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Development of Bovine Embryos after Vitrified-Thawed with Electron Microscope Grid and Open Pulled Straws

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The objective of this study was to optimize the vitrification method of *in vitro* produced bovine embryos. Thus, *in vitro* produced embryos at 8 cell, morula and blastocyst stages were vitrified on electron microscope grids (EM grids) or in open pulled straws (OPS) with EG5.5 (5.5 M ethylene glycol, 1.0 M sucrose and 10% FBS in m-DPBS medium) freezing solution and their survival rates after thawing were compared. The embryos on EM grids or in OPS were briefly exposed to EG5.5 freezing solution and plunged directly into liquid nitrogen within 30 to 35 sec. Post-thawed embryos were serially diluted in 0.5, 0.25 and 0.125 M sucrose in m-DPBS, each for 1 min, and then cultured in CRI aa medium supplemented with 10% FBS. Embryonic survival rate was assessed as re-expanded and hatched rates of those embryos after warming. The rates of re-expansion embryos did not significantly different between EM grid (8 cell: 42.10%, morula: 66.66% and blastocyst: 77.08%) and OPS (8 cell: 47.36%, morula: 61.90% and blastocyst: 83.33%) methods. In addition, the hatched rates in EM grid (8 cell: 31.57%, morula: 57.14% and blastocyst: 72.91%) were similar to those in OPS (8 cell: 34.21%, morula: 50.00% and Blastocyst: 77.08%). Interestingly, even at the same blastocyst stage, the *in vitro* survival of day 7 embryos (EM grid: 79.48 and OPS: 87.18%) was higher than those of day 8 embryos (EM grid: 72.10 and OPS: 82.06%). The total cell number of blastocyst developed *in vitro* after vitrification was examined with Hoechst 33342 staining to compare the embryo quality among different treatment groups. The total cell number of blastocyst was not significantly different between vitrified groups (EM grid: 162.4±8.0 and OPS: 158.4±7.1) and unvitrified control (168.0±5.6). These results indicate that both vitrification containers can provide the high rate of embryo survival. Moreover, the OPS container may not need a cap to protect the container from floating after immersion in LN_2. Therefore, this study suggest that bovine embryos can be cryopreserved easily, effectively and successfully by vitrification method using EM grid or OPS with EG5.5 freezing solution. In the future, the pregnancy rate would be investigated after transfer of our vitrified embryos into the appropriated recipients.

(Key words) ***Vitrification, Bovine, Embryos, EG5.5, EM grid, OPS***