pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 36(5) : 253-258 (2019)
http://dx.doi.org/10.17555/jvc.2019.10.36.5.253

Journal of Veterinary Clinics
www.ksvc.or.kr

Improved Preimplantation Development of Cloned Porcine Embryos through Supplementation of Histone Deacetylase Inhibitor MS-275

Xun Fang, Ahmad Yar Qamar, Sang Tae Shin and Jongki Cho[1]

College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Korea

(Received: September 18, 2019 / Accepted: October 11, 2019)

Abstract : The objective of this study was to analyse the effects of MS-275 (Class I and II histone deacetylase inhibitor) supplementation on the development of porcine *in-vitro* somatic nuclear transfer embryo production. During *in-vitro* development, early embryos were exposed to different concentrations of MS-275 (0, 5 μM, 10 μM, and 20 μM). In *in-vitro* culture supplemented group, the blastocyst development rate was significantly enhanced by 10 μM concentration than other groups (24.0% vs. 19.3%, 21.8%, 11.5%; $P < 0.05$). Additionally, the 6 h supplementation group, significantly improved the blastocysts production than 24 h, 48 h and control groups (26.1% vs. 17.0%, 15.2%, 2.8%; $P < 0.05$). Following supplementation with optimal concentrations and time (10 μM-6 h group), the blastocyst production was significantly higher than control (25.7% vs 15.8%; $P < 0.05$). The optimal concentrations of MS-275 significantly enhanced the percentages of ICM:TE than control (43.6% vs. 38.4%; $P < 0.05$) accompanied with significantly higher expression levels of reprogramming related genes (*POU5F1*, *Naong*, and *SOX2*). In conclusion, the optimal concentrations of 10 μM MS-275 and 6 h supplementation during *in-vitro* culture can significantly improve the quality of porcine *in-vitro* somatic nuclear transfer embryos through histone acetylation and epigenetic modification. Increasing the efficiency of clonal animal production will greatly promote the development of animal disease models and xenotransplantation.

Key words **:** SCNT embryo, HDACs inhibitor, *in-vitro* development, blastocyst development, reprogramming.

Introduction

Somatic cell nuclear transfer (SCNT) is a widely-used technique in the diverse fields of life science, such as animal production and biotechnology (15), xenotransplantation (12), and animal disease models (10,23). In addition, SCNT technology is also being applied in embryonic stem cell research and genetic engineering (9,19,21,35). Extracellular environment damages decrease the cloning efficiency through negative influence on oocyte maturation and culture (22,25,37). However, many internal problems lead to a decrease in the development rate of SCNT embryos and the quality of embryos, such as histone hypo-acetylation, DNA methylation, and abnormal reprogramming gene expression (2,4,6,28). Thus, nuclear reprogramming serves as a key to improved cloning efficiency.

Histone deacetylases (HDACs) catalyze the removal of acetyl group from lysine residues in histone amino termini (33) and play an important role in epigenetics. It is well known that the nuclear reprogramming process mainly involves various epigenetic modifications. Research evidences have revealed that HDACs-mediated histone acetylation/deacetylation is crucial for the development of mammalian oocytes (5,11,20,31,34). In terms of cloning and epigenetics relationship, there is still a need to search out the mechanisms associated with HDACs and HDACs inhibitor.

Entinostat SNDX-275 (MS-275), is a novel specific inhibitor belonging to the class I and II HDACs. Several studies related to cell biology involving the use of MS-275 resulted in significant inhibition of tumors in *in-vivo* conditions (18). However, the role of MS-275 in nuclear reprogramming for SCNT embryo production has not yet been reported and its mechanisms of action remained unclear.

The objective of this experiment was to investigate the improvement in the developmental competence of porcine SCNT embryos as a result of MS-275 supplementation and explore its relationship with DNA reprogramming and apoptosis. For this purpose, the different parameters including the developmental rates of embryos, and expression levels of genes related to apoptosis & reprogramming (*BAX*, *Bcl-2*, *POU5F1*, *SOX2*, and *NANOG*) were investigated.

Materials and Methods

Chemicals and reagents

The chemicals and reagents utilized were purchased from Sigma-Aldrich Company (St. Louis, MO, USA) unless otherwise indicated.

Production of embryos

All the experiments were conducted according to our laboratory protocols as previously explained (27). Transportation of ovaries from the slaughterhouse to the laboratory was done in a temperature-controlled thermos containing

[1]Corresponding author.
E-mail : cjki@cnu.ac.kr

0.9% saline. The follicular fluid was aspirated from follicles within a diameter range of (3-8 mm) using 10 mL syringes with the aid of 18 gauges' needle. The cellular part was allowed to settle down and washed utilizing HEPES-buffered Tyrode's (TLH) media, having 0.05% (w/v) polyvinyl alcohol (TLH-PVA) (1). Cumulus-oocyte complexes (COCs) with at least three compact cumulus cell layers were selected for the experimental trails. Selected COCs were washed three times with TLH-PVA, and once in in-vitro meturation (IVM) media drop. The base medium for IVM of oocytes was Medium-199 (Gibco, USA). Medium-199 was supplemented with 10% (vol/vol) porcine follicular fluid, 0.6 mM cysteine, 0.91 mM pyruvate, 10 ng/mL epidermal growth factor, 75 mg/mL kanamycin. Selected COCs (50-80) were placed per well of 4-well multi dishes (Nunc, Denmark) containing 500 mL of IVM media including 10 IU/mL PMSG/hCG (Intervet International BV, Holland). Then, COCs were incubated at 39°C temperature for 22 h with hormones in 5% CO_2 humidified atmosphere. Finally, change the COCs to IVM media without the hormones.

Donor cells were isolated from the porcine tissues. The primary fibroblast cell were cultured until 3/4 passages in CO_2 incubator at 38.7°C and 5% CO_2. Fibroblasts were cultured until the formation of complete monolayer cells in 60 mm culture dish using Dulbecco's Modified Eagle Medium (DMEM) (Corning, USA) containing 10% (v/v) fetal bovine serum (FBS) (36). The donor cell cycle was synchronized at Go/G1 stage for 48-72 h by serum starvation and a similar number of passages were used in each replicate (3-8 passages). Prior to nuclear transfer, trypsinization of donor cells was performed and cellular suspension was prepared in 0.4% (w/v) BSA with TLH. Denuded the oocytes by hyaluronidase (Sigma, USA) and oocytes were washed three times in hormone-free IVM medium and incubated for 15 min in 5 μg/mL Hoechst 33342 medium (Sigma, USA) overlaid by mineral oil. Enucleation of metaphase II and first polar body (PB) was performed with the help of 17 μm beveled glass pipette (Humagen, Charlottesville, VA, USA) and enucleation was confirmed by using epifluorescence microscope. Enucleation was followed by the injection of a single donor cell into the perivitelline space. Electric cell fusion of reconstructed oocytes was done in 280 mM mannitol solution with low Ca^{2+} concentration (0.001 mM), using 2 DC pulses of 160 V/cm for 40 μsec, alternative current of 2 V/cm, 2 sec using a BTX 2001 Electro-cell Manipulator (BTX, San Diego, CA, USA). Activation of reconstructed oocytes was performed in 280 mM mannitol solution, using 120 V/cm of direct current, two pulses for 60 μsec. Post activation and incubation of reconstructed oocytes were carried out at same condition and environment as explained SCNT embryos.

For *in-vitro* culture, a 25-μL droplet of PZM-5 (porcine zygote medium) was used and 10-12 embryos per drops. Prior to culture, embryos were washed three times in PZM-5 medium. Incubation was performed for a period of 6-7 days at 39°C temperature and in humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Designating nuclear transfer day as day 0, cleavage and blastocyst rates were evaluated on day 2 and day 6, respectively.

Experimental design

In experiment 1, the optimal concentration of MS-275 during IVC of porcine oocytes or embryo was determined. The developmental rates of the SCNT embryo were compared among the different concentration groups (0, 5 μM, 10 μM, 20 μM, respectively). The experiment was repeated 8 times. In experiment 2, the optimal exposure time of MS-275 suitable for the enhancement of development rates of porcine SCNT embryos supplemented with optimal concentration of MS-275 (0, 6 h, 24 h, and 48 h, respectively). In experiment 3, the developmental competence of porcine SCNT embryos was compared in terms of cleavage rate, blastocysts rate between the most effective concentration and exposure time of MS-275 with control group. In experiment 4, developmental competence of SCNT embryos compare with the MS-275 group and control group through different staining. In experiment 5, analyzed the extent of nuclear reprogramming of porcine SCNT embryos induced by the superior performance group of MS-275. The expression levels of different reprogramming and apoptosis-related genes including BAX, Bcl-2, POU5F1, SOX2, NANOG, and control gene β-Actin were compared between optimal concentration & exposure time of MS-275 and control group.

Differential staining

Triton X-100 (Sigma, USA) was diluted in PBS (T solution) to make final concentration of 0.1% (v/v). Blastocysts were incubated in Hoechst 33342 solution for 40 min. Blastocysts were incubated in 500 μL of the T solution at room temperature for 1 min. Then blastocysts were placed in 500 μL of Propidium iodide (Sigma, USA) for 35-40 sec followed by PBS washing. Ultimately, the blastocysts were transferred onto microscope slides, covered with a drop of glycerol solution and mounted by a coverslip. Blastocysts were observed immediately under UV light (200-400 nm) (8).

Gene expression analysis by quantitative polymerase chain reaction (qPCR)

Embryos sample were used day 6 blastocyst. Total RNAs were extracted to analyze gene expression, using TRIZOL reagent (Invitrogen, USA) was used. according to the manufacturer's protocol, and the total RNA concentration was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA). Following the manufacturer's instructions cDNA was synthesized from RNA. (cDNA synthesis kit, iNtRON Bio Inc., Korea).

The analysis to evaluate the gene expression levels was carried out for reprogramming and apoptosis-related genes (BAX, Bcl-2, POU5F1, SOX2, and NANOG). Quantitative real-time PCR (CFX connect; BIO-RAD, USA) was performed using 2X Real-Time PCR Pre-Mix (BioFACT, Korea) containing specific primers using 1 μL of the cDNA template. Reactions were conducted till 40 cycles and the cycling parameters were as follows: denaturation at 95°C for 15 minutes 20 seconds, annealing and extension at 60°C

for 40 seconds. The expression of each target gene was quantified relative to that of the internal control gene (β-Actin). Melting curve analysis was used to check PCR specificity. The relative quantification was based on the comparison of threshold cycle (Ct) at constant fluorescence intensity. The relative mRNA expression was calculated using the equation, mRNA expression = $2^{-(\Delta Ct\ sample - \Delta Ct\ control)}$. To determine a normalized arbitrary value for each gene, every value was normalized to β-actin.

Statistical analysis

Each experiment was repeated at least eight times and data was analyzed by SPSS 21.0 software (SPSS Inc., Chicago, USA) with a general linear model by one-way ANOVA. A probability of a *p*-value at < 0.05 was used. All experimental data percentages were presented as the mean ± SEM (standard error of the mean).

Results

***In- vitro* development of SCNT embryos after MS-275 supplementation (experiment 1-3)**

In-vitro developmental rates of porcine SCNT embryos following MS-275 supplementation were shown in Table 1 and 2. MS-275 supplementation of IVC media did not resulted in any improvement in cleavage rate between the groups. However, the blastocyst development rate was significantly higher ($p < 0.05$) in the 10 μM group than 20 μM and control group but no significantly different with 5 μM group (16.8 ± 1.6% vs. 21.8 ± 2.2%, 11.5 ± 0.9%, 19.3 ± 1.1%, respectively). In IVC supplemented time groups, the cleavage percentages were not significantly different ($p > 0.05$) among 6 h, 24 h and 48 h groups (89.8 ± 0.7% vs. 85.4 ± 1.6%, 88.1 ± 0.4%, respectively). Whereas, the percentage of blastocysts production rate was significantly higher ($p < 0.05$) in the 6 h group than other groups (26.1 ± 1.1% vs. 17.0 ± 1.2%, 15.2 ± 1.9%, 2.8 ± 1.4%, respectively). In Table 3, the percentage of MS-275 treatment group blastocysts rate was significantly higher ($p < 0.05$) than control group (25.7 ± 1.0% vs. 15.8 ± 0.8%).

Differential staining of SCNT blastocysts (experiment 4)

The total cell number and percentages of inner cell mass (ICM) and trophectoderm (TE) cells in cloned porcine embryos following MS-275 supplementation were shown in Table 4. The total cell number of MS-275 supplemented group was significantly higher ($p < 0.05$) than control (37.0 ± 1.0% vs. 43.4 ± 1.2%). Similarly, the percentages of ICM and TE cells were significantly higher ($p < 0.05$) in the optimal concentration and exposure time of MS-275 than the control group (38.4 ± 2.1% vs. 43.6 ± 1.9%) (Fig 1).

Gene expression of MS-275 treatment embryo (experiment 5)

The supplementation with optimal levels and exposure time of MS-275 significantly reduced ($p < 0.05$) the expression level of *BAX*, but *Bcl2* gene expression was significantly enhanced ($p < 0.05$). On the other hand, the expression levels of reprogramming related genes *POU5F1*, *SOX2* and *NANOG* were significantly improved ($p < 0.05$) by MS-275

Table 1. Effect of MS-275 supplementation into IVM media with 4 different concentrations on development of porcine SCNT embryos

Treatment (μM)	No. of embryos		
	Cultured	Cleaved (% ± SEM)	Develop to Bl. (% ± SEM)
0	171	150 (87.7 ± 0.8)^{a}	33 (19.3 ± 1.1)^{a}
5	174	157 (90.2 ± 1.2)^{a}	38 (21.8 ± 2.2)^{ab}
10	175	152 (86.9 ± 1.0)^{a}	42 (24.0 ± 2.1)^{b}
20	174	136 (78.2 ± 1.6)^{b}	20 (11.5 ± 0.9)^{c}

Means in the same column with different superscripts were significantly different (P < 0.05).
Values are listed as Mean ± S.E.M.

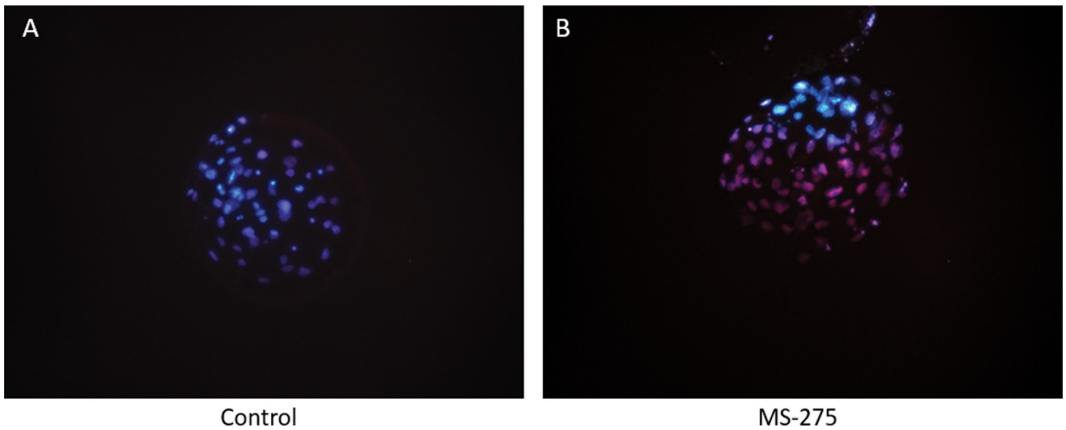

Fig 1. Different staining to compare with the control group (A) the MS-275 treatment group (B). Staining the ICM and TE by Hoechst 33342 and Propidium iodide. The experiment was replicated eight times.

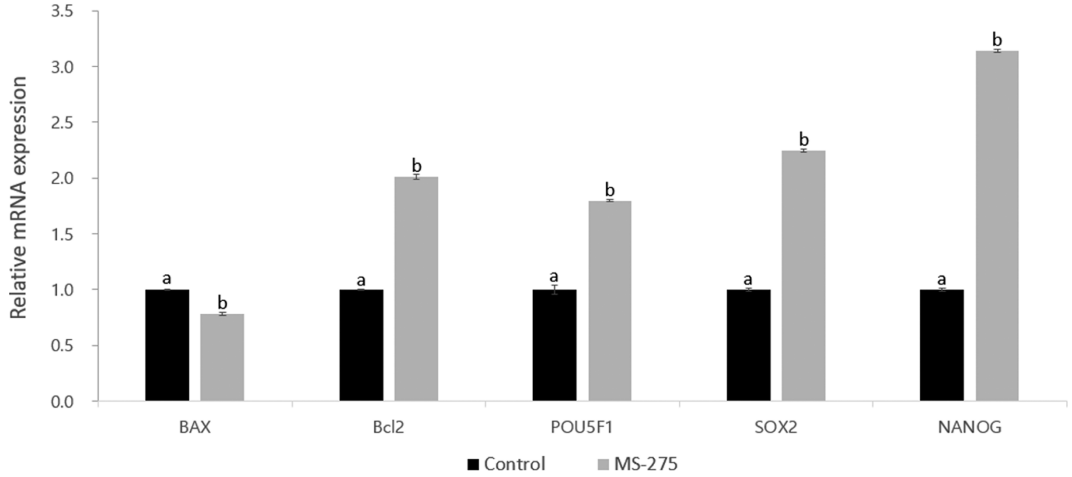

Fig 2. Relative expression of *BAX*, *Bcl-2*, *POU5F1*, *SOX2* and *NANOG* genes in cloned blastocysts using oocytes supplemented with 10 μM and 6h of MS-275 in culture media. [a-b]Within the same mRNA values with different superscript letters differ significantly ($P < 0.05$).

supplementation (Fig 2).

Discussion

The donor cell nucleus in MII oocyte reprograms to a pluripotent state after SCNT. However, not all of the SCNT oocytes can complete the nuclear reprogramming process. Abnormal degradation of mRNA in the cytoplasm of MII oocyte causes incomplete expression of reprogramming related genes (17). Therefore, the donor cell nucleus has incomplete or aberrant epigenetic reprogramming that mainly leads to low cloning efficiency by SCNT (38).

The use of HDACs inhibitors in IVC treatment is increasing both in SCNT and parthenogenetic embryos (14,17,32). At present, many studies have gradually shown that HDACs inhibitor treatment can improve the development of SCNT embryos. In this study, we sought to use MS-275 (class I and II HDACs inhibitor) treatment to improve the development of porcine SCNT embryos. We have found that MS-275 (10 μM for 6 h) can regulate and promote gene reprogramming and gene expression. In addition, we have detected a significant increase in the cell number of the SCNT embryo by increasing the associated gene expression (Table 4).

Histone acetylation is a key factor in the epigenetic reprogramming, it modifies and regulates chromatin configuration to participate in nuclear reprogramming. It is well-known that HDACs inhibitors restrain the expression of HDACs, and resulting in reduced deacetylation and increased acetylation levels (13). In this study, we tested the treatment of SCNT embryos with HDACs inhibitor. Compared with the control group, different concentrations and treat-

Table 2. Effect of MS-275 different supplementation time into IVC media with 4 different times on development of porcine SCNT embryos

Treatment (h)	No. of embryos		
	Cultured	Cleaved (% ± SEM)	Develop to Bl. (% ± SEM)
Control	176	155 (88.1 ± 0.4)[a]	30 (17.0 ± 1.2)[a]
6	176	158 (89.8 ± 0.7)[a]	46 (26.1 ± 1.1)[b]
24	178	152 (85.4 ± 1.6)[a]	27 (15.2 ± 1.9)[a]
48	177	110 (62.1 ± 1.7)[b]	5 (2.8 ± 1.4)[c]

Means in the same column with different superscripts were significantly different ($P < 0.05$).
Values are listed as Mean ± S.E.M.

Table 3. Effect of MS-275 supplementation into IVC media on the development of porcine SCNT embryos

Treatment (μM)	No. of embryos		
	Cultured	Cleaved (% ± SEM)	Develop to Bl. (% ± SEM)
Control	221	192 (86.9 ± 1.0)	35 (15.8 ± 0.8)[a]
MS-275	218	194 (89.0 ± 1.2)	56 (25.7 ± 1.0)[b]

Means in the same column with different superscripts were significantly different ($P < 0.05$).
Values are listed as Mean ± S.E.M.

Table 4. Developmental competence of porcine embryos of MS-275 treatment

Treatment (μM)	No. of cells			
	ICM	TE	Total Cell (mean ± SEM)	ICM/TE Cell (% ± SEM)
Control	10.2	26.8	37.0 ± 1.0^{a}	38.4 ± 2.1^{a}
MS-275	13.1	30.2	43.4 ± 1.2^{b}	43.6 ± 1.9^{b}

Means in the same column with different superscripts were significantly different ($P < 0.05$).
Values are listed as Mean ± S.E.M.

ment time of MS-275 did not have any effect on in cleavage of embryos (Table 1). At the same time, we found that long-term treatment will reduce embryo formation (Table 2). The reason behind the adverse effects of long-term treatment is that HDACs inhibitor will make chromatin relaxation, which will affect spindle formation and arrest mitosis (24). However, the treatment of MS-275 at the blastocyst stage significantly improves embryo quality, due to the increased acetylation of histones that can loosen the structure of chromatin and open the binding of DNA and nucleosomes to enhance gene expression (29). Previous research data also indicate that *in-vitro* development of SCNT embryos may be inhibited by HDACs activity (26) (Table 3). Our results also support the view that inhibition of HDACs activity is a key factor in the *in vitro* development of SCNT embryos.

The quality of embryo produced was assessed by analysis of mRNA expression levels of different genes associated with development (POU5F1, NANOG and SOX2) and apoptosis (BAX and Bcl2) in MS-275-treated SCNT blastocysts. During mammalian embryonic development, the cell mass will be divided into the inner cell mass (ICM) and trophectoderm (TE) (16) (Fig 1). In this study, the expression of POU5F1 and NANOG in the MS-275 treatment group was significantly increased. Oct4/POU5F1 is at the center of a pluripotency regulatory network and plays a vital role in the formation of the embryo (30). Oct4, NANOG and SOX2, which are known to form an auto regulatory network for pluripotency in other systems, are co-expressed also by porcine epiblasts, and by undifferentiated primary colonies in culture (7). The expression of SOX2 in the MS-275 treatment group was significantly increased (Fig 2). Therefore, the quality of SCNT embryos is also associated with apoptosis (3). MS-275 treatment significantly decrease the expression of the apoptotic gene (BAX) and increase expression of anti-apoptotic gene (*Bcl2*) in blastocyst (8). These results demonstrated that MS-275 treatment not only increased the expression of reprogramming-related genes but also improved embryo quality.

Conclusions

The present study indicated that 10 μM MS-275 treatment for 6 h positively regulates the epigenetic expression and increases histone acetylation, thereby increasing the development of SCNT embryos. Furthermore, MS-275 treatment enhanced ICM/Total cell number and the quality of blastocysts by increasing expression of Bcl2, POU5F1, NANOG and SOX2, and decreasing expression of BAX. Increasing the efficiency of clonal animal production will greatly promote the development of animal disease models and xenotransplantation. Therefore, further investigations are required for analyzing the effect of MS-275 on the development of fetuses after implantation from MS-275 treated embryos.

Acknowledgments

We thank all the members of our laboratory for technical support and helpful discussion. This work was supported by research fund of Chungnam National University 2018.

References

1. Bavister BD, Leibfried ML, Lieberman G. Development of preimplantation embryos of the golden hamster in a defined culture medium. Biol Reprod 1983; 28: 235-247.
2. Bo F, Di L, Qing-chang F, Liang R, Hong M, Liang W, Zhenhua G, Zhong-qiu L. Effect of trichostatin A on transfected donor cells and subsequent development of porcine cloned embryos. Zygote 2011; 19: 237-243.
3. Buemo CP, Gambini A, Moro LN, Hiriart MI, Fernandez-Martin R, Collas P, Salamone DF. Embryo aggregation in pig improves cloning efficiency and embryo quality. PLoS One 2016; 11: e0146390.
4. Cao Z, Li Y, Chen Z, Wang H, Zhang M, Zhou N, Wu R, Ling Y, Fang F, Li N, Zhang Y. Genome-wide dynamic profiling of histone methylation during nuclear transfer-mediated porcine somatic cell reprogramming. PLoS One 2015; 10: e0144897.
5. Cervera RP, Marti-Gutierrez N, Escorihuela E, Moreno R, Stojkovic M. Trichostatin A affects histone acetylation and gene expression in porcine somatic cell nucleus transfer embryos. Theriogenology 2009; 72: 1097-1110.
6. Deshmukh RS, Ostrup O, Ostrup E, Vejlsted M, Niemann H, Lucas-Hahn A, Petersen B, Li J, Callesen H, Hyttel P. DNA methylation in porcine preimplantation embryos developed in vivo and produced by in vitro fertilization, parthenogenetic activation and somatic cell nuclear transfer. Epigenetics 2011; 6: 177-187.
7. du Puy L, Lopes SM, Haagsman HP, Roelen BA. Analysis of co-expression of OCT4, NANOG and SOX2 in pluripotent cells of the porcine embryo, in vivo and in vitro. Theriogenology 2011; 75: 513-526.
8. Fang X, Qamar AY, Yoon KY, Cho JK. Improved preimplantation development of porcine cloned embryos by flavone supplement as antioxidant. J Emb Trans 2018; 33: 255-264.
9. Hassan BMS, Fang X, Roy PK, Shin ST, Cho JK. Effect of alpha lipoic acid as an antioxidant supplement during in vitro maturation medium on bovine embryonic development. J Emb Trans 2017; 32: 123-130.

10. Holm IE, Alstrup AK, Luo Y. Genetically modified pig models for neurodegenerative disorders. J Pathol 2016; 238: 267-287.
11. Huang Y, Yuan L, Li T, Wang A, Li Z, Pang D, Wang B, Ouyang H. Valproic acid improves porcine parthenogenetic embryo development through transient remodeling of histone modifiers. Cell Physiol Biochem 2015; 37: 1463-1473.
12. Hwang JH, Kim SE, Gupta MK, Lee H. Gnotobiotic miniature pig interbreed somatic cell nuclear transfer for xenotransplantation. Cell Reprogram 2016; 18: 207-213.
13. Jin JX, Lee S, Taweechaipaisankul A, Kim GA, Lee BC. The HDAC inhibitor LAQ824 enhances epigenetic reprogramming and in vitro development of porcine SCNT embryos. Cell Physiol Biochem 2017; 41: 1255-1266.
14. Jin L, Guo Q, Zhang GL, Xing XX, Xuan MF, Luo QR, Luo ZB, Wang JX, Yin XJ, Kang JD. The histone deacetylase inhibitor, CI994, improves nuclear reprogramming and in vitro developmental potential of cloned pig embryos. Cell Reprogram 2018; 20: 205-213.
15. Keefer CL. Artificial cloning of domestic animals. Proc Natl Acad Sci U S A 2015; 112: 8874-8878.
16. Kim G, Roy PK, Hassan BMS, Cho JK. Improved preimplantation development of porcine somatic cell nuclear transfer embryos by caffeine treatment. J Vet Sci 2019; 20: e31.
17. Lagutina I, Fulka H, Lazzari G, Galli C. Interspecies somatic cell nuclear transfer: advancements and problems. Cell Reprogram 2013; 15: 374-384.
18. Ma S, Liu T, Xu L, Wang Y, Zhou J, Huang T, Li P, Liu H, Zhang Y, Zhou X, Cui Y, Zang X, Wang Y, Guan F. Histone deacetylases inhibitor MS-275 suppresses human esophageal squamous cell carcinoma cell growth and progression via the PI3K/Akt/mTOR pathway. J Cell Physiol 2019; 12: 22400-22410.
19. Malaweera DBO, Ramachandra S, Wu JB, Oh SK, Kim SH, Kim SJ, Shin ST, Cho JK. Establishment of efficient microinjection system in the porcine embryos. J Emb Trans 2014; 29: 59-66.
20. Mao J, Zhao MT, Whitworth KM, Spate LD, Walters EM, O'Gorman C, Lee K, Samuel MS, Murphy CN, Wells K, Rivera RM, Prather RS. Oxamflatin treatment enhances cloned porcine embryo development and nuclear reprogramming. Cell Reprogram 2015; 17: 28-40.
21. Matoba S, Zhang Y. Somatic Cell Nuclear Transfer Reprogramming: Mechanisms and Applications. Cell Stem Cell 2018; 23: 471-485.
22. Min KH, Na SW, Lee EC, Kim GY, Fang X, Hassan BMS, Cho JK. Study on Chemicals for post-activation in porcine somatic cell nuclear transfer. J Emb Trans 2016; 31: 131-136.
23. Na SW, Lee EC, Kim GY, Min KH, Yu YK, Roy PK, Fang X, Hassan BMS, Yoon KY, Shin ST, Cho JK. Study on embryo transfer system for production of transgenic pigs. J Emb Trans 2015; 30: 345-350.
24. Nalawansha DA, Gomes ID, Wambua MK, Pflum MKH. HDAC inhibitor-induced mitotic arrest is mediated by Eg5/KIF11 acetylation. Cell Chem Biol 2017; 24: 481-492.
25. Oh SK, Malaweera DBO, Ramachandra S, Shin ST, Cho JK. Effect of 7,8-dihydroxyflavone on in vitro maturation of oocytes in pigs. J Emb Trans 2014; 29: 67-72.
26. Pasque V, Jullien J, Miyamoto K, Halley-Stott RP, Gurdon JB. Epigenetic factors influencing resistance to nuclear reprogramming. Trends Genet 2011; 27: 516-525.
27. Roy PK, Fang X, Hassan BMS, Shin ST, Cho JK. Effects of roscovitine on in vitro development of porcine oocyte using brilliant cresyl blue. J Emb Trans 2017; 32: 111-122.
28. Roy PK, Kim KY, Fang X, Hassan BMS, De Zoysa M, Shin ST, Cho JK. Optimization of post-activation systems to improve the embryonic development in porcine parthenogenesis and somatic cell nuclear transfer. J Emb Trans 2017; 32: 95-104.
29. Rybouchkin A, Kato Y, Tsunoda Y. Role of histone acetylation in reprogramming of somatic nuclei following nuclear transfer. Biol Reprod 2006; 74: 1083-1089.
30. Simmet K, Zakhartchenko V, Philippou-Massier J, Blum H, Klymiuk N, Wolf E. OCT4/POU5F1 is required for NANOG expression in bovine blastocysts. Proc Natl Acad Sci U S A 2018; 115: 2770-2775.
31. Song Y, Hai T, Wang Y, Guo R, Li W, Wang L, Zhou Q. Epigenetic reprogramming, gene expression and in vitro development of porcine SCNT embryos are significantly improved by a histone deacetylase inhibitor--m-carboxycinnamic acid bishydroxamide (CBHA). Protein Cell 2014; 5: 382-293.
32. Taweechaipaisankul A, Jin JX, Lee S, Kim GA, Suh YH, Ahn MS, Park SJ, Lee BY, Lee BC. Improved early development of porcine cloned embryos by treatment with quisinostat, a potent histone deacetylase inhibitor. J Reprod Dev 2019; 65: 103-112.
33. Wang H, Cui W, Meng C, Zhang J, Li Y, Qian Y, Xing G, Zhao D, Cao S. MC1568 enhances histone acetylation during oocyte meiosis and improves development of somatic cell nuclear transfer embryos in pig. Cell Reprogram 2018; 20: 55-65.
34. Whitworth KM, Mao J, Lee K, Spollen WG, Samuel MS, Walters EM, Spate LD, Prather RS. Transcriptome analysis of pig in vivo, in vitro-fertilized, and nuclear transfer blastocyst-stage embryos treated with histone deacetylase inhibitors post-fusion and activation reveals changes in the lysosomal pathway. Cell Reprogram 2015; 17: 243-258.
35. Yin Y, Hao H, Xu X, Shen L, Wu W, Zhang J, Li Q. Generation of an MC3R knock-out pig by CRSPR/Cas9 combined with somatic cell nuclear transfer (SCNT) technology. Lipids Health Dis 2019; 18: 122.
36. You J, Lee J, Hyun SH, Lee E. L-carnitine treatment during oocyte maturation improves in vitro development of cloned pig embryos by influencing intracellular glutathione synthesis and embryonic gene expression. Theriogenology 2012; 78: 235-243.
37. Yu YK, Roy PK, Min KH, Na SW, Lee EC, Kim GY, Fang X, Hassan BMS, Cho JK. Effect of insulin supplement on development of porcine parthenogenetic embryos. J Emb Trans 2016; 31: 123-129.
38. Zhao J, Whyte J, Prather RS. Effect of epigenetic regulation during swine embryogenesis and on cloning by nuclear transfer. Cell Tissue Res 2010; 341: 13-21.