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Effects of interval between fusion and activation, cytochalasin B treatment, and number of transferred embryos, on cloning efficiency in goats

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Abstract

To improve the efficiency of somatic cell nuclear transfer (SCNT) in goats, we evaluated the effects of the interval between fusion and activation (1 to 5 h), cytochalasin B (CB) treatment after electrofusion, and the number of transferred embryos on the in vivo and in vitro development of cloned caprine embryos. The majority of the reconstructed embryos had condensed chromosomes and metaphase-like chromosomes at 2 and 3 h after fusion; cleavage and blastocyst rates from those two groups were higher ($P < 0.05$) than those of embryos activated 1, 4, or 5 h after fusion. Treatment with CB between fusion and activation improved in vitro and in vivo development of nuclear transfer (NT) goat embryos by reducing the fragmentation rate ($P < 0.05$). Although there were no significant differences in NT efficiency, pregnancy rate and kids born per recipient were increased by transfer of 20 or 30 embryos per recipient compared with 10 embryos. We concluded that CB treatment for 2 to 3 h between fusion and activation was an efficient method for generating cloned goats by somatic cell NT. In addition, increasing the number of embryos transferred to each recipient resulted in more live offspring from fewer recipients.

Keywords: Nuclear transfer; Clone; Developmental potential; Nuclear remodeling; Embryo transfer

1. Introduction

The successful production of transgenic animals by somatic cell nuclear transfer (SCNT) has important implications in agriculture and medicine [1]. However, the efficiency of this technique remains low. Therefore, numerous modifications and improvements in SCNT techniques have been reported [2].

The interval between fusion and activation is an important factor affecting the developmental competence of cloned embryos [3,4]. Prolonged exposure of the donor nucleus to oocyte cytoplasm before activation might promote developmental capacity of nuclear transfer (NT) embryos in both cattle [5,6] and mice [7]. On the contrary, prolonged intervals between fusion and activation might impair embryonic development [8–12]. Moreover, live offspring were obtained from NT embryos activated immediately after nuclear transfer [13–15]. Thus, the optimum interval between fusion and activation should be determined.

Inhibiting the extrusion of pseudo-polar body (pPB) after artificial activation is thought to be important to maintain diploid NT embryos. Therefore, cytochalasin B (CB) has been widely used to prevent pseudo-polar

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body extrusion in mice [7,13], cattle [16,17], sheep [18,19], pigs [20], and goats [21]. Incubation of reconstructed embryos in postfusion medium supplemented with CB improved embryo development in sheep and rabbits [22,23]. In goats, fragmentation rate of cloned embryos was diminished in the presence of CB in the postfusion medium [24]. However, additional experiments are required to confirm these observations.

To produce live cloned goats, reconstructed embryos must be transferred into the oviduct or uterus of recipients. Although cultured caprine SCNT embryos can develop to the blastocyst stage in vitro, few resulted in birth of live kids [25]. To our knowledge, most viable cloned goats were derived from transfer of one- to four-cell embryos to the oviduct of recipients. The number of NT embryos transferred to each oviduct was generally from 7 to 10; pregnancy rates 30 to 50 d later ranged from 17% to 50%, depending on the types of donor cells, oocyte origin, and culture conditions [24,26–29]. Moreover, pregnancy rate and kids born per recipient were comparable between transfer of an average of 6.2 microinjected in vitro zygotes or 3.6 microinjected in vivo zygotes per recipient [30]. Therefore, we inferred that approximately 10 SCNT embryos per recipient were not enough to achieve high pregnancy rates. Hence, we tested the hypothesis that pregnancy rate and kids born per recipient could be improved by increasing the number of transferred embryos.

In the present study, we evaluated the effects of: (1) the interval between fusion and activation; (2) CB treatment versus no CB treatment; and (3) number of transferred embryos per recipient on the production of cloned progeny from SCNT program in goats. First, nuclear remodeling types and in vitro development of goat NT embryos held for 1, 2, 3, 4, or 5 h between fusion and activation were examined. Second, the effects of CB treatment between fusion and activation on in vitro and in vivo development of cloned goat embryos were assessed. Third, pregnancy rates and NT efficiencies resulting from transfer of 10, 20, 30, or 40 NT embryos per recipient were evaluated.

2. Materials and methods

Unless indicated otherwise, all chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA), and the media used in preparation of donor cells were obtained from Gibco (Grand Island, NY, USA). All trials were conducted in accordance with the Guidelines for the Care and Use of Animals of College of Veterinary Medicine, Northwest A&F University.

2.1. Preparation of donor cells

Goat fetal fibroblast cells (GFF) were isolated from a female fetus at Day 40 of gestation from a Saanen dairy goat (Yangling Keyuan Biotechnology Inc., Yangling, China). After removal of the head and internal organs, remaining fetal tissues were minced by scissors, and explants (approximately 1 mm^3) were seeded on 60-mm dishes (Nunc, Roskilde, Denmark) which were cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (Ham) supplemented with 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine and 100 IU/mL penicillin-streptomycin. After 3 to 4 d of incubation, explants were removed and a confluent monolayer of primary fetal cells was harvested by trypsinization (0.025% trypsin, 0.5 mmol/L EDTA), which was then passaged or cryopreserved. After freezing and thawing, goat fetal fibroblast cells at passage 8 to 10 were seeded in 24-well dishes (Nunc) and cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (Ham) with 10% FBS until confluence. The cells were kept in 100% confluence for 3 to 5 d prior to preparation for NT.

2.2. In vitro maturation (IVM)

Ovaries were collected from a local abattoir and transported to the laboratory in 0.9% NaCl. Cumulus-oocyte complexes (COCs) were recovered (from follicles 2 to 6 mm in diameter) by cutting with a scalpel. The cumulus-oocyte complexes were washed three times and then cultured in TCM-199 supplemented with 10% FBS, 0.075 U/mL human menopausal gonadotropin (Livzon, Ningbo, China), 1 μg/mL 17 β-estradiol, 10 ng/mL EGF, 1% (v:v) insulin-transferrin-selenium (ITS), and 0.2 mmol/L sodium pyruvate at 38.5°C, in 5% CO_2 in air. After 22 to 24 h of IVM, cumulus cells were removed from the matured oocytes by repeated pipetting in PBS supplemented with 0.1% hyaluronidase. Denuded oocytes with a first polar body were selected for enucleation.

2.3. Nuclear transfer (NT)

The NT manipulations were performed according to the method of Liu et al. [32], with minor modifications. Briefly, oocytes were transferred to a 30 μL droplet of micromanipulation medium (HTCM-199 with 10% FBS and 7.5 μg/mL CB) overlaid with mineral oil. Both the first polar body and the metaphase plate were removed with a 20 to 25 μm diameter pipette. Enucle-