Available online at www.sciencedirect.com
ScienceDirect
Small Ruminant Research 73 (2007) 246–251
Small Ruminant Research
www.elsevier.com/locate/smallrumres

Technical note

Optimization of electrofusion protocols for somatic cell nuclear transfer

Feng-Jun Liu, Yong Zhang *, Yue-Mao Zheng, Ming-Tao Zhao, Yu-Ling Zhang, Yong-Sheng Wang, Guo-Hua Wang, Fu-Sheng Quan, Zhi-Xing An

Institute of Biotechnology, Northwest Sci-Tech University of Agriculture & Forestry, Yangling, Shaanxi 712100, China

Received 5 January 2007; received in revised form 25 April 2007; accepted 11 May 2007
Available online 7 August 2007

Abstract

Electrofusion is one of the critical steps used in somatic cell nuclear transfer (SCNT). This low effectiveness of electrofusion of the somatic donor cell into the recipient oocyte limits the cloning success in certain mammals. In this study, chamber fusion (CF) and micro-electrode fusion were compared in goat SCNT. A 15 μm tip-end, 100 μm frustum-end and 200 μm parallel micro-electrodes were employed to perform micro-electrofusion with the aid of a micromanipulator. Different combinations of micro-electrodes, tip-end plus tip-end (TT), tip-end plus frustum-end (TF), frustum-end plus frustum-end (FF) and parallel micro-electrodes (PM) were evaluated. To improve fusion efficiency a couple of fibroblast karyoplast and oocyte cytoplast cells were pressurized or unpressurized during fusion in each group. No significant differences in the fusion rate of the unpressurized groups and chamber fusion (73.1% in TT, 75.3% in TF, 73.3% in FF, 74.2% in PM and 74.8% in CF) were recorded. The fusion rates in the TT (94.9%) and TF (92.2%) were significantly higher than in the FF (83.0%) and PM (83.9%) groups. The highest fusion rate and the lowest degeneration rate were obtained in the TT group. Compared with chamber fusion, the fusion rate was increased from 72.2 to 89.0% for the granulosa cells, 77.1 to 94.6% for fetal fibroblast cells and 51.2 to 78.0% for mammary gland epithelial cells in goat SCNT. These results showed that the pressurized fusion protocol carried out by a pair of tip-end micro-electrodes is optimal to improve the fusion efficiency of SCNT.

Keywords: Electrofusion; Chamber; Micro-electrode; Micromanipulator; SCNT; Goat

1. Introduction

Somatic cell nuclear transfer (SCNT) thus far has been successfully used in producing transgenic animal, creating mammary gland bioreactors and therapeutic cloning. To date, although some progress has been reported (Wilmut et al., 1997; Cibelli et al., 1998; Kubota et al., 2000), the overall cloning efficiency still remains very low (Cibelli et al., 1998; Wells et al., 1998; Kuhholzer et al., 2000). Optimal SCNT procedures still need to be improved for the production of transgenic animals and other purposes. Fusion of the karyoplast–cytoplast couplet, as an important link in nuclear transfer (NT), may partly influence the success

* Corresponding author. Tel.: +80 29 87080085; fax: +80 29 87080085.
E-mail addresses: Zhy1956@263.com, yzhang@bwh.harvard.edu (Y. Zhang).

0921-4488/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.smallrumres.2007.05.005

rate of SCNT. Compared to other fusion methods, electrofusion is most extensively used, because of lower toxicity, good repeatability and high efficiency. Electrofusion could also be performed by chamber fusion (Kubota et al., 2000; Shiga et al., 1999; Wakayama and Yanagimachi, 1999; Wells et al., 1999; Cho et al., 2002), or by micro-electrode fusion (Takeuchi et al., 1999; Kishi et al., 2000; Du et al., 2006; Shen et al., 2006). However, none of these studies have focused on a comparison regarding the superiority of those methods. In this study, chamber fusion and micro-electrode fusion were compared in goat SCNT. In order to improve fusion efficiency, 15 μm tip-end (TE) and 100 μm frustum-end (FE) micro-electrodes were used to fuse the somatic cell into the oocyte with the aid of a micromanipulator in micro-electrode fusion experiments.

2. Materials and methods

2.1. Micro-electrodes

Three kinds of micro-electrodes were used in this study—a 15 μm tip-end, 100 μm frustum-end and 200 μm rod micro-electrodes, respectively. The tip-end (15 μm o.d.) and frustum-end (100 μm o.d.) micro-electrode was made of a 200 μm platinum wire (Narishige, Tokyo, Japan). The 200 μm platinum wire acted as the 200 μm rod micro-electrode. The platinum wire was welded to a stainless steel wire (1 mm o.d.) that acted as electrode holder; the holder bent in the shape of "Z". Before use, both micro-electrodes holders were positioned in the pipette holders of the *xyz*-micromanipulators (Narishige MM-188NE, Tokyo, Japan), and the pair of micro-electrodes (anode and cathode) were in line—at an angle of 0° and 180° with respect to the microscope stage. Both pipette holders were connected to cell fusion system (Voltain EP-1, Australian) with the aid of two thin wires.

2.2. Chemicals

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

2.3. Preparation of the donor cells for nuclear transfer

Three kinds of somatic cells were used for the goat SCNT in this study. Granulosa cells (GC) were isolated from cumulus–oocyte complexes (COCs) after maturation culture, fetal fibroblast cells (FF) were derived from a Saanen fetus recovered at an autopsy on day 35 of pregnancy and mammary gland epithelial (MGE) cells were derived from colostrum by the modified method as described by Kishi et al. (2000). All these cells were cultured at 38.5 °C in 5% CO_2. After three to five passages, the confluent cells were starved in 0.5% FBS for 3–6 days before SCNT, and then trypsinized using 0.25% trypsin–EDTA. The dissociated cells were washed three to four times by centrifugation (1500 rpm/5 min) and 100–200 cells inserted into the injection droplets.

2.4. Production of karyoplast–cytoplast couplets

Cumulus–oocyte complexes (COCs) were recovered from the abattoir-derived ovaries and incubated in the maturation medium at 38.5 °C in 5% CO_2 for 20–22 h. The cumulus cells were then removed from the oocytes by repeated pipetting. The oocytes containing a first polar body were selected for enucleation as recipient cytoplasts. Enucleation was carried out in a droplet (30 μl) and a single donor cell (GC, FF or MGE) was then injected into the perivitelline space of each enucleated oocyte.

2.5. Electrofusion

Karyoplast–cytoplast couplets were transferred into Zimmermann cell fusion media (Zimmermann and Vienken, 1982) to equilibrate for 5 min before electrofusion. Fusion was performed using the following five protocols and fusion rates determined 30–60 min after each fusion pulse.

2.5.1. Tip-end plus tip-end (TT) fusion

Fusion was performed under an inverted microscope (×150). Both tip-end micro-electrodes were used for fusion (Plate 1A; ×150). A site diametrically opposite to the donor cell of the couplet was propped with the tip of one micro-electrode, and the site aimed, and gently pressurized or not with the tip of another micro-electrode. Pressurization meant that one micro-electrode touched the oocyte's zona pellucida and was pushed so as to establish and tighten the membrane contact and non-pressurization denoted that one micro-electrode just touched the zona pellucida but was not pushed or pressurized. Fusion was achieved using two DC pulses (32 V for 20 μs in the pressurized group; 34 V in the non-pressurized group) delivered by the cell fusion system.

2.5.2. Tip-end plus frustum-end (TF) fusion

A tip-end micro-electrode and a frustum-end micro-electrode were used for electrofusion (Plate 1B; ×150). The side diametrically opposite to the donor cell of the couplet was propped with the end of frustum-end micro-electrode and the site aimed, and gently pressurized or not-pressurized with the tip of tip-end micro-electrode. Fusion was achieved using the same parameters as in the TT fusion method.

2.5.3. Parallel micro-electrodes (PM) fusion

Both the rod micro-electrodes (200 μm o.d.) were arranged parallel in and used for fusion (Plate 1C; ×150). The side diametrically opposite to the donor cell of the couplet was propped with one micro-electrode; the other side of the couplet was aimed, and gently pressurized or not-pressurized with