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Preadipocytes as a novel nuclear donor cell for pig cloning

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ABSTRACT

The aim of the present study was to determine whether porcine preadipocytes can be an efficient donor cell for somatic cell nuclear transfer (SCNT) in pigs. Incidence of premature chromosome condensation following NT of preadipocyte was as high as that observed after NT with fetal fibroblasts. In vitro developmental rates of the NT embryos reconstructed with preadipocyte was equivalent to that of fetal fibroblast derived embryos. Transfer of NT embryos with preadipocytes to recipients gave rise to cloned piglets. These data demonstrate that preadipocyite collected from an adult pig is a promising nuclear donor cell for pig cloning.

Key Words: *somatic nuclear transfer, cloned pig, preadipocyte*

INTRODUCTION

The choice of a donor cell is the key to successful production of SCNT clones, because the type of donor cell determines the developmental potency of reconstructed embryos [1]. It is, therefore, crucial to find an ideal cell type. Donor cells used in pig SCNT are limited to fetal fibroblasts [2-7] and a few other cell lines [1, 8–11].

In this study, we focused on porcine preadipocytes as a donor for SCNT.

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Porcine preadipocytes are established by de-differentiation of mature fat cells taken from an adult pig [12].

The aim of the present study was to determine whether porcine preadipocytes can be an efficient donor cell for somatic cell nuclear transfer (SCNT) in pigs.

MATERIALS AND METHODS

Chemicals

Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise indicated.

Preparation of preadipocytes and fetal fibroblasts

Primary culture of preadipocytes were established by the procedures reported by Yagi et al [12] (Fig.1). Primary culture of fetal fibroblasts was established from day 25 porcine fetuses by routine cell culture technology. After 3-7 passages, preadipocytes or fetal fibroblasts were frozen. For each experiment, cells were thawed, cultured to sub-confluency, and then serum-starved in 0.5% FBS media.

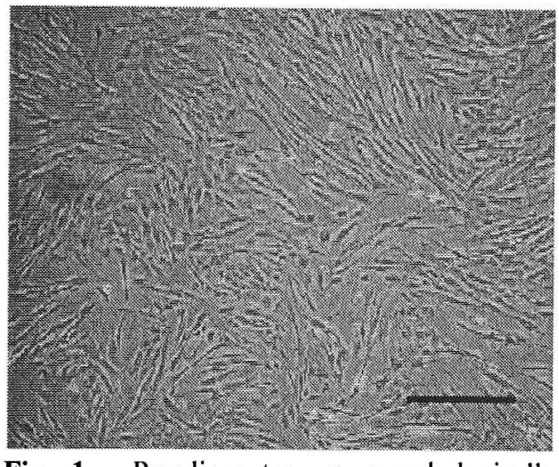

Fig. 1. Preadipocytes are morphologically similar to fibroblasts. Scale bar = 500μm.

Nuclear transfer

In vitro-matured oocytes were denuded of cumulus cells. Enucleation was performed using a chemically assisted method developed by Yin et al. [12].

Preadipocytes and fetal fibroblasts were used as nuclear donors after serum starvation for 48 h. Nuclear transfer was performed as described previously [13]. Briefly, a single donor cell was inserted into the perivitelline space of an enucleated oocyte. Membrane fusion was induced with a somatic hybridizer by applying a single direct current pulse in 0.28 M mannitol (Nacalai tesque, Kyoto, Japan) solution (Fig.2).

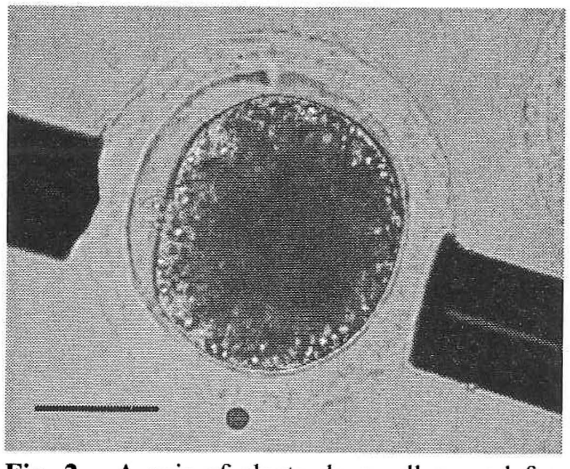

Fig. 2. A pair of electrode needles used for electrofusion of the donor cell and recipient oocyte. Scale bar = 50μm.

Some reconstructed embryos were fixed 1.5–2.0 h after nuclear transfer to examine for nuclear envelop breakdown (NEBD) and premature chromosome condensation (PCC). Embryos were fixed in aceto-alcochol (1:3) for 2-4 d at r.t. and stained with 1% aceto-orcein.

Activation of reconstructed embryos was induced by electrical stimulation [13]. Activated oocytes were treated with 5 μg/ml cytochalasin B for 3 h to suppress extrusion of the pseudo-second polar body.

Developmental ability of resultant NT embryos reconstructed with preadipocytes or fetal fibroblasts was examined by in vitro culture in NCSU23 [14] supplemented with 4 mg/ml BSA in a humidified atmosphere of 5% CO_2 in air at 38.5°C. Blastocyst formation of the reconstructed embryos were monitored during culture for 7 d.

Crossbred (Large White / Landrace × Duroc) prepubertal gilts synchronized with injections of 1000IU eCG and 1500IU hCG were used as recipients of NT embryos. Cloned embryos cultured for 1 or 2 d were surgically transferred into the oviduct of the recipient 24 or 48 h after hCG injection, respectively.

RESULTS AND DISCUSSION

We compared nuclear remodeling patterns of preadipocytes and fetal fibroblasts. Following NT using preadipocytes as nuclear donors, high levels of NEBD and PCC were observed (88%). Similar results were obtained with fetal fibroblasts (97%).

Blastocyst formation rate of the embryos reconstructed with preadipocytes (21%) was equivalent to that of fetal fibroblast derived embryos (12%).

Four live and one stillborn male piglets (Fig.3a, b) were obtained after transfer of the NT embryos reconstructed by preadipocytes to five recipient (162 ~289 embryos / recipient).

These data demonstrate that preadipocytes derived from adult tissue are potent nuclear donors for production of cloned pigs.

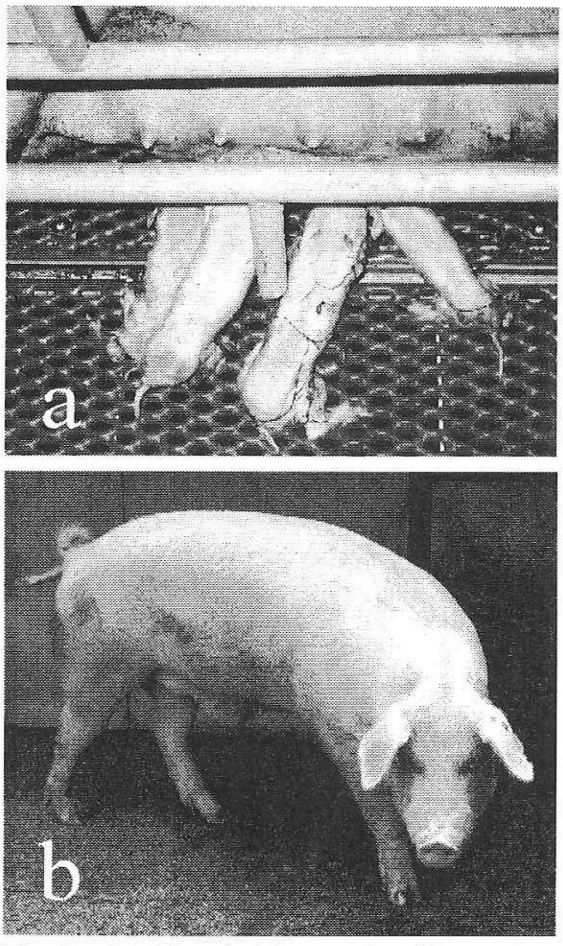

Fig. 3. (a) Cloned piglets produced by nuclear transfer of preadipocytes. (b) A cloned pig one year after birth.

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