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Production of a cloned calf using zona-free serial nuclear transfer

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Abstract

The efficiency of generating cloned animals following somatic cell nuclear transfer appears to have reached a plateau, despite ongoing research to improve developmental outcomes. A major limitation appears in the restricted nature of the adult/donor cell to de-differentiate to form a totipotent nucleus. Serial nuclear transfer, a modified cloning technique, has increased the developmental competence of amphibian, murine and porcine cloned embryos. This procedure involves a second nuclear transfer step; pronuclear-like cloned nuclei are transferred into pronuclear stage zygotic cytoplasts. The present study reports on the development of a serial nuclear transfer technique in the bovine, based on a zona-free method (hand-made cloning), resulting in the birth of a cloned calf. Comparisons were made between embryos produced by hand-made cloning and serial nuclear transfer. There were no differences between in vitro development or differential cell counts in the blastocysts produced. Transfer of 16 serial hand-made cloned blastocysts resulted in the production of one healthy calf (6%), whereas hand-made cloning resulted in the birth of 1 calf from 23 transferred blastocysts (4%). One serial nuclear transfer pre-term fetus had renal and hepatic abnormalities (previously observed in clones from this cell line). Although it may not be as beneficial in the bovine as in other species, normal placentation (size, placentomes and umbilicus) was encouraging.

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Refinement of this technique may help to identify species-specific differences in zygotic competence that affect reprogramming of donor cell nuclei and that may improve efficiency.

Keywords: Nuclear transfer; Pronuclear exchange; Bovine; Early development; Nuclear reprogramming

1. Introduction

Since the first report of live offspring produced by somatic cell nuclear transfer in 1997 [1], numerous modifications for producing cloned embryos have been investigated, including, different donor cell types, altered cell cycle stages of the donor cell, variations in the maturation stage of the recipient oocyte/host cell, and alterations to fusion and activation protocols [2–5]. Although these modifications have essentially fine-tuned the nuclear transfer procedure, enabling increases in cloned embryo production, the efficiency of producing live healthy clones remains extremely low. The development of a serial pronuclear transfer technique has generated offspring in amphibians [6], the pig [7] and the mouse [8–11]. Serial nuclear transfer involves two rounds of nuclear transfer and differs from serial transfer where embryo blastomeres are refused with enucleated mature oocytes for the multiplication of mammalian embryos. The first round is identical to that of standard nuclear transfer, whereby an unfertilized, enucleated oocyte is used as a recipient host for the donor cell. Thereafter, a second round of nuclear transfer (into a fertilized, enucleated zygote) is performed during pronuclear development. Until now, serial nuclear transfer has been restricted to two mammalian models (the mouse and pig), and has not been applied to the well-defined bovine system.

In 1975, Gurdon et al. [6] showed that nuclei from adult frog skin cells resulted in tadpoles with heartbeats in 3% of transfers; this proportion was increased to 5.3% by the use of serial nuclear transfer. The use of zygote cytoplasm as a recipient cytoplast in mammalian nuclear transfer is not novel. In 1981, Illmensee and Hoppe reported full-term development of cloned mice by microinjection of inner cell mass cells into enucleated, pronuclear-stage zygotes [12]. However, numerous attempts by other research groups failed to replicate this method [13–16]. McGrath and Solter suggested that Illmensee's and Hoppe's enucleation procedure may not have been complete, thus allowing remnants of DNA to persist and initiate embryonic development [15]. Wakayama et al. further reported that cumulus cell chromosomes became heavily pulverized following transfer into enucleated zygotes, with most embryos arresting at early stages of cleavage [16]. However, in 1996, Kwon and Kono reported the birth of cloned mice derived from embryonic blastomeres following two successive rounds of nuclear transfer, with the second round involving the transfer of pronuclei into enucleated zygotes [9]. These results confirmed that zygote cytoplasm could support additional nuclear reprogramming if the donor nucleus was in a pronucleus-like state. Ono et al. modified this technique to successfully produce live offspring from embryonic stem cells [11] and fetal fibroblasts [10]. In a more recent study in the mouse, serial nuclear transfer improved blastocyst development rates when compared with standard nuclear transfer [8]. Serial nuclear transfer was first reported in the pig in 2000 [6] and suggested that serial nuclear transfer could improve cloning efficiencies