ABSTRACTS

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Species specific challenges in cloning dogs

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OBJECTIVES AND METHODS: Somatic cell nuclear transfer (SCNT) is multiple steps including the removal of the nuclear material, injection of donor cell, fusion, activation of the reconstructed oocytes, and finally transfer to a synchronized female recipient. That’s why many factors contribute to the cloning efficiency in dog. By performing a retrospective analysis of 2005-2011 published papers regarding dog cloning, we define the optimum procedure and summarize the unique feature for dog cloning.

RESULTS: The first unique feature of dog cloning is to use in vivo matured oocytes due to the low efficiency rate of in vitro oocyte maturation and absence of superovulation/inducing ovulation. The fully matured oocytes are useful because it have the superior reprogramming ability compared to immature/aged oocytes. The protocol for donor cell cycle synchronization is the second feature of dog cloning. Roscovitine treatment resulted in the high pregnancy and pup viability rate compared to other methods such as confluence, serum starvation. Especially, we developed the optimal protocol, cell synchronization with high concentration of 15ug/ml for 24hr differently with one used in other species.

Similar to other mammals, membrane fusion between the intact cells and enucleated oocytes can be achieved by electric pulses using needle method or chamber method. However, in dogs, needle method with high voltage (3.8~4.0 kV/cm), the third unique feature of dog cloning, is a prerequisite for high fusion rates and the full term development of fused oocytes. The last feature is synchrony between oocyte donor dog and recipient, the parity of recipient. Estrus synchronization and in vitro culture protocol has not yet been established in dogs, the fused oocytes were surgically transferred to the naturally synchronized recipient immediately chemical activation. Although synchrony between oocyte donor dog and recipient within one day did not influence cloning efficiency, the parity of recipient affected the cloning efficiency, the nulliparous was associated with higher delivery rate (3% vs 1.7%, p<0.05) than to that of multiparous recipients.

Recently, canine specific cloning technology has been applied for producing transgenic models. The canine embryonic stem cells were established with extremely low efficiency unlike other mammals; alternatively, SCNT is the best option in transgenesis of dogs. Using SCNT technique, continuously red fluorescent protein (RFP) gene expressed cloned dog and conditionally green fluorescent protein (GFP) gene expressed cloned dog were generated with a high pregnancy rate (up to 34%) and these were proved by germ line transmission using natural breeding.

CONCLUSION: Although successful technical optimization of SCNT was implemented in dog cloning, we need to strive for in vitro maturation, in vitro culture, and estrous synchronization to improve SCNT techniques. Especially, dog, considered as human genetic disease model will be potential biomedical source by application of dog SCNT technique.

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