

Studies on *In Vitro* Developmental Rate of Activated Bovine Oocytes by Intracytoplasmic Sperm Injection with Frozen-Thawed Epididymal Spermatozoa

Dongsoo Lee, Sangkeun Kim

College of Vet. Med., Chungnam National University

The objective of this study was to determine the developmental competence of *in vitro* matured bovine oocytes after intracytoplasmic sperm injection (ICSI) with frozen-thawed epididymal spermatozoa. The ovaries were obtained from slaughtered Korean native cows. Oocytes matured *in vitro* for 24 hrs were fertilized by ICSI with frozen-thawed epididymal spermatozoa. After ICSI, one group of oocytes was activated with 7% ethanol for 5 min, and second group was not activated. The follicular oocytes were cultured in TCM-199 medium containing hormones and 10% FCS for 24~30 hrs in a incubator with 5% CO₂ in air at 38.5°C.

1. Results of IVM showed that the percentage of oocytes reaching M II after 24 hrs and 30 hrs of incubation were significantly higher ($p < 0.05$) after culture with TCM-199 media (80.0% and 88.3%) than M I (8.3% and 6.7%).
2. The rate of cleaved embryos to blastocyst obtained by ICSI treated activation oocytes was significantly higher ($p < 0.05$) than that of nonactivation oocytes (22/46, 47.8% vs 10/39, 25.6%).
3. The rates of embryos development to blastocyst obtained by ICSI treated sperm of fresh, epididymal and frozen-thawed epididymal were 24/45 (53.3%), 15/40 (37.5%), 11/43 (25.6%), respectively. and these values of fresh sperm injection were higher than frozen-thawed epididymal sperm. We also concluded that embryos can be produced with ICSI of *in vitro* matured oocytes by ICSI using frozen-thawed epididymal semen.

Key Words) *ICSI, frozen epididymal sperm, activated oocytes*