

THE EFFECTS OF EMBRYO CULTURE MEDIUMS ON REPROGRAMMING OF CARTILAGE CELLS FROM MALE AND FEMALE COW.

S Arat¹, A Tas¹, G Cetinkaya¹, T Akkoc¹; H Bagis¹,

¹.TUBITAK, Research Institute for Genetic Engineering, Gebze, Kocaeli, Turkey

Contact E-mail: sezen.arat@mam.gov.tr

There are several parameters affecting reprogramming of somatic cell in oocyte cytoplasm. The objective of this study was to examine the effect of cell type, sex of cell and embryo culture medium on somatic cell cloning (SSC). In the first experiment, five different medium combinations were compared to examine the effect of clon embryo development. In the second experiment, we investigated the effect of source and sex of cells on SSC. Bovine oocytes isolated from slaughterhouse ovaries were matured in TCM199 supplemented with fetal bovine serum (FBS), sodium pyruvate, penicillin/streptomycin EGF bFSH, and bLH. After maturation, cumulus cells were removed and oocytes previously stained with Hoechst were enucleated by aspirating the first polar body and the metaphase II plate. A single cell derived from cartilage tissue of two different strain of cow was inserted into the perivitelline space of the enucleated oocyte. Oocyte-cell couples were fused by a DC pulse of 133V/500 μ m for 30 μ s in the Zimmerman's medium. After fusion, fused NT units were activated using a combination of CaI (5 μ M for 5 min), CD (2.5 μ g/ml) + cycloheximide (CHX, 10 μ g/ml) for 1 h and CHX alone for 4 h. After activation, NT units were cultured in Sage medium for 72 h and then additional 4-5 days in five different medium combinations (Group 1: Sage with 8mg/ml BSA; Group 2: Sage with 10% FCS; Group 3: Sage with 10% Serum replacment; Group 4: Sage with 8mg/ml BSA and 5% FCS; Group 5: Sage with 4mg/ml BSA and 5% FCS). The differences among groups were analyzed. In the fifth group, blastocyst rate (39,3%) was higher than the other groups. When the compared the blastocytes rates between the first (18,36%) and the fifth groups (39,3%); the significant difference was found. In the second experiment, cell sources and sex were analyzed. Donor cells from two different native bovine strains (Anatolian black and grey) were used. When used cells from the Anatolian black cow; there was not found significant differences between development of female and male cloned embryos (28,5% versus 26,6%) to blastocyst stage. However; when the Anatolian black and Grey cloned embryos from male cells were compared, the significant differences were visualized on blastocysts (26,6% versus 38,2%, respectively) development. This results showed that blastocysts developed higher in the fifth group than the other groups. In addition source of cells were affected that SSC blastocysts rates. This study was supported by a grant from TUBITAK KAMAG, Turkey (106G005).

Correspondence; S. ARAT, sezen.arat@mam.gov.tr