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Morphological Characteristics of Pig Blastocysts Produced by Somatic Cell Nuclear Transfer

Y.M. Han, D.B. Koo, Y.H. Choi, J.S. Park, H.N. Kim, Y.K. Kang, W.K. Chang[1], and K.K. Lee

Animal Developmental Biotechnology Lab. Korea Research Institute of Bioscience & Biotechnology (*ymhan@mail.kribb.re.kr*), [1]National Livestock Research Institute

Blastocyst formation, consisting of the inner cell mass (ICM) and trophectoderm (TE), is the first differentiation process during embryonic development in mammals. It has been hypothesized that the proportion of ICM to TE in the blastocyst may be crucial for subsequent developmental competence of early embryos, which it may be expressed as a sensitive indicator for evaluating in vitro systems. In this study ICM/total cell ratio of nuclear transfer (NT) embryos was compared with IVF-derived and in vivo embryos. Somatic cell nuclei obtained from a fetus at Day 40 of gestation were transferred into the enucleated oocyte and then cultured in NCSU 23 medium for 6 days as previously described (Koo et al., Biol. Reprod. 2000; 63:986-992). ICM and TE cells of blastocysts were determined by using a differential staining method (Han et al., Biol. Reprod. 1999; 60:1110-1113).
Development rate (9.8± 2.5%, 23/225) to the blastocyst stage of NT embryos was lower than IVF embryos (23.8± 2.7%, 53/223). Thus, a difference was detected in the in vitro developmental rate to blastocyst stage between NT and IVF-derived embryos ($P<0.05$). In the next experiment, we investigated ICM and TE nuclei to assess the quality of blastocysts that produced by NT, IVF and in vivo, respectively. NT blastocysts (27.6± 8.3) showed a smaller total cell number than IVF-derived (42.6± 17.4) and in vivo embryos (283.9± 103.5) ($P<0.05$). Ratios of ICM/total cells in NT, IVF and in vivo blastocysts were 15.1± 18.6% (n=56), 12.3± 9.2% (n=57) and 30.4± 6.8% (n=40), respectively. Individual blastocysts for the ratio of ICM/total cells were assigned to 3 groups (I; <20%, II; 20 to 40% and III; >40%). As the results, most in vivo blastocysts (97.5%, 39/40) were distributed into group II while most NT (78.6%, 44/56) and IVF-derived blastocysts (82.5%, 47/57) were allocated to group I. Thus, our data show that NT or IVF-derived embryos have aberrant morphology during early development in in vitro systems, suggesting that these anomalies may result in developmental failures of the NT embryos to term.

(Key words) nuclear transfer, differential staining, blastocysts, porcine