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Developmental potential of human oocytes reconstructed by transferring somatic cell nuclei into polyspermic zygote cytoplasm

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ABSTRACT

The generation of patient-specific nuclear transfer embryonic stem cells holds huge promise in modern regenerative medicine and cell-based drug discovery. Since human in vivo matured oocytes are not readily available, human therapeutic cloning is developing slowly. Here, we investigated for the first time whether human polyspermic zygotes could support preimplantation development of cloned embryos. Our results showed that polyspermic zygotes could be used as recipients for human somatic cell nuclear transfer (SCNT). The preimplantation developmental potential of SCNT embryos from polyspermic zygotes was limited to the 8-cell stage. Since ES cell lines can be derived from single blastomeres, these results may have important significance for human ES cells derived by SCNT. In addition, confocal images demonstrated that all of the SCNT embryos that failed to cleave showed abnormal microtubule organization. The results of the present study suggest that polyspermic human zygotes could be used as a potential source of recipient cytoplasm for SCNT.

Introduction

Therapeutic cloning, whereby embryonic stem cells (ESCs) are derived from patient-specific, cloned blastocysts via somatic cell nuclear transfer (SCNT), holds great promise for treating many human diseases in regenerative medicine. Indeed, experiments in mice have shown that nuclear transplantation, combined with gene and cell therapy, represents a valid strategy for treating genetic disorders [1]. However, there are several obstacles to successful human therapeutic cloning. One major hindrance is the availability of human oocytes. To our knowledge, only three studies have described successful generation of human SCNT blastocysts [2–4]. In these studies, however, the cloned blastocysts are derived from fresh oocytes recovered from artificially stimulated volunteers.

Human oocytes are difficult to obtain and their collection raises ethical issue concerning the potential risk to female donors. This issue could be circumvented if the discarded oocytes or embryos from in vitro fertilization (IVF) procedures could be used. Several preliminary studies have generated early cleavage stage, nuclear transfer (NT) embryos using cytoplasts obtained from both in vitro and in vivo, matured oocytes that failed to fertilize in clinical IVF procedures [5–7]. Recently, Egli et al. reported that mouse zygotes that are temporarily arrested in mitosis can support somatic cell reprogramming, the production of embryonic stem cell lines and the full-term development of cloned animals [8]. Therefore, human polyspermic zygotes, which have no clinical use and are routinely discarded, may be a potential source of human oocytes for the creation of patient customized embryonic stem cells.

In the present study, we have investigated the use of mitotic human polyspermic zygotes as recipients for injection of heterologous donor somatic cells and observed the preimplantation development of these human NT embryos.

Material and methods

All chemicals used for SCNT were purchased from Sigma–Aldrich unless otherwise stated.

Source of polyspermic zygotes. All of the polyspermic zygotes were donated for this research from stimulated patients undergoing IVF treatment in the Reproductive Medical Center, The Third Affiliated Hospital of Guangzhou Medical College between October 2007 and December 2008. The patients were clearly informed of all the research details and were approved by the ethical committee of the hospital.

Preparation of donor cells. Donor cells were obtained from a foreskin excision of a 5-year-old boy. The cells were cultured in DMEM supplemented with 10% FBS and 1% non-essential amino acids. Cells were arrested in mitosis by culturing with 0.1 μg/mL nocodazole (Sigma M1404) for 6 h before nuclear transfer. Cells were

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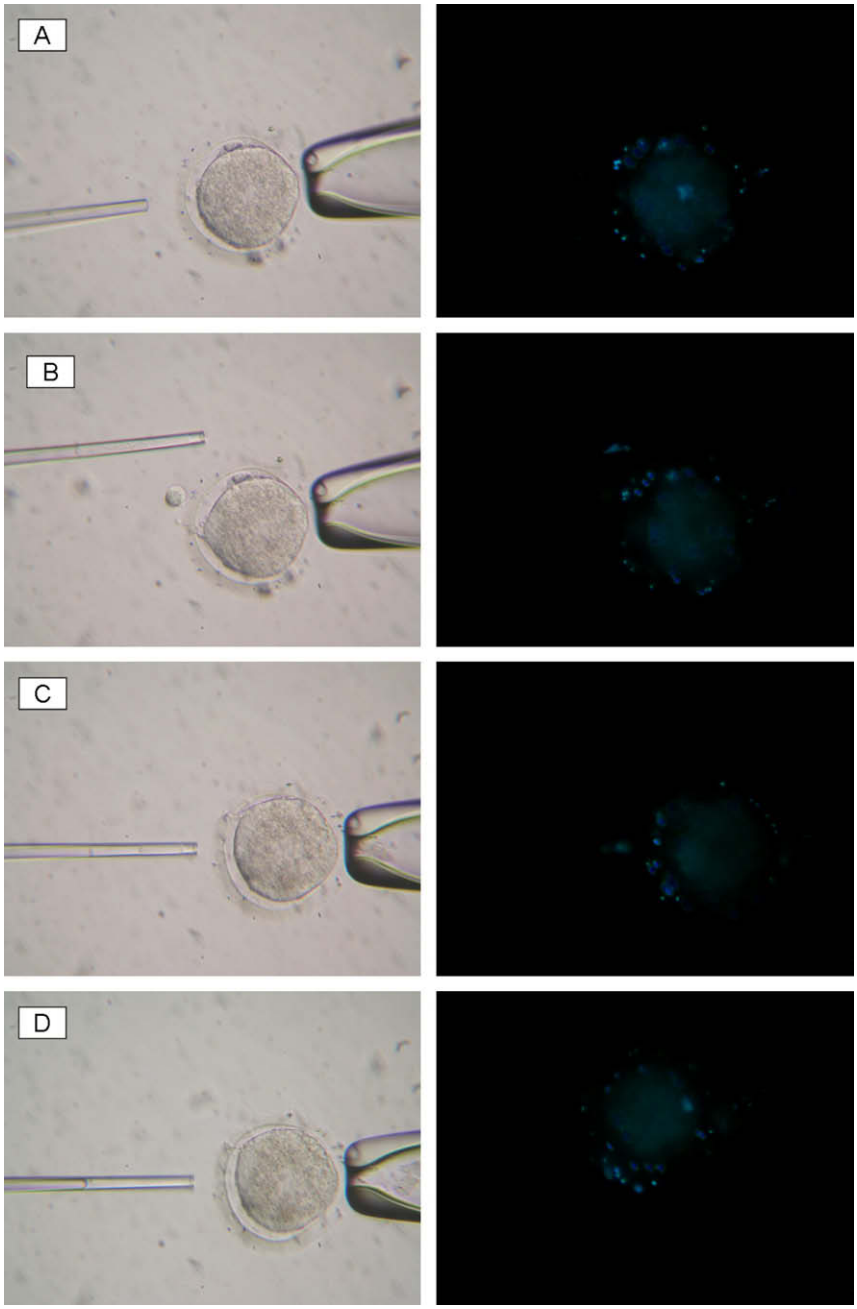

Fig. 1. The method of nuclear transfer into polyspermic zygotes arrested in mitosis. (A) Arrested polyspermic zygote before spindle and chromosome removal, 5 min after the shift from nocodazole to HTF with CB, Hoffman modulation contrast. (B) Removal the polyspermic zygote spindle and chromosomes by micromanipulation. (C) Piezo-actuated injection of a nocodazole-arrested somatic cell into a mitotic polyspermic cytoplasm. (D) Somatic cell chromosomes in the polyspermic zygote immediately after transfer (×200 under an inverted microscope).

Table 1
Preimplantation development of polyspermic zygotes after cell cycle arrest by nocodazole.

Drug treatment (total egg number)	2-cell (%)	8-cell (%)	Blastocyst (%)
No drug treatment (11)	11 (100)[a]	7 (63.6)[a]	1 (9.1)[a]
0.1 μg/mL nocodazole, arrested 5 h (8)	8 (100)[a]	6 (75)[a]	1 (12.5)[a]

Numbers with the same superscript denote that values that do not differ significantly within that column ($P > 0.05$).

obtained from culture dishes using the mitotic shake-off technique and were then mixed with HTF medium (Quinn's AdvantageTM Medium with HEPES) containing 0.1 μg/mL nocodazole.

Nuclear transfer into enucleated polyspermic zygotes arrested in mitosis. Polyspermic zygotes were obtained 18–20 h after insemination by conventional IVF. Zygotes were transferred into G1.5 media (Vitrolife Sweden AB) containing 0.1 μg/mL nocodazole for 5 h. Zygotes arrested in mitosis were washed with three drops of G1.5 to remove residual nocodazole and then transferred into oil-covered droplets of HTF supplemented with 7.5 μg/mL Cytochalasin B (CB, Sigma, C6762).

All manipulations were done on a heated stage of a Nikon microscope equipped with Hoffman modulation contrast optics. A 12 μm inner diameter (ID) blunt-tip pipette was passed through the zona using a piezo device. The pipette was then slowly withdrawn into the proximity of the metaphase chromosomes as visu-