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Optimization of Post-Activation Systems to Improve the Embryonic Development in Porcine Parthenogenesis and Somatic Cell Nuclear Transfer

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ABSTRACT

This study was conducted to establish the optimal chemical post-activation conditions in porcine embryonic development after parthenogenesis (PA) and somatic cell nuclear transfer (SCNT) using 4 different chemical compositions (cytochalasin B (CB), cyclohexamide (CHX), demecolcine (DC), 6-dimethylaminopurine (DMAP). Porcine embryos were produced by PA and SCNT and then, cultured for post-activation with CB (7.5 ㎍/mL), CB (7.5 ㎍/mL) + CHX (10 ㎍/mL), CB (7.5 ㎍/mL) +DC (0.4 ㎍/mL), and CB (7.5 ㎍/mL) + DMAP (2 mM). In PA embryonic development, cleavage rates have been significantly higher in CB group (94.7%) and CB+DMAP group (94.1%) than that of CB+CHX and CB+DC group (88.1 and 84.3%, respectively). There have been no significant differences in blastocyst formation rates among the four groups. In cell number of blastocyst was shown in CB group (42.3%) significantly higher than CB+CHX and CB+DC group (40.6 and 40.6%, respectively). In SCNT embryonic development, CB+DMAP group (89.7%) significant differences were found on embryo cleavage rates when compared with other three groups. Blastocyst formation rates in CB+DMAP group (26.9%) were significantly higher when compared with CB, CB+CHX, and CB+DC groups (25.5, 20.2, and 22.1%, respectively). In blastocyst cell number, CB+DMAP group (41.4%) was found higher significant difference compared with other three groups. Additionally, we have investigated survivin expression in early development stages of porcine SCNT embryos for more confirmation. Our results establish that CB group and CB+DMAP group for 4 h during post-activation improves pre-implantation improvement of PA and SCNT embryos.

(Key words: Embryo, Cloning, Parthenogenesis, Pig, Post-activation)

INTRODUCTION

Somatic cell nuclear transfer (SCNT) is a process whereas somatic cell of nucleus transfers into perivitelline space of enucleated oocytes, which is used for transgenic animal production and scientific research for xenotransplantation. SCNT embryos in early embryonic developmental stages have a role for nuclear reprogramming and remodeling of donor nuclei. Nowadays, cloned offspring production are very poor with low efficiency with SCNT embryos viability is very low (Betthauser et al., 2000a). To improve embryos quality, focus on post-activation on parthenogenesis (PA) and SCNT.

Physiologically and genomics properties in pigs are similar with human and also play importance role for different disease model and biological research with compared other species (Chieppa et al., 2014; Ito et al., 2014; Polejaeva et al., 2000; Umeyama et al., 2013). Porcine somatic cell cloning is essential for the conservation of endangered species, improved livestock animals, animals as bioreactors and hybrid bio-artificial organs production (Guo et al., 2015; Holm et al., 2016; Ito et al., 2014; Whitelaw et al., 2016). In porcine somatic cell cloning, the proficiency measured with embryos developing rate to stages of a blastocyst or born offspring rate in a relationship with a reconstructed number of embryos remains drastically low. However, the production rate is very low due to the maintenance of improper environmental conditions, early development of fetal death, stillbirth, abnormal birth and after birth neonatal death immediately (Miyazaki et al., 2005) and severe combined immune deficiency (SCID)(Ito et al., 2014) . Therefore, porcine cloning is very important in practical field of SCNT technique for developing efficient procedure of embryo production (Song et

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al., 2009c). For developing the embryos quality and increasing the developmental rates have to focus on post-activation.

Post-activation is an important steps for developmental competence of porcine cloning (De Sousa et al., 2002; Kurome et al., 2003; Miyoshi et al., 2005). Thus, most of the researchers have been trying to find a valuable method to optimize different post-activation media with cultured media of oocytes *in vitro*. To minimize the problems different post-activation media used to increase the production rates. Such as cytochalasin B (CB) (De Sousa et al., 2002), CB with cycloheximide (CHX) (Du et al., 2008), CB with 6-dimethylaminopurine (DMAP) (Yi and Park, 2005a) and CB with demecolcine (DC) (Song et al., 2009b). As a result, a better understanding of optimum post-activation media could enhance the embryos quality with positive effects on production of transgenic pigs. Cytochalasin B (CB) mechanism is to suppress the actin filament polymerization and has been widely used for suppression of the extrusion of the polar body in SCNT and PA embryos (Lee et al., 2004a; Meena and Das, 2006; Wilmut et al., 1997). It also suppresses pseudo polar body (PPB) extrusion, inhibits maturation of embryos, break up the spindle structure, and diploid embryos formation. Another researcher also reported that inhibit the extrusion of the polar body after activation of SCNT embryos (Song et al., 2009a). Cycloheximide (CHX) inhibits the protein synthesis and block the translation of eukaryotic and attached with ribosome (Schneider-Poetsch et al., 2010) reduced the maturation-promoting factor (MPF) with protein synthesis inhibition (Im et al., 2007). Demecolcine (DC) has less cytotoxicity (Cooper, 1987) and ability to microtubule depolymerization and limited formation of microtubule (Rieder and Palazzo, 1992). Post-activation treatment with DC, induce the single pronucleus (PN) formation and assist development to delivery of pig SCNT embryos (Lee et al., 2010). 6'DMAP also reduced the MPF levels (Szollosi et al., 1993) of pig embryos and used to induce embryo activation with electrically in pig SCNT embryos (Cervera et al., 2010; Vichera et al., 2010). Additionally, we investigated survivin expression during developmental stages in different groups of SCNT embryos for more confirmation, which is a member of the inhibitor of apoptosis (IAP) gene family. It suppresses the replication of apoptotic protein domains containing a single baculovirus (Altieri, 2004), cell division regulated by inhibition of apoptosis, which convey the attachment of kinetochore, process of cytokinesis and formation of bipolar spindle (Ruchaud et al., 2007).

The objectives of this study were; (i) To investigate the effects of the condition of post-activation with; CB, CB+CH, CB+DC, CB+DMAP development of parthenogenesis (PA) and somatic cell nuclear transfer (SCNT) changes nuclei of the donor *in vitro* pig embryos. (Miyazaki et al.) survivin gene expression during development of SCNT embryos using RT-PCR.

Materials and Methods

1. Culture Media

All chemicals and reagents purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) unless otherwise indicated. Oocytes cultured in maturation media consist of Tissue Culture Media-199 (TCM-199-Gibco). Culture media supplemented with 10% (v/v) of pFF (porcine follicular fluid), 10 μg/mL of eCG/hCG, 0.6mM cysteine, 0.91mM sodium pyruvate, and 75 μg/mL kanamycin, 10 ng/mL EGF (epidermal growth factor), 1 μg/mL insulin, 10 IU/mL human chorionic gonadotrophin (hCG; Intervet International BV, Holland) for 22 h (39°C, 5% CO_2). After 22 h of incubation, oocytes transferred to the 500 mL of culture media contains above formulation without hormone for another 22 h of incubation 39°C, 5% CO_2 conditions. 44 h of total incubation, oocytes stripped of cumulus cells by denuding of 0.1% (w/v) of hyaluronidase and oocytes with a visible first polar body was used for assessment of nuclear maturation for parthenogenesis and SCNT embryo production.

2. Oocytes Collection and IVM

Pig ovaries were collected from the local slaughterhouse within 4 h. The ovaries were maintained in a temperature-controlled thermos (38°C) and placed in 0.9% saline containing beaker whereas osmolality was regulated at 280 osmols/L. Collected ovaries were transported to the laboratory washed with pre-warmed saline water (38°C), and trimming the ovaries for collecting oocytes. The fluid in the follicles was aspirated using 10 mL syringes with the aid of 18 gauges' needle. The follicles, which have (3-8 mm) in diameter, selected for aspiration. Then the aspirate fluid collected into a 15 mL conical tube and kept for 5 min to allow them to settle down. After settling down the fluid washed with HEPES-buffered Tyrode's (TLH) media which containing 0.05% (w/v)polyvinyl alcohol (TLH-PVA) (Bavister et al.,

1983) then under stereomicroscope selected cumulus-oocyte complexes (COCs) which have at least thrice compact cumulus cells layers. COCs selected after three times with TLH-PVA, before IVM washed one time in IVM media. 50-80 COCs were selected for every 4-well multi dishes (Nunc, Denmark) for every well which containing 500 mL of IVM media including 10 IU/mL PMSG/hCG (Intervet International BV, Holland). Selected COCs were cultured at 5% CO_2 humidified atmosphere with temperature 39°C. After 22 h of maturation, transferred into hormone free IVM media after washing IVM media without hormone and cultured in additionally 18-21 h in without hormone IVM media.

3. Production of PA embryos

COCs were transferred into IVM medium without hormone by using 0.1% (w/v) hyaluronidase with pipetting gently repeated for removing cumulus cells after 44 h cultured in the maturation medium. After denuding, matured and good quality oocytes were activated with 120 V/cm of direct current with 2 pulses for 60 μsec where was media used 280 mM mannitol solution containing a low concentration of 0.01 mM $CaCl_2$ and 0.05 mM $MgCl_2$ using a BTX 2001 Electro-cell Manipulator (BTX, San Diego, CA, USA) for parthenogenetic activation (PA). After electrically activated PA oocytes were cultured with CB, CB+CHX, CB+DC, and CB+6'DMAP for 4 h with 5% CO_2 humidified atmosphere at 39°C.

4. Production of SCNT embryos

1) Donor cells preparation

Primary cell culture prepares from Sinclair's kidney cut into small pieces and centrifuge several times and culture in the incubator until 3/4 passages. Sinclair kidney fibroblasts were placed in 60 mm tissue culture dish that cultured with DMEM (Dulbecco's Modified Eagle Medium) from Sigma-Aldrich (De Sousa et al.) containing 10% (v/v) fetal bovine serum (FBS) from a single group before the formation of complete monolayers cells. G0/G1 stages of donor cells cycle synchronized for 48-72 h and a similar number of passages were used for each replicate (3-8 passages). Prior to nuclear transfer by using 0.4% (w/v) BSA with TLH prepared donor cells resuspension from trypsinization of cultured cells.

2) Transfer of nucleus

COCs were transferred into IVM media without hormone by using 0.1% (w/v) hyaluronidase with pipetting gently repeated for removing cumulus cells after 40 h cultured in maturation media. After denuding oocytes washed thrice in without hormone IVM media and put in an incubator for 15 min with 5 μg/mL Hoechst 33342 media of manipulation, then put into manipulation media which overlaid by mineral oil. Metaphase II and first polar body (PB) were removed with 17 μm beveled glass pipette (Humagen, Charlottesville, VA, USA) from metaphase II oocytes enucleating, after that enucleation confirmed by using epifluorescence microscope. After enucleating, inserted a fresh clean single cell into the space between zona pellucida and cell membrane.

3) Fusion and electrical activation

After enucleation, mature and good quality embryos were activated with 120 V/cm of direct current with 2 pulses for 60 μsec where was media used 280 mM mannitol solution containing a low concentration of 0.01 mM $CaCl_2$ and 0.05 mM $MgCl_2$ for SCNT and parthenogenetic activation (PA). When enucleation was completed for SCNT oocytes, electric cell fusion by 2 DC pulses of 160V/cm with 40 μsec, alternative current of 2V/cm, 2 sec (BTX, ECM 2001) 280 mM mannitol solution with low Ca concentration (0.001 mM). After electrically activated PA and SCNT oocytes were cultured with CB, CB+CH, CB+DC, CB+6'DMAP for 4 h with 5% CO_2 humidified atmosphere at 39°C.PA and SCNT embryos culture *in vitro.*

5. *In vitro* culture of embryos

PZM-5 (porcine zygote medium) was used for IVC medium that was made by 25 μL IVC droplet covered with mineral oil. Embryos washed thrice in PZM-5 medium and put into an incubator for 6 days with 39°C, 5% CO_2 humidified atmosphere, 5% O_2, and 90% N_2. Day of PA or SCNT designated at day 0, whereas cleavages and formation of blastocysts evaluated on day 2 and 6, respectively. By using Hoechst 33342 staining with the stereomicroscope total cells number in blastocysts were counted.

6. Comparison of gene expression

1) Total RNA Extraction and cDNA Synthesis

Embryos were harvested at different stages for analysis of total RNA transcript of survivin and GAPDH genes. For homogenization of the sample, used 10% volume of TRI

REAGENT (MOLECULAR RESEARCH CENTER, Ohio, USA). Store the homogenate 2-3 min at RT (room temperature). Supplement with 500 µL chloroform/1mL TRI REAGENT, vigorously shakes by hand for 15 sec spin at 12000 rpm, 4°C at 15 min. Transfer the 60% of colorless upper phase in a clean eppendorf tube. Adding 500 µL 0.5 mL isopropyl alcohol and 20 µg glycogen. Mixed well by hand and store at 4°C overnight. Centrifuge the store sample 12000 rpm for 10 min, 4°C. Discard the upper fluid slowly and wash with 75% of EtOH of RNA pellet. Following the manufacturer's instructions RNA were converted to cDNA 20 µL with 10 µL of 2X RT Reaction Solution, 1 µL Enzyme mix solution, 5 µL Template RNA, 4 µL DNase/RNase free water (cDNA synthesis kit, iNtRON Bio Inc.). The cDNA synthesis was completed reverse transcription at 50°C, 30 min, and RTase inactivation at 95°C, 5 min.

2) RT-PCR

Different numbers of cycles were amplified by using aliquots (1 µg) for target mRNA different conditions of PCR amplification logarithmic phase. RNA degradation possibility was ruled out and different concentration of mRNA was used for gene PCR amplification. The Survivin gene was quantified using 35 cycles. The cDNA extended with 20 µL of PCR reaction supplemented with 2.5 U i-StarTaqTM DNA polymerase, 2.5 mM dNTPs (iNtRON Bio. Inc.) including 10 pmol/µL specific primer. Initially, denaturation at 94°C for 2 min, denatured at 94°C with 20 sec, annealing at 58°C with 10 sec, extended at 72°C with 30 sec and finally extended at 72°C with 5 min. PCR reaction for oligo primers was listed Table 2. By using 1.5 %, agarose gel PCR reactions fractionated and stained by ethidium bromide and illumination under UV light. Pictures were taken, analyzed by Gel Doc EQ system (Bio-Rad Laboratories, Inc.).

7. Experimental Design

Experiment 1: Development of *in vitro* PA embryos treated with CB (cytochalasin B); CB+CHX (cycloheximide); CB+DC (demecolcine); CB+6'DMAP (6-Dimethylaminopurine) on embryonic development. Experiment 2: Development of *in vitro* SCNT embryos treated with CB (cytochalasin B); CB+CHX (cycloheximide); CB+DC (demecolcine); CB+6' DMAP (6-Dimethylaminopurine) on embryonic development. Experiment 3: Expression of survivin gene in different SCNT embryos using RT-PCR. In this study, four different post-activation media such as CB, CB+CHX, CB+DC, and CB+6'DMAP compared during post-activation in porcine embryos. Maturation rates were evaluating after denuding oocytes with a visible first polar body was used assessment and mature oocytes taken for the parthenogenetic and SCNT activation. Then activated embryos post-activated and cultured in PZM-5 medium for further development. After 2 and 6 days of incubation, embryos were examined, counted cleavage, blastocysts rates, and cells number, respectively. Blastocysts were stained for counting total cell numbers.

Table 1. Different post-activation treatment groups, time duration (h) and concentrations

Treatments	Time (Duration)	Concentration
CB	4 h	Cytochalasin B (7.5 µg/mL)
CB+CHX	4 h	Cytochalasin B (7.5 µg/mL) Cyclohexamide (7.5 µg/mL)
CB+DC	4 h	Cytochalasin B (7.5 µg/mL) Demecolcine (0.4 µg/mL)
CB+6'DMAP	4 h	Cytochalasin B (7.5 µg/mL) 2 mM 6-Dimethylaminopurine

Table 2. Primers with a base pair (bp) used for reverse transcriptase RT-PCR.

No.	Gene Name	Primer	Size (bp)
1	Survivin (F)	5′-GAC GAC GAC CCC ATA GAA GA-3′	149
2	Survivin (R)	5′-TTT GAC GTT TCT TTC AGG CG-3′	149
4	GAPDH (F)	5′-TCG GAG TGA ACG GAT TTG-3′	219
5	GAPDH (R)	5′-CCT GGA AGA TGG TGA TGG-3′	219

8. Statistical Analyses

Every experiment repeated at least eight times for embryonic development and data analyzed by SAS version 9.4 (Statistical Analysis System, USA) with a general linear model with one-way ANOVA. A probability of *p-value at <0.05*. All experimental data percentage presented as the mean ± SEM (standard error of the mean).

Results

1. Effects of post-activation treatments on development of PA *in vitro* embryos

In table 3, treatment of post-activation of PA embryos with CB, CB+CHX, CB+DC and CB+6'DMAP on embryo cleavage rates (84.3~94.7%), blastocyst formation rates (31.8~43.5%) and mean cell number of the blastocyst (40.6~42.3 cells/blastocyst). Embryo cleavage rates were significantly higher in CB group (94.7%) compared to CB+CHX group (88.1%) and CB+DC group (84.3%) but no significant difference in CB+6'DMAP group (94.1%) was observed. Similarly, in mean cell number of blastocyst but were not found significant differences in blastocyst formation among the four groups.

2. Effects of post-activation treatments on development of SCNT *in vitro* embryos

In table 4, cleavage rates were significantly higher in CB+6'DMAP group (89.7%) when compared to CB, CB+CHX, and CB+DC group (86.3, 81.7, and 84.0%, respectively) but not found significantly differences in CB+CHX group (81.7%)ss, and CB+DC group (84.0%) among two groups. Similarly, between two groups there were not found significant differences in the formation of blastocyst rates. In the case of blastocyst mean cell number CB+6'DMAP group (41.4%) was significantly higher when compared with CB, CB+CHX, and CB+DC group (40.1, 39.65, and 39.7%, respectively).

3. Survivin gene expression in SCNT embryos using RT-PCR

Survivin has both apoptosis and cell cycle progression regulators because of the IAPs (inhibitor of apoptosis protein family). We observed SCNT blastocysts influenced by survivin expression with high developmental competence during the treatment of different post-activation condition in porcine SCNT

Table 3. Effects of post-activation treatments on development of PA embryos with different media of CB, CB+CHX, CB+DC) and CB+6`DMAP on PA porcine embryos *in vitro.*

Treatment groups	No. of embryos cultured	% of embryos developed to*		Blastocysts cell no.
		≥2 cells	Blastocysts	
CB	295	94.7±1.5[a]	43.5±4.0[a]	42.3±0.9[a]
CB+CHX	295	88.1±4.0[ab]	32.2±5.5[a]	40.6±0.7[b]
CB+DC	295	84.3.±3.8[b]	31.8±3.3[a]	40.6±0.7[b]
CB+6`DMAP	295	94.1±1.6[a]	41.5±2.4[a]	41.7±0.8[a]

The experiment was replicated 8 times (*n*=8).

*Percentage of the number of embryos cultured.

[a, b]Values in the same column with different superscript letters are different (P<0.05).

Table 4. Effects of post-activation treatments on development of SCNT embryos with different media of CB, CB+CHX, CB+DC) and CB+6`DMAP on SCNT porcine embryos *in vitro.*

Treatment groups	No. of embryos cultured	% of embryos developed to*		Blastocysts cell no.
		≥2 cells	Blastocysts	
CB	187	86.3±1.6[ab]	25.5±1.9[ab]	40.1±0.3[b]
CB+CHX	187	81.7±1.6[b]	20.2±1.7[b]	39.6±0.3[b]
CB+DC	187	84.0±2.0[b]	22.1±1.7[ab]	39.7±0.3[b]
CB+6`DMAP	187	89.7±1.7[a]	26.9±1.7[a]	41.4±0.4[a]

The experiment was replicated 8 times (*n*=8).

*Percentage of the number of embryos cultured.

[a, b]Values in the same column with different superscript letters are different (P<0.05).

blastocysts. Survivin mRNA level expression in 6'DMAP treated group was significantly higher than that of other treatment groups of SCNT blastocysts as shown in Fig. 1.

Discussion

Activation is a possible way for cloning research that's why artificial activation of oocytes is a necessary component of nuclear transfer procedure (Kim et al., 1996). Although production of piglets was achieved after nuclear transfer and ICSI, still lower the efficiency of nuclear transfer and ICSI. As a result incomplete activation of oocytes is the main cause of low success rate of porcine nuclear transfer and ICSI (Betthauser et al., 2000b). To minimize the cause different combinations of activation method were used to induce artificial activation of porcine oocytes for improving the development competence of embryos of different species (Cheng et al., 2007). Such as CB combined with other chemicals CHX, DC, 6-DMAP would improve the development competence of activated porcine embryos to more successful the production of cloning (Yi and Park, 2005b). In the present study, we examined the various cytoskeletal regulators effects of different chemical stimuli combined with CB, CB+CHX, CB+DC, and CB+6-DMAP on the development competence of *in vitro* matured PA and SCNT porcine embryos (Table 1).

In the first experiment, we found that CB treatment group compared with other treated groups of PA oocytes during early developmental stages would improve the efficiency of developmental capacity of activated porcine oocytes. In previous studies, CB is a chemical reagent stabilized cytoskeleton regulator, inhibited the polymerization of microfilaments and spindle rotation and prevented extrusion of second polar body (Zhu et al., 2003). It was established that treatment with CB, CB+CHX, CB+DC and CB+6'DMAP groups during early embryonic development of PA and SCNT embryos improved pre-implantation development of porcine activated embryos. Another reporter also has been shown that treatment with CB increased the developmental rates of PA embryos whereas cleavage, blastocyst rates and total cell number in blastocysts were in CB (84.0, 47.0%, and 38.0, respectively), DC (78.0, 39.0%, and 37.0, respectively), and CB+DC (76.0, 39.0%, and 36.0, respectively) (Song et al., 2009a). Compared with the CB, DC treatment improved the developmental competence of porcine embryos by suppressing extrusion of pseudo-polar body and increasing the development rates. DC is a microtubule inhibitor that disrupts microtubule polymerization and makes mitotic and meiotic cells to loss dynamic spindle microtubules by binding tightly to tubulin dimers (Ibanez et al., 2003). He also reported in mouse oocytes that DC treatment inhibit the extrusion of second polar body in parthenogenetically activated mouse oocytes by suppressing of polymerization of new microtubules (Ibanez et al., 2003). Cycloheximide, a protein synthesis inhibitor has been used in matured oocytes to induce oocytes activation by preventing the reactivation of MPF and the production of cyclin B (Cheng et al., 2007). 6-DMAP, a protein kinase inhibitor, can trigger the meiotic resumption in different species by blocking the protein phosphorylation and inhibit the activation of MPF and second polar body extrusion (Cheng et al., 2007; Motlik et al., 1998).

In the second experiment, we found that CB+6-DMAP treated group increased the developmental rates of SCNT embryos. In previous study has been shown that combined treatment CB with 6-DMAP increased the cleavage and

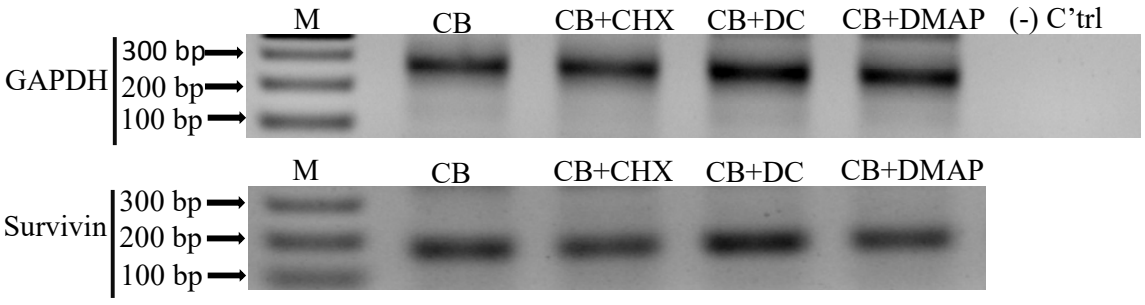

Fig. 1. Comparative expression of Survivin gene (149bp) between treatment groups porcine SCNT blastocysts. Survivin gene was expression whereas control gene GAPDH (219bp) measured by RT-PCR.

blastocysts rates and total cell numbers in porcine SCNT embryos when compared with others chemicals CB (48.0, 12.2%, and 23.3); CB+CHX (57.6, 19.3%, and 24.6); CB+6-DMAP (52.6, 14.5%, and 24.5) on the basis of cleaved oocytes (Yi and Park, 2005b). As a result, simultaneously treatment with CB group compared with CB+CHX, CB+DC and CB+6'DMAP groups (Table 3). In previous result also shown that like that CB+ 6-DMAP treated embryos group significantly increased the cleavage and blastocysts rates and total cell numbers when compared with other groups CB+CHX (46.3, 7.4%, and 25.0, respectively); CB+6-DMAP (57.3, 16.1%, and 25.3, respectively) (Cheng et al., 2007), another reporter has been shown in CB (85.0, 26.0%, and 36.0, respectively), CB+DC (79.0, 28.3%, and 37.0, respectively) (Song et al., 2009a). This finding has been agreed with preceding result (Grupen et al., 2002) single treatment with CB and 6'DMAP groups increased blastocysts formation rates but another consistent CB group is less effective on pre-implantation development than during post-activation of PA porcine embryos. Although the mechanism of increased embryonic development in presence of CB and 6'DMAP groups not completely known, but interestingly, that contradicted result in PA embryos, whereas no stimulatory effects on SCNT embryos treated with CB group. On behalf of formation of blastocyst rates were not found significant differences within four groups (Table 3), which has been consistent with preceding result (Himaki et al., 2010). However, the mean cell number of blastocyst was significantly higher in CB group (42.3%) when compared with CB+CHX, CB+DC groups were (40.6, 40.6%, respectively) as shown in Table 3. In SCNT, as shown in Table 4 cleavage rates were significantly higher in CB+6'DMAP group (89.7%) when compared with CB, CB+CHX, CB+DC groups were (86.3%, 81.7%, and 84.0%, respectively) but were not found significant differences in CB+CHX, and CB+DC groups (81.7, and 84.0%, respectively) among two groups. It was also not found significant differences in the formation of blastocyst rates within CB and CB+DC groups. In the case of blastocyst mean cell number CB+6'DMAP group (41.4%) was higher significant when compared with CB, CB+CHX, and CB+DC groups were (40.1, 39.65, and 39.7%, respectively). The researcher cannot find why same chemical treatment with PA and SCNT oocytes showed similar action. PA oocytes have maternal chromosomes but SCNT oocytes were treatment with differentiated somatic cells of G0/G1 stages (Lee et al., 2004b).

We also investigated reprogramming related gene survivin, which suppresses replication of apoptotic protein domains containing a single baculovirus (Altieri, 2004). Survivin is a bi-functional protein, cell division regulate by inhibition of apoptosis (Park et al., 2007). It is ubiquitously expressed in bovine murine and human during embryonic development (Adida et al., 1998). Increased in the apoptosis and decreased developmental blastocysts rates in survivin mRNA-targeted embryos (Park et al., 2007). Our research established that presence of survivin is very crucial for the development of early embryos. It is an inhibitor of apoptosis (IAP) family, which has two mechanisms: apoptosis inhibition and regular division of cell (Jeon et al., 2008). It has also the negative effect of apoptosis or programmed cell death. In the previous study was also described whether the abnormal embryos negative effect on nuclear reprogramming. At present in the murine model, survivin was an anti-apoptotic gene and during preimplantation development of mouse embryos of all stages was expressed (Kawamura et al., 2003). After activation gradually increased survivin mRNA expression in mature oocytes. In SCNT embryos 2-4 cell stage [14], survivin levels were significantly higher 6'DMAP treated group than other treatment groups of SCNT embryos. Significant differences found when compared with normally cleaved embryos than abnormal embryos i.e., arrested and fragmented in survivin mRNA expression. Survivin expression demonstrates that quality of embryos during developmental stages whereas previous studies reported that other species of embryos. More than 80% of embryos showed abnormalities in cell division and developmental stages in mice when injected survivin. Similarly, decreased developmental blastocysts rates higher apoptosis expressed survivin mRNA targeted embryos in bovine. During preimplantation of embryos in mouse were showed survivin null and lethality of embryonic development such as micronuclei formation, giant nuclei, aberrant nuclei and irregular morphology in embryonic developmental condition. Additionally, we have investigated survivin expression during early developmental stages embryos significant higher of 6'DMAP treatment group than other treatment groups. In our research established that CB group for parthenogenesis and 6'DMAP group for SCNT best condition of post-activation.

Our results establish that treatment with post-activation

cytoskeletal modifiers CB, CB+CHX, CB+DC and CB+6'DMAP treatment groups increase the development of pre-implantation of PA and SCNT embryos whereas CB group for parthenogenesis and 6'DMAP for SCNT the best condition for embryo production. Additionally, early embryonic developmental stages have a positive effect on different cytoskeletal regulators on PA and SCNT embryos of pigs. Survivin was express in all of the treatment groups. In future researches, survivin is a key factor of measured quality of embryos and established where the target point of embryos is *in vivo*.

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