

## Introduction

Nuclear transfer (NT) has several applications on mammalian biology as cloning of high genetic merit of livestock, transgenic animal production, saving the endangered animals etc. In addition of those, this technology gives us some important information of reprogramming of somatic cells. There are several parameters affecting reprogramming of somatic cell in oocyte cytoplasm (SSC). The objective of this study was to examine the effect of cell type and cell synchronization on somatic cell cloning (SSC).

In the first experiment, prior to NT, all somatic cells were allowed to grow to confluency (G1/G0) in DMEM-F12 supplemented with 10% FBS. The cartilage and fibroblast cells were used for nuclear sources. In the second experiment, before NT, cartilage cells were cultured either in DMEM-F12 supplemented with 10 % FBS to confluency ( group 1) or in DMEM-F12 supplemented with 10 % FBS and 15 µM roscovitine for 24 hr (group 2). The results showed that cartilage cells and confluency have beneficial effect on NT embryo development.

## Materials and Methods

Bovine cumulus-oocyte complexes were recovered by aspiration of follicles and matured in TCM 199 supplemented with 10% FCS, sodium pyruvate, bLH, bFSH, EGF, IGF-1 and penicillin/ streptomycin, at 39 °C in a humidified 5% CO<sub>2</sub> in air for 18 hrs. 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 cells derived from cartilage and fibroblast cells were inserted into the perivitelline space of the enucleated oocyte. Oocyte-cell couples were fused by a DC pulse of 133V/500 µm for 30 µs in the Zimmerman's medium. After fusion, fused NT units were activated using a combination of Cal(5µM for 5 min), CD(2.5 µg/ml)+cycloheximide(CHX, 10µg/ml) for 1 h and CHX alone for 4 h.

In the first experiment; cell sources were compared. Cartilage and fibroblast cells obtained from the ear tissue of a male Anatolian grey bull were used as the source of nuclear material.

In the second experiment, cartilage cells were cultured either in DMEM-F12 supplemented with 10 % FBS to confluency ( group 1) or in DMEM-F12 supplemented with 10 % FBS and 15 µM roscovitine for 24 hr (group 2).

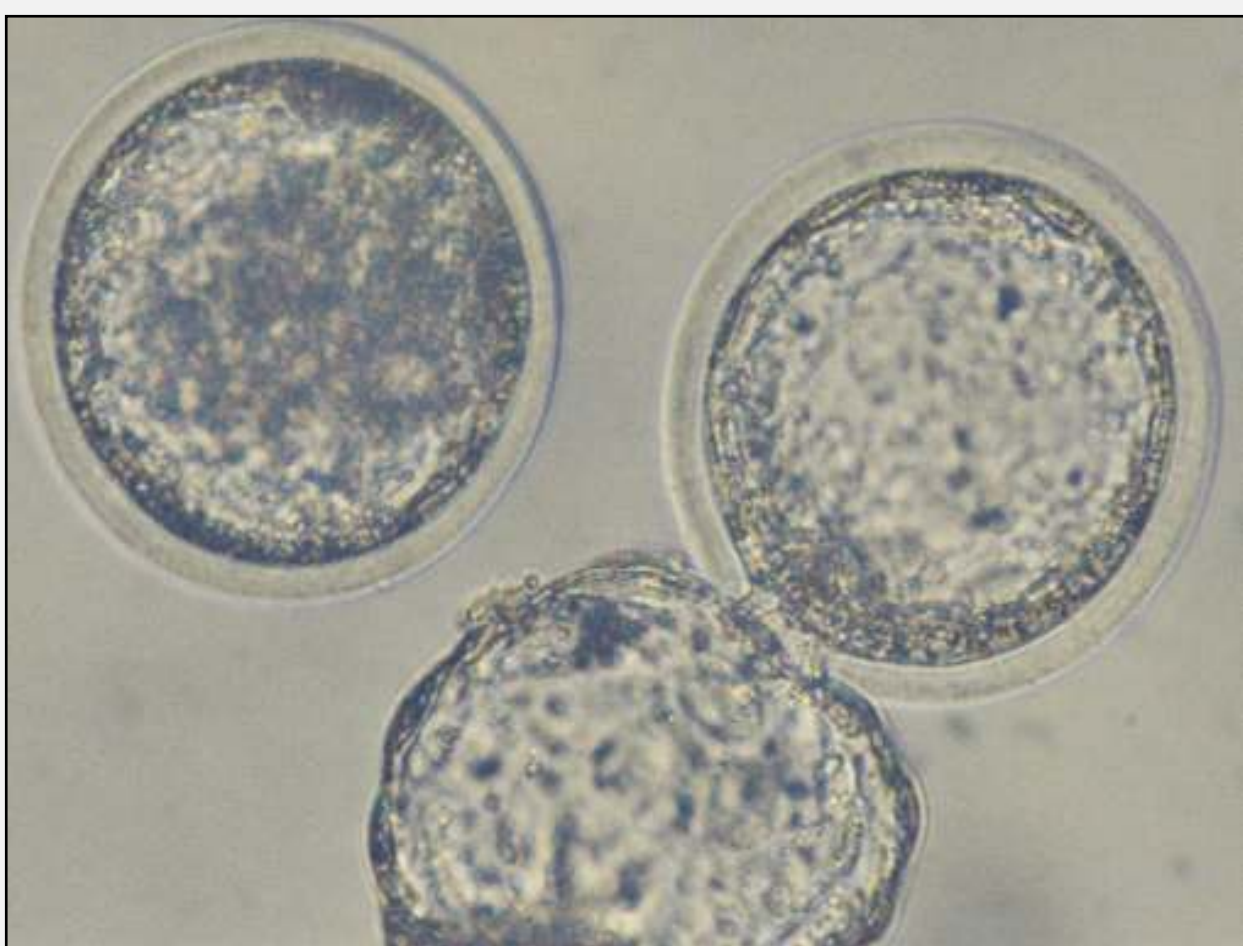


Figure 1. Anatolian Grey's blastocysts from cartilage cell



Figure 2. Anatolian Grey's blastocyst from fibroblast cell

## Conclusion

The cell types were compared cartilage cells showed the higher blastocyst rates than fibroblast cells. The cells synchronization were effective into the blastocysts rates. Confluency was showed the best results. The results indicate that cartilage cells and confluency have beneficial effect on NT embryo development. However, the first Anatolian Clon Calf was produced from fibroblast cell. In addition, two pregnant cows carrying clon grey calves from cartilage cells are waited to give birth at the beginning of next year.



Figure 3. First Clon Anatolian Grey Calf (19 August, 2009)

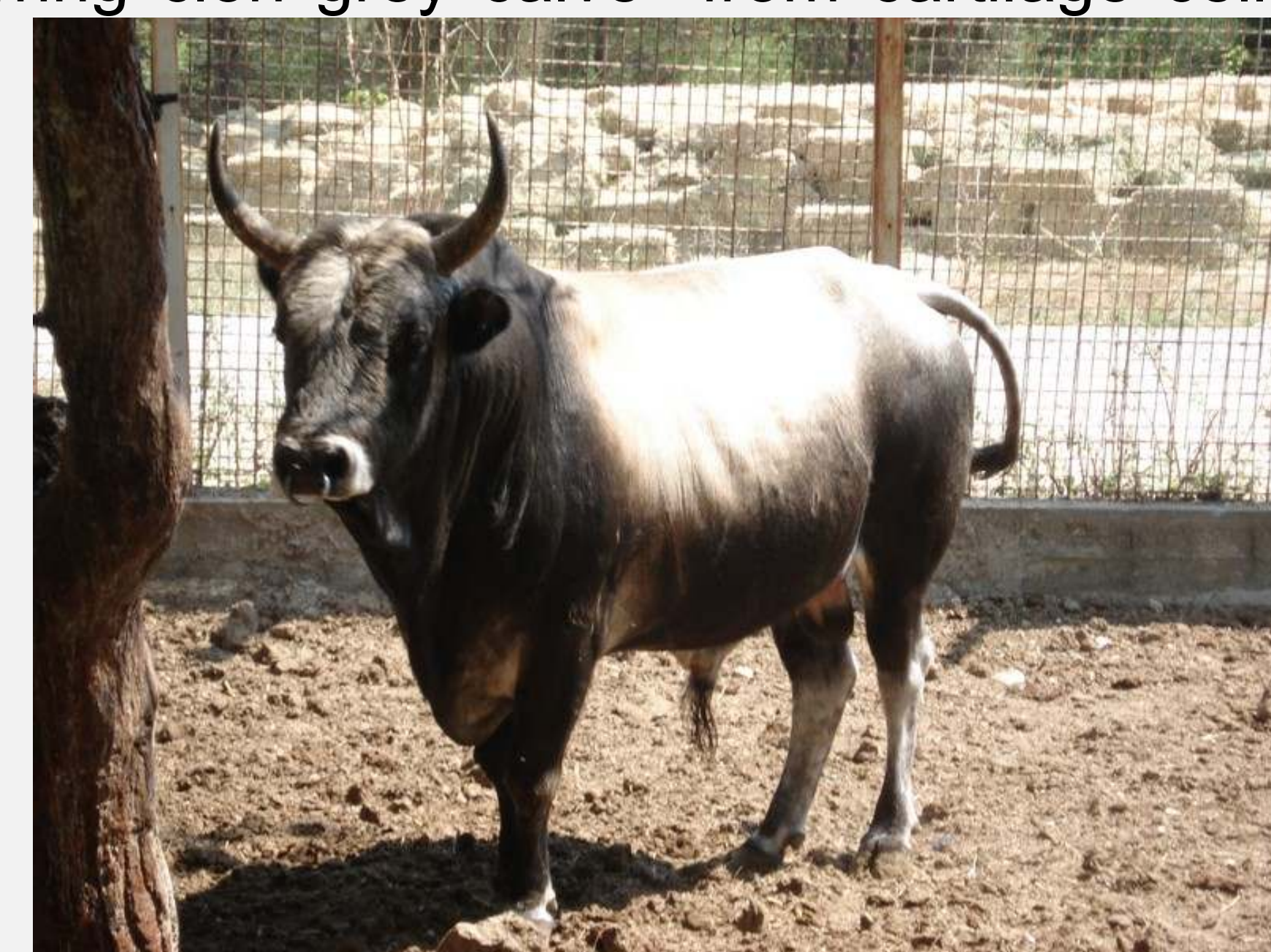


Figure 4. Cell Donor Anatolian Grey Bull

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## Results

Cartilage and fibroblast cells obtained from the ear tissue of a male Anatolian grey bull were used as the source of nuclear material.

In the first experiment, before Nuclear transfer (NT), all somatic cells were allowed to grow to confluency (G1/G0) in DMEM-F12 supplemented with 10% FBS. (Table 1). The result of the first experiment; blastocyst formation rate was higher on NT embryos from cartilage cells (26.8%) than from fibroblast cells (17.8).

In the second experiment, before NT, cartilage cells were cultured either in DMEM-F12 supplemented with 10 % FBS to confluency ( group 1) or in DMEM-F12 supplemented with 10 % FBS and 15 µM roscovitine for 24 hr (group 2). In the second experiment, blastocyst formation rate was higher on NT embryos from group 1 (20 %) than from group 2(8 %). (Table 2)

The results indicate that cartilage cells and confluency have beneficial effect on NT embryo development.

Table 1 : Effect of Cell Types

Differences of Different Cell Types		
Groups*	Cleavage Rates (%)	Blastocysts rate (%)
Anatolian Grey Bull Male Cartilage Cells	59/108 % (54,6)	29/108 (26.8)
Anatolian Grey Bull Male Fibroblast Cells	48/84 (57,14)	15/84 (17,83)

Table 2 :Cell synchronization table

Development Rates of Cell Preparation			
Groups	Oocytes Numbers	Cleavage Rates (%)	Blastocysts rate (%)
1	92	58/92 (63,04)	20/92 (20,74)
2	118	71/118 (60,16)	10/118 (8,47)

### The Combinations of Cell Synchronization

Group 1: Normal (Confluent) Cells

Group 2: Cells of Roscovitin Synchronization