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Short communication

The analysis of chromatin remodeling and the staining for DNA methylation and histone acetylation do not provide definitive indicators of the developmental ability of inter-species cloned embryos

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Abstract

The restricted supply of oocytes in the domestic dog limits the development of reproductive biotechnologies in this species. Inter-species somatic cell nuclear transfer could be an alternative for cloning animals whose oocytes are difficult to obtain. In this study, the possibility of cloning dog embryos using pig oocytes was investigated by evaluating nuclear remodeling. Chromatin remodeling, assessed by premature chromosome condensation, pseudo-pronuclei formation, DNA methylation and histone acetylation, along with the developmental ability was compared between intra- and inter-species cloned embryos. The incidence of premature chromosome condensation was significantly higher in intra-species cloned embryos relative to inter-species cloned embryos (87.2% vs. 61.7%; $P<0.05$), but comparable pseudo-pronuclei formation was observed in both (85.3% vs. 75.8%). None of the inter-species cloned embryos developed beyond the 8-cell stage while 18.3% of intra-species cloned embryos developed to the blastocyst

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stage. The relative level of both DNA methylation and histone acetylation was similar between intra- and inter-species cloned embryos at all times examined. These results suggest that although partial chromatin remodeling occurs, further investigation is needed to be able to use pig oocytes as recipient oocytes in dog cloning.

Keywords: Inter-species somatic cell nuclear transfer; Chromatin remodeling; DNA methylation; Histone acetylation

1. Introduction

Culturing of gametes and embryos *in vitro* has been achieved in many mammalian species, and has been useful for various reproductive biotechnologies, such as somatic cell nuclear transfer (SCNT) (Campbell et al., 1996b) and establishment of embryonic stem cells (Thomson et al., 1998). These techniques can be useful for research in the domestic dog and for assisted reproduction in valuable canine species including endangered ones (Hewitt and England, 2001).

Despite successful production of the first cloned dog (Lee et al., 2005), the application of SCNT in canines is limited, primarily as a result of the restricted supply of recipient oocytes due to the low efficiency of *in vitro* maturation (IVM) in dog oocytes compared to other mammalian species (Farstad, 2000). Oocytes matured *in vivo* can be collected surgically, however, this approach is technically difficult and the number of oocytes that can be obtained is very limited (Luvoni et al., 2006).

Inter-species SCNT, which involves the transfer of a somatic nucleus from one species into the ooplasm of a different species, could be an alternative approach when the supply of recipient homologous oocytes is limited, as in the canine. Previous studies have shown that cow oocytes received somatic cell nuclei from sheep, monkey and pig (Dominko et al., 1999) and rabbit oocytes received somatic cell nuclei from panda and cat (Chen et al., 2002; Wen et al., 2003) supported *in vitro* development of reconstructed embryos to blastocysts. Previous work has specifically shown that dog–cow inter-species SCNT embryos can proceed to morula stage and a blastocyst (0.4% of the total embryos) have been obtained (Westhusin et al., 2001; Murakami et al., 2005). However, pig oocytes have not been used as recipient oocytes in inter-species SCNT studies, despite the abundant supply of *in vitro* matured oocytes and well established SCNT procedures.

In this study, we investigated the possibility of producing cloned dog embryos using pig oocytes, by evaluating the extent of nuclear remodeling, at the level of structural chromatin modifications of the donor nuclei. Chromatin remodeling was compared following SCNT of pig and dog fibroblast nuclei into the pig ooplasm, by evaluating premature chromosome condensation (PCC), pseudopronuclei (PPN) formation, DNA methylation and histone acetylation. Developmental ability was also compared between intra- and inter-species cloned embryos.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, chemicals were purchased from Sigma–Aldrich Corp. (St. Louis, MO).