

Development of Bovine Embryos Reconstructed by Microinjection of Cultured Fetal Fibroblast Cells into *In-Vitro* Matured Oocytes

Sungmin Kim, Sangkeun Kim

College of Vet. Med., Chungnam National University

Bovine cumulus-oocyte complexes were recovered from ovaries at a slaughter and then divided into five groups : control group(unvitrified oocytes), 0 hr. group(composed of oocytes vitrified before the onset of maturation) and 10, 14, and 20 hrs groups(vitrified respectively at 10, 14 and 20 hrs after the onset of maturation). The oocytes remained vitrified for 24 hrs, and then were thawed in 30°C water bath. Survival and cleavage rates were defined as development rate on *in vitro* culture and stained with aceto-orcein or FDA test.

1. Small cells were more likely to result in nuclear formation(30%) than large cells(15%, $p<0.05$). Small, confluent and serum starved cells resulted in nuclear formation more often than did cycling cells.
2. The rate of nuclear formation was not dependent upon the media nor upon the duration of exposure to the media(1 to 4 hr.) after microinjection but before activation.
3. While such treatments did not have an effect on nuclear formation, treatment of parthenogenetically activated oocytes with Ca-free TL-Heapes reduced the percentage of blastocysts(11.2% vs. 27.6%) and increased the percentage of morula stage embryos (27.6% vs. 15.7%) as compared with culture in TCM-199 media.
4. Small confluent cells were used for nuclear transfer and resulted in two presumptive blastocyst stage embryos(5.2%) successful injections. These results show that presumptive blastocyst stage embryos can result from microinjection of fibroblast cells to enucleated oocytes and thus may provide a method to create transgenic knockout animals.

Key words) *microinjection, fibroblast cells, development*