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Serum-free Culture System Enhances Survivability Following Cryopreservation of Bovine Embryos

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This study was carried out to evaluate the survivability of post-thawed bovine embryos produced in vitro by different culture conditions with or without serum in IVM/IVC medium.

Cumulus-complex-oocytes collected from a local abattoir ovaries were matured in IVM medium (M199 + hormones) supplemented with or without 10% fetal bovine serum (FBS) at 39℃ in a humidified atmosphere of 5% CO2 in air. After 22 h of culture, the oocytes were inseminated with frozen-thawed semen (2 × 106 sperm/ml of final concentration) prepared with a Percoll-density gradient in IVF-TALP medium for 16 h. Later, sets of 15 presumptive zygotes were transferred into 50 l droplets of IVC medium (M199 10% FBS) and were co-cultured with bovine oviductal epithelial cells. At 48 h and 120 h post-insemination, the culture were 'fed' with 25 l of fresh IVC medium to each drops and were maintained for 182 h post-insemination to assess the rates of development to blastocyst stage and the number of cells among groups. Blastocysts were equilibrated in PBS supplemented with 1.5 M ethylene glycol (EG), and loaded individually into 0.25-ml straws. After 10 min of equilibration, the straws were transferred into a liquid nitrogen embryo freezer (Biogenics, USA) adjusted -7C and kept for 10 min. After 5 min, seeding was made and the straws were kept at -7C for a further 5 min. Freezing was accomplished by a cooling temperature of 0.6C from -7C to -35C, then holding 10 min, and plunging into liquid nitrogen for storage. Thawing of the straws was performed in a 35C water bath for 15 sec. Differences were analyzed by ANOVA program after arc-sine transformation of proportional data ($P<0.05$).

Both culture groups with and without serum developed to mataphase II (72~79%) without significant difference. No difference of cleavage rate (59~69%) was seen between with and without serum supplement. Significantly higher ($P<0.05$) development rate into blastocyst stage was seen in serum supplemented group than that of serum-free group (21% vs. 13%). After frozen the blastocysts cultured with or without serum, better survivability, by an assessment of hatching

rate, of frozen embryos was seen in serum-free group (77% vs. 53%, respectively). Cell number and the ratio of ICM to trophoblast of blastocysts in culture group with serum similar to those of without serum (105～120, 16～23%, and 103～118, 17～21%, respectively). However, at 36 h culture after thawing, the blastocysts cultured without serum had significantly ($P<0.05$) higher cell number (180± 5) than those of cultured with serum (159± 4).

The results suggest that serum-free culture system may enhance the survivability of frozen-thawed bovine embryos. Furthermore, serum addition in culture system has a pivotal role on reducing the cell number after thawing of frozen embryos, especially significantly lower the ratio of ICM.

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