

Development of Reconstituted Embryos with Fetal Fibroblast Cells in Rabbit

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To produce reconstituted rabbit embryos with fetal fibroblasts, the present study was evaluated the efficiencies of the activation conditions as assessments of subsequent development and chromosome in the embryos. New Zealand White rabbits were used throughout the study. Fetal fibroblasts collected from 22-d of fetuses were cultured in DMEM +10% FBS in 5% CO₂ in air. The culture was maintained for 10 passages. In every passage half of cell suspension were kept in frozen. From rabbits treated with FSH+30% PVP and hCG, oocytes were surgically collected from oviducts at 14 h post-hCG injection and stripped of their cumulus cells by a 300 IU hyaluronidase solution. Oocytes with an extruded first polar body and dense cytoplasm were enucleated by micromanipulation in Ham's F-10 medium + 7.5 $\mu\text{g/ml}$ cytochalasin B. Enucleation was confirmed under a fluorescence microscope after staining with 5 $\mu\text{g/ml}$ bisbenzimidazole for 2 min. Each enucleated oocyte was injected with a fetal fibroblast into a preivitelline space, and subsequently applied DC pulses (1.6 KV/cm, 60 μsec , twice) for both of fusion and activation. Reconstituted eggs were then cultured in CR1aa medium + 10 $\mu\text{g/ml}$ cycloheximide (CHX) for 3 h (Group 1), 2 mM 6-dimethylaminopurine (6-DMAP) for 3 h (Group 2), 2 mM DMAP + 5 $\mu\text{g/ml}$ CHX for 3 h (Group 3) and 2 mM Na-pyrophosphate for 3 h (Group 4), and compared to control that were unexposed to either of CHX, DMAP or Na-pyrophosphate for the efficiencies of development and ploidy. Culture of the eggs was maintained in CR1aa medium until day 3, and then moved to TCM-199 + 10% FBS.

The rates of cleavage and blastocyst development in control and Group 1 to 4 were: 22% and 9%, and 67% and 35%, 68% and 38%, 67% and 35%, and 65%

and 33%, respectively. Most reconstituted embryos (~80%) in control, Group 1 and Group 4 were diploid whereas in Group 2 and 3 the majority (~50%) were mixoploid.

These results indicate that the uses, as a MPF downstream chemical, of either CHX or Na-pyrophosphate after electric pulses are suitable treatments and suggest that this can be adapted as an effective method of oocyte activation during reconstitution of rabbit oocytes.

(Key words: cycloheximide, 6-dimethylaminopurin, Na-pyrophosphate, fetal fibroblast, rabbit, reconstituted embryo)

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