A-0104

Development of Vitrified Bovine Oocytes following Intracytoplasmic Sperm Injection (ICSI)

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Oocyte freezing has become a prevalent source for related reproductive technologies. This study was carried out to evaluate viability of post-thawed bovine oocyte injected DTT-treated sperm following by two different activation stimuli (Group 1, 5 M ionomycin, 5 min + CR1aa, 3 h + 1.9 mM dimetylaminopurine (DMAP), 3 h; group 2, ionomycin + 10 μg/ml cycloheximide (CHX), 5h). The techniques of ultra-rapid freezing used in this study were essentially similar to those of described by Vajta et al (Theriogenology 1999; 52:939-948). Denuded oocytes at 22 h of culture were exposed to cryoprotectant (3.2 M Ethylene glycol, 2.36 M DMSO, 0.6 M sucrose), and followed by freezing in electron microscopic grid. After thawing the oocytes were transferred back into the drop of maturation medium and cultured for additional 2 h before being subjected to ICSI. All eggs were then cultured in CR1aa medium, and transferred into M199+10% FCS on day 4. The culture was maintained until day 9. In Experiment 1, frozen-ICSI eggs were compared on development into blastocyst to those of unfrozen and IVF control. Those eggs were activated with the method of group 2. A higher proportion of unfrozen-ICSI and IVF eggs developed into cleavage and blastocysts than of frozen-ICSI eggs (65% and 13%; 71% and 23% vs. 39% and 8%; $P<0.05$). In Experiment 2, development and ploidy of embryos made from group 1 were compared to those from group 2. Between groups there did not differ on the rates of development, however, chromosomal abnormality in group 1 was significantly higher than in group 2 (49% vs. 30%; $P<0.05$). The present result suggests that frozen bovine oocytes can be used for ICSI.

Supported by Korean Research Foundation (KRF-99-041-G00150G7013)

(Key words) cycloheximide, frozen, ICSI, embryos, bovine