Animal Reproduction Science 121 (2010) 78–83

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Study on the inter-subspecies nuclear transfer of river buffalo somatic cell nuclei into swamp buffalo oocyte cytoplasm

C.Y. Yang, R.C. Li, C.Y. Pang, B.Z. Yang, G.S. Qin, M.T. Chen, X.F. Zhang, F.X. Huang, H.Y. Zheng, Y.J. Huang, X.W. Liang*

Guangxi Buffalo Research Institute, Chinese Academy of Agricultural Sciences, 24-1 Yongwu Road, Nanning 530001, Guangxi, PR China

ARTICLE INFO

Article history:
Received 13 June 2009
Received in revised form 23 April 2010
Accepted 12 May 2010
Available online 20 May 2010

Keywords:
Swamp buffalo
River buffalo
Subspecies
Somatic cell nuclear transfer

ABSTRACT

The objective of this study was to explore the feasibility of inter-subspecies somatic cell nuclear transfer (SCNT) of river buffalo (50 chromosomes) somatic cell nuclei into swamp buffalo (48 chromosomes) oocyte cytoplasm. The enucleated swamp buffalo oocytes were fused with four different types of river buffalo cells: freshly thawed ear fibroblasts, serum-starved ear fibroblasts, cumulus cells and ear fibroblasts from a cloned buffalo calf. As a result, the developmental competence of embryos reconstructed with freshly thawed ear fibroblasts was the poorest ($P < 0.01$), while those of the other three types were not different from each other. Furthermore, the efficiency of swamp–swamp buffalo, swamp–river buffalo and bovine-buffalo SCNT were also compared. The results showed that the blastocyst rate of swamp–river reconstructed embryos was not different from swamp–swamp embryos, while significantly higher than that of bovine-buffalo embryos ($P < 0.01$). A total of thirty cloned blastocysts derived from freshly thawed ear fibroblasts were transferred into thirteen recipient buffalos, four recipients established pregnancy, while three of them aborted on Days 65, 75 and 90 of gestation, respectively. One cross-bred buffalo (Murrah × swamp, 49 chromosomes) receiving three embryos delivered a 39 kg female calf on Day 335 of gestation. These results indicate that the inter-subspecies SCNT is feasible to produce swamp–river buffalo embryos, and these can develop to full term and result in live buffalo calves.

1. Introduction

Water buffalo (Bubalus bubalis) is broadly classified into river and swamp subspecies. The swamp buffalo, which is small-sized and mainly raised for work power, has 48 chromosomes. The river buffalo, which is large-sized and primarily used for milk production, has 50 chromosomes. Mitochondrial DNA analyses of water buffalo support a distinct genetic origin of river and swamp buffalos (Kumar et al., 2007). The population of buffalos in China ranks third in the world and are all of swamp type. China has introduced river buffalo to improve the productivity of swamp buffalo, and several reproductive technologies have been adopted with SCNT being a potential valuable tool to increase the number of river buffalos. However, although there are a few reports on SCNT in buffalo (Meena and Das, 2006; Parnpai et al., 2002; Saikhun et al., 2004; Shah et al., 2009; Shi et al., 2007; Simon et al., 2006; Srirattana et al., 2009; Yang et al., 2009), only two live cloned buffalos have been reported (Shi et al., 2007; Yang et al., 2009).

Bovine, rabbit, sheep and goat cytoplasm have been shown to support the development of somatic cell nuclei from various mammalian species (Chen et al., 2002; Dominko et al., 1999; Lanza et al., 2000; Loi et al., 2001; White et al., 1999; Xu et al., 2008), inter-species SCNT was proven successful with the birth of a cloned mouflon

* Corresponding author. Tel.: +86 771 3338631; fax: +86 771 3338814.
E-mail address: liangbri@126.com (X.W. Liang).

0378-4320/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2010.05.011

created by transferring a mouflon granulosa cell into an enucleated sheep oocyte (Loi et al., 2001). Inter-subspecies SCNT has also been reported to be successful in bovine, cat and goat (Gómez et al., 2004; Jian-Quan et al., 2007; Meirelles et al., 2001; Pope et al., 2000; Steinborn et al., 2002). However, attempts to reconstruct animals by inter-species and inter-subspecies SCNT have led to limited success. As foreign mitochondria of somatic cells are introduced into the oocytes, some degree of heteroplasmy can occur after SCNT (Steinborn et al., 2000). The incompatibility of nuclear and mitochondrial encoded components from different species and subspecies is likely to hinder the normal development of reconstructed embryos and affect the efficiency of SCNT. Therefore, it is more feasible to perform SCNT among the closely related subspecies to produce viable offspring. Accordingly, cytoplasm of swamp buffalo is a potential host for somatic cell nuclei from river buffalo.

The main objective of this study was to investigate the ability of somatic cell nuclei of river buffalo to differentiate in swamp buffalo cytoplasm. Furthermore, the feasibility of cell cryopreservation and the SCNT efficiency of ear fibroblasts and cumulus cells were examined, and serial cloning was firstly employed to produce the second-generation nuclear transferred (NT) embryos in buffalo. In addition, the developmental competence of inter-subspecies NT embryos was also compared with that of inter-species embryos.

2. Materials and methods

The petri dishes were produced by Nunc (Wiesbaden, Germany). All chemicals and media used in this study were purchased from Sigma (St. Louis, MO), with the exception of fetal bovine serum (FBS), which was purchased from Gibco BRL (Paisley, Scotland, U.K.).

2.1. In vitro maturation (IVM) of swamp buffalo oocytes

Oocytes were recovered from swamp buffalo and bovine ovaries which were transported to the laboratory in normal saline at 25 °C within 2 h from the abattoir. The cumulus–oocyte complexes (COCs) were matured in IVM medium (TCM199 + 10% FBS + 5 μg/mL FSH + 10 μg/mL LH + 0.2 mM sodium pyruvate + 1 μg/mL E_2 + 50 μM cysteine + 25 ng/mL EGF) in a 5% CO_2 incubator at 38.5 °C for 22–24 h. Cumulus cells were removed from oocytes by repeated pipetting after brief exposure to 2 mg/mL hyaluronidase. Oocytes that only had extruded the first polar body were used as cytoplasm for SCNT.

2.2. Preparation of donor cells

Several pieces of ear skin were obtained by biopsy from a female river buffalo calf (6 d) and a cloned buffalo calf (15 d), respectively. Tissues were sterilized and manually cut into small pieces, and then enzymatically digested with 0.25% trypsin and 0.05% EDTA at 4 °C for 18–20 h, the preliminary digested tissues were further digested at 37 °C for 40 min. The disaggregated cells were washed 3 times in cell culture medium (DMEM + 10% FBS + 100 IU/mL penicillin + 50 IU/mL streptomycin) by centrifugation at 260 × *g* for 5 min, and cultured under a humidified atmosphere of 5% CO_2 in air at 38.5 °C.

COCs recovered from an abattoir ovary (swamp type) and COCs derived from a river buffalo by ovum-pick-up (OPU) were cultured for 24 h, the cumulus cells were isolated from oocytes by repeated pipetting, then the separated cells were cultured in cell culture medium under a humidified atmosphere of 5% CO_2 in air at 38.5 °C.

The confluent monolayers were obtained and routinely passaged. After three passages, cells were cultured until approximately 70–90% confluent before enzymatic digestion, the digested cells were blended with DMEM supplemented with 10% FBS and 10% dimethyl sulfoxide (DMSO) and transferred into 0.25 mL plastic straws and cell tubes, the straws and tubes were store at −4 °C for 2 h and then at −80 °C for another 12 h, and cryopreserved in liquid nitrogen. The thawed cells were diluted with DMEM and centrifuged at 260 g for 5 min to remove cryoprotectants, and four types of donor cells were used in this experiment as follows:

Freshly thawed ear fibroblasts: the thawed cells were re-suspended in DMEM and directly used for SCNT.

Serum-starved ear fibroblasts (G0 ear fibroblasts): the thawed fibroblasts were cultured in DMEM supplemented with 10% FBS for 24 h before confluent, and then in DMEM supplemented with 0.5% FBS for another 5 days. The treated cells were trypsinized and re-suspended in DMEM as donors.

Cumulus cells: cells were thawed and treated by serum starvation.

Ear fibroblasts from a clone (G1 ear fibroblasts): the thawed ear fibroblasts derived from a cloned river buffalo calf were serum-starved and digested for SCNT.

2.3. Enucleation of buffalo oocytes

The oocytes were placed in 30–40 μL droplets of enucleation medium (TCM199 + 7.5 μg/mL CB + 10% FBS) for 5 min before micromanipulation. The first polar body and metaphase-II plate were removed by aspiration with a bevelled pipette (inner diameter approximately 20–25 μm) under the Nikon TE300 inverted microscope equipped with a micromanipulator (NT-88-V3, Narishige, Tokyo, Japan) and the Spindle-View System (CRI, Woburn, MA, USA).

2.4. Cell transfer, fusion, activation and embryo culture

A single fibroblast cell was introduced into the perivitelline space of the enucleated recipient oocyte with a micropipette through the slit in the zona pellucida made during enucleation. Three types of couplets were reconstructed as follows:

Swamp–river couplets: the serum-starved cumulus cells from a river buffalo were transferred into the enucleated swamp buffalo oocytes.

Swamp–swamp couplets: the serum-starved cumulus cells from a swamp buffalo were transferred into the enucleated swamp buffalo oocytes.

Bovine–buffalo couplets: the serum-starved cumulus cells from a river buffalo were transferred into the enucleated swamp buffalo oocytes.