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THE POTENTIAL PRODUCTION OF RECONSTRUCTED EMBRYO OF LOCAL GOAT THROUGH INTRACYTOPLASMIC DIRECT NUCLEAR INJECTION USING CUMULUS DONOR CELL

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ABSTRACT

The aim of this research is to develop methods for producing reconstructed embryo of local goat after intra cytoplasmic direct nuclear injection with cumulus donor cells into enucleated M-II oocyte recipient. Reconstructed embryo was performed by injection of cumulus donor cell intracytoplasmic. Donor cells were cultured in standard protocol until confluence with 10 % FBS supplementation. Cells culture done using CO_2 incubator 5 % 38 ^{o}C with maximum humidity, cultured in 2 – 3 passages and then it's used as donor cells after trypsinized with 0.1 % trypsin-EDTA. Enucleation was done by blind enucleation method of 10 – 25 % cytoplasmic near second polar body of M-II oocyte recipient. Reconstructed cells were activated chemically by Ethanol + 6-DMAP and Ca ionophore A-23187 + 6-DMAP. Variable observed are cleavage rate and embryo development compared with parthenogenetic activation as a control. Results showed that reconstructed embryo still very low success to reach morula stage (1.2 – 1.7 %) in in vitro development culture system using TCM 199. The cleavage rate of this reconstructed embryos are 12.41 % (ethanol + 6-DMAP) and 14.58 % (Ca ionophore A 23187). Viability of donor cell is about 78.30 %, selected for their medium and small size of population that probably in G0/G1 phases (4 – 6 um). It was concluded that intra cytoplasmic direct nuclear injection could be use as method of reconstructed embryo production of local goat. It is necessarily to done further research on analyze of cellular damage after enucleation and injection

Key words: Reconstructed Embryo, Intracytoplasmic, Enucleation, Cumulus Cells, Activation.

INTRODUCTION

The general nuclear transfer procedure of animal cell to produce a reconstructed embryo is to inject a donor cell into the perivittelin space in an enucleated oocyte and then fusion cell is performed. Intracytoplasmic direct nuclear Injection (IDNI was done by injection of donor cell intracytoplasm directly, then activated artificially because calcium-oscillation does not occurred in reconstructed oocytes as in normally fertilized oocytes.

Nuclear transfer has the potential to produce a number of cloned progeny and would greatly benefit current research effort. After a report of birth of Dolly in 1997 (Wilmut *et al*, 1997), many offspring of mammals have been obtained by nuclear transfer with various donor cells using somatic cells as well as embryonic cells. The

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nuclear transfer will bring many advantages in sex selection, improvement of production, breeding and feeding efficiencies. Furthermore, this technique is very valuable for medical application and biodiversity conservation for rare and endangered animals (Ikumi et al, 2003; Ciptadi, 2005,) .

MATERIALS AND METHODS

Cumulus oocyte complexes (COCs) were collected by aspirating follicles 2 – 6 mm in diameter, cultured for 18 – 20 hours in 100 ul drops of TCM199 stock supplemented with 10 % FBS at 38.5 °C in 5 % CO_2 incubators with maximum humidity. After in vitro maturation (IVM) the COCs were used for recipient oocytes. Enucleation was performed using micromanipulator attached with inverted microscope, blindly aspirated the first polar body and the metaphase plate with approximately 10 – 25 % cytoplasm surrounding the polar body (Tanaka, 2001, Ciptadi, 2005).

The donor cells of cumulus were obtained from culturing these cells until reaching confluence. Donor cells were cultured in standard protocol in DMEM until confluence with 10 % FBS supplementation. Cells culture done using CO_2 incubator 5 % 38 °C with maximum humidity. Cells are cultured in 2 – 3 passages and then using as donor cells after trypsinized with 0.1 % trypsin-EDTA. The cells were trypsinized, washed in DPBS(-) and centrifuged at 1500 rpm for 5 minutes. Cells were selected for small size diameter (5 – 7 mm) that probably in G0/G1 phases.

Reconstructed embryo was performed by injection of cumulus donor cell. Reconstructed cell was activated chemically by Ethanol + 6-DMAP and Ca ionophore A-23187 + 6-DMAP.(Ongeri *et al,* 2000). Variable observed are cleavage rate and embryo development compared with parthenogenetic activation as a control.

RESULT AND DISCUSSION

Results showed that reconstructed embryo still very low success to reach morula stage (1.2 – 1.7 %) on in vitro development culture system using TCM 199. The cleavage rate of this reconstructed embryos are 12.41 % (ethanol + 6-DMAP) and 14.58 % (Ca ionophore A 23187). Table 1. .

The low development of reconstructed of goat oocytes in this research may showed that effective activation protocols need to be developed. In contrary, Ongeri *et al.* (2000) reported that activation using Ethanol + 6-DMAP resulted in higher blastocyst development than in vitro fertilization (IVF). In goat, ethanol activation induced a 56 % cleavage rate in reconstructed oocytes with somatic cells (Baguisi *et al.,* 1999).

Table 1. Development of reconstructed oocytes of local goat activated by different methods.

Treatment of activation	Number of reconstructed oocytes	Cleavage rate (%)	Morula stage (%)
Parthenogenetic (%Ethanol + 6-DMAP)	168	62.0	25.0
7 % Ethanol + 6-DMAP	176.	12.41	1.7
Ca ionophore A-23187 + 6-DMAP	82	14.58	1.2

Viability of donor cell used is about 78.30 %, selected for their medium and small size of population (3 – 6 um) that probably in G0/G1 phases resulted from 2 – 3

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passages. The successful reprogramming of the donor nuclear material from differentiated cell may be related to the phase arrest in G0/G1 in the cell cycles (Das *et al.,* 2003). Im et al. (2001) reported there was no significant difference among passages when the 1 – 6 passages of cumulus cell were used in nuclear transfer. But, Campbell *et al* (1996) reported production of live lambs only from 1 to 3 passage cells without cell cycle synchronization.

Treatment with inhibitors protein synthesis, protein phosphorilation or histone kinase improved the efficiency of oocyte activation (Navara *et al*, 1994, Ongeri *et* al., 2000). In this research 6-DMAP were added to accelerate pronuclear formation and parthenogenetic development by inhibiting protein kinase function and promoting mitosis. The combination treatment (Ethanol/Ca ionophore with 6-DMAP) resulted in higher rate of pronuclear formation and significant increases the rate of cleavage and blatocyst development compared with single single treatment of each chemical artificial activation (Ongeri *et a*l. 2000; Ciptadi 2005).

Ethanol and Ca ionohore are commonly used for mammalian oocyte activation to elevate intracellular Ca^{++} concentration. Liu *et al.,* (1998) reported that optimal parthenogenetic development of matured oocyte treated with chemical agents could only be obtained if followed by incubation in 6-DMAP, cycloheximide or cytohalasin D.

In this study we also established the method on blind enucleation of goat oocyte recipient using standard micropipette(Ushijima *et al.*, 2002; Wakayama, 1998). This method may be a good tool for goat nuclear transfer in the case absent of fusion cell machine using conventional method of reconstructed oocyte. Because, use the intracytoplasmic injection allow the transfer of nuclei into cytoplast directly, makes electrofusion unnecessary.

CONCLUSION

It was concluded that INDI with cumulus donor cells into enucleated M-II goat oocyte recipient. Could be use as method of reconstructed embryo production of local goat. The effective activation of goat oocyte reconstructed need to be improved. It is necessarily to carried out researches on improving of activation method, analyze of cellular damage after enucleation and injection

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