

CLONING OF ANATOLIAN GREY BULL

Sezen Arat *¹, Arzu Tas¹, Haydar Bagis¹, Hakan Sagirkaya², Yavuz Nak², Deniz Nak², Tolga Akkoc¹, Gaye Cetinkaya¹; TUBITAK, MRC, Genetic Engineering and Biotechnology Institute, Kocaeli, Gebze, Turkey¹, Uludag University, Faculty of Veterinary Medicine Department of Reproduction and Artificial Insemination , Bursa, Gorukle, Turkey²

One of the application fields of nuclear transfer (NT) is to increase the population of endangered mammals. In the present study, cloning of native Anatolian Grey Cattle living semi-wildly especially in Marmara Region was aimed. Bovine oocytes isolated from slaughterhouse ovaries were matured in TCM199 supplemented with 10% fetal bovine serum (FBS), 50 µg/ml sodium pyruvate, 1% v:v penicillin-streptomycin (10.000 U/ml penicillin G, 10.000 mg/ml streptomycin), 50 ng/ml EGF, 0.5 µg/ml FSH, 5 µg /ml LH (Arat et al., 2004, *Reprod Fertility Dev* 16(1,2) 135-135) . After removing the cumulus cells of matured oocytes (MII), meiotic spindles and first polar bodies were removed. As nuclear material source, cartilage and fibroblast cells obtained from the ear tissue of 3 years old Anatolian grey bull were used. This animal is kept under the National Conservation Program of Turkish Government. Prior to NT, all somatic cells were allowed to grow to confluency (G1/G0) in DMEM-F12 supplemented with 10 % FBS (Arat S et al., 2002 *Biol Reprod* 66, 1768-1774). After transfer of single cells to enucleated oocytes, oocyte-cell couples were fused by a DC pulse of 2.66 kV/cm for 30 µs in the Zimmerman medium. After fusion, fused NT units were activated using a combination of calcium ionophore (5 µM for 5 min), cytochalasin D (2.5 µg/ml) and cycloheximide (CHX, 10 µg/ml) for 1 h and CHX alone for 4 h. In the first experiment, after activation, NT units were cultured in Sage cleavage medium (Cooke S et al., 2002, *Fertility and Sterility* 78(6) 1254-60, Tang R et al., 2006, *Human Reproduction* 21(5) 1179-1183) supplemented with 8 mg/ml BSA for 72 h and then developing embryos were divided into four groups. After 72 h culture, all cleaved embryos from cartilage cells in groups I, II, III and IV were respectively cultured in Sage blastocyst media supplemented with 8 mg/ml BSA, 8 mg/ml BSA + 5% FCS, 4 mg/ml BSA + 5% FCS and 4 mg/ml BSA + 100 ng/ml IGF for additional 4 days. Differences among groups were analyzed by one-way ANOVA after arcsin square transformation (p-value 0.05). In group III, development rate to blastocyst (27.3 %) was significantly higher than the other groups (18.2 %, 21.2 % and 15.0 % for groups I, II and IV, respectively). In the second experiment, development rates of embryos from cartilage or fibroblast cells, cultured in the group III medium treatment above were compared. Development rate to blastocyst of one cell embryos from cartilage cells (30.8 %) was significantly higher than the rate of embryos from fibroblast cells (20.8 %). Good quality 42 embryos from cartilage cells and 11 embryos from fibroblast cells were transferred into 23 holstein dairy recipient cows (2-5 blastocysts/cow). Day 35

pregnancies were diagnosed in 10 cows from cartilage cells (53% 10/19; one of them was over to 100 days) and in one cow from fibroblast cells (50 % 1/2). Two recipient cows carrying embryos from fibroblast cells were not yet examined for pregnancy. This cloning study is the first on Anatolian Grey Cow and still going on. Correspondence: Sezen.Arat@mam.gov.tr

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1111 N. Dunlap Ave., Savoy, IL USA 61874

Phone: +1-217-356-3182 Fax: +1-217-398-4119

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