韓國家畜繁殖學會誌 24(4) : 429~437 (2000)
Korean J. Animal Reprod.

Production of Transgenic Bovine Embryos Following Nuclear Transfer of Bovine Fetal Fibroblasts Transfected by Foreign Genes

Kil, K. S.[1], S. J. Uhm[1], E. H. Kim[1], H. J. Chung[1], T. Kim[2], H. Park[3], H. T. Lee[1] and K. S. Chung[1†]

Animal Resource Research Center, Konkuk Univ., Seoul

ABSTRACT

This study investigated the successful introduction of genes of erythropoietin (EPO) and enhanced green fluorescent protein (EGFP) in bovine embryos following nuclear transfer of bovine fetal fibroblasts (bFF), which were transfected by retrovirus vector system. Non-starved bFF were transferred into perivitelline space of enucleated oocytes. The bFF-oocyte units were accomplished by cell to cell fusion and activated with calcium inophore and 6-dimethylaminopurine. Reconstructed embryos were co-cultured with bovine oviduct epithelial cells in CR1aa medium for 8 days. Out of 187 (EPO) and 210 (EGFP) bovine eggs reconstructed by nuclear transfer, 149 (EPO : 80.0%) and 158 (EGFP : 75.2%) embryos were cleaved, and among them 36 (EPO : 24.2%) and 35 (EGFP : 22.2%) embryos developed to the blastocyst stage. Of these blastocysts, 100% integration of EPO gene in 36 embryos was determined by PCR, and 100% expression of EGFP gene in 35 embryos was observed under the fluorescent microscope. This result indicates that bovine oocytes reconstructed by nuclear transfer of transfected bFF can successfully develop to the blastocyst stage. Furthermore, this novel procedure may be presumably an attractive method efficiently to produce the transgenic cattles.

(Key words : Nuclear transfer, Fetal fibroblast, EPO, EGFP, Bovine)

Ⅰ. INTRODUCTION

Nuclear Transfer (NT) technique in mammals has been used as a powerful tool for production of cloned and transgenic animals. Recently, the development of somatic cell NT has become more successful in generating cloned animals (mouse - Wakayama et al., 1998; goat - Baguishi et al., 1999; sheep - Wilmut et al., 1997; pig - Polejaeva et al., 2000; cow - Kato et al., 1998). Moreover, the approach of somatic cell-mediated gene transfer enabled production of cloned transgenic animals from embryos reconstructed with sheep and cow fetal fibroblasts transfected with foreign genes (Schnieke et al., 1997; Cibelli et al., 1998). In

† Corresponding author : Animal Resource Research Center, Konkuk Univ., Seoul. E-mail : cks123@kkucc.konkuk.ac.kr

[1] 건국대학교 동물자원연구센터(Animal Resource Research Center, Konkuk University, Seoul)

[2] 대구효성가톨릭대학교 의과대학 생리학교실(Dept. of Physiology, Catholic University of Taegu-Hyosung, Taegu)

[3] 대구대학교 생물공학과(Dept. of Biotechnology, Taegu University, Taegu)

recent, gene-targeted sheep was produced by nuclear transfer with fetal fibroblasts (McCreath et al., 2000). These results suggest that the NT method using genetically modified somatic cell lines overcome the problems of mosaicism and low efficiency of transgenic livestock production when using conventional pronucleus DNA microinjection.

In this study, therefore, we investigated the production of transgenic bovine embryos using NT with genetically modified donor cells before embryo transfer. For transfection of donor cells used EGFP gene as marker and EPO gene as an important factor regulating the production of red blood cell. We successfully introduced foreign genes to bovine embryos reconstructed with bovine fetal fibroblasts (bFF) transfected by retrovirus vector for genetic modification of donor cells. Embryonic development and gene expression and integration of EGFP and EPO in bovine embryos reconstucted by nuclei cells transfected with EGFP and EPO genes were used as parameters that determine the success of this study.

Ⅱ. MATERIALS AND METHODS

1. *In Vitro* Maturation

Ovaries were obtained from slaughtered female cows, and the oocyte-cumulus complexes (OCCs) were recovered by aspiration from the follicles (2～6 mm in diameter) using a 10ml disposable syringe fitted with an 18-gauge needle. The OCCs were washed three times with TL-HEPES (1mg/ml BSA, low carbonate TALP; Parrish et al., 1988) and the maturation medium, respectively. Ten OCCs were transferred into a 50 μl of maturation medium under warm paraffin oil in a petri dish equilibrated for 2 hr in 5% CO_2 and 95% O_2 in air. The maturation medium for bovine OCCs was TCM-199 (with Earle's salts; Gibco, Grand Island, NY) supplemented with 25 mM $NaHCO_3$, 10% FBS, 0.2mM pyruvate, 5 μg/ml FSH, 1 μg/ml estradiol-17β and 25 μg/ml gentamycin. Culture was carried out at 39℃ in 5% CO_2 in air for 22～24 hr.

2. Nuclear Transfer

1) Preparation of Cells

The virus-producing cell line (pLNβ-EGFP and pLNC-EPO) was constructed by transfection of PA317 and PG13 cells (Miller and Buttimore, 1986; Miller et al., 1991) with plasmid pLNβ-EGFP and pLNC-EPO, followed by selection with 800 μg/ml of G418 (Gibco/BRL) for 2 weeks. The resulting neomycin resistant cells were continuously grown in Dulbecco's Modified Eagle's Medium (DMEM; Gibco/BRL) supplemented with 10% FBS, penicillin (100 μg/ml), and streptomycin (100 μg/ml). For targeting cell line, primary bFFs were isolated from fetuses of pregnant female at 60-days after mating. The cells were maintained in the same media as described by the virus-producing cell line up to passage 2 to 7. After infection of bFFs with the pLNC-EPO and pLNβ-EGFP vector retrovirus, respectively, the cells were selected with medium containing 400 μg/ml of G418 for two weeks. The PG13 LNC-EPO or PA317 LNβ-EGFP transfected cells were grown at 37℃ in an atmosphere of 5% CO_2 in air.

2) Retrovirus Vector Construction

Construction of pLNβ-EGFP (Fig. 1) was done by inserting a 780 bp fragment of the EGFP gene isolated from pEGFP-N1 (purchased from Clontech) after *Not* Ⅰ, Klenow and *Hind* Ⅲ treatment into pLNβZ (Kim et al., 1993) treated with Sal Ⅰ, Klenow and Hind Ⅲ. Construction of pLNC-EPO was done by inserting CMV promoter and a 719 bp fragment of cloned EPO. For eukaryotic cell transfection, purification of plasmid DNA from 500

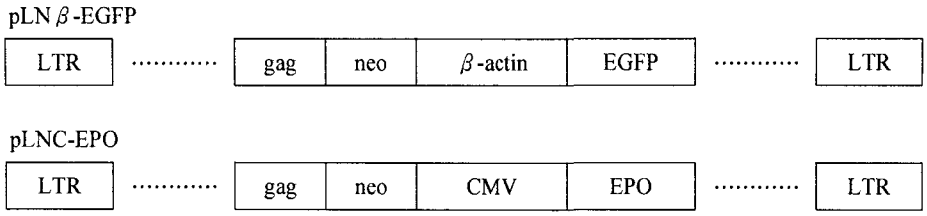

Fig. 1. Construction of pLNβ-EGFP and pLNC-EPO retrovirus vectors. LTR, long terminal repeat; gag, 5' portion of Moloney murine leukemia virus (MoMLV) gag gene; neo, neomycin resistance gene; β-actin, rat β-actin promotor; EGFP, enhanced green fluorescent protein gene; CMV, human cytomegalovirus immediate early promoter; EPO, erythropoietin gene.

ml LB culture of transformed HB101 *E. coli* competent cells was done by using a Qiagen column (Qiagen Inc.). All the enzyme for DNA manipulation were purchared from New England Biolabs, Inc.

3) Transfection and Infection of Cells

Polybrene/DMSO (dimethyl sulfoxide) mediated transfection (Kawai and Nishizawa, 1984) was performed as follows: 10 μg of plasmid DNA in 1 ml medium supplemented with ploybrene (30 μg/ml; Aldrich, WI) was added to amphotropic MLV-based PA317 packing cells plated on the previous day (5×10^5 cells/60mm dish). After 6 hrs of incubation at 37℃ in an atmosphere of 5% CO_2 in air, the DNA-medium mixture was aspirated, and DMSO shock was applied by adding 2ml of 25% DMSO in medium to a 60mm dish for 1 min. Following three washes with medium, cells were fed with 4 to 5ml of medium and incubated overnight before trypsinization of split the transfected cells.

Infection of bFF was performed following the modified procedure of Miller and Rosman (1989); 3 ml of a mixture of fresh non-selection medium (filtered through a 0.22 μl pore-size filter) of virus-containing medium, and polybrene (5 μg/ml of final concentration) were added to target cells which were plated on the previous day. Exposure of the target cells to the mixture was allowed for only 1 hr. The virus-containing medium was harvested from virus-producing cells which had been fed with non-selection medium on the previous day. Following day 1 of culture, infected cells were trypsinized and split in non-selection medium. In both transfection and infection medium (G418) was done on the next day after splitting.

4) Enucleation of Oocytes

Matured oocytes were stripped of cumulus cells in PBS supplemented with 0.1% hyaluronidase and washed three times in PBS containing 0.1% BSA. Oocytes were enucleated by aspirating the first polar body and adjacent cytoplasm (approximately 30% of ooplasm) using a beveled pipette (30mm in diameter) in PBS containing 0.1% BSA and 7.5 μg/ml Cytochalasin B (CB; Sigma). Enucleated oocytes were incubated in CR1aa containing 0.3% BSA until injection of donor cells. Enucleation was confirmed by staining aspirated portion of cytoplasm with 5 μg/ml Heochst 33342 (Sigma).

5) Nuclear Transfer

The isolated single bFF was inserted into perivitelline space of each enucleated oocyte. Reconstructed eggs were washed three times in fusion solution. The solution composed 0.3 M manitol, 0.1mM $CaCl_2$ and 0.1 mM $MgSO_4$. Fusion was

performed at room temperature, in a chamber with two stainless steel electrodes 1mm apart overlaid with fusion solution. The reconstructed eggs were manually aligned with a fine pasteur pippete, so that the contact surface between the bFF and oocyte was parallel to the electrodes. Membrane fusion was induced with two D.C. pulses of 2.5 KV/cm for 30 μsec delivered by a BTX Electro Cell Manipulator 200 (BTX, San Diego, CA). Fused bovine oocytes and denuded matured oocytes as control were activated by treatment of 5 μM calcium inophore and 2.0mM 6-dimethylaminopurine (6-DMAP).

3. *In Vitro* Fertilization (IVF)

Matured COCs were washed twice with Sp-TALP and subsequently with Fert-TALP (Rosenkrans et al., 1993). After washing, ten mature COCs were pooled in a 44 μl of Fert-TALP droplet under paraffin oil. Bull spermatozoa recovered from frozen-thawed semen were separated on a discontinuous percoll gradient. Highly motile spermatozoa were added to Fert-TALP at a final concentration of 1.0×10^6 sperm/ml. Then 2 μl of heparin stock solution to induce sperm capacitation and 2 μl of PHE stock solution (2mM Phenicillamine, 20 μM hypotaurine and 1 μM epinephrine) to stimulate sperm motility were added into a Fert-TALP droplet. Sperm and COCs were co-incubated in a 50 μl drops of Fert-TALP under paraffin oil for 24 hrs at 39℃, 5% CO_2 in humidified atmosphere.

4. *In Vitro* Culture of Reconstructed Embryos

All embryos were co-cultured with bovine oviduct epithelial cells in 500 μg of CR1aa containing 10% FBS for 8 days at 39℃ in an atmosphere of 5% CO_2 in air.

5. PCR Analysis

Transgenic embryos with EPO gene were identified by using gene specific primers. The primers used for amplification of the EPO-specific sequences were as follows: 3'-GATGAGGGCCCCGGG-TGTGG-5' (5' primer) and 3'-CGGGGGA- GGG-TGTGGCACAA-5' (3' primer). Reaction mixtures for PCR were as follows: 2.5 U of TaKaRa Ex-Taq DNA polymerase, 0.5 μl of 10×Ex Taq buffur, 8.5 μl of dNTP mixture, 1 μl of each primers, and almost 100 ng of DNA. Amplification was repeated for 35 cycle: denaturation at 94℃ for 40 sec, annealing at 63.5℃ for 40 sec and extension at 72℃ for 35 sec. PCR products were applied to a 2.0% agarose gel, electrophoresed, stained with ethidium bromide, and photographed under ultraviolet light.

6. Statistical Analysis

The data from at least four replications were pooled. Differences in the percentages of oocytes developed to particular stages were determined by Chi-square analysis.

Ⅲ. RESULTS

Expression of EGFP in bFF transfected by PA317 LNβ-EGFP retrovirus vector was shown in Fig. 2. The EGFP and Neo^R genes in the provirus were to be expressed under the promoters of β-actin and LTR promoters, respectively. The green area (Fig. 2B) shows the expression of EGFP gene. Even in the cells of one day post-infection some green fluorescent cells were detected in EGFP expression. After selection with G418 for 2 weeks, all bFFs expressed EGFP (Fig. 2B). Thus, these transfected bFF were used as donor cells in following experiments.

In vitro development of reconstructed bovine embryos produced by these two different transfected bFFs was showed in Table 1. Out of 187 (EPO) and 210 (EGFP) bovine eggs reconstructed

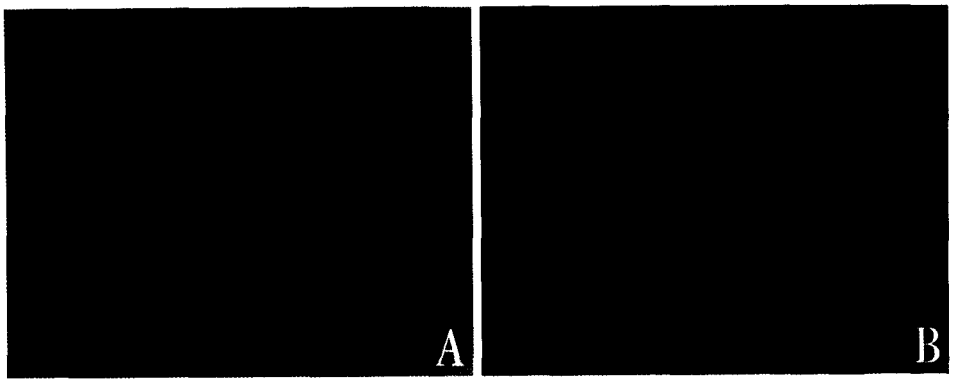

Fig. 2. Expression of EGFP gene in bovine fetal fibroblast transfected by PA317 LN β-EGFP retrovirus vector; A) under light microscope, B) under fluorescent microscope.

Table 1. *In vitro* development of reconstructed embryos following nuclear transfer with transfected bFF

Treatment		No. of oocytes examined	No. of embryo			Cell number of blastocysts
			Fusion(%)	Cleaved(%)	Blastocysts(%)	
IVF		192	-	157(81.8)	73(46.5)[a]	128±2.3[a]
Activation		137	-	94(68.6)	26(27.6)[b]	45±1.9[b]
NT	EGFP	210	167(79.5)	158(75.2)	35(22.2)[b]	111±2.4[a]
	EPO	187	154(82.4)	149(80.0)	36(24.2)[b]	109±2.6[a]
Total		397	321(80.8)	307(77.3)	71(23.1)	110±2.5

[a,b] Values within columns with different superscripts differ ($P<0.05$).

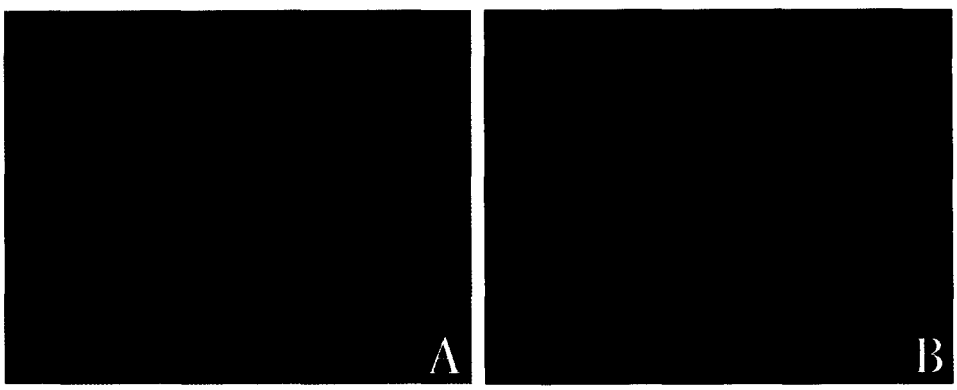

Fig. 3. Expression of EGFP gene in bovine hatching blastocyst stage embryo from nuclear transfer; A) under light microscope, B) under fluorescent microscope.

by nuclear transfer, 149 (EPO : 80.0%) and 158 (EGFP : 75.2%) embryos were cleaved, and among them 36 (EPO : 24.2%) and 35 (EGFP : 22.2%) embryos developed to the blastocyst stage. The blastocyst rates of these NT embryos were significantly lower ($P<0.05$) than that (46.5%) of IVF embryos, but were similar to that (27.6%) of activated embryos. However, total cell numbers (EGFP : 111 ± 2.4, EPO : 109 ± 2.6) of NT-blastocysts were not significantly different to that (128 ± 2.3) of IVF-blastocysts, but were significantly higher ($P<0.05$) than that (45 ± 1.9) of activated blastocysts.

Moreover, reconstructed bovine embryos developing *in vitro* were examined to evaluate the expression of EGFP under fluorescence microscopy using a standard FITC filter set (Fig. 3). All NT blastocyst stage embryos with bFF transfected by EGFP gene emitted the green fluorescence without mosaicism (Fig. 3B). In addition, PCR analysis of or the integration of EPO gene in blastocysts following NT with transfected bFF was shown in Fig. 4. Each genomic DNA from single blastocyst was applied to PCR analysis. Transfected bFFs was used as positive control and non-transfected bFFs was used as negative control. As expected, only the predicted 702 bp fragment for EPO gene was detected in the samples of transfected bFFs and single NT blastocyst. Furthermore, amplified product was not detected from non-transfected bFF. Therefore, these results showed that reconstructed embryos were all transgenic embryos.

Ⅳ. DISCUSSION

The main objective of this study was to transfer the foreign genes in bovine embryos employing nuclear transfer approach. Usually, microinjection of DNA into pronuclei of fertilized oocytes has been used for production of transgenic animals. However, the integration rate of exogenous DNA into their genomes is under 1% in livestock (reviewed from Wall, 1996). In addition, the most of transgenic animals showed mosaicism because the timing and site of integration were random (reviewed from Wall, 1996). These problems, however, could be solved by applying the somatic cell nuclear transfer approach, because it allowed evaluating the expression of the transgene in the somatic cells *in vitro* before creation of the embryo. Another significant advantage of the somatic cell nuclear thansfer approach is that it takes only one generation to produce multiple numbers of transgenic livestock of our expectation, compared to at

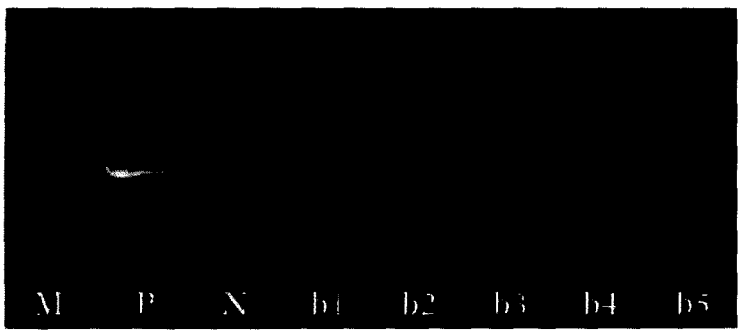

Fig. 4. Integration of EPO gene in blastocyst from nuclear transfer. M: size marker (100 bp ladder), P: positive control (transfected bovine fetal fibroblasts), N: negative control (non-transfected bovine fetal fibroblasts), b1~b5: single NT blastocyst.

least two generations with the traditional micro-injection approach. In this study, for NT of bovine oocytes, EPO or EGFP gene induced into bFF as donor cells. We could successfully developed to the blastocyst stage from NT embryos produced by NT with these donor cells. Moreover, these target genes were detected in these all blastocysts.

We used EGFP and EPO genes for introduction of foreign genes into bFF. EGFP (Zhang et al., 1996) has been used as a reporter gene instead of wild-type GFP, because wild-type GFP from jellyfish is expressed at low levels in mammalian cells. The β-galactosidase gene has been widely used as a reporter gene for monitoring gene expression especially *in vivo,* but the EGFP system may be a useful alternative because expression of EGFP can be detected in living cells (Shimada et al., 1999). Erythropoietin (EPO) as a important factor regulating production of red blood cell is produced in the kidney and circulated to bone marrow (Judith, 1984, Wolfgang, 1992). On the bone marrow, it is acting to induce the differentiation of hematopoietic stem cells (Benjamine et al., 1999). Its formation greatly increases in response to hypoxia.

For the initial step, we transfected the EGFP and EPO gene to the bFF cells by using a retrovirus vector system. Use of retrovirus vectors for gene transfer was mainly due to several failures of traditional calcium phosphate transfection method. All surviving cells after infection with retrovirus vector followed by G418 selection emitted green fluorescence. In the cells one day post-infection, the difference between positive and negative cells in terms of EGFP expression was evident. In addition, the embryos reconstructed by the transfected bFFs successfully developed to the blastocyst stage. These embryos resulted in cytoplasmic expression of EGFP at blastocyst stage without showing mosaicism.

Recently, transgenic sheep and cows from the embryos reconstructed with fetal fibroblasts transfected with the foreign genes have been produced (Schmieke et al., 1997; Cibelli et al., 1998). These cloned animals were all transgenic, and these investigations indicated that cloned embryos produced by transfected somatic cells could produce 100% transgenic animals. Also, we found 100% expression of EGFP gene and integration of EPO gene in NT blastocyst stage in this study. Therefore, this study is a significant result and supports that use of the EGFP and EPO genes in transgnic animal production may significantly enhance the efficiency of transgenic animal production because expression and integration of these genes allows us to select positive embryos before transfer to the surrogate mother. Further studies evaluating developmental and physiological effects by the expression of the EGFP gene are ongoing. Moreover, for the expression of exogenous genes of interest in the transgenic cows, the rat β-actin and CMV internal promoter in the retrovirus vector seems to be an appropriate promoter. We previously proved excellence of the rat β-actin and CMV promoter in the retrovirus vector in bovine embryos (Kim et al., 1993).

In conclusion, the results of this study show the possibility of efficient transgenic cow production by applying a nuclear transfer approach. Based on the experiences and knowledge acquired from this study, the same experiments will be continued using the somatic cells of adult cow as nuclei donors. This will provide a powerful research tool for studying developmental events in domestic animals and marked cell lines for other genetic manipulations.

V. REFERENCES

1. Baguishi, A., Behboodi, E., Melican, D. T.,

Pollock, J. S., Destrempes, M. M., Gammuso, C., Williams, J. L., Nims, S. D., Porter, G. A., Midura, P., Palacios, M. J., Ayres, S. L., Denniston, R. S., Hayes, M. L., Ziomek, C. A., Meade, H. M., Godke, R. A., Gavin, W. G., Overstrom, E. W. and Echelard, Y. 1999. Production of goats by somatic cell nuclear transfer. Nature Biotech. 17:456-461.

2. Benjamine, L. E. and Bunn, H. F. 1999. Regulation of the erythropoietin gene. Blood 94: 1864-1877.
3. Cibelli, J. B., Stice, S. L., Golueke, P. J., Kane, J. J., Jerry, J., Blackwell, C., Ponce de Leon, F. A. and Robl. J. M. 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280:1256-1258.
4. Judith, B. S. 1984. The chemistry and physiology of Erythropoietin. Vit. Horm. 41:161-211.
5. Kato, Y., Tani, T., Sotomaru, Y., Kurokawa, K., Kato, J., Doguchi, H., Yasue, H. and Tsunoda, Y. 1998. Eight calves cloned from somatic cells of a single adult. Science 282: 2095-2098.
6. Kawai, S. and Nishizawa, M. 1984. New procedure for DNA transfection with polycation and dimethyl sulfoxide. Mol. Cell. Biol. 4: 1172-1174.
7. Kim, T., Leibfried-Rutledge, M. L. and First, N. L. 1993. Gene transfer in bovine blastocysts using replication-defective retroviral vectors packaged with gibbon ape leukemia virus envelopes. Mol. Reprod. Dev. 35:105-113.
8. McCreath, K. J., Howcroft, J., Campbell, K. H. S., Colman, A., Schnieke, A. E. and Kind, A. J.. 2000. Production of gene-targeted sheep by nuclear transfer from cultured somatic cells. Nature 405:1066-1069.
9. Miller, A. D. and Buttimore, C. 1986. Redesign of retrovirus packaging cell line to avoid recombination leading to helper virus production. Mol. Cell. Biol. 6:2895-2902.
10. Miller, A. D., Garcia, J. V., Von Suhr, N., Lynch, C. M., Wilson, C. and Eiden, M. V. 1991. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. J. Virol. 65:2220-2224.
11. Miller, A. D. and Rosman, G. J. 1989. Improved retroviral vectors for gene transfer and expression. Biotechniques 7:980-990.
12. Polejaeva, I. A., Chen, S., Vaught, T. D., Page, R. L., Mullins, J., Ball, S., Walker, S., Ayares, D. L., Colman, A. and Campbell, K. H. S. 2000. Cloned pig produced by nuclear transfer from adult somatic cells. Nature 407:505-509.
13. Rosenkrans, C. F., Zeng, G. Q., McNamara, G. T., Scfoff, O. K. and First, N. L. 1993. Development of bovine embryos *in vitro* as affected by energy substrates. Biol. Repord. 49:459-462.
14. Schnieke, A. E., Kind, A. J., Ritchie, W. A., Mycock, K., Scott, A. R., Ritchie, M., Wilmut, I., Colman, A. and Campbell, K.H.S. 1997. Human factor Ⅸ transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 278:2130-2133.
15. Shimada, H., Kaname, T., Suzuki, M., Hitoshi, Y., Araki, K., Imaizumi, T. and Yamamura, K. 1999. Comparison of ES cell fate in sandwiched aggregates and co-cultured aggregates during blastocysts formation by monitored GFP expression. Mol. Reprod. Dev. 52:376-382.
16. Wakayama, T., Perry, A. C. F., Zuccotti, M., Johnson, K. R. and Yanagimachi, R. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 394:369-374.
17. Wall, R. J. 1996. Transgenic livestock: progress and prospects for future. Theriogenology 45:57-68.
18. Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J. and Campbell, K. H. S. 1997. Viable

offspring derived from fetal and adult mammalian cells. Nature 385:810-813.
19. Wolfgang J. 1992. Erythropoietin: structure, control of production, and function. Physiol. Rev. 72:449-489.
20. Zhang, G., Gurtu, V. and Kain, S. R. 1996. An enhanced green fluorescent protein allows densitive detection of gene transfer in mammalian cells. Bioch. Biophys. Res. Commun. 227:707-711.

요 약

외래유전자를 도입한 소 태아세포의 핵치환에 의한 형질전환 소 수정란 생산

길광수[1] · 엄상준[1] · 김은하[1] · 정학재[1] · 김태완[2] · 박흠대[3] · 이훈택[1] · 정길생[1†]

[1]건국대학교 동물자원연구센터
[2]대구효성가톨릭대학교 의과대학 생리학교실
[3]대구대학교 생물공학과

본 연구는 retrovirus vector system에 의해서 EPO와 EGFP 유전자가 전이된 소 태아세포를 이용하여 핵치환된 소 난자에서의 이들 유전자의 성공적인 도입을 조사하였다. Non-starved 소 태아세포는 탈핵된 소 난자의 위란강내로 주입되었다. 소 태아 세포와 난자는 세포간 전기자극에 의해 융합시켰으며, 이후 calcium ionophore와 6-dimethylaminopurine를 이용하여 난활성을 유도하였다. 핵치환에 의해 재구성된 난자는 8일 동안 CR1aa 배양액에서 소 난관상피세포와 함께 공배양하였다. 핵치환에 의해 재구성된 187개와 210(EPO, EGFP)개의 소 난자 중에서, 149개와 158(EPO : 80.0%, EGFP : 75.2%)개의 난자가 분할되었고, 이들 분할된 난자 중 36개와 35(EPO : 24.2%, EGFP : 22.2%)개의 난자가 배반포까지 발달하였다. 이들 배반포에서, EPO 유전자는 PCR에 의해 36개의 모든 난자에서 삽입을 확인하였고, EGFP 유전자의 발현은 형광현미경 하에서 35개의 모든 난자에서 확인하였다. 이 결과는 외래유전자가 삽입된 소 태아 세포를 이용하여 핵치환된 난자는 배반포까지 성공적으로 발달할 수 있다는 것을 나타낸다. 더우기, 이러한 방법은 효율적인 형질전환 소를 생산하는데 이용될 수 있으리라 사료된다.

(접수일자 : 2000. 11. 3. / 채택일자 : 2000. 11. 20.)