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ARTICLE

Increased cleavage rate of human nuclear transfer embryos after 5-aza-2′-deoxycytidine treatment

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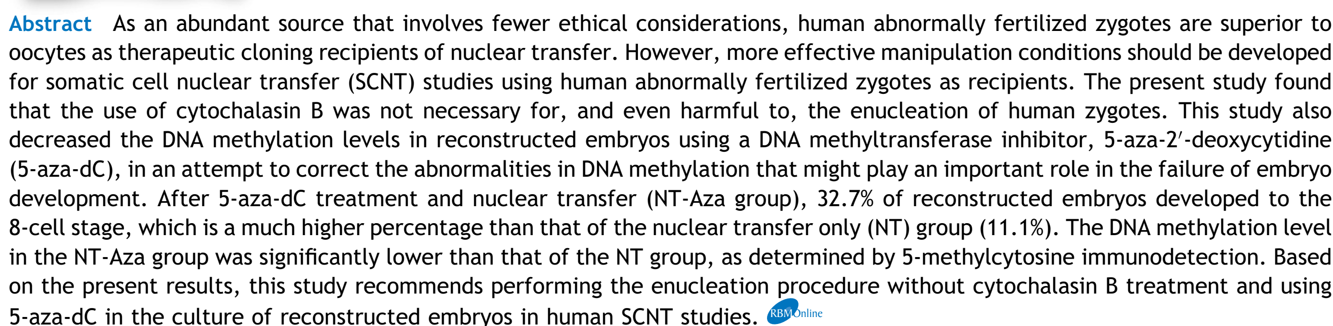

Abstract As an abundant source that involves fewer ethical considerations, human abnormally fertilized zygotes are superior to oocytes as therapeutic cloning recipients of nuclear transfer. However, more effective manipulation conditions should be developed for somatic cell nuclear transfer (SCNT) studies using human abnormally fertilized zygotes as recipients. The present study found that the use of cytochalasin B was not necessary for, and even harmful to, the enucleation of human zygotes. This study also decreased the DNA methylation levels in reconstructed embryos using a DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (5-aza-dC), in an attempt to correct the abnormalities in DNA methylation that might play an important role in the failure of embryo development. After 5-aza-dC treatment and nuclear transfer (NT-Aza group), 32.7% of reconstructed embryos developed to the 8-cell stage, which is a much higher percentage than that of the nuclear transfer only (NT) group (11.1%). The DNA methylation level in the NT-Aza group was significantly lower than that of the NT group, as determined by 5-methylcytosine immunodetection. Based on the present results, this study recommends performing the enucleation procedure without cytochalasin B treatment and using 5-aza-dC in the culture of reconstructed embryos in human SCNT studies.

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KEYWORDS: abnormally fertilized zygotes, 5-aza-2′-deoxycytidine, cytochalasin B, human, somatic cell nuclear transfer

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Introduction

Patient-specific stem cells originating either from induced pluripotent stem cells or somatic cell nuclear transfer (SCNT) hold considerable medical promise in the generation of differentiated cells for cell replacement without immunological rejection. Because stem cells derived from induced pluripotent stem cells and blastocysts exhibit differences in gene expression (Ghosh et al., 2010) and differentiation potential (Hu et al., 2010) and because there are safety issues associated with induced pluripotent stem cells, including compromised genomic integrity or mutations (Gore et al., 2011; Mayshar et al., 2010), SCNT provides a promising alternative to these methods.

Compared with the successes in animal cloning, the progress in human SCNT studies has been slow due to the legal and social considerations that limit the availability of human oocytes for research. To date, only a few studies have obtained human blastocysts using SCNT (French et al., 2008; Li et al., 2009; Noggle et al., 2011; Yu et al., 2009), with the successful establishment of human nuclear transfer (NT) embryonic stem cell lines in only one study; however, these cell lines could not be used in clinical practice due to their triploid origin (Noggle et al., 2011).

In addition to oocytes, mitotic zygotes can support nuclear reprogramming, as was shown in previous studies (Egli et al., 2007; Greda et al., 2006; Schurmann et al., 2006). Assuming that human zygotes develop in a similar manner, abnormally fertilized zygotes from human IVF programmes (Munné and Cohen, 1998), which are routinely discarded, could be used in NT studies as a comprehensive source of NT recipients. A study was conducted with this goal, but the preimplantation developmental potential of the SCNT embryos from polyspermic zygotes was limited to the 8-cell stage (Fan et al., 2009).

Incomplete reprogramming is at least in part responsible for the limited developmental potential of cloned embryos (Armstrong et al., 2006). Aberrant expression and methylation was shown to occur in imprinted genes in cloned embryos (Mann et al., 2003; Suzuki et al., 2011), and the methylation pattern of pluripotency-related genes was shown to exhibit abnormalities (Lan et al., 2010). The partial erasure of pre-existing epigenetic markers by a DNA methyltransferase inhibitor, such as 5-aza-2′-deoxycytidine (5-aza-dC), would be beneficial to cloned embryos (Ding et al., 2008; Enright et al., 2003; Wang et al., 2011).

The present study used mitotic human abnormally fertilized zygotes as recipients for NT. The effects of cytochalasin B (CB) treatment on the survival rate of the manipulated zygotes and the effects of 5-aza-dC treatment on the cleavage rate of the reconstructed embryos were observed.

Materials and methods

All chemicals were purchased from Sigma-Aldrich Chemical (St Louis, MO, USA) unless otherwise stated.

Experimental groups

Six experimental groups were included as follows: (i) abnormally fertilized zygotes control (AFZ-C), cultured without any manipulation to investigate the efficiency of the culture system; (ii) abnormally fertilized zygotes Hoechst (AFZ-H), stained with Hoechst 33342 and briefly exposed to UV light to eliminate any adverse effects of DNA staining and UV exposure on embryo development; (iii) enucleation of one pronucleus (EO), with one of the three pronuclei extracted in interphase to investigate the effects of micromanipulation on embryo development; (iv) nuclear transfer with CB treatment (NT-CB), abnormally fertilized zygotes treated with 7.5 μg/ml CB (cat. no. C6762) during the enucleation process; and (v) nuclear transfer with 5-aza-dC treatment (NT-Aza) and (vi) nuclear transfer without 5-aza-dC treatment (NT), respectively, for the comparison of the cleavage rate.

Source of human abnormally fertilized zygotes and cumulus cells

All of the human abnormally fertilized zygotes and cumulus cells for this research were donated anonymously without any payment by patients younger than 40 undergoing IVF treatment at the Centre for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, between March 2011 and January 2012. The patients were informed of all of the research details. The use of the human zygotes was permitted by the Ethical Committee of the Provincial Hospital Affiliated to Shandong University (approval number 2011-05, approval date 20 January 2011) and written consent was obtained from all of the patients.

Preparation of donor cells

Human cumulus cells from cumulus—oocyte—complexes awaiting intracytoplasmic sperm injection (ICSI) were used as donor cells. The cumulus cells were removed from the complexes with hyaluronidase (80 U/ml in HEPES-human tubal fluid; SAGE IVF, USA). The dispersed cumulus cells were collected and resuspended in G-MOPS medium (Vitrolife, Sweden) after centrifugation at 150 ***g*** for 10 min. The cumulus cell suspension (2 μl) was used in the NT experiment.

Enucleation of one pronucleus of the abnormally fertilized zygote

Abnormally fertilized zygotes with three pronuclei were enucleated to verify the influence of micromanipulation on the development of the embryo. Of the three pronuclei, one was extracted from each zygote in interphase using an injection pipette of 7-μm outer diameter. The enucleated zygotes were then cultured routinely and embryo development was observed daily.

Nuclear transfer into enucleated abnormally fertilized zygotes

All of the manipulations were performed on the heated stage of an Olympus microscope equipped with Hoffman modulation contrast optics. Abnormally fertilized zygotes

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