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Cloned kids derived from caprine mammary gland epithelial cells

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Abstract

The use of nucleus transfer techniques to generate transgenic dairy goats capable of producing recombinant therapeutic proteins in milk could have a major impact on the pharmaceutical industry. However, transfection or gene targeting of nucleus transfer donor cells requires a long in vitro culture period and the selection of marker genes. In the current study, we evaluated the potential for using caprine mammary gland epithelial cells (CMGECs), isolated from udders of lactating F1 hybrid goats (Capra hircus) and cryopreserved at Passages 24 to 26, for nucleus transfer into enucleated in vivo–matured oocytes. Pronuclear-stage reconstructed embryos were transferred into the oviducts of 31 recipient goats. Twenty-three (74%), 21 (72%), and 14 (48%) recipients were confirmed pregnant by ultrasonography on Days 30, 60, and 90, respectively. Four recipients aborted between 35 and 137 d of gestation. Five recipients carried the pregnancies to term and delivered one goat kid each, one of which subsequently died due to respiratory difficulties. The remaining four goat kids were healthy and well. Single-strand conformation polymorphism analysis confirmed that all kids were clones of the donor cells. In conclusion, the CMGECs remained totipotent for nucleus transfer.

Keywords: Developmental biology; Embryo; Goat; Mammary gland epithelial cell; Nucleus transfer

1. Introduction

Since the successful production of the first cloned sheep, "Dolly" [1], somatic cell nucleus transfer (SCNT) has resulted in a variety of mammalian clones. In vitro transfection of cultured cells combined with nucleus transfer (NT) is an effective means of producing transgenic animals [2,3]. An important aspect of using SCNT technology to produce transgenic animals is the source and efficacy of the donor cells. Fetal fibroblast cells have been used as sources of donor nuclei in the production of transgenic animals because they grow rapidly and have the potential for multiple cell divisions before undergoing senescence [4,5]. Among adult cells used for donor nuclei, only cumulus cells and ear fibroblasts have been used to produce transgenic offspring [6–8]. However, to improve the production of transgenic cloned animals, a superior type of donor cell must be identified. In addition, to improve the efficiency of NT, there is a requirement for more accurate methods of identifying genetically modified donor cells that express high levels of recombinant protein and reconstructed embryos prior to embryo transfer.

Caprine mammary gland epithelial cell (CMGEC) cultures can provide a simple, biologically meaningful in vitro test system for recombinant DNA constructs expressed in the mammary glands of transgenic animals [9]. If genetically modified CMGECs (derived from a single colony) that express a high level of recombinant

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protein can be selected for NT, the transgenic cloned offspring will produce high levels of the recombinant protein of interest in the milk [10]. However, the manipulations required for genetic modification and selection of suitable donor cells are time intensive. In addition, due to the difficulty involved in fusing CMGECs to enucleated oocytes, few studies have used CMGECs as donor cells for NT [11]. To date, little is known about the developmental competence of reconstructed embryos using long-term–cultured CMGECs as donor cells for NT. Kishi et al. [12] produced cloned cattle using colostrum-derived mammary gland epithelial cells for NT; however, the overall efficiency of animal production based on the number of NT embryos produced and transferred was <1%. In a recent study, all recipients aborted within 120 d after transfer of cloned blastocysts derived from CMGECs (3 to 5 passages) [11].

The objective of the current study was to determine whether embryos generated by NT from CMGECs could support development to term.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all media and components were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

2.2. Isolation of donor lines

Caprine mammary gland epithelial cells were isolated from lactating F1 hybrids (Jinin Blue dam × Boer sire or Xuhuai dam × Boer sire, Capra hircus), as previously described by Kishi et al. [12] and Pantschenko et al. [13]. Approximately 5 g of secretory tissue was removed from one of the two udders and was trimmed of visible fat and connective tissue. After two washings with Ca^{2+}-, Mg^{2+}-free phosphate-buffered saline (DPBS), the tissue sections were minced finely and digested in Dulbecco's Modified Eagle's Medium (DMEM)/F-12 (11300-037; Gibco, Grand Island, NY, USA) with 200 IU/mL collagenase, 100 IU/mL hyaluronidase, 1,200 IU/mL penicillin-G, and 200 μg/mL streptomycin sulfate, with agitation at 37 °C for 1 h to recover CMGECs. The digestion was stopped by the addition of one-fourth volume of fetal bovine serum (FBS; Gibco). After centrifugation, the cells were washed three times with DPBS. The isolated CMGECs were then cultured on a collagen matrix in basal medium composed of DMEM/F-12 (Gibco), 10% FBS (Gibco), 10 μg/mL insulin, 5 μg/mL transferrin, 5 μg/mL hydrocortisone, 200 IU/mL penicillin-G, and 200 μg/mL streptomycin sulfate at 37 °C in a humidified atmosphere containing 5% CO_2. Attached CMGECs were washed several times with DPBS after 24 h, and flash basal medium was added for continuous culturing. When subculturing, the CMGECs were treated with 0.25% trypsin–ethylenediamine tetraacetic acid (EDTA) and incubated at 37 °C until the cells detached from the plastic dish. Then, the cells were centrifuged at 800 × g for 5 min and resuspended in basal medium for culturing. The epithelial population was enriched by selective detachment and attachment methods. After 40 to 50 d culture, cells were collected and frozen at a density of 6×10^6 cells/mL in DMEM/F-12 (Gibco) supplemented with 10% dimethyl sulfoxide (DMSO) and 20% FBS at 4 °C for 30 min, at −20 °C for 1.5 h, at −80 °C for 1 day, then stored in liquid nitrogen.

2.3. Preparation of donor cells

Ten lines of CMGECs were used as nucleus donors after 40 to 50 d culture (Passages 25 to 27). Donor cells were plated into 6-well plates, cultured in basal medium to 80% confluence, and then starved for 2 d in low serum media (DMEM/F-12 [Gibco] with 0.5% FBS [Gibco], 10 μg/mL insulin, 5 μg/mL transferrin, 5 μg/mL hydrocortisone, 200 IU/mL penicillin-G, and 200 μg/mL streptomycin sulfate) until the day of NT. Just prior to NT, the donor cells were collected by washing twice with DPBS and digesting with 0.05% trypsin-EDTA. Then, the cells were washed twice and resuspended in M_2 medium [14,15].

2.4. Synchronization of donor and recipient does

Donor goats (Xuhuai dam × Boer sire) were synchronized and superovulated as described previously by Zou et al. [14]. Briefly, estrus in these donors was synchronized by treatment with prostaglandin $F_{2\alpha}$ (Lutalyse, 4 mg im; Shanghai Institute of Planned Parenthood Research, Shanghai, China) at Days 1 and 11 of the treatment, respectively. At Day 20, superovulation was induced by follicle-stimulating hormone (FSH; Folltropin, 270 IU total im; Ningbo Hormone Factory, ZheJiang, China), given twice daily for 3 d, prostaglandin $F_{2\alpha}$ (Lutalyse, 4 mg im) at Day 22, and luteinizing hormone releasing hormone (LHRH; Cystorelin, 40 μg im; Ningbo Hormone Factory) at Day 24.

Recipient synchronization was achieved using the same protocol as for donor goats, without FSH.