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DEVELOPMENTAL CAPABILITY OF CAT EMBRYOS RECONSTRUCTED BY TRANSFERRING SOMATIC CELLS INTO ENUCLEATED M I OR M II OOCYTES IN VITRO

$$\label{eq:even M.*} \begin{split} \text{Evecen M.*}^*, & \text{Pabuccuoğlu S.}^!, \text{Demir K.}^!, \text{Yağcıoğlu S.}^!, \text{Can A.}^!, \text{Ertürk E.}^!, \\ & \text{Hamzaoğlu A.}^!, \text{Arıcı R.}^!, & \text{Öztürk G.}^!, \text{Ak K.}^!, \text{Birler S.}^! \end{split}$$

Animal production via SCNT provides a unique tool for protection of valuable individuals, conservation of vulnerable and endangered species and production of transgenic animals.

transgenic animals.

A total of 167 MI and 219 MII stage oocytes were used as the material of the study. The oocytes were enucleated at 44 h after in vitro maturation by aspiration of the polar body and the MI and MII plates. Cycling somatic cells (granulosa) were used for nuclear transfer. Cell fusion was induced with DC pulses of 2.0 kV/cm 60µs, 0.1s apart (x2) delivered by a BTX Electrocell Manipulator 200 (BTX, San Diego, CA, USA). After fusion, the embryos were activated by exposure to 1.0 kV/cm 20µs DC pulses 0.1s apart (2x) followed by 2 mM 6-DMAP (6-dimethylaminopurine) in culture medium for 4 h in a humidified atmosphere of 5% CO₂, 5% O₂, and 90% N, at 38°C. The nucleus transferred embryos were cultured with mSOF medium supplemented with % 0.4 BSA in a humidified 5% CO₂, 5% O₃, and 90% N, atmosphere at 38°C with 100% humidity. After the in vitro culture period, all the embryos transferred to hSOF containing Hoechst 33342 (5 µg/ml) for 30 minute and the cell numbers were counted under ultraviolet light using a fluorescent microscope.

The fusion (66.66-21.55%) and cleavage rates (15.75-11.11%) of MII stage oocytes

The fusion (66.66-21.55%) and cleavage rates (15.75-11.11%) of MII stage oocytes were significantly higher than MI stage oocytes (p <0.02). While SCNT embryos were developed to 8-16 cell stage in MII group 14 (9.58%), all the cleaved embryos arrested at the 2-4 cell stage in MI group and none of them was developed to morula and blastocyst stages.

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