

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

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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. The objective of this study was to examine the effect of cell source, sex of cell and embryo culture medium on somatic cell cloning (SSC). Mostly, fibroblast or granulosa cells were used for cloning. We use cartilage cells obtained from cartilage tissue from bovine ear and investigate behavior of those cells in bovine oocyte cultured in different culture condition.

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. As a result, when we compared the different medium composition, the fifth group (Sage with 4mg/ml BSA and 5% FCS) was the higher than the others. In the second experiment, cell sources and sex were analyzed. In the experiment two different native bovine strains (Anatolian black and grey) were used. When the Anatolian Black and Greys' male cells were compared, the significant differences were visualized by blastocysts development. This results showed that blastocysts developed higher in the fifth group than the other groups. Source and sex of cells were affected that SSC blastocysts rates.

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 and penicillin/ streptomycin, at 39 °C in a humidified 5% CO₂ 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 (Figure 1). A single cells derived from cartilage tissue of two different strain of cow was inserted into the perivitelline space of the enucleated oocyte (Figure 3). Oocyte-cell couples were fused by a DC pulse of 133V/500 μm for 30 μs in the Zimmermann's medium. After fusion, fused NT units were activated using a combination of Cal (5 μM for 5 min), CD (2.5 μg/ml) + cycloheximide (CHX, 10 μg/ml) for 1 h and CHX alone for 4 h.

In the first experiment; after activation, NT units were cultured in Sage medium for 72 h and then additional 4-5 days in five different medium combinations

In the second experiment, cell sources and sex were analyzed. Donor cells from two different native bovine strains (Anatolian black and grey) were used (Figure 2).



Figure 1. Enucleation of oocytes

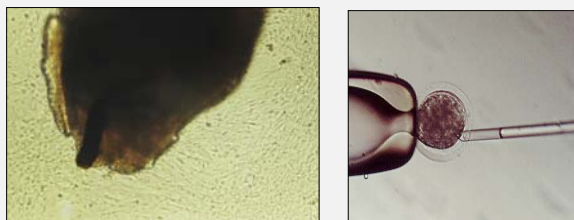


Figure 2. Cartilage cells from tissue of Anatolian black cow.

Figure 3. Transfer of donor cell into enucleated oocyte.

Results

In the first experiment, the five different medium combinations were used. The differences among groups were analyzed. In the fifth group, blastocyst rate (39,3%) was higher than the other groups. When the compared the blastocysts rates between the first (18,36%) and the fifth groups (39,3%); the significant difference was found (Table 1). 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 (Table 2, Figure 4). However; when the Anatolian black and Grey cloned embryos from male cells (Figure 5) 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.

Table 1 : Culture Conditions

Groups*	Culture Condition				
	Culture Condition Parameters	Number of Oocytes	Cleavage Rates	Blastocysts Rates	Number of Blastocyst cells
Partenogenetic	1	109	50/109 45,87 %	15/50 30 %	76
Partenogenetic	4	229	119/229 51,96 %	51/119 42,85 %	110
Partenogenetic	5	52	28/52 53,85 %	23/28 82,14 %	92,66
NT	1	209	98/209 46,88 %	18/98 18,36 %	-
NT	2	10	6/10 60 %	2/6 33,3 %	-
NT	3	7	4/7 57,14 %	-	-
NT	4	232	125/232 53,88 %	12/395 31,2 %	-
NT	5	120	56/120 46,67 %	22/56 39,3 %	99

The Combinations of Culture Medium

- 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.

Table 2 : Cell sources and sexes

Differences of Different Strain and Sex		
Groups	Cleavage rate (%)	Blastocysts rate (%)
Anatolian black female cells	21/30 70 %	6/21 28,52 %
Anatolian black male cells	15/35 42,85 %	4/15 26,6 %
Grey male cells	34/68 50 %	13/34 38,23 %

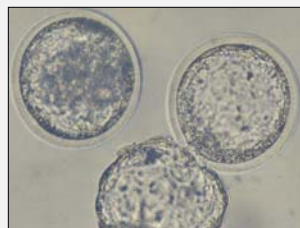


Figure 5. Anatolian Grey's male blastocysts

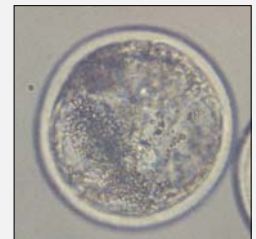


Figure 4. Anatolian Black's female blastocyst

Conclusion

This study showed that in vitro culture condition has very important effect on reprogramming of cells in oocyte cytoplasm as much as other factors mentioned for cloning.