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Bovine Nuclear Transfer using Ear Skin Fibroblast Cells Derived from Serum Starvation and Passage Numbers.

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To facilitate the widespread application of somatic cell cloning, improvements in blastocyst production efficiency and subsequent fetal viability are required. Area where technical improvements are needed include donor cell treatments, starvation and passage numbers. This study was carried out to investigate the effect of serum-starvation and passage on the development of ear skin fibroblast cells cloned embryos. A skin biopsy was obtained from the ear of a 2-year-old Korean Hanwoo female. The cells were cultured in 10% FBS+DMEM up to 2-3 months (up to 10 passages) and then used. In Experiment 1, the Korean bovine Ear Skin Fibroblast cells (KbESF) were either serum starved (culture in 0.05% FBS+DMEM) or serum fed (10% FBS+DMEM) for 4-7 days prior to NT. In Experiment 2, the KbESF cells used for nuclear transfer in these experiments were from passages 2 to 10. The development of 208 nuclear transfer (NT) embryos reconstructed from either serum starved or serum fed ear skin fibroblast was assessed. NT embryos reconstructed from serum starved and serum fed cells showed the same developmental rate (cleavage 80.16 vs. 85.37%; blastocyst 20.63 vs. 19,51%). The development of 590 nuclear transfer (NT) embryos reconstructed from passage 2 to 10 was assessed. We observed the same developmental rates for embryos derived from later passages as compared with those embryos from early passages (blastocyst from 16.69 to 27.91%, average 20.17%). There was no significant difference between serum-fed and serum-starved donor cells. We observed no difference in developmental rates for embryos derived from 2 to 10 passages. These data show that prolonged culture and serum starvation does not affects the cloning competence of adult somatic cells.

(Key words) ***Korean bovine Ear Skin Fibroblast cells (KbESF), passage, serum starvation, nuclear transfer***