

EFFECT OF TRICHOSTATIN A (TSA) TREATMENT OF DONOR CELLS ON DEVELOPMENT OF BOVINE NUCLEAR TRANSFER (NT) EMBRYOS.

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Nuclear reprogramming is the process by which a differentiated nucleus returns to a totipotent stage. It is possible that in somatic cell cloning, donor cells with less epigenetic modifications - lower levels of methylated DNA and/or higher levels of histone acetylation - may be better reprogrammed. To investigate the effect of histone acetylation of donor nucleus on NT-embryo development we treated adult bovine fibroblasts with trichostatin A (TSA) – a potent histone deacetylase inhibitor - during serum starvation (0.2 mM TSA) or in complete medium (0.08 mM TSA) 24 h prior to nuclear transfer. NT-embryos were constructed by zona-free method (Oback et al. 2003). Cell couplets were fused by single DC-pulse of 1.2 Kv/cm applied for 30 µsec. NT-embryos were activated 1.5 to 3.5 h after fusion with 5 µM ionomycin for 4 min followed by 4 h culture in 2 mM 6-DMAP in m-SOF. Zona-free NT-embryos were cultured individually in 3 µl drops under mineral oil at 38.5°C in 5% CO₂, 5% O₂ and 90% N₂. The rates of cleavage, morula compaction D6 (MCD6) and blastocyst formation D7 (BLD7) were recorded. The data were compared by Chi-square test.

The results of the preliminary experiments showed that TSA-treatment of donor cells during serum starvation did not affect developmental ability of NT-embryos till the stage of morula compaction but significantly decreased the rate of BLD7 (28.7 vs 42.1%, P<0.05).

In the second series of experiments we evaluated the development of NT-embryos derived from TSA (complete media)-treated fibroblasts using two different time between fusion and activation that is considered to be the time of active remodelling and reprogramming of the donor nucleus (Table 1).

Table 1. Effect of TSA-treatment in relation to different fusion-activation timing.

Group	Time *, h	N	Cleavage(%)	MCD6 (%)	BLD7 (%)
control	2 - 2.5	69	61 (88.4)	24 (34.8) a	14 (20.3) c
control	3 - 3.5	121	118 (97.5)	58 (47.9) ab	41 (33.9) d
TSA	1.5 - 2	83	78 (94)	43 (51.8) b	29 (34.9) d
TSA	3 - 3.5	112	107 (95.5)	57 (50.9) b	42 (37.5) d

Chi-square test, P<0.05. Time* : time between fusion and activation.

Our results indicate that TSA-treatment of donor cells allows NT-embryos to undergo reprogramming faster than in control (TSA 1.5-2 h vs control 3-3.5 h).

In conclusion we demonstrate that there is a significant decrease of blastocyst rate after TSA-treatment of donor cells during serum starvation but not during normal culture. In addition we show that TSA treated donor cell nuclei acquire the ability to be reprogrammed faster. This work was supported by FIRB.