

***In Vitro* Development of Porcine Nuclear Transfer Embryos Constructed by the Microinjection of Fetal Fibroblast Cells into Oocytes**

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This study was conducted to examine *in vitro* development of porcine embryos constructed by the microinjection of cultured fetal fibroblast cells into porcine oocytes matured *in vitro*. Single fetal donor cells were deposited into the perivitelline space of enucleated oocytes, followed by electrical fusion and activation. Activated embryos were cultured in NCSU-23 medium supplemented with 5% FBS, at 38.5°C for 6 to 8 days in 5% CO<sub>2</sub> and air. In experiment 1, fusion rates of nuclear transfer embryos did not differ for fetal fibroblast cells incubated in 5% FBS + NCSU-23 vs 5% FBS + TL Heaps medium, nor did fusion rates of donor cells differ between 1 vs 8 hr incubation durations. Fusion rates for the four treatment subclasses ranged from 72.1 to 78.0%. In experiment 2, fusion rates did not differ significantly for nuclear transfer embryos constructed using donor cells cultured in 5% FBS + NCSU-23 medium for 1~2, 6~8 or 12~14 days (60.0, 73.3, and 62.5%), respectively. The cleavage rate for nuclear transplant embryos using fetal fibroblast cells cultured for 1~2 days was 44.0%, significantly less than 56.7% and 50.0%, for 6~8 or 12~14 days duration of culture, respectively. In experiment 3, the proportions of nuclear transfer embryos that developed to the =2 cell and to the blastocyst stage were not affected significantly by culture medium (5% FBS + NCSU-23 vs 5% FBS + TL-Heaps) or by O<sub>2</sub> concentration of the culture (5 vs 10%). Rates of development to the =2 cell stage ranged from 65.9 to 70.1%, and development rates to the blastocyst stage ranged from 9.8 to 12.5% for the

four treatment subclasses. Developmental rate was highest for embryos cultured in 5% FBS + NCSU-23 under a gas atmosphere of 5% O<sub>2</sub> in air. In experiment 4, the fusion rate of donor cells in nuclear transfer embryos using fetal fibroblast cells was 68.4%. The cleavage rate of nuclear transferred embryos to the blastocyst stage was 21.1%, which was lower than that of control IVF embryos (34.2%).

Key words) *nuclear transfer, porcine fetal fibroblast, fusion, cleavage rate*