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Original research article

Cloned buffalo (*Bubalus bubalis*) embryos from adult cumulus cells and cytoplasts prepared by demecolcine-assisted enucleation of meiotically matured oocytes

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Abstract

We report a simplified chemically-enhanced enucleation of in vitro matured buffalo oocytes for generating cytoplasts for producing nuclear transfer (NT) embryo production. Oocytes aspirated from the ovaries from abattoir were subjected to in vitro maturation for 22h. In the first experiment, the in vitro matured (IVM) oocytes (22h) were denuded and treated with demecolcine (0.50 µg/ml IVM medium) for additional 2h. Oocytes exhibiting characteristic extrusion cones were enucleated using traditional or standard micromanipulator-guided enucleation. In the second experiment, the IVM (22h) oocytes were denuded and treated with demecolcine (0.50 µg/ml IVM medium) for 2h. The zona pellucidae of denuded oocytes were then removed using prteinase K (2µg/ml in M-199 with 0.4% FBS). Cytoplasts were prepared by micromanipulator-free or manual bisection of extrusion cones bearing zona-free oocytes. Chemically-assisted cytoplasts formation efficiency was found to be 59.99% using micromanipulator-guided enucleation (experiment-1), and 68.23% in zona-free IVM oocytes (experiment-2). The demecolcine-derived cytoplasts formation efficiency was higher ($P<0.5$) in zona-free oocytes compared to the micromanipulator-guided enucleation of zona-included in vitro matured oocytes. The cytoplasts generated were viable, and found suitable for reconstituting cytoplast-somatic cell couplets from prolonged cultured, serum starved adult cumulus cells, and supported the development of pre-implantation NT embryos to different stages of developments. Of the 36 (24 zona-included and 12 zona-free) NT embryos, 23 morulae (20 zona-included and 3 zona-free), and 4 hatched zona-included blastocysts were obtained at day 8 of the in vitro culture. Blastomere counts of the NT- and iv vitro fertilization (IVF)-derived embryos were comparable. The results demonstrate that demecolcine-induced enucleation would greatly facilitate the process of producing NT cloned quality embryos in water buffaloes.

Keywords: *Nuclear transfer; Induced enucleation; Demecolcine; Water buffalo*

1. Introduction

Water buffalo (*Bubalus bubalis*) is an economically important multipurpose livestock species and is the mainstay of Indian livestock agriculture. In view of the inherent reproductive problems (weak/ silent estrus signs, seasonal anestrous, a long post-partum anestrous, delayed age of puberty and low conception rates) (Nandi et al., 2002), and significantly limited adoption of the reproductive technologies namely, superovulation and embryo transfer (Madan *et al*., 1996), there is an increasing demand of large-scale production of buffalo embryos for faster dissemination, genetic improvement and conservation of valuable germplasm. Buffaloes have been subjected to various advanced reproductive biotechnological applications including production of cloned embryos (Meena and Das, 2006; Simon *et al*., 2006; Muenthaisong *et al*., 2007), derivation of embryonic stem cells (Verma *et al*., 2007) for producing transgenic embryos and studying the developmental molecular biology of the species. Nuclear transfer technology could be of high significance in buffalo for faster multiplication of valuable sires and elite dams, not only for enhanced productivity, but also for their applications in various nutritional and immunological investigations, and repopulations of indigenous buffalo breeds that are being endangered.

One of the initial and most critical steps in mammalian NT process is the generation of viable cytoplasts by removing the nuclear genetic material from IVM oocytes (Bordignon and Smith 1998), and using these cytoplasts as recipients for competent donor somatic cells. Using the

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