OPEN ACCESS
DOI: 10.5455/javar.2014.a11

J. Adv. Vet. Anim. Res., 1(2): 65-69, June 2014
Available at- *http://bdvets.org/JAVAR*

JAVAR Journal of Advanced Veterinary and Animal Research

TECHNICAL NOTE

Applications of somatic cell nuclear transfer in goats

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ABSTRACT

A number of animals with genetically identical appearance can be produced by somatic cell nuclear transfer (SCNT). From current advancement of SCNT and molecular techniques, production of a transgenic animal becomes easier. Although cloning efficiency in goat is low, the ability to propagate genetically identical animals, with a gene or genes of interest, would be important for increasing productivity and ultimately the economic livelihood. In this paper, the potential applications and uses of SCNT technology like production of transgenic goat for production of quality milk and meat are discussed.

Keywords:
Somatic Cell Nuclear Transfer, Goat, Applications

Received : 06 March 2014, **Revised**: 30 March 2014,
Accepted : 06 April 2014, **Published online**: 27 April 2014.

INTRODUCTION

Animal cloning is a potential technology for the production of genetically identical animals. Wilmut et al. (1997) could produce the first cloned sheep, namely "Dolly" by the transfer of a nucleus from a differentiated somatic cell into enucleated oocyte by the process of nuclear transfer (NT). Now, SCNT technology is expected to be useful for farm animal breeding and research, production of transgenic animals for biomedical purposes, and the conservation of endangered species. This technology needs more improvement due to the high rate of loss occurred during gestation and the abnormalities observed in cloned animals (Constant et al., 2006; Heyman et al., 2002; Hoffert et al., 2005; Oishi et al., 2006). The milk obtained from the SCNT derived transgenic goat could produce antithrombin III (Baguisi et al., 1999). Thus, goats are important for the transgenic production of therapeutic recombinant proteins because of their high yield of purified product, relatively shorter generation interval and low incidence of the disease (Meade et al., 1998). Up to 700 liters of milk per year can be obtained from a single transgenic goat, capable of expressing between 1 and 10 g of protein per liter. Although milk yields are higher in dairy cows, dairy goats offer a shorter generation interval and thus better-looking for use in competitive transgenic programs (Ziomek, 1998). The production of transgenic animal derived from SCNT technology is more efficient method than pronuclear DNA-microinjection (Schnieke et al., 1997; Cibelli et al., 1998a; Ziomek, 1998; McCreath et al., 2000).

Transfected cells with gene of interest can be screened prior to nuclear transfer (NT) to detect both the presence and location of the transgene in the donor cell DNA. In addition, the advantage of this approach is that 100% of the resulting offspring will be transgenic (Niemann and Kues, 2000). Some of the proteins currently being produced in transgenic animals via SCNT are human clotting factor IX (Schnieke et al., 1997) and antithrombin III (Baguisi et al., 1999). The advantage of SCNT techniques in dairy goats to generate the human recombinant proteins and monoclonal antibodies in the milk of transgenic offspring could potentially contribute to the pharmaceutical industry (Baguisi et al., 1999). In this article, SCNT technology in goat has been discussed.

WHAT IS SCNT

Somatic cell nuclear transfer is a complex technique, involving donor cell selection, oocyte collection and enucleation, nuclear transfer, and embryo transfer. The most cells in normal mammalian development are gradually losing their pluripotency when develop into differentiated cells. The critical aspect of SCNT is reprogramming of differentiated cells, so the reconstructed donor cells and recipient cytoplasm can develop into live clones. Therefore, type of donor cell and quality of receptor cytoplasm affect efficiency of SCNT.

DONOR CELLS

Cloned goat have been generated from several cell types including cumulus cells (Zou et al., 2002; Keefer et al., 2002), fetal fibroblasts (Baguisi et al., 1999; Keefer et al., 2002; Melican et al., 2005; Liu et al., 2011; An et al., 2012; Soh et al., 2012; Liu et al., 2013), adult fibroblasts (Dutta et al., 2011), lymphocyte cells (Dutta et al., 2011), bone marrow-derived mesenchymal stem cells (Kwong et al., 2014), ear fibroblast cells (Wang et al., 2007; Abdullah et al., 2011; Liu et al., 2012; Kwong et al., 2012; Wan et al., 2012; Kwong et al., 2014), mammary gland epithelial Cells (Zhang et al., 2008; Yuan et al., 2009; Zhang et al., 2010; Yuan et al., 2014). However, the cell cycle of the donor cells usually affects reprogramming of the reconstructed embryos and their development. The G0 phase is a quiescent stage of cell cycle and after fusion into oocyte cytoplasm, the G0 nuclei of the oocytes were synchronized for DNA replication. The possibility of chromosomal aberrations during development of nuclear-transplanted cells could help cells to reprogram the normal cell cycle. In previous SCNT experiments, serum starvation, contact inhibition (Song et al., 2009; Jang et al., 2005), or chemical treatment (Gibbons et al., 2002; Yu et al., 2003; Vacková et al., 2003), were generally used to induce the cells into the G0/G1 phase. Melican et al. (2005) suggested that the kidding rate of the serum starvation treatment group was greater than contact inhibition treatment, indicating that the serum starvation treatment was more beneficial than the contact inhibition treatment.

RECIPIENT OOCYTES

Most current SCNT studies use MII-phase enucleated oocytes as recipient cytoplasm for NT. There are two sources for these stage oocytes: *in vivo* mature oocytes collected directly from animals or aspirated oocytes collected from the ovaries of live or slaughtered animals and then cultured *in vitro* for maturation. In sheep, the different oocyte sources did not affect the fusion of reconstructed embryos, but blastocyst formation, recipient sheep pregnancy, and lambing rates of *in vivo* matured oocytes were significantly higher than those of *in vitro* matured oocytes (Well et al., 1997). However, in goats, there was no significant difference in developmental capabilities of *in vivo* and *in vitro* matured oocytes as recipient cytoplasm (Reggio et al., 2001). Thus, the most recipient oocytes for SCNT in goats are derived from slaughter house.

APPLICATION OF SCNT IN GOATS

SCNT is a relatively simple and inexpensive technique for increasing production of commercial value in dairy goat by using transgenic goats for the production of many recombinant therapeutic proteins for treatment of diseases. In fact, the first drug called ATryn, a human antithrombin protein approved for human use was derived from a genetically engineered goat (Dutta et al., 2011). Thus, goat is also an adapted domestic species for biological investigation and application, because of its short gestation period and easy maintenance. Moreover, SCNT technique was greatly improved, and widely successful in other animal species. Previously, the technology to produce transgenic goat was the microinjection of the DNA construct into the pronuclear embryos. However, this method shows a low efficiency and cannot predict outcome (Baldassarre and Karatzas, 2004). For SCNT, donor cell can be genetically modified by introducing the gene of interest and screened before use in SCNT.

SCNT in goats for milk production: Cloned goats express many proteins in their milk that cannot be chemically synthesized or manufactured by bacterial or cell culture systems (Baguisi et al., 1999; Reggio et al., 2001). Thus, the current progress of the SCNT technology has successfully produced healthy transgenic goats for the production of α-fetoprotein (AFP), suggesting that purified AFP derived from the milk of SCNT goat was similar to human AFP isolated from human cord blood (Parker et al., 2004). The milk of the four transgenic goats developed by SCNT could produce $MSP1_{42}$-malaria antigen (Behboodi et al., 2005). Recombinant lysosomal human acid β-glucosidase, the enzyme involves for catabolism of glucosylceramide into ceramide and glucose, has been successfully expressed in milk of cloned transgenic goat (Zhang et al., 2010). Human lactoferrin (hLTF), one of the components of the immune system of the body that provides antibacterial activity to human infants, can be produced from milk of SCNT goat (An et al., 2012; Wan

et al., 2012; Meng et al., 2013). Milk from cloned transgenic goat produced by SCNT could express human lysozyme (*hLYZ*), which possesses antimicrobial properties causing increase in safety and storage time of milk (Liu et al., 2013). Moreover, Yekta et al. (2013) showed great success in SCNT transgenic goat using fetal fibroblasts with a linearized marker-free construct in which the transgene was juxtaposed to b-casein promoter designed to secret the recombinant protein in goat milk. Greater expression of recombinant human alpha-lactalbumin derived from transgenic goat using SCNT was observed in goat milk (Yuan et al., 2014).

SCNT in goats for meat production: Goat meat is an important food worldwide and it has high-quality protein and low cholesterol; thus providing health benefits to humans. SCNT using myostatin-targeted 2-month-old goat fibroblasts as donor is a potential method for producing myostatin-targeted goats for meat purpose (Zhou et al., 2013).

CONCLUSIONS

The ability of SCNT technology to propagate genetically identical animals with superior genotypes would be important for increasing the number of economically important animals. Importantly, this technology can be applied with cell culture system and animal molecular technology to produce transgenic animal. However, the efficiency of animal production derived from SCNT is still low. Moreover, the production of animals by this technology could lead to more efficient models for biomedical research.

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