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Review

Current status and applications of somatic cell nuclear transfer in dogs

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Abstract

Although somatic cell nuclear transfer (SCNT) technology and applications are well developed in most domesticated and laboratory animals, their use in dogs has advanced only slowly. Many technical difficulties had to be overcome before preliminary experiments could be conducted. First, due to the very low efficiency of dog oocyte maturation *in vitro*, *in vivo* matured oocytes were generally used. The nucleus of an *in vivo* matured oocyte was removed and a donor cell (from fetal or adult fibroblasts) was injected into the oocyte. Secondly, fusion of the reconstructed oocytes was problematic, and it was found that a higher electrical voltage was necessary, in comparison to other mammalian species. By transferring the resulting fused oocytes into surrogate females, several cloned offspring were born. SCNT was also used for producing cloned wolves, validating reproductive technologies for aiding conservation of endangered or extinct breeds. Although examples of transgenesis in canine species are very sparse, SCNT studies are increasing, and together with the new field of gene targeting technology, they have been applied in many fields of veterinary or bio-medical science. This review summarizes the current status of SCNT in dogs and evaluates its potential future applications.

Keywords: SCNT; Dogs; Oocytes; Embryo transfer; Cloned offspring

Contents

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1. Introduction

Throughout the world, many people keep pets, and dogs (*Canis familiaris*), as one of the favorite species, have played an important role in human society. After their domestication, dogs shared their environment with human beings and, because of their compatible natures, were bred for specific purposes from security, hunting, field and farm work to companions, and lately to law enforcement. Currently, 63% of households in the United States have companion animals and more than half of these owners have more than one animal (American Pet Product Manufacturers Association; APPMA, 2006). In South Korea, 23% of the population now owns a pet such as a cat or dog, and the numbers increase each year (http://www.petian.com).

The reproductive system of dogs is well-known for its many unique characteristics. First, the ovaries of dogs are enveloped by an ovarian bursa, a specialized covering made up of adipose tissue, making endoscopy approach techniques difficult. Second, their pattern of estrous cycles is non-seasonal and monoestrous, and the inter-estrus interval is longer than in other animals (dogs, 4.5–8 months; mice, 5 days; and cows, 21 days). Third, the reproductive system of dogs is peculiar and due to the steep angle of the cranial vagina to the cervix, non-surgical access to the uterus for artificial insemination, transcervical catheterization or transferring embryos is technically difficult and because of this, expensive laparoscopic equipment is needed [1].

Furthermore, in most species, oocytes are ovulated at metaphase II but dog oocytes are ovulated at the germinal vesicle (immature) stage; final maturation of the oocytes to metaphase II requires 48–72 h in the oviducts [2]. Finally, because dog oocytes are rich in lipids they are homogeneously dark in color, impeding manipulations such as removal of nuclear materials [3]. Due to these unique aspects of dog reproduction, the application of assisted reproductive technologies (ARTs) such as *in vitro* fertilization, embryo transfer, cryopreservation, and somatic cell nuclear transfer (SCNT) are less well developed compared to other species. To date, obtaining a puppy from *in vitro* fertilization or intracytoplasmic sperm injection (ICSI) has still not been achieved in a dog.

However, since the first dog was cloned in 2005 [4], there has been increased interest in canine SCNT application in veterinary medical science and biomedical research. When dogs live with us, they develop similar lifestyle diseases to humans including diabetes and obesity. Furthermore, because dogs are often inbred to preserve breed characteristics, genetic mutations such as hip dysplasia and predispositions to certain cancers become evident. For these reasons and because they are more evolutionarily similar to humans than rodents, dogs are ideal models for studying human diseases [5–10]. Somatic cell nuclear transfer, the transfer of a donor cell from one individual into an enucleated unfertilized oocyte of another, is an essential tool in the geneticist's toolbox. This technique has been evaluated as a possible tool for propagating elite livestock, preserving endangered animals and for biomedical research applications. Thus, mastering SCNT and other ARTs in dogs will lead to numerous benefits for both human and veterinary medical sciences.

2. Current status of SCNT in the dog

Since Dolly, the first cloned sheep, was born in 1997, live cloned offspring or transgenic animals have been produced by SCNT in eleven species [11]. In order for SCNT to be successful, every step, from oocyte maturation, enucleation, micro-injection of the donor cell, activation, and *in vitro* culture to embryo transfer and birth of cloned offspring, must be very well harmonized.

2.1. Oocyte maturation

Mature oocytes are a prerequisite for SCNT, whether they are produced *in vivo* or *in vitro*. In general, the first cloned offspring of a species are born from *in vitro* matured oocytes because they are of higher quality and developmental competence. In the case of livestock, large numbers of immature oocytes are available from abattoirs, and can be matured *in vitro* during culture for 24–44 h. Moreover, the efficiency of *in vitro* oocyte maturation systems is high in cows, sheep and pigs (~70–80%) [12–14], and as a result the success of ARTs including SCNT in large animals has improved dramatically.

Many researchers have tried to optimize *in vitro* maturation (IVM) of dog oocytes collected from ovaries obtained by ovariohysterectomy from local veterinary clinics. In contrast to farm animals, the efficiency of IVM in dogs is still very low, with success rates of 0–25% [15–23]. Our research team achieved an IVM efficiency of around 20% [22,23], which varied depending on the reproductive stage of the ovaries collected (follicular, luteal and anestrus) with the follicular phase showing the best results [23,24]. In addition to the estrous stage, benefits of hormones such as FSH, progesterone and estrogen [22,25] have been evaluated as well as antioxidants [26], protein supplementation