

## **THE EFFECT OF ELECTRICAL PULSE ON DEVELOPMENT OF NUCLEAR TRANSFER (NT) BOVINE EMBRYOS FROM CARTILAGE CELLS**

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In some previous NT studies, it was reported that oocyte activation starts with electrical stimulation applied before chemical activation (Rzucidlo SJ et al.,2004 *Reprod Fertility Dev* 16(1,2)157-157, Arat S et al 2006 *Reprod Fertility Dev* 18(1,2)119-119). Therefore; one of the several parameters affecting reprogramming of somatic cells is the fusion parameter and time. Electrical stimulation has also an effect on embryo quality (Milazzotto MP et al.,2008 *Reprod Dom Anim* 43,319-322). The objective of this study was to examine the effect of fusion parameter and time on blastocysts development rates on somatic cell nucleus transfer(SCNT).Bovine oocytes isolated from slaughterhouse ovaries were matured in TCM199 supplemented with 10% fetal bovine serum(FBS), sodiumpyruvate, penicillin/streptomycin, EGF, bFSH, and bLH in a humidified atmosphere of 5% CO<sub>2</sub> in air for 18 h. Single cells derived from cartilage tissue of Anatolian Black Cow were inserted into the perivitelline space of the enucleated oocytes. In the first experiment, NT couples were fused by 2.66 kV/cm, 30  $\mu$ s, 1 pulse(Group1) in sorbitol fusion buffer (0.25 M sorbitol, 0.1 mM Calcium acetate,0.5 mM Mg Acetate). After one hour of the first fusion nonfused NT couples were refused by 1.40 kV/cm , 40  $\mu$ s, 1 pulse(Group 2) .After fusion, all fused NT couples in group 1(1.5 h after the first fusion) and 2 (1.5 h after the second fusion) were activated using a combination of cytochalasin D(2.5  $\mu$ g/ml) and cycloheximide (CHX,10  $\mu$ g/ml) for 1 h and CHX alone for 4 h. Following activation, reconstructed oocytes were cultured in Sage cleavage medium supplemented with 8 mg/ml BSA for 72 h and then developing embryos were cultured in Sage blastocyst media (Tang R et al., 2006, *Human Reproduction* 21(5) 1179-1183) supplemented with 4 mg/ml BSA + 5% FCS for additional 4 days. Differences among groups were analyzed by one-way ANOVA after arcsin square transformation(p-value 0.05). There was no significant difference on fusion rates observed between Group 1(49.4%) and Group 2(47.5%). However, the result showed that the blastocyst development was seriously decreased after second fusion(Group 1: 30.8% and group 2: 2.5%). This was considered as a negative effect of the electrical stimulation which was applied twice on NT couples. In the second experiment; development rates of embryos were compared from NT couples fused 24 or 28 hours after maturation. In this experiment fusion was applied one time. NT couples fused approximately 24 and 28 h postmaturation were considered as early and late, respectively. The results showed that there was no significant differences between two groups on the blastocysts development rates (31.6% early and 28.4%

late). According to the these results, 4 h difference at oocyte ages was not an effective parameter on the blastocyst deveopment rates of NT embryos. This study was supported by a grant from TUBITAK TOVAG 104O360 and KAMAG, Turkey (106G005). Correspondence; S. ARAT, sezen.arat@mam.gov.tr

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