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Author(s)	MOHAMED SAYED, Mohamed Nour
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selection of cells with increased metastatic properties from the parent POS canine osteosarcoma cells. The procedure selected medium sized and polygonal cells, and were named highly metastasizing POS (HMPOS) cells. HMPOS cells produced numerous and large masses of lung metastases with various sizes and replacement of lung tissues 12 weeks after implantation as compared to POS cells.

Secondly, treatments of HMPOS cells *in vitro* morphologically elongated and increased ALP activity and staining of cells. HMPOS tumor growth *in vivo* were significantly inhibited when OCT and ATRA were given subcutaneously three times a week for 5 weeks (1.0 ( $\mu\text{g}/\text{kg}$  bw)). The subcutaneous tumors of the control mice consisted of osteoblastic

cells and isolated chondroblastic cells, but formed several areas of osteoid and increased amount of collagen tissue in all treated mice. Microscopic metastatic nodule developed only in two from six mice treated with ATRA. Metastasis was not seen in the mice treated with OCT or OCT + ATRA.

In conclusion, this study demonstrated that inhibition of growth *in vitro* and *in vivo* of the POS canine osteosarcoma cells and its clonal cells, and its pulmonary metastasis *in vivo*, was induced by these drugs and suggest that both its differentiating and resulting apoptotic inducing activities may be responsible for the antitumor effects. These drugs may be useful in the clinic as an adjunct to canine osteosarcoma therapy.

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## Bovine nuclear transfer using embryonic blastomeres and cumulus cells

Mohamed Sayed Mohamed Nour

Laboratory of Theriogenology,  
Department of Clinical Sciences,  
Graduate School of Veterinary Medicine,  
Hokkaido University, Sapporo 060-0818, Japan

This study was conducted to improve the recipient cytoplasm preparation, and to find out the most suitable donor-recipient cell-cycle phase coordination for nuclear transfer (NT).

First, the effect of recipient oocyte quality on the development after NT using embryonic blastomeres as nuclear donors was investigated. Embryos reconstituted from good

quality oocytes showed significantly higher developmental rate to the blastocyst stage than those from poor ones. The second study was designed to develop a new efficient enucleation technique. The chromatin material of 41% of metaphase II and 100% of the activated oocytes was located adjacent to the first and second polar body, respectively. Enucleation after activation resulted in a higher enu-

cleation rate (92%) than that before activation (60%) without detrimental effect on development after embryo reconstruction with blastomeres. Third, the relationship between the size and cell-cycle phase of cumulus cells was investigated. Most of trypsinized cells derived from serum-starvation and confluent cultures were in the medium-size range (15-20 $\mu$ m). The medium- and small-sized cells had significantly higher percentages (95-98%) of G<sub>0</sub>/G<sub>1</sub>-phase nuclei than the larger cells (82%) regardless of the culture conditions.

NT embryos produced from serum-starved cells of different sizes had the same developmental potential. Embryos reconstituted from medium-sized cells of both cultures showed the same developmental rate.

The fourth study was conducted to determine the most suitable donor-recipient cell-cycle phase coordination using the cumulus cells as nuclear donors. Confluent (G<sub>0</sub>/G<sub>1</sub> phase) and aphidicolin-treated (S phase) cumulus cells were used as nuclear donors. Using metaphase II (M phase) and activated (S phase) cytoplasts, 3 donor-cytoplast combinations (G<sub>0</sub>/G<sub>1</sub> + M, G<sub>0</sub>/G<sub>1</sub> + S, and S +

S) were produced to examine the effect of cell-cycle phase coordination. The G<sub>0</sub>/G<sub>1</sub> + M combination resulted in higher rate of development to the blastocyst stage (38.3%) than the other combinations (1.5 and 2.1%). Last, the normality of embryos reconstituted from cumulus cells (G<sub>0</sub>/G<sub>1</sub> + M combination) and embryonic blastomeres (S + S combination) were examined by comparing to *in vitro* fertilized (IVF) embryos. The three sets of embryos showed the same developmental potential. The time between the initial cleavage and blastocoel formation was shorter in both NT embryos. Blastocysts with normal chromosomal complements were the same for the cumulus cell NT embryos and IVF embryos.

In conclusion, this study described a new enucleation procedure with high efficiency using activated (S phase) oocytes. In somatic cell NT, the G<sub>0</sub>/G<sub>1</sub> + M combination results in normal development to blastocysts with normal ploidy. The G<sub>0</sub>/G<sub>1</sub>-phase donor nuclei source can be obtained from the most available medium-sized cells derived from both serum-starvation and confluent cultures.

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