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Effects of Trichostatin A on *In Vitro* Development of Porcine Parthenogenetic and Nuclear Transfer Embryos

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ABSTRACT

Developmental potential of cloned embryos is related closely to epigenetic modification of somatic cell genome. The present study was to investigate the effects of applying histone deacetylation inhibitor, trichostatin A (TSA) to activated porcine embryos on subsequent development of porcine parthenogenetic and nuclear transfer embryos. Electrically activated oocytes were treated with 5 nM TSA for different exposure times (0, 1, 2 and 4 hr) and then the activated embryos were cultured for 7 days. The reconstructed embryos were treated with different concentrations of 0, 5, 10 and 25 nM TSA for 1 hr. Also 5 nM TSA was tested with different exposure times of 0, 0.5, 1, 2 and 4 hr. And fetal fibroblast cells were treated with 50 nM TSA for 1, 2 or 4 hr and with 5 nM TSA for 1 hr. Cumulus-free oocytes were enucleated and reconstructed by TSA-treated donor cells and electrically fused and cultured for 6 days. In parthenogenetic activation experiments, 5 nM TSA treatment for 1 hr significantly improved the percentage of blastocyst developmental rates than the other groups. Total cell number of blastocysts in 1 hr group was significantly higher than other groups or control. Similarly, blastocyst developmental rates of porcine NT embryos following 5 nM TSA treatment for 1 hr were highest. And the reconstructed embryos from donor cells treated by 50 nM TSA for 1 hr improved the percentage of blastocyst developmental rates than the control group. In conclusion, TSA treatment could improve the subsequent blastocyst development of porcine parthenogenetic and nuclear transfer embryos.

(Key words : Porcine; reconstructed embryos, Trichostatin A, Parthenogenetic activation)

INTRODUCTION

Even though the successful production of offspring derived by somatic cell nuclear transfer (SCNT) have been demonstrated in various mammalian species (Wilmut and Schnieke, 1997; Wakayama *et al*., 1998; Kato *et al*., 1998; Baguishi *et al*., 1999; Onishi *et al*., 2000; Polejaeva *et al*., 2000; Shin *et al*., 2002; Chesne *et al*., 2002; Woods *et al*., 2003; Galli *et al*., 2003; Zhou *et al*., 2003), little is known about the mechanisms that somatic genomes are epigenetically reprogrammed in NT embryos. Incomplete reprogramming has been proposed to account for the inefficiency of somatic cell cloning by nuclear transfer technique (Niemann and Wrenzycki, 2000; Tanaka *et al*., 2001; Rideout *et al*., 2001). Epigenetic processes such as DNA methylation and histone acetylation can lead to regulation of gene expression (Dannenberg and Edenberg, 2006). Consequently, abnormal gene expression patterns in cloned embryos have been reported to result from incomplete reprogramming of somatic cell genome (Niemann and Wrenzycki, 2000; Tanaka *et al*., 2001; Rideout *et al*., 2001). Histone acetylation and deacetylation play important roles in eukaryotic gene expression regulation. Histone hyperacetylation results in increased expression of transgenes in early stage mouse embryos (Thompson *et al*., 1995), indicating that histone acetylation may be involved in embryonic gene expression. Abnormal acetylation reprogramming of somatic chromatins begins as early as the one-cell stage during the somatic cell nuclear transfer (Wee *et al*., 2006). Histone deacetylase inhibitor, trichostatin A (TSA), has been widely used to study the relationship between developmental potential of embryos and acetylation status of chromatin. TSA acts by preventing the deacetylation of histones, and is also known to inhibit DNA methylation levels associated with histone modifications in mammalian cells (Enright *et al*., 2003; Parekh-Olmedo *et al*., 2003; Kishigami *et al*., 2006a). Several reports showed beneficial effects

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of TSA treatment to donor cells with TSA which improved NT embryos development in bovine NT embryos (Enright *et al.*, 2003), and TSA treatment of mouse reconstructed embryos with somatic cells significantly increased blastocyst formation rates and in success rates of mouse cloning from cumulus cells (Kishigami *et al.*, 2006b). These results suggested that treatment of donor cells with TSA is able to increase level of histone acetylation and improve developmental potential of cloned embryos. Hyperacetylation might actually improve reprogramming of somatic nuclei during early reconstructed embryonic development. However, the efficiency of mouse cloning can be enhanced by up to 6-fold following treatment of mouse cloned embryos with TSA (Kishigami *et al.*, 2006b), even though high TSA treatment may be toxic as is a high dose TSA treatment in somatic cloning (Svensson *et al.*, 1998). Recently, with combined chemical treatments including cycloheximide and cytochalasin B and TSA after SCNT can significantly improved the *in vitro* production of porcine SCNT embryos (Zhang *et al.*, 2007). However, beneficial effects from low concentration of only TSA treatment in porcine parthenogenetic and cloned embryos have not been previously reported. Furthermore, the beneficial effects of treatment to donor cells with TSA in porcine cloned embryos have not been demonstrated. Here, the objective of this study was to determine if TSA treatment could improve the development of porcine parthenogenetic and reconstructed embryos to blastocysts *in vitro* following PA and NT.

MATERIALS AND METHODS

Preparation of Porcine Fetal Fibroblasts

Fetuses were obtained from a pregnant sow at day 35 after insemination, and the head and internal tissues of fetuses were removed using fine scissors, and soft tissues such as liver and intestine were discarded. The tissue was cut into small pieces with fine scissors and treated with 0.05% trypsin and 0.5mM EDTA (15050-065, Gibco) and shaken for 10 min at 38.5℃ in an incubator. This suspension was centrifuged at 500 g for 10 min. The cell pellet was resuspended in DMEM medium supplemented with 75 μg/ml penicillin G, 50 μg /ml streptomycin, 5% (v/v) fetal bovine serum (FBS) and 5% fetal calf serum (FCS) and cultured at 38.5℃. All cells were cryopreserved upon reaching confluence. The donor cells from passages 3 to 8 were used for nuclear transfer (NT). Cells were treated with 0.25% trypsin and 0.5 mM EDTA for single-cell isolation by 2 min incubation in 38.5℃ incubator before nuclear transfer.

Preparation of Oocytes

Ovaries were obtained from prepubertal gilts at a local slaughter house and transported to laboratory in PBS solution supplemented with 100 IU/ml penicillin and 50 μg/ml streptomycin at 35℃. Cumulus-oocyte complexes (COCs) were collected from follicles with a diameter of 2~6 mm using 10-ml syringe fixed with an 18-gauge needle. COCs were washed three times in TL-Hepes containing 0.1% (w/v) polyvinyl alcohol (PVA). The oocytes was matured in TCM-199 (M-4530, Sigma) supplemented with 0.1% (w/v) polyvinyl alcohol (PVA), 3.05 mM D-glucose, 0.91 mM sodium pyruvate, 0.57 mM L-cysteine, 0.5 μg/ml LH (L-5269, Sigma Chemical Co, St. Louis, MO), 0.5 μg/ml FSH (F-2293, Sigma), 10 ng/ml epidermal growth factor (E-4127, Sigma), 75 μg/ml penicillin, 50 μg/ml streptomycin, and 0.05% (v/v) MEM vitamins (Sigma, M-6895). COCs were transferred into 500 μl of maturation medium in a 4-well multidish (Nunc, Roskide, Denmark) and incubated for 44 hr at 38.5℃ in an atmosphere of 5% CO_2 in air with maximum humidity. After 22 hr of *in vitro* maturation, the oocytes were washed three times and transferred into 500 μl of the maturation medium without hormone for additional 22 hr of culture.

Parthenogenetic Activation of Porcine Oocytes

Cumulus-free oocytes were transferred to activation solution consisting of 0.3 M D-mannitol, 0.1 mM $MgSO_4$, 0.05 mM $CaCl_2$ and 0.01% PVA, and washed three times. Cumulus-free oocytes were activated by a direct current pulse of 1.5 kV/cm for a duration of 100 μsec using an BTX Elector-Cell Manipulator 2001 (BTX, San Diego, CA). Parthenogenetic activation experiments were replicated six times. The rates of embryos at cleavage and blastocyst stages were evaluated at 3 days and 7 days after activation, respectively.

Nuclear Transfer and Fusion

Nuclear transfer and fusion were carried out as outlined previously described by Park *et al.* (2001). After 44 hr of maturation, cumulus cells were treated briefly with 0.1% hyaluronidase and then freed from cumulus cells by pipetting. Cumulus-free oocytes were enucleated by aspirating the first polar body and adjacent cytoplasm with a fine glass pipette in TCM-199 supplemented with Hepes, 0.3% BSA (Sigma, A-8022), and 7.5 μg/ml cytochalasin B at 38℃. A single donor cell was placed in the perivitelline space of the enucleated oocytes. Injected oocytes were placed between two wire electrodes (1mm apart) in the cell fusion medium, consisting of 0.3 M mannitol and 1.0 mM $CaCl_2 \cdot H_2O$ and 0.1 mM $MgCl_2 \cdot 6H_2O$ and 0.5 mM Hepes, and fused with two DC pulses of 1.1 kV/cm for 30 μsec

on BTX Elector-Cell Manipulator 2001 (BTX, San Diego, CA). Nuclear transfer experiments were replicated at least four times. The rates of embryos at fusion and cleavage and blastocyst stages were evaluated at 3 to 18 hr and 3 days and 6 days after fusion and activation, respectively.

TSA Treatment

TSA treatment was performed according to a protocol as outlined previously (Kishigami *et al*., 2006b). 1 mg of TSA (Sigma, T-8552) was dissolved in 1ml DMSO. The stock solution was stored at −20 ℃. TSA stock solutions were added to the PZM-3 medium or DMEM medium supplemented with 5% (v/v) FBS and 5% FCS before use for find concentrations. In PA experiments, activated oocytes were treated with PZM-3 containing 5 nM TSA for 0, 1, 2 and 4 hr and washed three times in PZM-3 culture medium. Porcine fetal fibroblast cells were treated with different exposure times (0, 1, 6 and 12 hr) of 5 or 50 nM TSA and analyzed by Western blotting. In NT experiments, the reconstructed embryos were treated with PZM-3 medium containing TSA (5 nM; 0, 0.5, 1, 2 and 4 hr) and treated with TSA (1 hr; 0, 10 and 25 nM) and washed three times in PZM-3. Porcine fetal fibroblast cells were treated with 50 nM TSA for 1, 2 or 4 hr and with 5 nM TSA for 1 hr before NT.

Western Blot Analysis

Analysis of histone modification in porcine fetal fibroblasts following TSA treatment were subjected to Western blotting with the antibodies against histone H3 protein. The band images were scanned with GT-6000 Scanner (Epson-Seiko) and densitometric analyses were performed using NIH Image (version 1.56). After TSA treatment, cells were washed in PBS. Samples were sonicated in a lysis buffer and fractionated on 12% SDS-polyacryamide gels. Gels was transferred to a PDVF membrane (Bio-Red Laboratories, Inc., CA., USA) and incubated in blocking buffer (5% non-fat dry milk) for 1 hr. Anti-acetyl-hostone H3 (Upstate) and mouse anti-beta-actin (Abcam Co. Ltd., UK) was used in a 1:5,000 dilution added to the TBS-T buffer. Anti-rabbit antibody and Anti-mouse antibody by diluted 1:5000 was used as secondary antibody during 1 hr at room temperature. Blots were developed by using ECL Western blotting detection reagents (GE Healthcare) by manufacturer's recommendations. The membrane was exposed with a sheet of diagnostic film in the film cassette.

Experimental Design

In PA experiments, to investigate the effect of 5 nM TSA with different exposure times on *in vitro* development of porcine activated oocytes after electric activation (EA), cumulus-free oocytes were activated by electrical pulses (150 kV/cm, 100 μsec). After EA, 5 nM TSA was tested with different exposure times of 0, 1, 2 and 4 hr and cultured in PZM-3 (Yoshioka *et al*., 2002) for 7 days.

In Western blotting experiments, to examine histone acethylation levels in porcine fetal fibroblast cells, porcine fetal fibroblast cells with different exposure times (0, 1, 6 and 12 hr) of 5 or 50 nM TSA and subjected to Western blotting.

In NT experiments, to investigate the effect of TSA with different concentrations and exposure times on *in vitro* development of reconstructed embryos after nuclear transfer, and cumulus-free oocytes were enucleated and reconstructed by donor cells and fused with two DC pulses (1.1 kV/cm, 30 μsec). After NT, different TSA concentrations of 0, 5, 10, and 25 nM was tested for 1 hr and different exposure times of 0, 0.5, 1, 2 and 4 hr in 5 nM TSA was evaluated. Also, to examine the effect of TSA treatment for somatic cells on *in vitro* development of porcine NT embryos, fetal fibroblast cells were treated with 50 nM TSA for 1, 2 or 4 hr and with 5 nM TSA for 1 hr and then washed in PBS. The enucleated oocytes were reconstructed by TSA-treated donor cells, fused with two DC pulses (1.1 kV/cm, 30 μsec), and cultured in PZM-3 for 6 days. Blastocysts were stained with Hoechst 33342 to determine the number of nuclei by fluorescent microscope (Olympus, Japan).

Statistical Analysis

All data were expressed as mean±standard error (SE). The significant difference among treatment groups was determined by ANOVA using the GLM procedures of SAS (SAS Institute Inc., Cary, NC) and Duncan's multiple range after analysis of variance. Differences were considered to be statistically significant when a value was less than 0.05.

RESULTS

Effects of TSA Exposure Times on *In Vitro* Parthenogenetic Pevelopment of Porcine Oocytes

To examine the effects of TSA with different exposure time on parthenogenetic development, matured oocytes were treated with 5 nM from 1 to 4 hrs following electric activation. In PA experiments, there was no significant difference in cleavage rates between TSA-treated groups and the control group. However, 1

Table 1. Effects of TSA with different exposure times on *in vitro* parthenogenetic development of porcine oocytes

Treatment (hr)[1]	N^2	Cleavage rate (mean±SE) (No.)	Blastocyst rate (mean±SE) (No.)	Nuclei no. per blastocyst (mean±SE)
0	149	83.2±2.3 (125)^{ab}	19.5±1.3 (29)^{b}	(37.4±0.6)^{a}
1	153	92.8±2.1 (142)^{b}	34.0±2.1 (52)^{a}	(42.6±1.3)^{b}
2	150	84.7±2.8 (127)^{ab}	22.7±2.8 (34)^{b}	(36.0±0.8)^{a}
4	150	79.3±2.3 (119)^{a}	16.7±1.5 (25)^{b}	(36.3±0.8)^{a}

^{a,b} Values within a columns with different superscripts are significantly different (p<0.05).
[1] Oocytes were treated with 5 nM TSA at different exposure times (0, 1, 2 and 4 hr) after electrical activation.
[2] Number of activated oocytes examined.

hr TSA-treated group significantly increased (p<0.05) the best of blastocyst rate compared with other groups (Table 1). Also, total cell number of blastocysts in 1 hr groups was significantly higher than that in other groups and control. Cleavage rates and blastocyst developmental rates and total cell number of blastocysts in 2 hr group were similar to the control group.

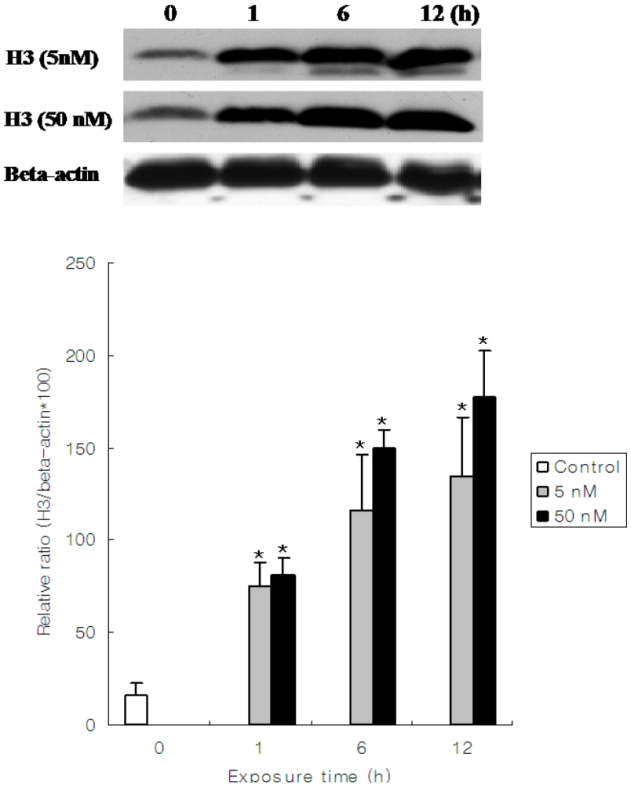

Fig. 1. Effects of 5 nM or 50 nM TSA with different exposure times (0, 1, 6 and 12 hr) and for fetal fibroblast cells on levels of histone H3 aceltylation. The anti-acetyl-histone H3 and beta-actin antibodies were used for Western blotting. The acetyl-histone H3 and beta-actin protein were detected. The graphs show expression status of acetyl-histone H3 protein levels are presented as a ratio of band intensity in fetal fibroblast cells following TSA treatment. The error bars represent the standard deviation (S.D.) from three independent experiments. * p<0.05 compared with control group.

Effects of TSA Treatments on the Levels of Histone H3 Aceltylation of Fetal Fibroblast Cells

To investigate whether increased histone H3 acethylation levels could be induced by TSA, fetal fibroblast cells were treated with 5 or 50 nM TSA for various times (0, 1, 6 and 12 hr) and subjected to Western blotting. The histone H3 acethylation levels were markedly increased after treatment with TSA even for 1 hr compared with control and fetal fibroblast cells treated with TSA tended to be hyperacethylated in H3 (Fig. 1). The histone H3 acethylation levels of fetal fibroblast cells treated with 5 nM TSA for 1 hr were similar to those with 50 nM TSA.

Effects of TSA Treatments on the Development of Porcine Nuclear Transfer Embryos Cultured *In Vitro*

In NT experiments, where different concentrations of TSA were exposed to activated embryos, 5 nM TSA-treated groups significantly increased the percentage of blastocyst developmental rates compared with the control group (Table 2). Total cell number in 5 nM group was highest, although there was no significant different. Cleavage rates and blastocyst developmental rates and total cell number of blastocysts in 10 nM and 25 nM groups were similar to those in the control group. When different exposure times with 5 nM TSA for the potential of reconstructed embryos were tested, 1 hr group of TSA-treated reconstructed embryos significantly increased blastocyst rates compared with other groups (Table 3). And there was no significant difference in cleavage rates and total cell number of blastocysts between TSA-treated groups and the control group. When fetal fibroblast cells used treated with 50 nM TSA for different exposure times, 1 hr group with TSA-treated cells significantly increased (p<0.05) blastocyst developmental rates compared with the control

Table 2. Effects of TSA with different concentrations on *in vitro* development of porcine nuclear transfer embryos

Treatment (nM)[1]	*N*[2]	Fusion rate (mean±SE) (No.)	Cleavage rate (mean±SE)[3] (No.)	Blastocyst rate (mean ±SE)[3] (No.)	Nuclei no. per blastocyst (mean±SE)
0	175	84.0±1.0 (147)	85.7±2.3 (126)	14.3±1.5 (21)[b]	30.0±0.8
5	90	83.3±0.7 (75)	89.3±3.8 (67)	26.7±1.9 (20)[a]	34.8±1.7
10	88	85.2±2.2 (75)	90.7±2.1 (68)	13.3±2.1 (10)[b]	31.6±1.6
25	90	82.2±4.1 (74)	83.8±4.3 (62)	14.9±2.8 (11)[b]	30.2±1.6

[1] Porcine reconstructed embryos were treated with TSA of different concentrations (0, 5, 10 and 25 nM) for 1 hr after electrical activation.
[2] Number of reconstructed embryos examined.
[3] Rates of cleavage (Day 3) and blastocyst (Day 6) were adjusted for fusion rates.
[a,b] Values within a columns with different superscripts are significantly different ($p<0.05$).

Table 3. Effects of TSA with different exposure times on *in vitro* development of porcine nuclear transfer embryos

Treatment (hr)[1]	*N*[2]	Fusion rate (mean±SE) (No.)	Cleavage rate (mean±SE)[3] (No.)	Blastocyst rate (mean±SE)[3] (No.)	Nuclei no. per blastocyst (mean±SE)
0	174	86.8±1.1 (151)	85.4±3.1 (129)	11.9±1.4 (18)[b]	29.3±0.8
0.5	90	83.3±2.8 (75)	84.0±4.2 (63)	13.3±1.1 (10)[b]	31.8±1.6
1	92	87.0±2.7 (80)	93.8±3.0 (75)	22.5±1.3 (18)[a]	33.3±1.2
2	85	88.2±1.8 (75)	90.7±1.7 (68)	10.7±2.0 (8)[b]	30.4±1.2
4	84	85.7±3.0 (72)	93.1±2.2 (67)	13.9±1.8 (10)[b]	29.0±0.7

[1] Porcine reconstructed embryos were treated with 5 nM TSA at different exposure times (0, 0.5, 1, 2 and 4 hr) after electrical activation.
[2] Number of reconstructed embryos examined.
[3] Rates of cleavage (Day 3) and blastocyst (Day 6) were adjusted for fusion rates.
[a,b] Values within a columns with different superscripts are significantly different ($p<0.05$).

Table 4. Effects of TSA treatment for fetal fibroblasts on *in vitro* development of porcine nuclear transfer embryos

Treatment (nM/hr)[1]	*N*[2]	Cleavage rate (mean±SE) (No.)	Blastocyst rate (mean±SE) (No.)	Hatching blastocyst rate (mean±SE) (no.)	Nuclei no. per blastocyst (mean±SE)
Control	201	70.7±2.5 (142)	18.4±0.8 (37)[bc]	11.9±1.2 (24)[bc]	29.5±0.8
50/1	103	77.7±3.1 (80)	28.2±0.8 (29)[a]	23.3±1.4 (24)[a]	33.1±1.0
50/2	101	72.3±6.1 (73)	23.8±2.0 (24)[ab]	17.8±1.7 (18)[ab]	28.3±0.8
50/4	101	71.3±4.8 (72)	10.9±1.1 (11)[d]	6.9±1.1 (7)[c]	28.7±2.4
5/1	100	77.0±2.6 (76)	18.0±1.2 (18)[c]	14.0±0.8 (14)[b]	28.4±0.9

[1] Fetal fibroblast cells were treated with 50 nM TSA for 1, 2 or 4 hr and with 5 nM TSA for 1 hr before NT.
[2] Number of reconstructed embryos examined.
[a~d] Values within a columns with different superscripts are significantly different ($p<0.05$).

group (Table 4). Also, hatching blastocyst formation rates of the reconstructed embryos with donor cells treated by 50 nM TSA for 1 hr was significantly improved compared with the control group (Table 4). However, the blastocyst rates of the reconstructed embryos with donor cells treated by 5 nM TSA for 1 hr were similar to those in the control group. There was no significant difference in cleavage rates and total cell number of blastocysts. In contrast, 4 hr group of 50 nM TSA-treated donor cells showed significantly lower percentages of developed blastocysts compared to the control group. Parthenogenetic blastocysts (A) were produced by activation with one pulse (1.5 kV/cm, 100 μsec) on day 7. And, NT blastocysts (B) were pro-

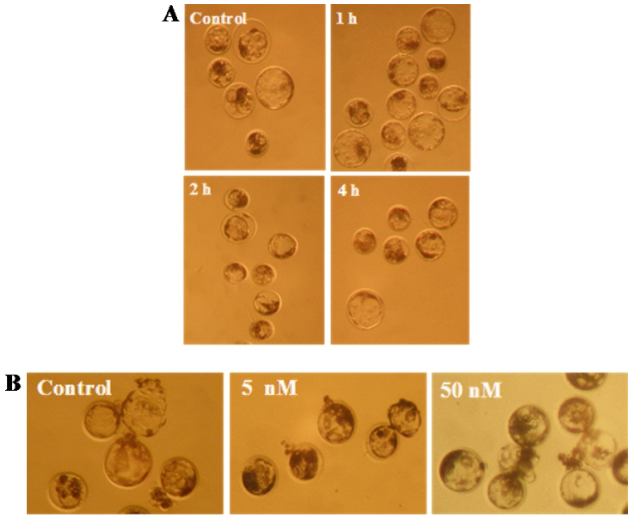

Fig. 2. Porcine parthenogenetic blastocysts. (A): 0 (control), 1, 2 and 4 hr groups of activated oocytes treated with 5 nM TSA. Porcine NT blastocysts (B): Control (non-treated group) and 1 hr group of 5 nM TSA-treated reconstructed embryos, and 1 hr group of reconstructed embryos with 50 nM TSA-treated donor cells.

duced with two DC pulse (1.1 kV/cm, 30 μsec) on day 6 (Fig. 2).

DISCUSSION

Mammalian cloning by somatic cell nuclear transfer resulted in high gestational or neonatal failure with a few percent of live birth from reconstructed embryos (Rideout *et al.*, 2001). Efficiency in nuclear transfer depend upon many factors. One important factor that may affect the successful development of nuclear transfer embryos is the epigenetic reprogramming of reconstructed embryos, such as DNA methylation, chromatin remodeling, imprinting, X chromosome inactivation, and telomere maintenance (Shi *et al.*, 2003). Abnormal DNA methylation patterns in embryos derived by nuclear transfer is known to the low efficiency of nuclear transfer (Li and Engelhardt, 2003). Moreover, *in vitro* development to blastocyst stage following nuclear transfer (NT) is dependent on the oocyte's ability to reprogram donor cell genome to that of zygotes (Enright *et al.*, 2003; Kishigami *et al.*, 2006b). The efficiency of mouse cloning can be enhanced by up to 6-fold through treatment of the histone deacetylation inhibitor trichostatin A (TSA) in the oocyte activation media (Kishigami *et al.*, 2006b), suggesting that TSA enhances reprogramming of transferred somatic nuclei in oocytes, even though high concentration of TSA are toxic to embryonic development (Svensson *et al.*, 1998).

In this study, we examined whether TSA treatment can improve the development of porcine parthenogenetic and NT embryos *in vitro*. In PA experiments, shorter exposure (1 hr) of porcine embryos to TSA after electrical activation significantly increased the blastocyst formation rates and total cell number of blastocysts. It can be speculated that 1 hr of exposure to 5 nM TSA may affect the activated embryo's ability to reprogram its genome. Activated oocytes treatment with 5 nM TSA for at least 1 hr can induce high rates of blastocyst formation and increase total cell number of blastocysts. On the other hand, exposure to TSA for 4 hr may be ineffective or have a detrimental effect. Thus, 1 hr of TSA (5 nM) supported the subsequent blastocyst development of porcine parthenogenetic embryos. Similarly, in NT experiments, 1 hr group of 5 nM TSA-treated reconstructed embryos significantly increased the percentage of blastocyst developmental rates compared with TSA-non-treated groups, that is quite different from mouse case where treatment of 5 nM TSA for 10 hr were improved significantly blastocyst formation in cloned mouse embryos (Kishigami *et al.*, 2006b). Moreover, it was lower concentration (5 nM) that improved development of porcine reconstructed embryos than 10 nM of TSA which was relatively ineffective in our study. Modifying the epigenetic status of a donor genome may be species-dependent. It can be speculated that histone deacetylation inhibitor TSA treatment during early reconstructed porcine embryonic development may be easily affected the change of histone acetylation or DNA methylation state of donor cells after epigenetic alteration compared with other species. TSA treatment of porcine reconstructed embryos can be an effective method to high rates of blastocyst formation of porcine NT embryos. Furthermore, porcine reconstructed embryos from donor cells treated by 50 nM TSA for 1 hr could enhance the subsequent blastocyst development and improved embryo quality than 4 hr of 50 nM TSA which was relatively ineffective in our study, while 50 nM TSA treatment of porcine reconstructed embryos for 24 hr after NT were improved significantly blastocyst formation (Zhang *et al.*, 2007). Lower concentrations (5 nM) of TSA, this treatment of fetal fibroblast cell cells for 1 hr significantly increased the histone H3 acetylation levels (Fig. 1), increased development of blastocysts derived from TSA-treated reconstructed embryos (Table 2 and 3). 50 nM TSA treatment of fetal fibroblast cells for 1 hr also significantly increased the histone H3 acetylation levels (Fig. 1), increased development of blastocysts derived from TSA-treated donor cells (Table 4). Epigenetic changes in donor cells are sensitive to low levels of a histone-modifying drug, and it is possible that histone modifica-

tions changes occurred in porcine reconstructed embryos from low concentration and short exposure of TSA (Table 2 and 3). It is concluded that TSA treatment should lead to improvements on the efficiency of the nuclear transfer procedures. Further investigation is needed to determine whether other chemical regents during early reconstructed porcine embryonic development could further enhance subsequent embryo development and affect the epigenetic reprogramming events.

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