韓國家畜繁殖學會誌 24(4) : 365~373(2000)
Korean J. Animal Reprod.

Effect of Fusion Procedure on the Development of Embryos Produced by Somatic Cell Nuclear Transfer in Hanwoo (Korean Cattle)

Im, G. S[1]., B. S. Yang[1], S. J. Park[1], W. K. Chang[1] and C. S. Park[2†]
Department of Animal Science, Chungnam National University

ABSTRACT

The purpose of this study was to investigate the effects of the fusion pulses and fusion media on fusion rate and the development of embryos produced by somatic cell nuclear transfer in Hanwoo (Korean cattle).

Nuclear donor cumulus and fetal fibroblast cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum at 38.5℃ in a humidified atmosphere of 5% CO_2 in air. The *in vitro* matured oocytes were enucleated and then the isolated donor cells were introduced. The cumulus cell and cytoplast were fused using one pulse of 70 volts for 40 μs, two pulses of 70 volts for 40 μs and one pulse of 180 volts for 15 μs. The fetal fibroblast cell and cytoplast were fused using one pulse of 180 volts for 15 μs or 30 μs. The cumulus cell and cytoplast were fused using mannitol and Zimmermann cell fusion medium (ZCFM) as a fusion medium. The fused embryos were activated after the fusion with 10 μM calcium ionophore for 5 min and 2 mM 6-dimethyl- aminopurine for 3 h. The nuclear transfer embryos were cultured in 500 μl well of modified CR1aa supplemented with 3 mg/ml BSA in the four well dish covered with mineral oil. After 3 days culture, culture medium was changed into modified CR1aa medium containing 1.5 mg/ml BSA and 5% FBS for 4 days. The incubation environment was 5% CO_2, 5% O_2, 90% N_2 at 38.5℃.

When the cumulus cells were fused with enucleated oocytes by three different fusion pulses, one pulse of 180 volts for 15 μs yielded the highest fusion rate and developmental rate to blastocyst among the pulses ($P<0.05$).

When the fetal fibroblast cells were fused with enucleated oocytes, one pulse of 180 volts for 30 μs yielded significantly higher fusion rate compared with that for 15 μs($P<0.05$). The present result indicates that the fusion rate between karyoplast and cytoplast was affected by the cell type and the optimal fusion condition was different according to cell type or size. When the fusion was conducted by the use of mannitol and ZCFM, the fusion rate was 71.2% and 65.8%, respectively. The

[1] National Livestock Research Institute, RDA, Suwon 441-350, Korea.
[2] Department of Animal Science, Chungnam National University, Daejon 305-764, Korea.
[†] Corresponding author : Department of Animal Science, Chungnam National University, Daejon 305-764, Korea. E-mail: parkcs@cuvic.cnu.ac.kr

developmental rates to blastocyst were 37.8% and 39.8%, respectively. There was no significant difference between two fusion media in the developmental rate of cumulus cell nuclear transfer embryos. These results indicate that optimal electric current should be selected according to cell type.
(Key words : Somatic cell nuclear transfer, Fusion pulse, Enucleated oocytes)

Ⅰ. INTRODUCTION

Nuclear transplantation is to introduce the exogenous nucleus into the cytoplast. The introduced nucleus undergoes nuclear envelope breakdown (NEBD) and premature chromosome condensationn (PCC), which are then followed by nuclear reformation (Campbell et al., 1993). Accordingly, cell to cell fusion is the essential procedure. In the beginning, the inactivated Sendai virus (HVJ) was used in rabbit (Bromhall, 1975; Stice et al., 1987), mouse (McGrath and Solter, 1983), sheep (Willadson, 1986) and cattle (Robl et al., 1987). Sendai virus was not effective for bovine oocytes. So, bovine virus, bovid herpesvirus known to cause fusion of cultured cells, was used. This method, however, was not effective (Robl et al., 1987). Willadson (1986) and Robl et al. (1987) reported that electrofusion was the efficient method compared with Sendai virus. Since then, electrofusion have been commonly used.

Electrofusion of mammalian cells induces the destablization of closely associated bilayer lipid membranes between adjacent two cells. The precise way in which a direct current pulse disrupts cell membranes is not clear. After electrofusion treatment, the membranes recover to minimize the surface tension of the lipid membrane bilayer. This results in a point membrane fusion between two cells (Zimmermann and Greyson, 1983; Zimmermann, 1986; Hofmann and Evans, 1986).

Researchers reported that factors affecting fusion rate were cell size, voltage and fusion medium. Tatham et al. (1996) reported that the optimal electrofusion parameters should be selected according to the various cell types. Zakhartchenko et al. (1995, 1997) also reported that the fusion rate was different according to different karyoplast-cytoplast ratios and cell size, and a higher voltage was needed to induce the disruption of the membrane of small cell (Zimmermann and Vienken, 1982). Vignon et al. (1998) reported that 60~65% fusion rate was accomplished when somatic cells were fused with recipient oocytes using two pulses of 1.2 KV/cm for 50 μs, and Shiga et al. (1999) reported that 54.7~65% fusion rate was obtained when the fusion between karyoplast and cytoplast using two pulses of 700 to 800 V/cm for 50 μs was carried out.

Willadson (1986) first reported the production of sheep by nuclear transfer using mannitol solution as the fusion medium. Robl et al. (1987) reported that Zimmerman cell fusion medium (ZCFM) showed higher fusion rate compared with TL Hepes. Since then, Zimmermann cell fusion medium (Robl et al., 1987; Stice and Keefer, 1993; Kato et al. 1998) and mannitol (Kono et al., 1994; Heyman, 1994; Baguisi et al., 1999) have been commonly used as the fusion medium. Therefore, this study was carried out to investigate the optimal fusion condition.

Ⅱ. MATERIALS AND METHODS

1. Oocyte Collection and Maturation

Ovaries were collected from the local slaughter house and transported using the 25~30℃ saline in a thermos to the laboratory. Ovaries were washed three times with the saline and stored until aspira-

tion. Cumulus-oocyte complexes (COCs) were recovered by aspiration of 2~6 mm follicles using a 10 ml syringe with 18 gauge needle and stored in Dulbecco's phosphate buffered saline (D-PBS, Gibco, USA) supplemented with 5% fetal bovine serum (FBS, Gibco, USA). The aspirated follicular fluid was put in a 60 mm petri dish (Falcon, USA) and COCs were evaluated under the stereo-microscope (×40).

Only the COCs surrounded with at least three or four compact layers of cumulus cells and with evenly distributed cytoplasm were selected for *in vitro* maturation. The maturation medium was tissue culture medium 199 (TCM199, Gibco, USA) supplemented with 10% FBS and 1% antibiotic-antimycotic solution (Gibco, USA). The COCs were washed three times with maturation medium and then cultured for 20~22 h in preincubated 500 μl drop of maturation medium in a 35 mm petri dish (Falcon, USA) covered with mineral oil (E. R. Squib & Sons, Inc., USA). The incubation environment was 5% CO_2 and 95% humidified air at 38.5℃.

2. Nuclear Transfer Procedure

1) Enucleation

Cumulus cells were removed from the oocytes matured for 18~20 h by vortexing in calcium and magnesium free PBS supplemented with 0.1% hyalruronidase (Sigma, USA) for 5 min. Only oocytes with the first polar body were selected as recipient oocytes. All micromanipulations were carried out at room temperature using micromanipulator (Nikon Narishige, Japan) and inverted microscope (Nikon, Japan). The recipient oocytes were placed in 30 μl drop of TCM199 supplemented with 20% FBS and 50 μg/μl phytohemaglutinin (PHA, Sigma, USA) (Keefer et al., 1994).

The oocytes were held by holding pipette (outer diameter was approximately 90~120 μm). Enucleation of oocytes was carried out by cutting the part of zona pellucida near the first polar body, and then a small volume of cytoplasm surrounding the first polar body was squeezed out through the slit made at enucleation with a cutting needle. Enucleated oocytes were transferred to TCM199 supplemented with 20% FBS and washed three times.

2) Donor Cell Preparation

(1) Cumulus Cell

After the COCs were collected by the same method as that of *in vitro* maturation, the COCs were washed three times in calcium and magnesium free PBS supplemented with 5% FBS by centrifugation at 1,200 rpm for 5 min and then resuspended with Dulbecco's modified Eagle medium (D-MEM, Gibco, USA) supplemented with 10% FBS and 1% antibiotic-antimycotic solution. The suspended cumulus cells were placed in 100 mm petri dish (Falcon, USA) and cultured at an atmosphere of 5% CO_2 in 95% humidified air at 38.5℃.

After 24 h culture, the oocytes were washed by replacing the culture medium. The culture medium was replaced with fresh medium at every 48~72 h. The attached cumulus cells were passaged by the trypsinization or cryopreserved when the cumulus cell became confluent. Single cell isolation was undertaken by the incubation in 0.5% trypsin (Sigma, USA) solution for 5 min.

(2) Fetal Fibroblast Cell

The fetal fibroblast cells to be used were derived from 40~50 days old fetus from the local slaughter house. The fetus was transported to the laboratory with the ice. A brain and red tissue were removed. After washing with calcium and magnesium free

PBS, single cell suspension was undertaken by the trypsinization of fetal tissue with 0.04% trypsin and 0.02% EDTA (Sigma, USA) in D-MEM at 38.5℃ for 10 min. Cells were washed by centrifugation at 1,000 rpm for 10 min, and then resuspended with D-MEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution. Resuspended cells were placed into 100 mm petri dish and incubated at 5% CO_2 in 95% humidified air at 38.5℃. The culture medium was replaced at firstly 24 h and every 48~72 h. When the cell became confluent, cells were passaged by the trypsinization or cryopreserved. Single cell isolation was undertaken by the incubation in 0.5% trpysin solution for 5 min.

3) Nuclear Transfer

Enucleated oocytes were placed in 30 μl drop of the same medium as that of enucleation, and donor cells were placed in 20 μl drop of calcium and magnesium free PBS supplemented with 5% FBS. Donor cells were introduced into the perivitelline space of the recipient oocytes through the hole made at enucleation by the injection pipette (approximately inner diameter was 10~15 μm). The nuclear transferred oocytes were washed three times in TCM199 supplemented with 20% FBS.

4) Fusion

Fusion between karyoplast and cytoplast was accomplished by the use of electric pulse. The karyoplast and cytoplast complexes were placed in the fusion medium for equilibration and then placed between the 1 mm gap electrofusion chamber (PN 450-1) filled with the fusion medium. The karyoplast and cytoplast complexes were aligned manually with mouth pipette and then electric current was applied by an Electro Cell Manipulator ECM® (BTX Inc, USA). After the fusion treatment, the karyoplast-cytoplast complexes were washed in TCM199 supplemented with 20% FBS, and the fusion was evaluated after 1 h.

5) Activation of Embryos Produced by Nuclear Transfer

To activate the fused embryos, the embryos were treated with 10 μM calcium ionophore for 5 min and immediately followed by 2 mM 6-dimethylaminopurine for 3 h.

3. *In vitro* Culture

After the activation treatment, fused embryos were washed three times with culture medium and then cultured on the cumulus cell feeder layer of $1\sim1.2\times10^5$/ml in 500 μl well of modified CR1aa supplemented with 3 mg/ml BSA in the four well dish (Nunclon, USA) covered with mineral oil. Co-culture cumulus cells were prepared by the treatment with 10 μg/μl mitomycin C for 2.5 h. The culture media were changed according to experiment and details were described in experimental design. The incubation environment was 5% CO_2 in 95% humidified air or 5% CO_2, 5% O_2, 90% N_2 at 38.5℃.

4. Experimental Design

Experiment 1 was designed to evaluate the effect of fusion pulse on fusion rate and the development of embryos produced by cumulus cell nuclear transfer. The fusion pulse between cumulus cell and cytoplast was one pulse of 70 volts for 40 μs, two pulses of 70 volts for 40 μs and one pulse of 180 volts for 15 μs.

Experiment 2 was designed to evaluate the effect of fusion pulse on fusion rate and the development of embryos produced by fetal fibroblast cell nuclear transfer. The fusion pulse between cumulus cell and cytoplast was one pulse of 180 volts for 15 μs or 30 μs.

Experiment 3 was designed to evaluate the effect

of fusion medium on fusion rate and the development of embryos produced by cumulus cell nuclear transfer. The fusion media were 0.3 M mannitol supplemented with 0.05 mM $CaCl_2$ and 0.1 mM $MgSO_4$, and ZCFM.

5. Statistical Analysis

Data obtained from this study was analyzed by using ANOVA. Differences between treatments were compared by using Duncan's multiple range test and Student's t-test of SAS (Version 6.12).

Ⅲ. RESULTS

1. Effect of the Fusion Pulse between the Donor Cumulus Cell and Recipient Oocyte

In order to investigate the effect of fusion pulse on the fusion rate between the donor cumulus cell and recipient oocyte, three fusion pulses were used. As shown in Table 1, the fusion rate was significantly higher ($P<0.05$) in one pulse of 180 volts for 15 μs compared with other treatment groups. Also, the developmental rate to blastocyst was significantly higher in one pulse of 180 volts for 15 μs($P<0.05$).

2. Effect of the Fusion Pulse between the Donor Fetal Fibroblast Cell and Recipient Oocyte

In order to investigate the effect of fusion pulse on the fusion rate between the donor fetal fibroblast cell and recipient oocyte, two fusion pulses were used.

As shown in Table 2, the fusion rate was significantly higher in one pulse of 180 volts for 30 μs compared with one pulse of 180 volts for 15 μs. However, there was no significant difference between two pulses in developmental rates of embryos.

3. Effect of the Fusion Medium on Fusion Rate and the Development of Embryos Produced by Cumulus Cell Nuclear Transfer

To investigate the effect of fusion medium on the development of embryos produced by cumulus cell nuclear transfer, the fusion was conducted by the use of mannitol and ZCFM as the fusion medium. The fusion rates in mannitol and ZCFM were 71.2% and 65.8%, respectively. The developmental rates to blastocyst were 37.8% and 39.8%, respectively.

There was no significant difference between two fusion media in the developmental rates of embryos. These results suggested that both mannitol and ZCFM could be used for cumulus cell fusion.

Ⅳ. DISSCUSSION

Table 1. Effect of fusion pulse on the fusion rate and development of embryos produced by cumulus cell nuclear transfer in Hanwoo

Fusion pulse[1]	No. of oocytes manipulated	No. (%) of oocytes fused	No. (%) of oocytes developed to	
			2 cell	Blastocyst
70 V, 40 μs, 1 time	32	0(0.0)^{c}	0(0.0)^{b}	0(0.0)^{b}
70 V, 40 μs, 2 times	59	15(25.4)^{b}	13(86.7)^{a}	2(13.3)^{b}
180 V, 15 μs, 1 time	62	36(58.1)^{a}	28(77.8)^{a}	13(36.1)^{a}

^{a,b,c} Means with different superscripts within the same columns were significantly different ($P<0.05$).

[1] Fusion voltage was applied by using 1 mm gap electrofusion chamber (PN450-1) of an Electro Cell Manipulator ECM® (BTX Inc, USA).

Table 2. Effect of fusion pulse on the fusion rate and development of embryos produced by fetal fibroblast cell nuclear transfer in Hanwoo

Fusion pulse[1]	No. of oocytes manipulated	No. (%) of oocytes fused	No. (%) of oocytes developed to	
			2 cell	Blastocyst
180 V, 15 μs 1 time	81	37(45.7)[b]	30(81.1)	9(24.3)
180 V, 30 μs 1 time	95	60(63.2)[a]	51(85.0)	15(25.0)

[a,b] Means with different superscripts within the same column were significantly different ($P<0.05$).

[1] Fusion voltage was applied by using 1 mm gap electrofusion chamber (PN450-1) of an Electro Cell Manipulator ECM® (BTX Inc, USA).

Table 3. Effect of fusion medium on the fusion rate and development of embryos produced by cumulus cell nuclear transfer in Hanwoo

Fusion medium[1]	No. of oocytes manipulated	No. (%) of oocytes fused	No. (%) of oocytes developed to	
			2 cell	Blastocyst
Mannitol	104	74(71.2)	56(75.7)	28(37.8)
ZCFM	149	98(65.8)	84(85.7)	39(39.8)

[1] Mannitol: 0.3 M Mannitol supplemented with 0.05 mM $CaCl_2$ and 0.1 mM $MgSO_4$, and ZCFM: Zimmermann Cell Fusion Medium.

When the cumulus cells were fused with enucleated oocytes using one pulse of 70 volts for 40 μs, two pulses of 70 volts for 40 μs and one pulse of 180 volts for 15 μs, one pulse of 180 volts for 15 μs yielded the highest fusion rate among fusion pulses ($P<0.05$). This result was consistent with the report of Cibelli et al. (1998) who produced fetal fibroblast cell nuclear transfer embryo using one pulse of 180 volts for 15 μs. Kato et al. (1998) also used two pulses of 150 volts for 25 μs and obtained the fusion rate of 47.5% for cumulus cell and 67.7% for oviduct cell. Zakhartchenko et al. (1999) also used two pulses of 2.1 KV/cm for 10 μs and obtained the fusion rate of 63% for mammary gland cells and 73% for ear cells. Vignon et al. (1998) used one pulse of 1.21 KV/cm for 50 μs and obtained fusion rate of 54.0 ~74.2% for muscle cell and 48.2~65.9% for skin cell.

In nuclear transfer using blastomere, the fusion voltage between blastomere and cytoplast was approximately from 0.7~1.3 KV/cm (Heyman et al., 1994;Kono et al., 1994;Westhusin et al., 1992). The pulse used in nuclear transfer using blastomere was lower than that using somatic cell. These facts demonstrated that for smaller cells, a higher voltage was needed to induce smaller cell membrane electrical breakdown for the accomplishment of higher fusion rate (Zimmerman and Vienken, 1982).

Unlike cumulus cell, when the fetal fibroblast cells were fused with enucleated oocytes using one pulse of 180 volts for 15 μs or 30 μs, one pulse of 180 volts for 30 μs yielded significantly higher fusion rate ($P<0.05$). The present result suggested that fusion rate between karyoplast and cytoplast could be affected by the cell type and the optimal fusion condition may be changed according to cell type or size (Zakhartchenko et al., 1995, 1997;

Tatham et al., 1996).

In this study, mannitol and ZCFM were used and obtained the fusion rate of 71.2% and 65.8%, respectively. There was no significant difference between fusion media for the fusion rate and the developmental rate to blastocysts. In bovine nuclear transfer using blastomere, ZCFM was most commonly used (Robl et al., 1987; Stice and Keefer, 1993; Van Stekelenburg-Hammers et al., 1993; Okoshi et al., 1997). Mannitol was commonly used in other species such as mouse, rabbit, pig and sheep (Kono and Tsunoda, 1988; Collas and Robl, 1990; Prather et al., 1989; Smith and Wilmut, 1989). Whereas, in somatic cell nuclear transfer for bovine, both of ZCFM and mannitol were commonly used (Kato et al., 1998; Vignon et al., 1998; Shin et al., 1999). This result suggested that both mannitol and ZCFM could be used as the fusion medium for the fusion of somatic cell nucleus.

V. REFERENCES

1. Baguisi, A., Behboodi, E., Melican, D. T., Pollock, J. S., Destempes, M. M., Cammuso, C., Williams, J. L., Nims, S. D., Porter, C. A., Midura, P., Palacios, M. J., Ayres, S. L., Denniston, R. S., Hayes, M. L., Ziomek, C. A., Meade, H. M., Godke, R. A., Galvin, W. G., Overstrom, E. W. and Echelard, Y. 1999. Production of goats by somatic cell nuclear transfer. Research 17:456-461.
2. Bromhall, J. D. 1975. Nuclear transplantation in the rabbit egg. Nature 258:719-721.
3. Campbell, K. H. S., Ritchie, W. A. and Wilmut, I. 1993. Nuclear cytoplasmic interactions during the first cell cycle of nuclear transfer reconstructed bovine embryos : Implications for deoxiribonucleic acid replication and development. Biol. Reprod. 49:933-942.
4. Cibelli, J. B., Stice, S. L., Goluke, P. J., Kane, J. J., Jerry, J., Blackwell, C., Abel Ponce de Leon, F. and Robl, J. M. 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280:1256-1258.
5. Collas, P. and Robl, J. M. 1990. Factors affecting the efficiency of nuclear transplantation in the rabbit embryo. Biol. Reprod. 43:877-884.
6. Heyman, Y., Chesne, P., Lebourhis, D., Peynot, N. and Renard, J. P. 1994. Developmental ability of bovine embryos after nuclear transfer based on the nuclear source : *in vivo* versus *in vitro*. Theriogenology 42:695-702.
7. Hofmann, G. A. and Evans, G. A. 1986. Electronic genetic physical and biological aspects of cellular electromanipulation. Engineering in medicine and biology magazine. December. pp. 6-24.
8. Kato, Y., Tani, T., Sotomaru, Y., Kurogawa, K., Kato, J. Y., Doguchi, H., Yasue, H. and Tsunoda, Y. 1998. Eight calves cloned from somatic cells of a single adult. Science 282: 2095-2098.
9. Keefer, C. L., Stice, S. L. and Matthews, D. L. 1994. Bovine inner cell mass cells as donor nuclei in the production of nuclear transfer embryos and calves. Biol. Reprod. 50:935-939.
10. Kono, T. and Tsunoda, Y. 1988. Effect of induction current and other factors on large scale electrofusion for pronuclear transplantation of mouse eggs. Gamete Res. 19:349-357.
11. Kono, T., Sotomaru, Y., Aono, F., Takahasi, T., Ogiwara, I., Sekizawa, F., Arai, T. and Nakahara, T. 1994. Effect of ooplast activation on the development of oocytes following nucleus transfer in cattle. Theriogenology 1463:1471 (Abstr.).
12. McGrath, J. and Solter, D. 1983. Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. Science 220:1300-

1302.

13. Okoshi, K., Hata, M., Kato, Y. and Tsunoda, Y. 1997. Effect of age of donor embryos on the developmental ability of nuclear transferred eggs. J. Reprod. Dev. 43:261-265.
14. Prather, R. L., Sims, M. M. and First, N. L. 1989. Nuclear transplantation in early pig embryos. Biol. Reprod. 41:414-418.
15. Robl, J. M., Prather, R., Barnes, F., Eystone, W., Northy, D., Giligana, B. and First, N. L. 1987. Nuclear transplantation in bovine embryos. J. Anim. Sci. 64:642-647.
16. Shiga, K., Fujita, T., Hirose, K., Sasae, Y. and Nagai, T. 1999. Production of calves by nuclear transfer of nuclei from cultured somatic cells aobtained from Japanese black bulls. Theriogenology 52:527-535.
17. Shin, T. Y., Rho, S., Kim, S. K., Lee, B. C. and Hwang, W. S. 1999. Successful nuclear transplantation of fetal fibroblast cells as donor nuclei in Korean Native Cattle. Theriogenology 51:214(Abstr.).
18. Smith, L. C. and Wimut, I. 1989. Influence of nuclear and cytoplasmic activity on the development *in vivo* of sheep embryos after nuclear transplantation. Biol. Reprod. 40:1027-1035.
19. Stice, S. L. and Keefer, C. L. 1993. Multiple generational bovine embryo cloning. Biol. Reprod. 48:715-719.
20. Stice, S. L., Bean, H. M. and Robl, J. M. 1987. Nuclear activation and transplantation in the rabbit oocyte. Biol. Reprod. 36(Suppl.1) : 93 (Abstr.).
21. Tatham, B. G., Giliam, K. J. and Trounson, A. O. 1996. Electrofusion parameters for nuclear transfer predicted using isofusion contours produced with bovine embryonic cells. Mol. Reprod. Dev. 43:306-312.
22. Van Stekelenburg-Hammers, A. E. P., Van Inzen, W. G., Van Achterberg, T. A. E., Kruip, T. A. M., De Latt, S. W. and Weima, S. M. 1993. Nuclear transfer and electrofusion *in vitro*- matured/ -fertilized bovine embryos : effect of media and electrical fusion parameters. Mol. Reprod. Dev. 36:307-312.
23. Vignon, X., Chesne, P., Bourhis, D. L., Flechon, J. E., Heyman, Y. and Renard, J. P. 1998. Developmental potential of bovine embryos reconstructed from enucleated matured oocytes fused with cultured somatic cells. Life Science 321:735-745.
24. Westhusin, M. E., Leavanduski, M. J., Scarborough, R., Looney, C. R. and Bondioli, K. R. 1992. Viable embryos and normal calves after nuclear transfer into hoechst stained enucleated demi oocytes of cows. J. Reprod. Fertil. 95: 475-480.
25. Willadson, S. M. 1986. Nuclear transplantation in sheep embryos. Nature 320:63-65.
26. Zakhartchenko, V., Wolf, E., Palma, G. A. and Brem, G. 1995. Effect of donor embryo cell number and cell size on the efficiency of bovine embryo cloning. Mol. Reprod. Dev. 42: 53-57.
27. Zakhartchenko, V., Stojkovic, M., Brem, G. and Wolf, E. 1997. Karyoplast-cytoplast volume ratio in bovine nuclear transfer embryos : effect on developmental potential. Mol. Reprod. Dev. 48:332-338.
28. Zakhartchenko, V., Scherthaner, W., Prelle, K., Stoijkovic, P., Brem, G. and Wolf, E. 1999. Nuclear transfer in the bovine embryo : developmental potential of cultured adult cells. Theriogenology 51:218(Abstr.).
29. Zimmermann, U. and Greyson, J. 1983. Electric field-induced cell fusion. Biotechniques 1:118 -122.
30. Zimmermann, U. and Vienken, J. 1982. Electric field-induced cell to cell fusion. J. Membrane Biol. 67:165-182.

31. Zimmermann, U. 1986. Electrical breakdown, electropermeablization and electrofusion. Rev. Physiol. Biochem. Pharmacol. 105:176-256.

요 약

한우에서 융합방법이 체세포 핵이식 수정란의 발달에 미치는 영향

임기순[1] · 양보석[1] · 박성재[1] · 장원경[1] · 박창식[2]

축산기술연구소[1] · 충남대학교 축산학과[2]

체세포 핵이식이 완료된 수정란을 70 volt 40 μs 1회, 70 volt 40 μs 2회, 180 volt 15 μs 1회 및 180 volt 30 μs 1회의 전압을 이용하여 융합을 실시하였으며, 융합배지로는 mannitol 및 ZCFM을 사용하였다. 70 volt 40 μs 1회, 70 volt 40 μs 2회 및 180 volt 15 μs 1회의 전압을 이용하여 공핵 난구세포와 수핵란 세포질간에 융합을 유도한 결과, 융합율은 각각, 0.0%, 25.4% 및 58.1%였으며, 배반포 발생율은 각각, 0.0%, 13.3% 및 36.1%였다. 180 volt 15 μs 1회의 전압을 이용하였을 때 융합율 및 배반포 발생율이 유의적으로 높았다($P<0.05$). 180 volt 15 μs 및 30 μs 1회의 전압을 이용하여 공핵 태아 섬유아세포와 수핵란 세포질간에 융합을 유도한 결과, 융합율은 각각, 45.7% 및 63.2%였으며, 배반포 발생율은 각각, 24.3% 및 25.0%였다. 융합율은 180 volt 30 μs 1회의 전압을 이용하였을 때 유의적으로 높았으나, 배반포 발생율에 있어서는 차이를 나타내지 않았다($P<0.05$). Mannitol 및 ZCFM을 이용하여 융합을 실시한 결과, 융합율은 각각, 71.2% 및 65.8%였고, 배반포 발생율은 각각, 37.8% 및 39.8%였다. 융합배지간 융합율 및 발생율에 있어서는 유의적인 차이를 나타내지 않았다.

(접수일자 : 2000. 10. 18. / 채택일자 : 2000. 11. 1.)