

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 growth factors of nuclear transfer embryos from Anatolian native cows.

In the first experiment; oocytes maturation were compared into maturation mediums with or without growth factors. In the second experiment, six different medium combinations (with or without growth factors) were compared to examine the effect of clon embryos from cartilage cells development. The results showed that growth factors have a beneficial effect on oocyte maturation and blastocyst development if they are added in culture medium with serum.

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 and fibroblast cells were inserted into the perivitelline space of the enucleated oocyte (Figure 2). 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(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; bovine oocytes isolated from slaughterhouse ovaries were matured in TCM199 supplemented with 10% FBS, sodium pyruvate, bFSH, and bLH without growth factors (group 1) or with EGF (group 2) or with 10ng/ml EGF and 100ng/ml IGF-1 (group 3) for 18 hours.

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

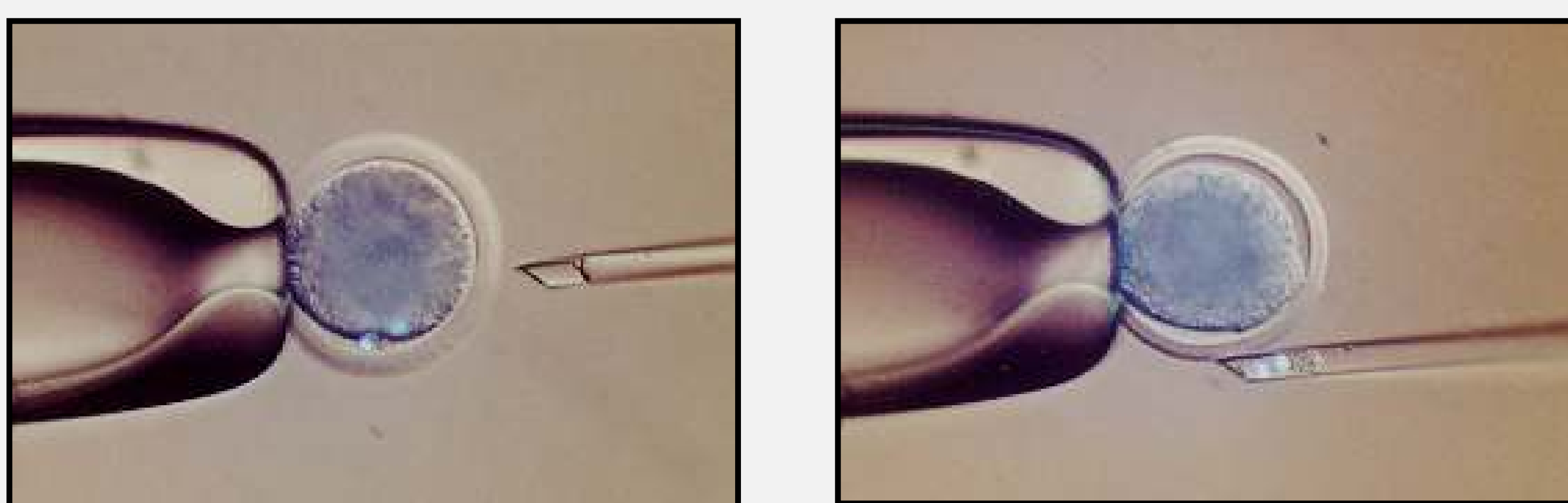


Figure 1. Enucleation of oocytes



Figure 2. Transfer of donor cell into enucleated oocyte.

Results

In the first experiment, bovine oocytes isolated from slaughterhouse ovaries were matured in TCM199 supplemented with 10% FBS, sodium pyruvate, bFSH, and bLH without growth factors (group 1) or with EGF (group 2) or with 10ng/ml EGF and 100ng/ml IGF-1 (group 3) for 18 hours. Maturation rates were higher in group 2 and 3 (75 % and 75% respectively) than in group 1 (64 %) (Table 1)

In the second experiment, after maturation, oocytes from group 3 as cytoplasm sources and cartilage cells obtained from the ear tissue of an Anatolian native cow as donor were used. NT units were cultured in Sage cleavage medium supplemented with 8 mg/ml BSA for 72 h and then developing embryos were divided into six groups and cultured in 1) Sage with 8mg/ml BSA, 2) Sage with 8 mg/ml BSA and 5% FCS, 3) Sage with 4 mg/ml BSA and 5% FCS, 4) Sage with 4mg/ml BSA and 100ng/ml IGF-1, 5) Sage with 4 mg/ml BSA, 100ng/ml IGF-1 and 5% FCS, 6) Sage with 8 mg/ml BSA and 100ng/ml IGF-1 for an additional 4 days. Development rates to blastocyst were higher in group 2,3,6 (19 %, 24 % 15% respectively) than in group 1,4,5 (7 %, 9%, 6%). The results indicate that growth factors have a beneficial effect on oocyte maturation and blastocyst development if they are added in culture medium with serum.

Table 1 : Maturation Culture Conditions

Oocytes Maturation Rates			
Groups	Maturation time	Alive oocytes number	Mature oocytes rates (%)
Only hormone and serum (Group 1)	18. saat	840	536 (63.8)
10 ng/ml EGF (Group 2)	18. saat	940	710 (75.5)
10 ng/ml EGF+100 ng/ml IGF (Group 3)	18. saat	841	633 (75)

Table 2 : Culture Conditions

Culture Conditions without growth factors -1				
Groups*	Culture Condition Parameters	Number of Oocytes	Cleavage Rates (%)	Blastocyst Rates (%)
NT	1	232	114/232 (47,13)	17/232 (7,32)
NT	2	262	143/262 (54,58)	49/262 (18,70)
NT	3	297	198/297 (66,66)	72/297 (24,24)

Culture Conditions with growth factors-2				
Groups*	Culture Condition Parameters	Number of Oocytes	Cleavage Rates (%)	Blastocyst Rates (%)
NT	4	63	39/63 %61,9	6/63 %9,52
NT	5	33	18/33 %54,54	5/33 %15,15
NT	6	33	12/33 %36,36	2/33 %6,06

The Combinations of Culture Medium

Group 1: Sage with 8 mg/ml BSA

Group 2: Sage with 8 mg/ml BSA and %5 FCS

Group 3 : Sage with 4 mg/ml BSA and %5 FCS

Group 4: Sage with 4mg/ml BSA and 100ng/ml IGF-1

Group 5: Sage with 4 mg/ml BSA and 100ng/ml IGF-1 and %5FCS

Group 6: Sage with 8 mg/ml BSA and 100ng/ml IGF-1

Conclusion

This study showed that growth factors have a beneficial effect on oocyte maturation and blastocyst development if they are added in culture medium with serum.