Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 75 (2011) 1516–1524
Theriogenology
www.theriojournal.com

Effect of roscovitine treated donor cells and different activation methods on development of handmade cloned goat (*Capra hircus*) embryos

Y.S. Akshey, D. Malakar*, A. Kumar De, M. Kumar Jena, S. Kumar Pawar, R. Dutta, S. Sahu

Animal Biotechnology Centre, National Dairy Research Institute, Karnal-132001, India

Received 22 October 2010; received in revised form 18 December 2010; accepted 21 December 2010

Abstract

The aim of the present investigation was to find out the effects of roscovitine treatment of donor cells and different activation methods on development of HMC goat embryos. Goat fetal fibroblast cells were cultured and divided into three treatment groups—contact inhibition group, roscovitine treatment group and serum starvation group. There was a significant decrease in blastocyst yield in serum starvation group (6.82%) compared to roscovitine treatment group (19.31%) and contact inhibition group (18.52%), however, no significant difference was found between roscovitine treatment group and contact inhibition group. To see the effect of different methods of activation, the reconstructed embryos were randomly divided into two groups and activated by two methods—one half by 2 μM Ca ionophore and another half by 2.31 kV/cm for 15 μSec electrical pulse. Subsequently, cloned embryos were cultured in TCM-199 based embryo development medium supplemented with 10 mg/mL bovine serum albumin in WOW culture system. There was a significant increase in the rate of cleavage and blastocyst production in electric pulse activation of 78.57% and 21.43% than Ca ionophore activation of 62.63% and 10.61% respectively. In conclusion, treatment of donor cells with roscovitine yields a significantly increased blastocyst than serum starved donor cells but equivalent blastocyst to contact inhibition group and electrical pulse activation (EPA) improves the production of HMC goat embryos.

Keywords: Ca ionophore; Cloned goat blastocyst; Electric pulse; Handmade cloning; Roscovitine

1. Introduction

Nuclear transfer of somatic cell is one of the most efficient techniques to propagate a highly valuable, existing and extinct species [1] and can be used for the production of genetically modified animals [2]. Two different approaches are currently available in somatic cell nuclear transfer. First one is traditional micromanipulator based embryo production by which cloned offspring of different species were born [3]. Second one is zona free handmade cloning (HMC) technique by which species like cattle [4,5], horse [6] and pig [7] were born. Goat blastocysts [8] and transgenic porcine blastocysts [9] were also produced by using Hand-made cloning approach. In spite of its widespread applications, the efficiency of blastocyst production in animals including goat by SCNT is less and a host of factors have been shown to contribute to this low level of efficiency such as stage of donor cell cycle, donor cell type, methods of embryo culture, imprinting defects, reprogramming failures, inefficient artificial methods of

* Corresponding author. Tel.: 91-9416741839.
E-mail address: dhrubamalakar@gmail.com (D. Malakar).

0093-691X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2010.12.015

activation, lab to lab variation as well as the oocyte source and quality at the beginning of maturation [10].

The stage of the donor cell cycle is a major factor in the success of nuclear transfer in mammals [11,12]. Donor cells arrested in the G_0 or G_1 stage of the cell cycle have been used to produce several cloned animal species like mice [13], pigs [14,15], sheep [16] and cattle [17]). Serum starvation [18,19,20] or contact inhibition [21,22] is used frequently for the synchronization of donor cells at the G_0 or G_1 phase. Recently roscovitine is reported to synchronize cells at the G_0 or G_1 phase of the cycle [23,24]. Whereas Gibbons et al (2002) [25] reported a significant decrease in blastocyst yield in cattle following treatment of donor cells with roscovitine than that of contact inhibition, reverse was found in porcine [26]. Although yield of blastocysts was reduced, the survivability of the cloned calves was enhanced after treatment of donor cells with roscovitine [25]. Similar result was reported in horse [27,28].

Activation is another important parameter affecting blastocyst production of handmade cloned embryos. It can be carried out by various agents like Ca ionophore, ethanol and cytochalasin [29,30]. Electric pulses were also applied for the production of cloned goat embryos using traditional method [31,32] in goat.

At present, there is no report available in goat, regarding the effect of roscovitine on production of handmade cloned embryo. Also, activation protocol for the zona free approach has not been standardized in goat till now. Couplet formation with optimize donor cell as well as proper activation protocol is not optimized in zona free approach in goat. Therefore, the present investigation was carried out to standardize the optimum activation protocol for HMC in goat and to find out whether roscovitine treatment of donor cells could improve the cloned blastocyst yield and quality of blastocysts in terms of total cell number of blastocysts.

2. Material and methods

All chemicals and media were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and disposable plastic wares were purchased from Falconware Becton-Dickinson (Bedford, MA, USA).

2.1. Establishment of fibroblast cell lines and treatment of donor cells

Fetal fibroblast cells were used as donor cells in the present study. Goat fetuses of around 60 days of age obtained from local abattoir were dissected out from uteri and washed 4 to 5 times in sterile Dulbecco's phosphate buffer saline (DPBS). Skin explants were taken and washed 7 to 8 times with sterile DPBS and tissue pieces were cultured in separate four well tissue culture dishes (4WD) (Nunc, Denmark) in Dulbecco's modified eagles medium (DMEM) supplemented with 10% FBS and 50 μg/mL gentamicin sulphate in 5% CO_2 in air with maximum humidity at 37 °C temperature. The explants were removed after proliferation and establishment of fibroblasts cells. Monolayer of fibroblasts was allowed to grow till 100% confluency. The fibroblast cells were sub-cultured by partial trypsinization after reaching 100% confluency. Trypsinized cells were allocated to new dishes for further passage. Prior to HMC, cells of 70–80% confluence were divided into three treatment groups. $T_{1;}$ contact inhibition group was cultured in DMEM supplemented with 10% FBS till 100% confluency. $T_{2;}$ roscovitine treatment group was cultured in DMEM supplemented with 10% FBS and 15 μM roscovitine for 24 h, and $T_{3;}$ serum starvation group was cultured in DMEM supplemented with 0.5% FBS for 72 h. Cells in passages 4–6 were used for further observations.

2.2. In vitro maturation of oocytes

In vitro maturation (IVM) of goat oocytes were carried out as described earlier by Malakar and Majumdar (2005) [33]. Briefly, goat ovaries were collected from local abattoir and transported to laboratory in a thermo flask containing 0.9% normal warm (32–35 °C) sterile saline fortified with antibiotics (50 μg/mL gentamicin sulphate) within 3 h. Then the ovaries were washed 4–5 times with normal saline. Cumulus oocyte complexes (COCs) were aspirated by puncturing method in aspiration medium consisting of TCM-199 (HEPES modified) and bovine serum albumin (BSA) (0.3% w/v). COCs with ≥3 cumulus layers and homogeneous ooplasm were taken for in vitro maturation. COCs were washed two times with washing medium containing TCM 199 (HEPES modified), 10% FCS, 50 μg/mL sodium pyruvate, 3.5 μg/mL L-glutamine and 50 μg/mL gentamicin sulphate and three times with maturation medium containing TCM-199 (HEPES modified), 10 μg/mL LH, 5 μg/mL FSH, 1 μg/mL estradiol-17β, 0.05 mg/mL sodium pyruvate, 5.5 mg/mL glucose, 3.5 μg/mL L-glutamine, 50 μg/mL gentamicin, 3 mg/mL BSA and 10% EGS (heat inactivated goat serum). COCs (15–20 oocytes) were placed in 100 μL of maturation medium, covered with embryo tested mineral oil and incubated in 5% CO_2 in air with maximum humidity at 38.5 °C for 22 h.