韓國家畜繁殖學會誌 25(4) : 399~407 (2001)
Korean J. Animal Reprod.

Effects of Sucrose Treatment on the Morphology and Integration of Foreign DNA into Bovine Oocytes

Kim, S. G., K. S. Kim, T. W. Kim[1], H. T. Lee and K. S. Chung[†]
College of Agriculture, Animal and Life Science, Konkuk University

ABSTRACT

The microinjection of retroviral vectors into the perivitelline spaces of MⅡ-stage oocytes increased production of transgenic bovine embryos. However, oocytes have various sizes of perivitelline space, and there is the tendency that the oocyte membranes are damageable by micropipettes during the injection of retroviral vectors into perivitelline spaces of oocytes. Thus, it was not always possible to stably inject retroviral vector into perivitelline spaces of oocytes. Here we used sucrose to minimize the damage of the oocyte membrane. When the oocytes were suspended in 0.5% sucrose, poor quality oocytes showed rough cytoplasmic membranes, while good quality oocytes maintained smooth membranes. However, when the latters were subjected to *in vitro* fertilization, no significant differences were observed in cleavage rates (82% of control Vs. 84% of sucrose treated oocytes). The Same trends were obtained from the oocytes fertilized after microinjection of LN β-EGFP and LNC-hGH genes into the perivitelline spaces. The rates of cleavage and blastocyst from microinjection of LN β-EGFP genes were 81 and 25%, and from microinjection of LNC-hGH genes were 83 and 30%, respectively. The result indicated that microinjected oocytes could develop to the blastocyst stages after *in vitro* fertilization with no significant difference from control group. Moreover, the integration of hGH-gene (by PCR analysis) was detected in 52% of infected cleaved embryos and the expression of EGFP-gene (under a fluorescence microscope) was also observed in 34% of infected blastocyst. These results indicated that 0.5% sucrose treatment could be an efficient method not only to select good quality embryos but also to inject retroviral vectors into perivitelline spaces without any harm and hence improving developmental rates.

Ⅰ. INTRODUCTION

In the previous studies, microinjections of foreign DNA into pronucleus were used for gene transfers into various mammalian embryos, but their efficiency were low in production of transgenic cattle and other livestock (Wall, 1996). Thus the interest in the production of transgenic animals by retroviral vectors is growing. Several mosaic transgenic fetuses were obtained by placing retroviral packaging cells into the perivitelline spaces of bovine embryos, but no live animals were produced (Haskell and Bowen 1995). However, the introduction of retroviral vector genes into metaphase Ⅱ oocytes of the second meiosis resulted in the higher probability of pre-in-

[†] Corresponding author : K. S. Chung, College of Agriculture, Animal and Life Science, Konkuk University.
[1] 대구효성카톨릭대학교 의과대학 생리학교실(Dept. of Physiology, Catholic University of Taegu-Hyosung, Taegu)

tegration complexes gaining access to the chromatin compared to the pronuclear injection (Chan et al., 1998) and therefore, this method led to production of transgenic cattles and monkeys (Chan et al., 1998 and 2001). Rescently, 3% sucrose pretreatment as an efficient and non-damaging method could not only easily remove the well-distinguished spindles and chromosomes but also provide a method for judging oocyte quality in nuclear transplantation (Wang et al., 2001). Most mammalian cells respond to hypertonic cell shrinkage and a phenomenon known as regulatory volume increase (RVI) maintaine cell volume. Bortner and Cidlowski (1996) also reported that the absence of volume regulatory mechanisms contributed to the rapid activation of apoptosis in thymocytes. We hypothesized that when hypertonic cytoplasmic membrane shrinkage was induced by sucrose treatment, retroviral vector could be stably injected into perivitelline spaces of M Ⅱ stage oocytes and therefore, production of transgenic bovine embryos should be increased.

These experiments were carried out to investigate the effects of sucrose pretreatment on morphology of oocytes, *in vitro* development of microinjected oocytes, and integration and expression of infected embryos.

Ⅱ. MATERIALS AND METHODS

1. Preparation of Retroviral Vector

For Introducing gene sequences of retroviral vector and vesicular stomatitis virus G glycoprotein (VSV-G; Burns et al., 1993), 293mGPHy cells containing the gag and pol genes were converted into virus-producing cells (Kim et al., 2001). At first, the virus-producing cell line (LN β-EGFP or LNC-hGH) was constructed by transfection of PG13 and PT67 cells with plasmid pLN β-EGFP and pLNC-hGH (Fig. 1), followed by selection with 600 μg/ml of G418 (Gibco/BRL) for two weeks (Miller et al., 1991). Secondly, to introduce VSV-G gene which is equivalent to the retrovirus *env* gene, the cells were undergone calcium phosphate transfection by adding 20 μg of plasmid pHCMV-G in 1 ml of calcium phosphate solution to the Neo^R (G418 resistant) 293mGPHy cells plated on the previous day (1×10^6 cells/100 mm dish) (Yee et al., 1994; obtained from Dr. Jane C. Burns). After 8 hours of incubation at 37℃ with 5% CO_2 in air, calcium phosphate solution was aspirated to culture the cells with 10 ml of medium. Because VSV-G is cytotoxic, the medium containing retrovirus vectors was harvested after 48 hours of transfection.

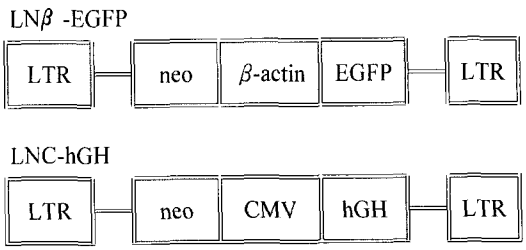

Fig. 1. Structures of replication-defective retroviral vectors. LTR; long terminal repeat, neo, G418 resistant gene, β-actin; rat β-actin promoter, EGFP; enhanced green fluorescent protein gene, CMV; human cytomegalovirus immediated early promoter, hGH; human growth hormone gene. Drawings are not to scale. The LN β-EGFP and LNC-hGH plasmids were constructed by replacing LacZ genes of pLNCZ with the 368 bp EGFP and 345 bp hGH cDNA fragment, respectively (Kim et al., 1993).

2. Concentration of Virus

The medium harvested from the virus-producing cells was centrifuged to pellet the viruses at 50,000 × g for 90 minutes at 4℃ using vertical rotor (Beckman 70Ti). Following complete removal of supernatant after centrifugation, the pellet was placed at 4℃ overnight with small volume of 0.1X

Hank's Balanced Salt Solution (HBSS) for suspension (Yee et al., 1994). For 1,000X concentration, virus stock of the first concentration cycle was pooled and centrifuged again. The concentrated virus stock was filtered through 0.45 μm pore-size filter before storage in aliquots at −70℃.

3. *In Vitro* Oocyte Maturation

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

4. Microinjection of Retroviral Vector into Perivitelline Space

For microinjection of retroviral vector, bovine oocytes were recovered from maturation medium after 17 hrs of incubation. These oocytes were stripped of cumulus in TL-HEPES stock supplemented with 0.1% hyaluronidase and washed three times in TL-HEPES containing 0.1% BSA. The micromanipulation medium was modified TCM-199 (mTCM-199) supplemented with 0.2 mM pyruvate, 0.5% sucrose, 15 mM Hepes, and 10% FBS. Vector stock (×1000) was concentrated to 3×10^8 G418 resistant colony forming units/ml on the EBTr (bovine trachea target cell), to permit microinjection of picoliters. All of them were microinjected while maintained in droplets of their respective handling medium (mTCM-199) under paraffin oil. When swelling of the zona pellucida was observed, microinjection was stopped. Although it was impossible to assess exactly what volume of solution was injected or retained into the perivitelline space after microinjection, the degree of swelling suggested that~10 to 100pl was introduced into the perivitelline space.

5. *In Vitro* Fertilizaion (IVF)

Matured and microinjected oocytes were washed twice with Fert-TALP (Rosenkrans et al., 1993) and then ten oocytes were transferred into a 44 μl of Fert-TALP droplet under paraffin oil. Insemination was achieved by adding a motile fraction of frozen-thawed bull sperm selected by discontinuous percoll gradient with a final sperm concentration of 2×10^6 cell/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 oocytes were coincubated in a 50 μl drop of Fert-TALP under paraffin oil at 39℃ for 24 hrs and 5% CO_2 in humidified atmosphere.

6. *In Vitro* Culture (IVC)

After 24 hrs insemination, the inseminated oocytes were stripped of any adherent sperm and transferred to the drops of culture medium, CR1aa (Rosenkrans and First, 1993) supplemented with 3 mg/ml fatty-acid-free BSA, 20 μl/ml MEN essential amino acid, 10 μl/ml MEN non-essential amino acid, 0.44 μg/ml Na pyruvate, 1.46 μg/ml glutamine, 25 μg/ml gentamycine under liquid paraffin at 39℃ in 5% CO_2 in air. Following culture for 48 hrs, all embryos were co-cultured with bovine oviduct epithelial cells in 500 μl of CR1aa containing 10% FBS for 4 days at 39℃ in an atmosphere of 5% CO_2 in air.

7. Investigating the EGFP Expression in the Embryos

The expression of EGFP in blastocyst placed in a microdrop of TL-HEPES medium (Sigma) was observed under the fluorescence microscope using an FITC filter.

8. PCR Analysis of Single Embryo

Embryos for PCR analysis were washed three times in sterile saline solution before transferring into a 0.2 ml PCR tube with 5 μl of autoclaved, deionized water. Embryo samples were stored at −20℃ until PCR analysis. Before PCR analysis, embryo samples were subjected to repeated freezing in liquid nitrogen and thawing at room temperature for three times. Each reaction material contained 1~2 μl cDNA solution, 50 picomol of each primer, 5 μl of 10 Ex Taq buffer, 8 μl of 25 mM dNTPs and 0.5 μl Ex Taq. The primer used for amplification of the hGH-specific sequences were as follows: 3'-GACAACCTCCCACGACCTTAT-5' (5' primer) and 3'-AGTCCTTCCTGTA CCTG-TTCC-5' (3' primer), which yielded a 345-bp fragment. The final volume of the PCR reaction was 50 μl and the amplification cycle was thirty-five cycles of 94℃ for 30 sec, 58℃ for 30 sec and 72℃ for 30 sec. After 35 cycles, PCR products were separated on a 1.7% agarose gel.

9. Statistical Analysis

The data from at least three replications were pooled. Differences in the percentages of oocytes developed to particular stages were estimated by Student's *t*-test.

Ⅲ. RESULTS

1. Morphological Classification Oocytes

By the treatment of 0.5% sucrose, oocytes were classified into two groups: oocytes with smooth plasma membranes and oocytes with rough plasma membranes (Fig. 2). Oocytes with smooth plasma membranes were regarded as good quality oocytes and retroviral vectors could be safely injected in their perivitelline spaces because of proper increase of perivitelline spaces by sucrose treatment. According to these results, good quality oocytes were only used in present studies.

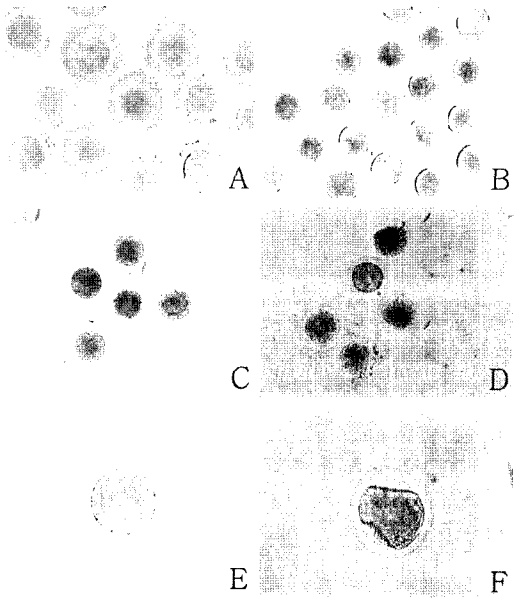

Fig. 2. Morphology of bovine oocytes treated with 0.5% sucrose after 17 hrs incubation in maturation medium. A; Oocytes before 0.5% sucrose treatment (×100), B; Oocytes after 0.5% sucrose treatment (×100), C; Good quality oocytes (×100), D; Poor quality oocytes (×100), E; Good quality oocyte (×200), F; Poor quality oocyte (×200).

2. Effects of Sucrose Treatment on *In Vitro* Fertilization of Oocytes

Oocytes were pretreated in mTCM-199 medium containing 0.5% sucrose and then subjected to *in vitro* fertilization. As shown in Table 1, the cleavage rates of sucrose non-treated and treated oocytes

Table 1. Effect of sucrose treatment on cleavage of oocytes after *in vitro* fertilization

Treatment on oocytes	No. of oocytes used for treatment	No.(%) of selected oocytes	No. of oocytes used for IVF	No.(%) of cleaved oocytes
Control	-	-	148	121(82)[a]
0.5% sucrose*	216	153(71)	153	128(84)[a]

* Matured oocytes were selected by the treatment in mTCM199 medium including 0.5% sucrose.

[a] Values with same superscripts within column mean no significant difference.

were 82 and 84%, respectively, with no significant difference. This result showed that the treatment of bovine M II oocytes with medium containing 0.5% sucrose was non-damaging.

3. Effects of Sucrose Treatment on *In Vitro* Fertilizaiton and Development of Oocytes Injected with Retroviral Vector

Retroviral vectors were microinjected into the perivitelline spaces of oocytes. The rates of cleavage and blastocyst in microinjection of LNβ-EGFP genes were 81 and 25%, and in microinjection of LNC-hGH gene were 83 and 30% (Table 2 and Table 3) and there was no significant difference ($p<0.05$) between control and microinjection group. Therefore, these results showed that embryos infected by retroviral vector after treatment of 0.5% sucrose could develop to blastocyst stage with no significant difference from control group.

Table 2. *In vitro* development of embryos injected with retroviral vectors, LNβ-EGFP into perivitelline spaces of bovine oocytes

Treatment on oocytes	No. of oocytes	No.(%) of cleaved embryos	No.(%) of blastocysts
Control	10^3	77(75)[a]	24(23)[b]
0.5% sucrose*	15^3	124(81)[a]	38(25)[b]

* Matured oocytes were selected by the treatment in mTCM199 medium including 0.5%sucrose.

[a,b] Values with same superscripts within column mean no significant difference.

Table 3. *In vitro* development of embryos injected with retroviral vectors, LNC-hGH into perivitelline spaces of bovine oocytes

Treatment on oocytes	No. of oocytes	No.(%) of cleaved embryos	No.(%) of blastocysts
Control	10^9	84(77)[a]	25(23)[b]
0.5% sucrose*	19^7	164(83)[a]	59(30)[b]

* Matured oocytes were selected by the treatment in mTCM199 medium including 0.5% sucrose.

[a,b] Values with same superscripts within column mean no significant difference.

4. Integration and Expression of Foreign Gene in Embryos Microinjected with LN β-EGFP and LNC-hGH Genes into Perivitelline Spaces

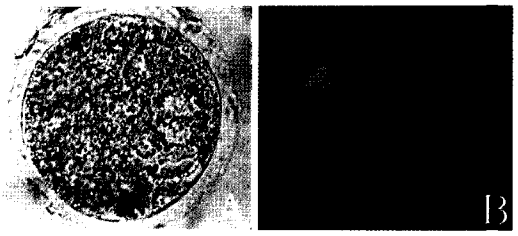

Fig. 3. Representative microscopic images of the day 7 blastocyst developed from the oocytes microinjected with LNβ-EGFP after 0.5% sucrose treatment. A; Light stereomicroscopic image, B; Fluorescence microscope image under the EGFP excitation light.

Embryos developed to blastocysts after microinjection of LNβ-EGFP and LNC-hGH genes into perivitelline spaces of oocytes were investigated to confirm the integration and expression of foreign DNA. As shown in Fig. 3 and Fig. 4, 34% (13/38) of blastocysts infected by LNβ-EGFP was observed under fluorescence microscope using a standard FITC filter set. No mosaicism was shown. As shown in Fig. 4 and Fig. 5, 52% (50/96) of cleaved embryos infected by LNC-hGH were also detected by PCR analysis. Therefore, these results indicated that although microinjected oocytes were pretreated in mTCM-199 medium containing 0.5% sucrose, retroviral vector genes were introduced into bovine embryos.

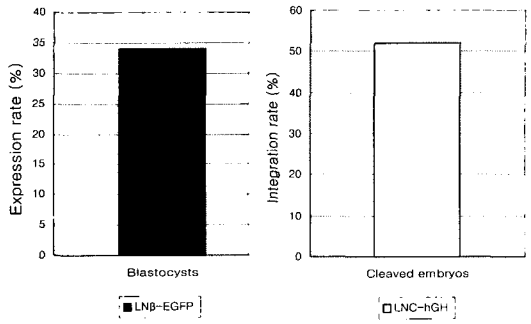

Fig. 4. Expression and integration rates in bovine embryos developed from oocytes microinjected with LNβ-EGFP and LNC -hGH. Expression of EGFP-gene was observed under fluorescence microscope in 34% (13/38) of infected blastocysts. Integration of hGH gene was detected by PCR analysis and 52% (50/96) of cleaved embryos were infected.

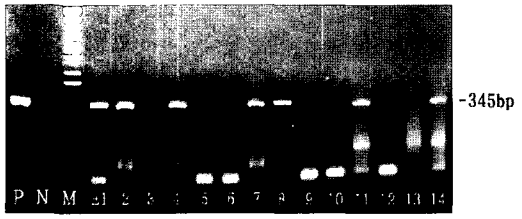

Fig. 5. PCR analysis of bovine embryos developed from oocytes after microinjection of LNC-hGH into perivitelline space. Lanes 1~14; LNC-hGH injected embryos, M; 100bp molecular size marker, P; positive control, N; negative control (non-injected bovine embryo).

Ⅳ. DISCUSSION

Sucrose has been widely used, particularly in embryo cryopreservation and nuclear transfer (Rall, 1987; Collas and Barnes, 1994). Its safety for further embryo development were also proven (Saito et al., 1994). Agca et al. (2000) reported oocytes at the M Ⅱ stage were no more sensitive to anisotonic stress than GV oocytes. Moreover, 11% of poor quality M Ⅱ oocytes could be selected by 3% sucrose treatment in mice (Wang et al., 2001).

The objective of this study was to investigate the effects of sucrose treatment on the morphology of oocytes, *in vitro* development of infected embryos and the integration and expression of foreign DNA microinjecited into perivitelline spaces of oocytes. When the oocytes were treated with medium containing 0.5% sucrose, non-harmful effect was observed and they were morphologically classified into two groups, good and poor quality oocytes. The former maintained smooth plasma membranes, but the latter showed rough plasma membrane and enlarged perivitelline space. Actually, about 71% were selected as good quality oocytes after 17hr incubation with 0.5% sucrose (Table 1). According to these results, good quality oocytes could be selected for the injection of retroviral vectors into perivitelline spaces. Same trends was obtained from the oocytes fertilized after microinjection of LNβ -EGFP and LNC-hGH into perivitelline spaces and no significant differences were observed in cleavage rates between control group and microinjection group (Table 2 and Table 3). Therefore, these re-

sults indicated that treatment of bovine MⅡ oocytes with medium containing 0.5% sucrose was a stable method for microinjection of retroviral vectors into perivitelline spaces and the embryos infected by this method could develop to blastocyst stages with no significant difference from the control group.

Saito et al. (1994) reported that the use of sucrose could induce the shrinkage of the cell. Bortner and Cidlowski (1996) indicated that the absence of volume regulatory mechanisms contribute to the rapid activation of apoptosis in thymocytes. Hyperosmotic shock induced both activation and translocation of glucose transporters in mammalian cells (Barros et al., 2001). Furthermore, recent work showed that apoptosis was detected in *in vitro* immature and matured oocytes (Matwee et al., 2000). These reports suggested that the proportion of apoptosis in oocytes of poor quality would be higher than that in good quality oocytes. Thus we obtained these results that *in vitro* development of selected oocytes after microinjection of retroviral vector could develop to blastocyst stage with no significant difference from the control group. Moreover, as compared with the previous report in which the expression rate of foreign DNA in oocytes and zygote microinjected with LacZ-gene into perivitelline spaces were 56 and 22%, respectively (Chan et al., 1998), gene transfer efficiency of foreign gene in bovine oocytes selected by sucrose treatment was similar as shown in Fig. 4.

From the results obtained in present studies, it could be the fact that good quality bovine MⅡ oocytes was selected by medium containing 0.5% sucrose, retroviral vectors were injected into perivitelline space increased by sucrose without damage and bovine embryos infected by retroviral vectors could support further developing to term. Therefore, this method will provide a powerful research tool for studying microoperation in other animals.

Ⅴ. REFERENCE

1. Agca, Y., Liu, J., Rutledge, J. J., Critser, E. S. and Critser, J. K. 2001. Effect of osmotic stress on the developmental competence of germinal vesicle and metaphase Ⅱ stage bovine cumulus oocyte complexes and its relevance to cryopreservation. Mol. Reprod. Dev., 55:212-219.
2. Barros, L. F., Barnes, K., Ingram. J. C., Castro, J.. Porras, O. H. and Baldwin, S. A. 2001. Hyperosmotic shock induces both activation and translocation of glucose transporters in mammalian cells. Eur. J. Physiol., 442:614 -621..
3. Burns, J. C., Friedmann, T., Driever, W., Burrascano, M. and Yee, J. K. 1993. Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: Concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. Proc. Natl. Acad. Sci, USA., 90:8033-37.
4. Bortner, C. D. and Cidlowski, J. A. 1996. Absence of volume regulatory mechanisms contributes to the rapid activation of apoptosis in thymocytes. Am. J. physiol., 271:C950- C961.
5. Chan, A. W. S., Horman, E. J., Ballou, L. U., Burns, J. C. and Bremel, R. D. 1998. Transgenic cattle produced by reverse- transcribed gene transfer in oocytes. Proc. Natl. Acad Sci. USA., 95:14028 -33.
6. Chan, A. W. S., Chong, K. Y., Martinovich, C., Simerly, C. and Schatten, G. 2001. Transgenic monkeys produced by retroviral gene transfer into mature oocytes. Science., 291:309-12.
7. Collas, P. and Barnes, F. L. 1994. Nuclear transplantation by microinjection of inner cell mass and granulosa cell nuclei. Mol. Reprod. Dev., 38:264-267.
8. Haskell, R. E. and Bowen, R. A. 1995. Effi-

cient production of transgenic cattle by retroviral infection of early embryos. Mol. Reprod. Dev., 40:386-390.

9. Matwee C., Dean, H. B. and King, W. A. 2000. Apoptosis in the early bovine embryo. Zygote, 8:57-58.
10. Muller U. 1999. Ten years of gene targeting: targeted mouse mutants, from vector design to phenotype analysis. Mech. Dev., 82(1-2):3-21.
11. Miller, A. D., Garcia, J. V., Suhr, N. V., Lynch, C. M., Wilson, C. and Eiden, M. V. 1991. Concentration and properties of retrovirus packaging cells based on Gibbon ape leukemia virus. J. Virol., 65:2220-24.
12. Kim, T., Lee, Y. M., Lee, H. T., Heo, Y. T., Yom, H. C., Kwon, M. S., Koo, B. C., Whang, K. and Roh, K. S. 2001. Expression of the E. coli LacZ gene in chicken embryos using replication defective retroviral vectors packaged with vesicular stomatitis virus G glycoprotein envelopes. to be published in Asian-Australasian J. Animal Sci., 14:163-169
13. Parrish, J. J., Susko-Parrish, J., Winer, M. A. and First, N. L. 1988. Capacitation of bovine sperm by heparine. Biol. Reprod., 38:1171-1180
14. Rall, W. F. 1987. Factors affecting the survival of mouse embryos cryopreserved by vitrification. Cryobiology, 24:387- 402
15. 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. Reprod., 49:459-462.
16. Saito, N., Imai, K. and Tomizawa, M. 1994. Effect of sugars addition on the survival of vitrified bovine blastocysts produced *in vitro*. Theriogenology, 41:1053-1060.
17. Soriano, P., Cone, R. D., Mulligan, R. C. and Jaenish, R. 1986. Tissue-specific and ectopic expression of genes introduced into transgenic mice by retroviruses. Science, 234:1409-1413.
18. Wall, R. J. 1996. Transgenic livestock: Progress and prospects for the future. Theriogenology, 45:57-68.
19. Wang, M. K., Liu, J. L., Li, G. P., Lian, L. and Chen, D. Y. 2001. Sucrose pretreatment for enucleation: An efficient and non-damage method for removing the spindle of the mouse M Ⅱ oocyte. Mol. Reprod. Dev., 58:432-436.
20. Yee, J. K., Friedmann, T. and Burns, J. C. 1994. Generation of high-titer pseudotyped retroviral vectors with very broad host range. Methods in Cell Biol., 43:99-112.

요 약

소 난자에서 형태와 외래 DNA Integration에 관한 Sucrose 처리의 효과

김선균 · 김강식 · 김태완[1] · 이훈택 · 정길생[†]

건국대학교 농축산생명과학대학

MⅡ단계 난자의 위란강에 retroviral vector를 주입하여, 형질전환 난자의 생산하려는 연구가 수행되고 있다. 그러나 이러한 난자의 위란강의 크기는 매우 다양하므로, 외래유전자를 위란강에 미세주입을 할 때, 난자의 세포질에 손상을 줄 수가 있다. 이에 본 연구에서는 외래유전자 주입시 발생할 수 있는 난자세포의 손상을 최소화하기 위하여 sucrose처리법을 채택하였다. 즉 난자를 0.5%의 sucrose가 첨가된 배양액으로 처리함으로써 일정한 형태의 세포질을 유지하지 못하는 난자와 일정한 형태의 세포질을 유지하는 난자로 분류할 수 있었으며, 후자의 경우 세포질의 큰 손상 없이 retroviral vector를 난자의 위란강내에 주입할 수 있었다. 그러나 sucrose처리에 의해 선별된 난자의 수정율과 대조군의 그것 사이에는 유의차가 없었다. 또 sucrose처리에 의해 선발된 난자에 있어서 retroviral vector (LNβ-EGFP and LNC-hGH) 주입 후의 분할율과 배반포발달율 같은 양상을 보였다. LNβ-EGFP이 주입된 경우, 분할율과 배반포율이 81 및 25% 보였으며, LNC-hGH이 주입된 경우, 83 및 30%를 보였다. 그 결과 미세주입된 난자는 대조군과 유의적인 차이 없이 발달할 수 있었다. 게다가, hGH-gene의 결합율이 PCR 분석에 의하여 분할된 난자에서 52%를 보였으며, 또한 EGFP-gene의 발현율이 현광현미경을 통해 배반포난자에서 34%가 관찰되었다. 이상의 결과를 종합할 때, 0.5%의 sucrose 처리는 우량난자의 선발을 가능하게 하였으며, 주입유전자의 발현율이 낮아지지 않았을 뿐만 아니라, 외래유전자의 위란강내 주입시 난자에 대한 물리적 손상을 줄일 수 있으므로 발달율을 개선할 수 있는 것으로 사료된다.

(접수일자 : 2001. 10. 22. / 채택일자 : 2001. 11. 20.)