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Technical note

Efficiency of cloned embryo production using different types of cell donor and electric fusion strengths in goats

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Abstract

The type of somatic cell used as a cell donor and the electric field strength (EFS) applied for membrane fusion of the reconstructed oocytes are the two important aspects that need to be standardized for somatic cell nuclear transfer (SCNT). In the present study two somatic cells types, namely fibroblast cell grown from ear tissue biopsies of Barbari female goats and cumulus cells were used as somatic donor cells. For fusion of oocyte reconstructed membranes following somatic cell transfer, a dc current of 3 electrical field strength (EFS), i.e., 1.0–1.5; 2.0–2.5; 3 and above 3, were applied. When cumulus cells were used as a nuclear donor, a maximum fusion rate of ($55.4 \pm 3.9\%$) was obtained by applying 2.0–2.5 kV/cm dc current. The fusion rate obtained was significantly ($P < 0.05$) higher than all the other EFSs treatments of cumulus, as well as fibroblast cell types. The maximum fusion rate ($31.9 \pm 2.4\%$) for the fibroblast cell line was observed when an EFS of 2.0–2.5 kV/cm was applied. It could be concluded that the difference in membrane surface properties between the cumulus and fibroblast cell may contribute to the higher fusion rate obtained in cumulus cells for cloned embryo production.

Keywords: Cumulus cell; Fibroblast cell; Enucleation; Cell fusion

1. Introduction

After the creation of Dolly from adult ewe epithelial cells (Wilmut et al., 1997), many other somatic cell types, including mammary epithelial cells, ovarian cumulus cells, fibroblast cells, various internal organ cells, Sertoli cells (Kato et al., 1998; Wakayama and Yanagimachi, 2001), macrophage and blood leukocytes (Galli et al., 1999) have been successfully utilized in nuclear transfer. However, clear consensus as to the superiority of a specific somatic cell type has not yet been reached. A number of research studies have observed cumulus cells to be the most effective cell type for somatic cell cloning with respect to both *in vitro* development, as well as the full-term survival rate (Kato et al., 2000; Forsberg et al., 2002).

During recent years the use of electrical stimuli for membrane fusion of the enucleated oocytes and transferred somatic cells has been the method of choice (Kubota et al., 2000; Betthauser et al., 2000; Du et al., 2002). However, the overall cloning efficiency has remained low (Cibelli et al., 1998; Wells et al., 1998; Kuhholzer et al., 2000). This may be due to the low fusion efficiencies currently being achieved between the somatic donor cells and the recipient oocyte, following somatic nuclear transfer (Cibelli et al., 1998; Kubota et al., 2000).

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The birth of live goat clones using different cell types have been reported viz., dwarf goat clones using fetal fibroblast cells (Keefer et al., 2001), Jining goat clones using cumulus cells (Zou et al., 2001), dairy goats using cumulus and fetal fibroblast cells (Lan et al., 2006), "Breed Early Lactate Early" (BELE) goat clones using adult granulosa cells (Keefer et al., 2002), and Asian Yellow goat clones using ear fibroblast cells (Chen et al., 2007). However, in India, research on cloning by somatic cell nuclear transfer (SCNT) is very limited and no live cloned animals have been reported to date (Das et al., 2003). The present study is a preliminary effort to standardize the cloning protocol by applying different electric strength dc currents (EFS) to increase fusion efficiency, using cumulus and adult fibroblast cell as donor cells in goats.

2. Material and methods

All the chemicals used in the present experiment were from Sigma–Aldrich, USA, unless otherwise specified.

2.1. Oocyte recovery and maturation in vitro

Caprine cumulus oocyte complexes (COCs) were aspirated from antral follicles of abattoir-derived ovaries of goats and good quality oocytes (2–4 intact layers of cumulus cells) were matured in Medium 199 (Hyclone)—including Earle's salts, L-glutamine, sodium bicarbonate, and 25 mM HEPES containing 7.5% (v/v) fetal bovine serum (FBS; Hyclone, USA), supplemented with 10 μg/ml ovine FSH 10 μg/ml ovine LH and 1.0 mg/ml estradiol. The COCs were matured in groups of 20–25 in 50 μl drops of maturation medium in a 35 mm culture dish (Falcon) at 38.5 °C, 5% CO_2 and 95% relative humidity for 26–27 h in a CO_2 incubator. The matured oocytes, with well-expanded cumulus layers were selected for cumulus cell denudation and further enucleation and nuclear transfer (NT).

2.2. Culture of skin fibroblast and cumulus donor cells

Ear tissue biopsies were collected from 4 female Barbari does and these ear tissue samples were cultured in Dulbecco's Minimum Earle's medium (DMEM) +15% FBS. The primary fibroblast cell lines were further processed up to 6th passage, before being used as cell donors for nuclear transfer . The cumulus cells were randomly collected from the *in vitro* matured oocytes and further cultured in DMEM, containing 10% FBS (Hyclone) in a 25 ml tissue culture flask (Nunc). The cumulus cells lines were further processed up to 10–12 passages, before being used as a cell donor (Fig. 1A). The cell lines were made viable donor cells according to the method as described by Du et al. (2006) for cumulus cells. Nuclear donor cells of both cell types were then disassociated by 2–3 min of trypsinization at 37.8 °C, and finally resuspended in 1 ml DMEM + 5% FBS.

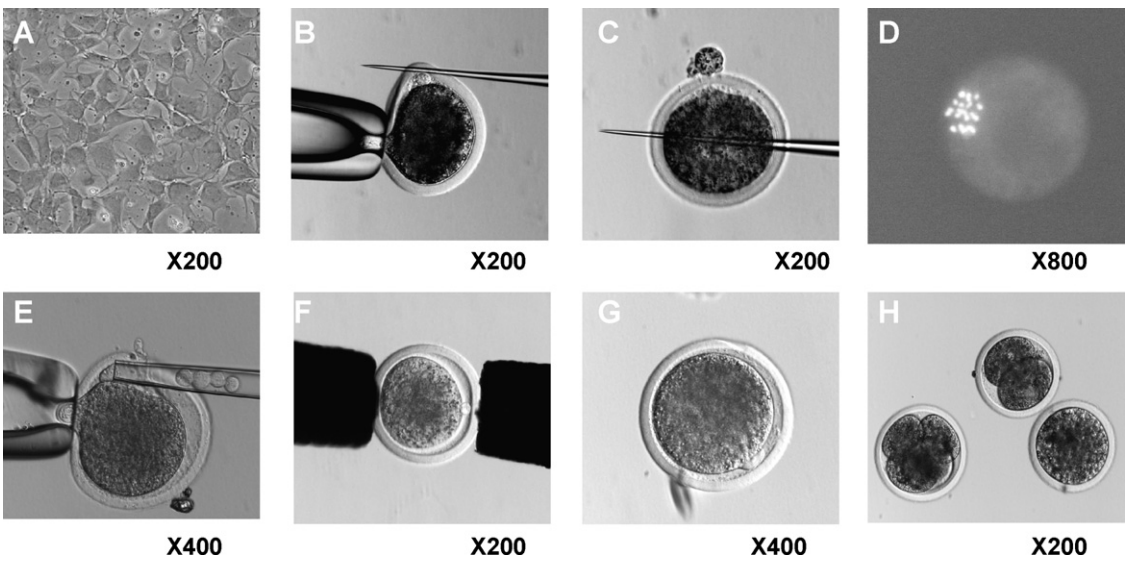

Fig. 1. Production of cloned embryos by somatic cell nuclear transfer in goat. (A) Cumulus cell line in 12th passage (B) Piercing of zona pellucida with enucleation needle covering polar body for creation of slit. (C) Enucleation by squashing and compressing out first polar body and MII chromosomes. (D) Confirmation of enucleation under fluorescence microscope by staining the extruded ooplasm with Hoechst 33342 stain. (E) Transfer of cumulus cell into perivitelline space of enucleated oocyte. (F) Fusion of oocytes-somatic cell by dc electric pulse through metal microelectrode. (G) The fusion of somatic cell with oocytes, as evident by notch formation. (H) Development of 2-cell and 6–8-cell embryos produced by SCNT on day 3 (72–96 h) of culture.