cooling chamber of programmable freezer precooled to -7° C. after 2 min, the straw was seeded, maintained at -7°C for 8 min more, and then cooled to -35°C at 0.3° C/min plunged and stored in liquid nitrogen for at least 3 days. for straw thawing, straws were warmed in air for 10 sec and exposed to 37°C water for 20 sec. straws were then removed from 37°C water bath. embryos were then held at room temperature for 3 min before placed into culture. embryos were evaluated at 24 to 72 h post-warming for survival (resumption of development into blastocyst) and hatching. The survivability of frozen-thawed embryos was no difference between 1.5 M EG and 1.8 M EG (71 and 70.%, respectively). Addition of 0.1 M sucrose to 1.5 M and 1.8 M ethylene glycol in the freezing solution did not differ significantly embryo survival (74 and 77%, respectively) whereas freezing solution contained 0.3 M sucrose to 1.5 M and 1.8 M ethylene glycol, the survivability in 1.8 M EG +0.3 M

sucrose group was significantly higher than in 1.5 M EG+0.3 M sucrose group (71 and 89%,  $p<0.05$ ). however, there were no difference in the overall total cell number between the two groups ( $122\pm1.8$  vs  $131\pm1.4$ , respectively). in conclusion, the results suggest that 1.8 M EG +0.3 M sucrose may be optimal for the survival of frozen-thawed embryos produced *in vitro* and that direct transfer method is applicable under on-farm conditions.

Key words) *Bovine, In vitro, Slow-freezing, Ethylene glycol, Sucrose*