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Effect of Sucrose and Polybrene on the Gene Transfer into Porcine Oocytes using Retroviral Vector

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레트로 바이러스 벡터를 이용한 돼지난자에의 유전자 전이에 있어 Sucrose와 Polybrene의 효과

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ABSTRACT

In vitro matured porcine oocytes have very small volume of perivitellinspace (PVS). In these respect, the effect of sucrose and polybrene on the efficiency of gene transfer was investigated. As a gene (hGH) transfer vehicle, vesicular stomatitis virus glycoprotein pseudotyped retroviral vector (VSV-G) was used. Sucrose treatment has no detrimental effect on the rates of cleavage and resulted in the enlargement of PVS for the efficient introduction of retroviral vector stocks. Introduction rates of retrovirus in 0.5, 1, 2, 3 % sucrose treatment group were higher than that of the non-treatment group (39.3, 43.3, 35.7, 40.7 % vs. 8.3 %), respectively. In addition, we observed that sucrose pretreatment during injection procedure significantly reduce the frequency of polyspermy. In general, polybrene is a polycation essential for retrovirus transduction. The groups with the addition of 0.5, 5, 50 μg/ml polybrene exhibited a significant effect on gene transfer compared to that of the non-addition group (56.5, 50.0, 57.1 % vs. 34.6 %), respectively. But, when the oocytes were co-injected with retrovirus and 50 μg/ml polybrene, the rates of cleavage and blastocyst development were 43.3 and 4.6%, respectively. This rates were lower than those of the non-addition group (70.0 and 17.3 %). In conclusion, sucrose pretreatment have increased efficiency of retroviral mediated gene transfer in porcine oocytes with no damage on *in vitro* fertilization and embryo development. In addition, sucrose pretreatment was beneficial in polyspermy inhibition. Presence of polybrene during microinjection showed a beneficial effect on the gene transfer in porcine oocytes, in low concentration. And these results will provide an useful tool for production of transgenic pigs by retroviral mediated gene transfer.

(Key wards : Transgenic porcine, Retrovirus, Sucrose, Polybrene)

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Ⅰ. INTRODUCTION

Since the first production of transgenic mice by pronuclear microinjection of foreign DNA into fertilized embryos (Gordon, 1980), various efforts have been made for the generation of transgenic livestock. Studies about transgenic animal have focused on alternative methodologies for efficient production of transgenic animal, because of the low efficiency of current protocols. Recently, Sperm mediated DNA transfer, infection of retrovirus vector into oocytes or embryos, and nuclear transfer with genetically modified donor cells are used as candidate for new transgenic technologies (Niemann et al., 2000).

Integration of a DNA copy of the viral genome into host cellular DNA is an essential step in the life cycle of most, if not all, retroviruses (Temin and Panganiban, 1984). With the application of this mechanism, many approaches attempted to establish retroviral-mediated gene transfer system thereby foreign gene was introduced into mammalian cells efficiently (Mullier, 1994). The main advantage of retroviral-mediated gene transfer is the technical ease of presenting a virus to embryos at various developmental stages (Wheeler and Walters, 2001). There are, however, some limitation for using retrovirus vector including difficulty in preparing an enough titer and restricted host cell range. Vesicular stomatitis virus G (VSV-G) glycoprotein pseudotyped retroviral vector could be used to overcome the limited host-cell range and could be concentrated by ultracentrifugation without loss of infectivity (Marsh and Helenius, 1989).

Recently, transgenic cattle (Chan et al., 1998) and monkey (Chan et al., 2000) were produced by microinjection into perivitelline space (PVS) using retroviral vector pseudotyped with VSV-G. These results suggested that retroviral vector could be a useful tool for studying the generation of genetic diversity and the generation of transgenic animals.

In production of transgenic animals, pigs seems to be more suitable than in other livestock species by reason of short pregnancy period and large litter size (Wheeler and Walters, 2001). However, a low incidence of cytoplasmic maturation (Abeydeera, 2002; Niwa, 1993) and a high frequency of polyspermic fertilization (Coy et al., 1999; Kouba et al., 2000) of pig oocytes *in vitro* is regarded as the major causes of low efficiency in using pig oocyte. For these reasons, many studies have been performed to improve *in vitro* developmental competence of porcine oocytes thereby enable to provide an ideal source for genetic micromanipulation and increase viability of the *in vivo* transferred embryos. It was reported that high titer vector stock was essential for successful transgenesis following injection of retroviral vector into PVS, because of the limited volume of the PVS (Chan et al., 1998). Morphologic differences between naturally ovulated pig oocytes and *in vitro*-matured pig oocytes exist in diameter of oocytes, thickness and hardness of zona pellucida, cytoplasmic density and particularly in size of PVS (Wang et al., 1998). The size of PVS of *in vitro* matured pig oocytes is limited to inject with retroviral vector stocks sufficiently.

Oocytes treated by sucrose have not any detrimental effect during IVF and support further embryo development (Collas and Barns, 1994; Saito et al., 1994). Addition of sucrose in the medium can increase the concentration of cytoplasmic protein and cause the shrinkage of the oocyte (Saito et al., 1994). More quantity of retroviral vector can be introduced into PVS with no impediment on the subsequent development of embryo by inducing enlarged PVS by addition of sucrose.

Infection of VSV-G was induced by binding between the viral envelope glycoprotein and phospholipid component of cell membrane (Mastromarino, 1993).

Polybrene is a positively charged polyelectrolyte that is believed to increase transduction by neutralizing the negative charges on the surface of the opposing retroviral and cellular bilayers (Coller, 1980; Coelen et al., 1983). Therefore, the improved transduction efficiency may be predicted following the co-administration of polybrene and retrovirus into PVS of porcine oocytes in the presence of sucrose.

The objective of the study was to investigate the effect of sucrose and polybrene on the efficiency of gene transfer during microinjection of retroviral vector in PVS in pig oocytes.

Ⅱ. MATERIALS AND METHODS

1. Preparation of Retroviral Vector

Introduction of gene sequences of retroviral vector and VSV-G (Burns et al., 1993) was done to convert 293mGPHy cell (Kim et al., 2001) containing the *gag* and *pol* genes to virus-producing cells. LNC -hGH retrovirus vector DNA sequence was introduced by infecting the cells with the medium taken from PG13 and PT67 packaging cells stably expressing LNC-hGH provirus, then the cells were allowed two weeks for G418 (600 μg/ml) selection (Miller et al., 1991). In order to introduce VSV-G gene which is equivalent to the retrovirus *env* gene, the cells underwent calcium phosphate transfection by adding 20 μg of plasmid pHCMV-G in 1ml 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; courtesy of Dr. Jane C. Burns). After 8 hrs of incubation at 37℃ in 5% CO_2 in air, calcium phosphate solution was removed to culture the cells with 10ml of medium. Because VSV-G is cytotoxic, the medium containing retrovirus vectors was harvested at 48 hrs of transfection after washed off.

2. Concentration of Virus

The conditioned medium containing virus producing cells was centrifuged to collect viruses at 50,000× g for 90 minutes at 4℃ using vertical rotor (Beckman). Following complete removal of supernatant after centrifugation, the pellet was placed at 4℃ overnight with small volume of 0.1× Hank's Balanced Salt Solution (HBSS, Yee et al., 1994). For the high titer [$10^8\sim10^9$ CFU (colony forming unit)/ml], virus stock of the first concentration cycle was pooled and centrifuged a second time. The concentrated virus stock was filtered through 0.45 μm pore-size filter before storage in aliquots at −70℃.

3. Oocyte Collection and *In Vitro* Maturation

Ovaries were obtained from prepubertal gilts at a local slaughterhouse and transported to the laboratory in physiological saline maintained 30℃ within 1 hr. The cumulus-oocyte complexes (COCs) were aspirated from the follicles (2~6 mm in diameter) using a 10 ml disposable syringe fitted with an 18-gauge needle. The COCs were washed three times with TL-HEPES

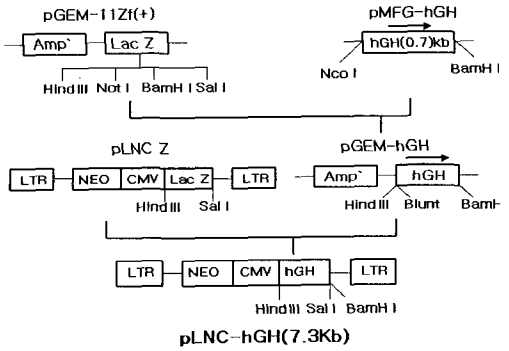

Fig. 1. Full strategy for producing replication defective retroviral vectors. LTR; long terminal repeat, NEO; G418 resistant gene, CMV; human cytomegalovirus immediated early promoter, hGH; human growth hormone gene. The LNC-hGH plasmids were constructed by replacing LacZ genes of pLNCZ with 345 bp hGH cDNA fragment (Kim et al., 1993).

(Parrish et al., 1988) and the maturation medium. Then 50 COCs were placed into a 500 μl of maturation medium under paraffin oil equilibrated at 39℃ in 5% CO_2 in air for at least 2 hrs prior to use. The maturation medium for porcine COCs was BSA-free North Carolina State University (NCSU) 23 medium supplemented with 0.56 mM cysteine, 25 μg/ml gentamycin sulfate, 10% (v:v) porcine follicular fluid, 10ng/ml epidermal growth factor, 0.5 μg/ml FSH (Follitropin V; Vetrepharm, Canada) and 1 μg/ml estradiol-17 β (Sigma Co., St. Louis, Mo). Twenty or twenty two hrs after culture for maturation, COCs were washed twice with TL-HEPES medium and then further cultured in 500 μl of the same maturation medium without hormonal supplement (FSH and estradiol-17 β) for 20~22 hrs.

4. Microinjection of Retroviral Vector into Peri-Vitelline Space

For microinjection of retroviral vector, porcine oocytes were recovered from maturation medium at 38 hrs after incubation. Cumulus cell enclosing oocytes were stripped in NCSU 23 medium supplemented with 0.1% hyaluronidase and washed three times in micromanipulation medium. The micromanipulation medium was hormon-free maturation medium supplemented with 15 mM Hepes equilibrated at 39 ℃ in 5 % CO_2 in air for 2 hrs prior to use. When swelling of the zona pellucida was observed, microinjection of retroviral vector was deemed successfully. Injected oocytes were cultured for 5~6 hrs in micromanipulation medium before fertilization.

5. *In Vitro* Fertilizaion (IVF)

After *in vitro* maturation, the oocytes were treated with 0.1% hyaluronidase solution to remove cumulus cells and washed three times with modified tris buffered medium (mTBM) containing 1 mM caffeine and 1 mg/ml BSA. After washing, 30~35 denuded oocytes were placed into 50 μl drops of the fertilization medium covered with paraffin oil. The dishes were kept in the incubator for 30 min until spermatozoa were added for fertilization. Semen were collected from pig cauda epididymis obtained from a local slaughterhouse. Semen was diluted into 5ml Sp-TALP (Rosenkrans et al., 1994), and the extender was removed by washing two times. At the end of the washing procedure, the sperm pellet was resuspended in the mTBM containing 1 mM caffeine and 1 mg/ml BSA. After appropriate dilution, 50 μl of the sperm suspension was added to a 50- μl drop of the fertilization medium containing oocytes to give a final sperm concentration of 1.5×10^5 cells/ml. Sperm and oocytes were coincubated in a 100 μl drops of mTBM under paraffin oil for 6 hrs at 39℃ in 5% CO_2 in humidified atmosphere. The day of insemination was designated as Day 0.

6. *In Vitro* Culture (IVC)

At 6 hrs after insemination, the fertilized embryos were washed in NCSU 23 medium prior to place in culture medium. About 15~20 embryos were transferred into a 50 μl of NCSU 23 medium supplemented with 3 mg/ml fatty-acid-free BSA. Following culture for 48 hrs, all embryos were cultured in 50 μl of NCSU 23 medium containing 10% FBS for 4 days.

7. 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 and 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: 5'-TATTCCGACACCCTCCAACAG-3' (5'primer) and 5'-CCTTGTCCATGTCC TTCCCGA-3' (3' primer), which yielded a 345-bp fragment. The final volume of the PCR reaction was 50 μl and the amplification reaction was 35 cycles following three step: at 94℃ for 30 sec (dissociation), at 58℃ for 30 sec (annealing) and at 72℃ for 30 sec (extension). After 35 cycles, PCR product were loaded and separated on a 1.7% agarose gel.

8. Statistical Analysis

The data were pooled from at least four replications. Differences in the percentages of oocytes developing to a particular stages were determined by Student's t-test. Statistical significance was determined at the $P<0.05$ level.

Ⅲ. RESULTS

1. Morphologic Difference of *In Virto* Matured Porcine Oocytes by Sucrose Treatment

Increased size of PVS was obtained by sucrose treatment (Fig. 2). Also, sucrose treated oocytes (B) exhibited clear areas in the cytoplasm cortex and the size of PVS was wider than that of *in vitro* matured oocytes (C). This morphology of sucrose treatment oocytes is similar to that of ovulated oocytes (A).

2. Effect of Sucrose Treatment on *In Vitro* Fertilization of Porcine Oocytes

When the oocytes were treated with 0.5 or 3 % sucrose, rates of sperm penetration were 88.4 and 85.9 %, respectively (Table 1). This rates were lower than the rates of the non-treated group (90.8 %). However, there was no statistical difference. In addition, oocytes treated with 0.5 or 3 % sucrose (60.3 %, 61.9 %) compared to non-treated oocytes (76.6 %) showed a significantly ($P<0.05$) lower percentage of polyspermy.

3. Effect of Sucrose Treatment on *In Vitro* Development of Porcine Oocytes and Transfer Rate of hGH Gene

The effect of sucrose treatment on the subsequent development and integration rate of hGH gene was investigated. When the oocytes were treated with 0.5, 1, 2 or 3 % sucrose, rates of cleavage and

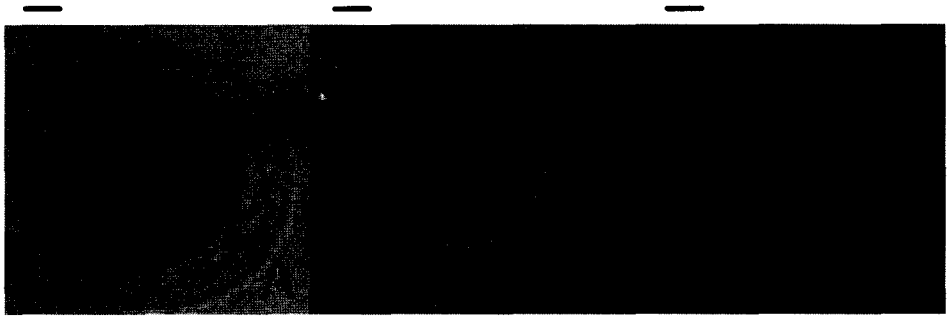

Fig. 2. Micrographs of porcine oocyte recovered from the oviduct (A), an *in vitro* matured oocytes after treatment of sucrose (B) and *in vitro*-matured oocytes (C). Note the size of the PVS was greater for ovulated oocytes than for *in-vitro* matured oocytes, also the oviductal oocyte and sucrose treatment oocyte have clear areas in the cortex. Bar = 26 μm.

Table 1. *In vitro* fertilization of porcine oocytes treated with various concentration of sucrose

	No. of matured oocytes	No. of penetrated oocytes (%)	No. of polyspermic oocytes (%)
Control	120	109 (90.8)	92 (76.6)[a]
0.5 % Sucrose	121	107 (88.4)	73 (60.3)[b]
3 % Sucrose	121	104 (85.9)	75 (61.9)[b]

[a,b] Different superscript within same column were significantly different (P<0.05).

Table 2. Effect of sucrose treatment on *in vitro* development of porcine oocytes and integration rate of hGH gene in blastocysts

Treatment	No. of oocytes	No. of embryos developed to		No. of blastocysts gene-Integrated (%)
		2-cell (%)	Blastocyst (%)	
Control	240	170 (70.8)	34 (14.1)	3 (8.3)[a]
0.5 % Sucrose	180	131 (72.7)	28 (15.5)	11 (39.3)[b]
1 % Sucrose	180	130 (72.2)	30 (16.6)	13 (43.3)[b]
2 % Sucrose	180	120 (66.6)	28 (15.5)	10 (35.7)[b]
3 % Sucrose	190	133 (70.0)	27 (14.2)	11 (40.7)[b]

[a,b] Different superscript within same column were significantly different (P<0.05).

blastocyst development were 66.6~72.7% and 14.2 ~16.6 % (Table 2). There was no statistical difference to the control- and treated-group and these data demonstrated that sucrose treatment has no detrimental effect on IVF and subsequent development. In addition, gene transfer rates in oocytes treated with sucrose were higher than that of the non-treated oocytes (39.3, 43.3, 35.7, 40.7 % vs. 8.3%, respectively, P<0.05), when gene transfer were observed by PCR analysis (Fig. 3). Integration

Fig. 3. PCR analysis of *in vitro* produced individual porcine blastocysts after microinjection of retroviral vector into perivitelline space. Lanes 1~14; LNC-hGH injected embryos, M; 100bp molecular size marker, P; positive control (hGH genomic DNA), N; negative control (non-injected embryo + retroviral vector).

Table 3. Effect of polybrene treatment on *in vitro* development of porcine oocytes and integration rate of hGH gene in blastocysts

Polybrene (μg/ml)	No. of oocytes examined	No. of embryo developed to		No. (%) of integration in blastocysts
		2-cell (%)	Blastocyst (%)	
0	150	108 (72.0)[a]	26 (17.3)[a]	9 (34.6)[a]
0.5	150	101 (67.3)[a]	23 (15.3)[a]	13 (56.5)[b]
5	150	103 (68.6)[a]	22 (14.6)[a]	11 (50.0)[b]
50	150	65 (43.3)[b]	7 (4.6)[b]	4 (57.1)[b]

[a,b] Different superscript within same column were significantly different ($P<0.05$).

rates were observed by PCR analysis (Fig. 3).

4. Effect of Polybrene Treatment on *In Vitro* Development of Porcine Oocytes and Gene Transfer Rate of hGH Gene

Dose-response effect of polybrene was examined using several concentrations of polybrene (Table 3). When the oocytes were co-injected with retrovirus and 0.5, 5, 50 μg/ml polybrene, the rates of cleavage and blastocyst development were 67.3, 68.6, 43.3 % and 15.3, 14.6, 4.6 %, respectively. The rates of cleavage and blastocyst development of co-injected group with 50 μg/ml polybrene were lower than that of the co-injected group with 0, 0.5, 5 μg/ml polybrene. The groups with the addition of polybrene exhibited a significant effect ($P<0.05$) on gene transfer rates compared to those of the control group (ranging 50.0~57.1% vs. 34.6 %).

Ⅳ. DISCUSSION

Anthony Chan et al. (1998, 2000) reported that production of transgenic animals following microinjection of retroviral vector (VSV-G) into perivitelline space at MⅡ stage. They suggested that this method could be used to produce transgenic animals for other species. But in *in vitro* matured porcine oocytes, very small volume of perivitellinspace (Fig. 2C), was limited to introduce retroviral vector stocks sufficiently. Therefore, we hypothesis that a critical requirement for retroviral- mediated gene transfer in *in vitro* matured pig oocytes is enlargement of PVS. Enlargement of PVS could be induced by incubating the oocyte into an high osmotic medium. Sucrose has been broadly used in embryo cryopreservation as a cryoprotectant, and it has no detrimental effect on IVF and subsequent development (Coalls and Barnes, 1994; Saito et al, 1994). More recently, Wang et al. (2001) reported the safety of sucrose pretreatment in mouse MⅡ oocytes. In the present study, oocytes having enlargement of PVS by sucrose treatment showed a significantly high rates of gene transduction than that of intact oocytes. However, the rates of cleavage and blastocyst development were not affected. In addition, sucrose treatment has the advantage of reducing the damage during micromanipulation. This simple method may thus be used in retroviral-mediated gene transfer in other species.

In the present study, we observed that sucrose pretreatment during retrovirus injection procedure significantly reduce the frequency of polyspermy, but the rates of cleavage and subsequent development were not affected. Polyspermy remains the major problem to obtain successful production of large numbers of embryos with high developmental competence. In general, cortical granule (CG) exocytosis is responsible for the block to polyspermy

in many species. In pig, incomplete CG exocytosis (Cran and Cheng, 1986; Wang et al., 1996), and low CG enzyme activity (Cran and Cheng, 1986) were reported as potential causes of polyspermy. Inhibitory effect of sucrose on polyspermy may be explained by physical and biochemical characteristics on CG. Physical characteristics of sucrose, such as dehydration, can increase the concentration of cytoplasmic protein and the shrinkage of the oocyte (Saito et al., 1994). Therefore, sucrose may have beneficial effect of CG exocytosis by which polyspermy could inhibited. The oocytes treated with sucrose exhibited enlargement of PVS and clear area in the cortex cytoplasm (Fig. 2B). This morphology of oocytes treated with sucrose is similar to that of *in vivo* maturated oocytes (Fig. 2A). These results were supported by the previous study (Wang et al., 2001) that morphologic differences exist not only in the density of cytoplasm but also in the extracellular between *in vitro*-maturated and *in vivo*-maturated pig oocytes, with reference to the potential relationship between oocytes morphology and occurrence of polyspermy after IVF. Sucrose can stabilized some protein by preventing the loss of enzyme activity (Bradbury and Jakoby, 1972), and inhibiting the irreversible aggregation (Frigon and Lee, 1972). Therefore, activities of cortical granule enzyme could be preserved by these biochemical characteristics of sucrose.

A beneficial effect of polybrene on the retrovirus -mediated gene transfer was observed. Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vector infection requires interaction between the viral envelope protein and a phospholipid component of the cell membrane to mediate viral entry by membrane fusion (Mastromarino et al., 1987). Therefore, the first step of retroviral infection is binding of viruses to the cell surface. But the negative charges on the surfaces of the opposing retroviral and cellular bilayer restrict the choice of binding (Coller, 1980). This limitation can be overcome by using the polymeric electrolytes such as polybrene. Polybrene is a polycation essential for retrovirus transduction (Andreaids and Palsson, 1997). Infectivity has been shown to increase with polybrene concentration (Aubin et al., 1994; Chuck, 1995). Thus, we tested the effects of various concentration of polybrene on gene tranduction and to our knowledge, this is the first report to compare the effect of polybrene on embryos.

Altough polybrene treatment during microinjection had beneficial effect on gene-transduction, the developmental potential of oocytes co-administrated with high concentration of polybrene was lower than that of oocyte injected with retrovirus alone. Toyoshima and Vogt (1969) observed that polybrene had toxic effect on the target cells, only when the target cell were exposed at a high concentration for a long time. Using the different polymeric electrolytes without detrimental effect on IVF and subsequent development may overcome this problem and improve on retroviral-mediated gene transfer.

The present study, we frequently observed that cloggy of injection pipette by cluster of some material, in contrast to previous study, introduction rate was not increased with the increase of polybrene concentration. This observation is explained by possible mechanism that high concentration of polybrene induce virus aggregation and results in the loss of introduction rate. Andreadis and Palson. (1997) supported this possibility. In their reports, polybrene may act electrostatically neutralize the negative charge of viral particles, thus increase the rate of aggregation formation. Therefore, the viral titer is reduced and consequently the efficiency of transduction decreases.

In conclusion, sucrose pretreatment have increased efficiency of retroviral mediated gene transfer in porcine oocytes with no damage on IVF and embryo development. In addition, sucrose pre-

treatment was beneficial in polyspermy inhibition. Presence of polybrene during microinjection showed a beneficial effect on the gene transfer in porcine oocytes in low concentration. And these results will provide a useful tool for production of transgenic pigs by retroviral mediated gene transfer.

Ⅴ. 요 약

본 연구에서는 레트로 바이러스를 이용하여 형질전환 돼지를 생산하기 위한 기초 연구로써 유전자 도입 효율을 증진시키기 위해 sucrose와 polybrene을 사용하여 그 효과를 조사하였다. 유전자(hGH) 전달체로는 vesicular stomatitis virus glycoprotein pseudotyped retroviral vector (VSV-G)를 사용하였다. 체외에서 성숙된 돼지 난자는 매우 협소한 위란강내 공간을 가지며 따라서 위란강내 공간의 확장은 필수조건이다. sucrose 처리는 수정율 및 배발달에는 영향을 미치지 않았으며 sucrose 처리를 통해 돼지난자는 위란강의 공간이 확장되어 충분한 양의 바이러스 벡터의 주입이 가능하였다. PCR 분석 결과 8.3 %의 유전자 도입율을 나타낸 무처리군에 비해 0.5, 1, 2, 3 % sucrose 처리군에서 39.3, 43.3, 35.7, 40.7 %의 높은 유전자 도입율을 나타내었다. 추가적으로 돼지난자는 sucrose 처리에 의해 다정자 침입의 억제 효과를 나타내었다. 일반적으로 레트로 바이러스를 이용한 세포에의 높은 유전자 도입 효과를 나타내는 것으로 알려진 polybrene을 바이러스와 공동 주입한 결과 0.5, 5, 50 μg/ml의 농도에서 각각 56.5, 50.0, 57.1 %의 도입율을 나타냄으로써 바이러스 단독 주입군의 34.6 %보다 높은 효율을 나타내었다. 그러나 50 μg/ml의 농도를 주입하였을 경우, 분할율과 배반포발달율이 43.3 및 4.6%를 나타냄으로써 바이러스 단독 주입군의 72.0, 17.3%보다 유의적으로 낮았다. 이상의 결과를 종합할 때, sucrose의 처리는 돼지 난자의 발달에 영향을 주지 않으며 유전자 도입효율을 증진시킬 수 있었으며, 추가적으로 다정자 침입 억제효과를 보였다. 또한 polybrene의 사용은 낮은 농도에서 유전자 도입효율을 증진시켰다. 이러한 결과들은 형질 전환 돼지의 생산 가능성을 높이는데 이용될 수 있다고 사료된다.

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