

Effect of the Treatment of Cytochalasin-B and the Loading Improvement of *In-Vitro* Porcine Embryos for Vitrification

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This study was examined the survival rate of vitrified blastocysts according to cytochalasin-B (CB) treatment, CB non-treatment, different concentration and exposed times of CB, equilibration cryoprotectant and freezing vessels. *In-vitro* maturation and fertilization were performed according to the procedures of Funahashi *et al.* Fertilized oocytes were cultured in glucose-free NCSU 23 supplemented with 5mM sodium pyruvate, 0.5mM sodium lactate and 4mg/ml bovine serum albumin for 2 days at 39°C and 10% fetal bovine serum was added to the culture medium thereafter. Embryos developed to the morula to blastocyst stage were treated with different concentration and exposed time of CB, centrifuged group (13,000 rpm, 13min) & non-centrifuged, cryoprotectant method (Beebe's method & Vajta's method) and loading vessels (OPS, EMG, NLS).

The results obtained in this study were summarized in follow; In experiment I: The survival rates of the embryos treated CB (60.5%) were significantly higher than non-treated (32.8%) ($p < 0.05$). However the recovered with normal morphology rates (84.2%) were not significantly different than non-treated (81.9%). In experiment II: The group of CB treated with 7.5 $\mu\text{g/ml}$ were significantly showed a higher of normal morphology (44/45; 98%) and viability rate (33/45; 73%) than other groups (morphology; 65~70%, viability; 15~74%) ($p < 0.05$). While the control was lower as 70% of morphology and 38% of viability. In experiment III: The group of exposed time for 20 minutes were significantly were observed a higher rates of normal morphology and viability than other exposed groups" ($p < 0.05$). And the group of 13 minutes, 13,000 rpm centrifuged (64%) were significantly

higher than non-centrifuged(36%). In experiment IV: The survival rates of Exp. 1(Beebe's method) and Exp.2(Vajta's method) were 81% and 86.4%, respectively after 24 hours of incubation and were 26.3% and 30.2%, respectively after 48 hours of incubation. There was no significant difference between treatments. In experiment V: The efficiency of freezing method and loading convenience were orderly higher in OPS(open pulled straw), NLS(nylon loop system) and EMG(electron microscope grid). The optimal capacity per vessel was orderly lowered in NLS, EMG and OPS, respectively. The survival rate of vitrified morula and blastocysts on OPS, EMG, NLS as freezing vessels was 47.0(78/166), 35.0(56/162) and 13.0%(26/206), respectively. OPS was significantly higher than EMG and NLS($p < 0.05$). And after morula and blastocyst were warmed, the recovery rate, morphology and rate of development were orderly higher in NLS, OPS and EMG respectively.

In conclusion, these results suggested that the survival rate of vitrified porcine embryos by 7.5 $\mu\text{g/ml}$ CB concentration, exposed times for 20 minutes, centrifuge treatment and using of OPS freezing vessels was relatively high and effective. Therefore, the development of this method is a useful technique for cryopreservation at blastocysts stage.

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Key words) *Cytochalasin-B(CB), OPS, EMG, NLS, Vitrification, Porcine embryos*