

## Assessments of Apoptosis in Bovine Embryos Reconstructed with Fetal Fibroblast

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Mainly due to deficiencies in nuclear reprogramming, gene expression and DNA fragmentation, which result in early and late embryonic losses, the overall success rate achieved by cloning techniques to date is low. This present study compared the incidences of DNA fragmentation during development of IVF, parthenotes (PT), nuclear transfer (NT) and transgenic (TG) embryos. Terminal deoxynucleotidyl transferase (TdT) nick-end labelling (TUNEL) with propidium iodide counter staining was used for determination of DNA fragmentation and total number, respectively. TG and NT donor cells were fetal fibroblasts with or without transfection with EGFP, and cultured in DMEM+15% FCS until confluent, for 5 days. At 19 h post-maturation (hpm), enucleated oocytes were reconstructed with donor cells and activated at 24 hpm with the combinations of ionomycin (5 M, 5 min) and cycloheximide (10 g/ml, 5 h) after electric fusion by a single DC pulse (1.6 KV/cm, 60 sec). Parthenotes were produced by the same activation protocol at 24 hpm. The eggs and control IVF embryos were cultured in CR1aa at 39C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Embryos at the 8-cell stage in all treatments, IVF, PT, NT and TG, showed DNA fragmentation. The apoptotic cell index (total number of apoptotic nuclei/total number of nuclei) of day 7 blastocysts was significantly ( $P < 0.05$ ) higher in TG and NT embryos (17/91,  $18.6 \pm 4.0\%$  and 13/94,  $13.8 \pm 4.7\%$ , respectively) compared to IVF and PT embryos (9/122,  $7.4 \pm 3.4\%$  and 8/93,  $8.6 \pm 2.9\%$ , respectively). TUNEL positive cells were detected in almost all blastocysts at day 7 and were mainly observed in the ICM. The DNA fragmentation ratio of the ICM in the blastocyst at day 7 was significantly ( $P < 0.05$ ) higher in TG embryos ( $64.7 \pm 21.4\%$ ) than in IVF ( $44.4 \pm 28.0\%$ ), PT ( $50.0 \pm 18.6\%$ ) or NT ( $53.0 \pm 32.5\%$ ) embryos. These results indicate that the high occurrence of DNA fragmentation observed in NT and TG embryos compare to IVF and PT embryos. In addition, ICM of TG blastocysts revealed a high DNA fragmentation ratio, which ma

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