

Production of *In Vivo*-derived Cat Blastocysts for Establishment of Cat Embryonic Stem Cells

Xian-Feng Yu, Kye-Young Park, Eu-Gene Choi, Xi-Jun Yin, Inhyu Bae,
Chul-Ju Yang, Seong-Gyun Cho and Il-Keun Kong*

*Department of Animal Science and Technology, College of Agriculture and Life Sciences, Sunchon National University, Suncheon, JeonNam Province
540-742, S. Korea*

Identification and characterization of spontaneously occurring genetic diseases in cats has permitted the development of valuable models for testing potential treatments of similar human diseases. With the near completion of the feline genome project, establishment of pluripotent cat embryonic stem (ES) cells would facilitate the targeting of specific genetic loci to produce new feline medical models. We produced *in vivo* blastocysts from stroller female cats by using surgical methods. Briefly, the stroller female cats were superovulated with an intramuscular (i.m.) injection of 200 IU pregnant mare serum gonadotropin (PMSG) apart 100 h by an i.m. injection of 100 IU of human chorionic gonadotropin (hCG) to induce follicular growth and ovulation, and then inter-uterus fertilization is accomplished by surgical methods at 24 h after hCG with fresh epididymal sperms. On day 7, the stroller female cats underwent ovariohysterectomy and blastocysts were flushed from the oviducts and uterine horns. Average 3.2 blastocysts were recovered from the reproductive tract of each stroller female cats. These blastocysts were containing a large and distinguishable inner cell mass(ICM). *In vivo*-matured blastocysts were subsequently cultured in mouse ES cell medium on inactivated stroller cat embryonic fibroblasts. ICM was isolated from the *in vivo*-matured blastocysts by mechanically. Primary colonies were formed within 3 days. Colonies were mechanically disaggregated into small clusters and re-plated on fresh feeders and were transferred after one week onto new feeder layers. After 3 passage,

colonies had either differentiated or died. *in vivo*-derived from blastocysts in cat can be use effectively an establishment of ES cell lines.

Key words) *Stroller female cat, Blastocyst, Embryonic stem cells, Superovulation*

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