Valproic Acid Improves Porcine Parthenogenetic Embryo Development Through Transient Remodeling of Histone Modifiers

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Key Words
Valproic acid \• Parthenogenetic \• Development \• Histone modifications

Abstract

\textbf{Background/Aims:} Parthenogenetic embryos are useful in many applications, such as being an alternative source of embryonic stem cells that would avoid ethical problems. Aberrance in epigenetic reprogramming is considered the major reason for the developmental failure of parthenogenetic embryos. Many histone deacetylase inhibitors have been shown to improve the reprogramming of stem cells and embryos. Here, the relationship between histone modification and parthenogenetic embryonic development was explored. \textbf{Methods:} Valproic acid (VPA) treatment was applied during the culture of parthenogenetic embryos. The abundance of histone modifiers was examined by immunofluorescence and quantified by Image-pro software. \textbf{Results:} The acH3K9 level in \textit{in vitro} fertilized embryos was significantly higher than parthenogenetic embryos. VPA treatment improved both the blastocyst formation rate and the acH3K9 level in parthenogenetic embryos. The signal intensities of acH4K5 and H3K4me2 were also enhanced in VPA treated embryos. The H3K27me2 level was decreased in the VPA treated embryos at the 2-cell stage. However, the enhancement in the acH3K9, acH4K5 and H3K4me2 level, or the decrease in the H3K27me2 level disappeared shortly after VPA withdrawal. \textbf{Conclusion:} Optimizing histone modifications for a short time following activation was sufficient to enhance the \textit{in vitro} development of parthenogenetic embryos.

Y. Huang and L. Yuan contributed equally to this work.
Introduction

Parthenogenesis is defined as an embryo developing from an unfertilized egg. Due to the lack of the paternal genome, the development of a parthenogenetic embryo would be terminated during gestation. Parthenogenetic models are valuable tools for basic research. Many researchers have concentrated on investigating the differences between parthenogenetic embryos and normally developed embryos [1], to transform parthenogenetic embryos and enable them to develop to term. For example, a large number of imprinted genes were found to be differentially expressed between parthenogenetic and fertilized embryos [2].

In addition to genomic imprinting, other epigenetic modifications (DNA methylation or histone modification) may also be dysfunctional and may become an obstacle during the development of parthenogenetic embryos. Histone acetylation alters the higher-order chromatin structure and provides the greatest opportunity for unfolding chromatin to recruit different transcriptional factors. Therefore, many HDAC inhibitors have been used to improve embryonic development [3-5]. However, the relationship between histone modifications and embryonic development, especially for the parthenogenetic embryo, requires further exploration.

In our previous studies, vitamin C [6], Scriptaid [7] and valproic acid (VPA) [8] have been shown to improve the developmental competence of porcine SCNT embryos and change their histone acetylation. In this study, we tried to improve the in vitro development of porcine parthenogenetic embryos with vitamin C, Scriptaid or VPA treatment, and analyze the abundance of acetylated histone-H3 at lysine-9 (acH3K9), acetylated histone-H4 at lysine-5 (acH4K5), dimethylation of histone-H3 lysine-4 (H3K4me2) and dimethylation of histone-H3 lysine-27 (H3K27me2).

Materials and Methods

All animal care and experiments in this study were conducted according to the guidelines of the Animal Care and Welfare Committee of Jilin University. All chemicals were purchased from Sigma Aldrich Co. (St. Louis, MO, USA), unless otherwise stated. All of the solutions and media were filtered using a 0.22-mm filter.

Oocyte collection, in vitro maturation, parthenogenetic activation, and in vitro culture

The protocol for the generation of porcine parthenogenetic embryos was previously described in detail [9]. Briefly, porcine ovaries were collected at a local slaughterhouse and kept in sterile saline water at 32–37°C. The follicular contents were aspirated from 3 to 6 mm follicles, and cumulus-oocyte complexes (COCs) with at least three uniform layers of cumulus cells were then selected using a sterile glass pipette.

After rinsing three times, COCs were matured in TCM-199 supplemented with 0.1% polyvinyl alcohol, d-glucose (3.05 mM), sodium pyruvate (0.91 mM), penicillin (75 µg/ ml), streptomycin (50 µg/ ml), epidermal growth factor (10 ng/ ml), cysteine (0.57 mM), follicle-stimulating hormone (0.5 µg/ ml), and luteinizing hormone (0.5 µg/ ml) at 39°C and 5% CO₂. After culturing for 42 to 44 h, the cumulus cells of COCs were denuded from the oocytes with 0.1% hyaluronidase. Only the oocytes with an extruded first polar body, round-shape and intact cytoplasm were selected and maintained in the manipulation medium for the further experiments.

Parthenogenetic activation was performed in medium containing 0.3 M mannitol, 1.0 mM CaCl₂·2H₂O, 1.0 mM MgCl₂·6H₂O and 0.5 mM HEPES. Denuded oocytes were activated using two 2-DC pulses with a voltage of 1.2 kV/ cm for 30 µsec on a BTX Electro Cell Manipulator 2001 (BTX, San Diego, CA, USA). Then, oocytes were cultured in PZM-3 (with 3 mg/ml BSA) supplemented with 7.5 µg/ ml cytochalasin B for 4 h.

After rinsing completely with PZM-3 (with 3 mg/ml BSA), embryos were further cultured in PZM-3 (with 3 mg/ml BSA) at 39°C in a humidified atmosphere of 5% CO₂. Embryos at the 1-cell, 2-cell, 4-cell, and blastocyst stages were collected at 18, 24, 36 and 132 h after electrical activation, respectively.
In vitro fertilization (IVF)

Before in vitro fertilization, mTBM medium (113.1 mM NaCl, 3 mM KCl, 7.5 mM CaCl\(_2\).2H\(_2\)O, 5 mM sodium pyruvate, 11 mM glucose, 1 mM caffeine, 20 mM Tris, 0.1% BSA, 75 µg/ml penicillin and 50 µg/ml streptomycin) was equilibrated at 39°C in a humidified atmosphere of 5% CO\(_2\) for 18 to 24 h. Denuded oocytes were rinsed three times with mTBM medium and 15 oocytes were then transferred into 50µl drops of mTBM medium. Fresh spermatozoa were subjected to centrifugation at 800 rpm for 10 min, and the supernatant was discarded. The precipitate was washed twice by centrifugation at 800 rpm for 10 min in DPBS supplemented with 0.1% BSA. Following the washing procedure, the spermatozoa were suspended in mTBM medium and incubated at 39°C in a humidified atmosphere of 5% CO\(_2\) for 1 h. An appropriate dilution was conducted with the swim-up spermatozoa, and 5 µl of this suspension was added per mTBM drop containing oocytes to yield a final concentration of 1.5 - 5 × 10^5 spermatozoa/ml. After co-incubation for 7 h, the oocytes were then washed three times with PZM-3 (with 3 mg/ml BSA). Forty to 50 oocytes were transferred into 100 µl drops of PZM3 (with 3 mg/ml BSA) for an additional culture at 39°C in a humidified atmosphere of 5% CO\(_2\). The time at which the oocytes were transferred into PZM-3 drops was considered 0 h. IVF embryos at the 1-cell, 2-cell, 4-cell, and blastocyst stages were collected at 15, 21, 33 and 129 h, respectively.

Drug treatment

The concentrated stock solution of VPA was made at 40 mM in PZM-3 and stored at -20°C. In accordance with different experimental procedures, the VPA stock solution was dissolved in PZM-3 (with 3 mg/ml BSA) at different concentrations. Following treatment, embryos were washed three times with PZM-3 (with 3 mg/ml BSA) and further cultured in PZM3 without VPA.

Vitamin C was dissolved in PZM-3 and prepared as a stock solution at 5 mg/mL, and the stock solution was stored at -20°C. The vitamin C concentrated stock solution was dissolved into the PZM-3 (with 3 mg/ml BSA) at different concentrations according to the different experimental procedures. At the end of the treatment, embryos were transferred into PZM3 (with 3 mg/ml BSA) without vitamin C.

Scriptaid was dissolved in dimethyl sulfoxide at 5 mg/mL, and the stock solution was stored at -20°C. The stock solution was added to PZM-3 (with 3 mg/ml BSA) at different concentrations according to different experimental procedures. After Scriptaid treatment, embryos were washed three times with PZM-3 (with 3 mg/ml BSA) and further cultured in PZM-3 without Scriptaid.

Immunofluorescence

Briefly, the zona pellucidae of embryos were removed by treating with 3.3 mg/ml pronase solution, and the embryos were rinsed in BSA-DPBS. Then, the embryos were fixed in 4% (v/v) paraformaldehyde for 30 min and permeabilized with 0.2% Triton X-100 for 30 min. After being blocked in 5% goat serum in PBS for 30 min, embryos were co-incubated with a rabbit polyclonal primary antibody (acH3K9, 1:400, Abcam; acH4K5, 1:400, Abcam; H3K4me2, 1:500, Cell Signaling Technology; H3K27me2, 1:500, Cell Signaling Technology) at 4°C overnight. After removing the primary antibodies, the embryos were washed intensively with 0.2% (v/v) Tween-20 in PBS. Alexa Fluor 594 goat anti rabbit IgG (Invitrogen, Carlsbad, CA, USA) diluted in 1:800 was then applied and incubated at 4°C overnight. After washing, the embryos was stained with Hoechst 33342 and mounted on slides. The fluorescence of embryos were then observed under an epifluorescence microscope (Nikon, Tokyo, Japan) equipped with a digital camera.

The fluorescence intensity of acH3K9, acH4K5, H3K4me2 and H3K27me2 were quantified with Image-pro plus software (Media Cybernetics, USA). Example for region of interested (ROI) at different stage could be seen in Fig. 1. The IOD (integral optical density) and area of immunofluorescence staining in the ROI was calculated. Briefly, the image was converted into Gray Scale and inverted. Then, the picture was snapped and converted into Gray Scale again. The average level of optical density outside of the nuclear region was taken as the incident level. The sum of IOD and area were then counted. In the present study, per unit area IOD was used for comparison, which was calculated by IOD sum/ area sum.

Statistical analyses

All experiments were performed at least three times. Comparison of the mean per unit area IOD between the control and the VPA treated embryos was conducted using the independent-samples t test
in SPSS. Data expressed as percentages were compared by chi-squared test. A probability of < 0.05 was considered statistically significant.

Results

Low signal intensity of acH3K9 in parthenogenetic embryos

Aberrant epigenetic regulation, especially of DNA methylation, genomic imprinting and histone modification, largely accounts for the developmental failure of parthenogenetic embryos. In the present study, the differences in acH3K9 abundance between parthenogenetic and IVF embryos were explored (Fig. 1). As shown in Fig. 2A, there were similar trends between IVF and parthenogenetic embryos and both displayed a trough at the 4-cell stage. However, acH3K9 showed significantly higher signal intensity in IVF embryos than in the parthenogenetic embryos from the 1-cell to blastocyst stage. Therefore, we propose that low levels of histone acetylation exert negative effects on the development of parthenogenetic embryos.

VPA enhanced the in vitro development of parthenogenetic embryos

Vitamin C, VPA and Scriptaid were found to improve the developmental competence of porcine somatic cell nuclear transfer (SCNT) embryos. Therefore, in the present study, Vitamin C, VPA and Scriptaid were applied to the culture of porcine parthenogenetic embryos according to the treatment of the porcine SCNT embryos. As shown in Table 1, 500 nM Scriptaid treatment for 16 h could significantly improve the cleavage rate at 24 h (73.2% vs. 49.3%, \( p = 0.000 \)), but not the blastocyst formation rate at 144 h (35.1% vs. 23.2%, \( p = 0.057 \)). As for Vitamin C, its treatment of 50 μg/ml for 16 h could significantly enhance both the cleavage rate at 24 h (76.1% vs. 49.3%, \( p = 0.000 \)) and the blastocyst formation rate at 144 h (36.3% vs. 23.2%, \( p = 0.026 \)). When the parthenogenetic embryos were treated with 1 mM VPA for 16 h, the cleavage rate at 24 h (78.0% vs. 49.3%, \( p = 0.000 \)) and the blastocyst formation rate at 144 h (48.8% vs. 23.2%, \( p = 0.000 \)) were significantly higher than in the control embryos. In addition, the blastocyst formation rate of embryos treated

Fig. 1. Immunofluorescence localization of acH3K9 (red) and DNA stain (blue) in the in vitro fertilized (IVF) embryos, and VPA treated and untreated embryos. (A to H) Parthenogenetic embryos without VPA treatment. (I to P) IVF embryos. (Q to X) Parthenogenetic embryos with VPA treatment. Examples for region of interest at different stage were circled in the enlarged pictures.
with VPA was even higher than that of the Scriptaid-treated embryos (48.8% vs. 35.1%, $p = 0.042$). Combined with the results regarding the acH3K9 levels between parthenogenetic and IVF embryos, it seems that VPA treatment would exert more advantageous effects on the development of porcine parthenogenetic embryos than Scriptaid or Vitamin C treatment.

To better determine the effect of VPA treatment on the in vitro development of parthenogenetic embryos, the development rate with VPA treatment at different concentrations or durations was studied. The in vitro development of parthenogenetic embryos treated with 1 mM VPA for 0, 8, 16, 24 and 48 h was analyzed. As shown in Table 2, the blastocyst formation rate of parthenogenetic embryos treated with 1 mM VPA for 16 h was significant higher than the rates of embryos treated for 0 and 8 h (43.8% vs. 21.1%, $p =$
Huang et al.: Valproic Acid Improves Parthenogenetic Development

The level of acH3K9 was increased at the early stage under VPA treatment

The above results showed that the signal intensity of acH3K9 in parthenogenetic embryos was lower than that of the IVF embryos, and the developmental competence could be enhanced with VPA treatment. Therefore, whether VPA treatment could restore the normal level of acH3K9 was assessed. As shown in Fig. 1 and 2A, the signal intensity of acH3K9 at the 1-cell stage was increased with VPA treatment, which was even higher than that of the IVF embryos (p = 0.014). However, the signal intensity of acH3K9 was decreased after VPA withdrawal. At the 2-cell stage, the signal intensity of acH3K9 in embryos with VPA...
treatment was higher than in the non-treated embryos ($p = 0.003$), but lower than the IVF embryos ($p = 0.000$). There were similar levels of acH3K9 between the VPA treated and non-treated parthenogenetic embryos at the 4-cell stage ($p = 0.802$); but there was high signal intensity in the IVF embryos compared to the VPA treated or untreated embryos ($p = 0.000$). Similarly, there were no significant differences in the signal intensity of acH3K9 between the VPA treated and untreated parthenogenetic embryos at the blastocyst stage ($p = 0.814$). The acH3K9 signal intensity of the IVF embryos was significantly higher than both the VPA treated ($p = 0.003$) and the untreated ($p = 0.004$) embryos at the blastocyst stage. Generally speaking, the signal intensity of acH3K9 was gradually decreased to similar levels as the untreated parthenogenetic embryos after VPA withdrawal.
acH4K5, H3K4me2 and H3K27me2 were transient and fluctuated with VPA treatment

Considering the phenomenon of acH3K9, we tried to determine whether other histone modifiers undergo similar changes after VPA treatment. The dynamic modulation of histone H4 acetylation was analyzed, and acH4K5 was selected to study (Fig. 3). The signal intensity of acH4K5 in parthenogenetic embryos with VPA treatment was significantly higher than the untreated embryos at the 1-cell ($p = 0.012$), 2-cell ($p = 0.006$) and 4-cell stages ($p = 0.000$) (Fig. 2B). However, when the embryos developed into the blastocyst stage, the acH4K5 signal in VPA treated embryos was lower than that of the untreated embryos ($p = 0.000$).

The fluctuation of histone methylation modification with VPA treatment was also studied. The H3K4me2 is considered to be an open chromatin marker, and its abundance was analyzed in the present study (Fig. 2C and 4). Similar to the presentation of acH3K9, the signal intensity of H3K4me2 in embryos with VPA treatment was higher than the untreated embryos at the 1-cell ($p = 0.000$) and 2-cell stage ($p = 0.000$). There were no significant differences in the levels of H3K4me2 between the VPA treated and untreated parthenogenetic embryos at the 4-cell stage ($p = 0.240$) or blastocyst stage ($p = 0.358$).

H3K27me2 is mainly taken as a marker of closed chromatin, and its abundance was also analyzed in the present study (Fig. 2D and Fig. 5). At the 1-cell stage, there were no significant differences in the level of H3K27me2 between the VPA treated and untreated parthenogenetic embryos ($p = 0.631$). At the 2-cell stage, the signal level of H3K27me2 in the embryos with VPA treatment was significantly lower than that of the untreated embryos ($p = 0.046$). The signal intensity of H3K27me2 in VPA treated embryos was restored to a similar level as the untreated embryos at the 4-cell stage ($p = 0.964$). Strikingly, H3K27me2 were nearly undetectable in both the VPA treated and untreated embryos at the blastocyst stage. Generally, though there were differences in the impacts of the abundance among acH4K5, H3K4me2 and H3K27me2 with VPA treatment, such impacts were largely eliminated in a short time after VPA withdrawal.
Discussion

It is well known that no piglet has been born through parthenogenesis to date. Abnormality in epigenetic modifications is considered the major reason for the failed development of parthenogenetic embryos. In the present study, the results revealed that the signal intensity of acH3K9 in the parthenogenetic embryos was significantly lower than the IVF embryos. Similarly, acH3K27, another active epigenetic modification, presented higher levels in the parthenogenetic embryos than in the IVF embryos [7]. The results urged us to consider whether the development of parthenogenetic embryos could be improved by rescuing their histone acetylation. Therefore, the histone deacetylase inhibitor VPA, which has been shown to significantly improve porcine SCNT embryos [5, 8], was applied in the present study.

Several histone deacetylase inhibitors have been shown to significantly improve the reprogramming of inducible pluripotent stem cells (iPSC) and somatic cell nuclear transfer (SCNT) embryos [10]. Furthermore, histone deacetylases were found to play a critical role in BMP9-mediated osteogenic signaling in mouse mesenchymal stem cells [11]. In the present study, the in vitro development of parthenogenetic embryos was enhanced with VPA treatment. Actually, in the induction of iPSC, the reprogramming rates could be increased up to 100-fold by the administration of VPA [12, 13]. However, the results indicated that VPA treatment to improve the blastocyst formation rate was time and concentration dependent. A previous study showed that low dose VPA exerts little effects on the cleavage rate and blastocyst formation rate of bovine embryo development, but their development was significantly impaired with 3 mM VPA treatment [14]. There may be some cytotoxicity of VPA to the embryos under longer treatment periods or with higher concentration treatments.

With VPA treatment, the signal intensity of both acH3K9 and acH4K5 was increased, indicating that the acetylation level was possibly increased across all H3 and H4 histones. Moreover, a previous study has shown that acH3K9 in SCNT embryos was increased with TSA treatment and reached similar levels to that of the IVF embryos [15]. As for acH4K5, it is found at low levels in bovine cloned embryos and can also be enhanced by TSA treatment [16]. Such enhancement in the level of histone acetylation might lead to more parthenogenetic embryos developing to the blastocyst stage. Histone acetylation could alter the chromatin structure into a more accessible state for many transcriptional factors, therefore, in favor of reprogramming. It seems that high a level of histone acetylation was correlated with high developmental competence. However, knocking down histone deacetylase 1 (HDAC1) decreased the blastocyst formation rate of bovine parthenogenetic embryos despite the increase in acH3K14 [17]. The relationship between acetylation and developmental competence still needs to be further studied.

Histone methylation is also an important form of histone modification and plays a critical role in embryonic development. However, few studies on embryos were focused on the correlation between VPA treatment and histone methylation. In this study, H3K4me2 underwent similar changes to acH3K9, which presented high levels at the 1-cell and 2-cell stages in parthenogenetic embryos with VPA treatment. In fact, one study on human breast cancer cells has suggested that inhibiting HDAC I/II results in significant increases of H3K4me2 [18]. H3K4me2 is considered an active chromatin marker [19]. The signal intensity of H3K4me2 increased with VPA treatment, and this may beneficially activate development-related genes thus contributing to the enhancement of developmental competence. H3K27me2 is typically considered to be a marker of passive chromatin [20]. A short decrease of H3K27me2 at the 2-cell stage after VPA treatment may also be beneficial to the subsequent development of parthenogenetic embryos.

Regardless of histone acetylation or histone methylation, these changes were only maintained for a short time after VPA withdrawal. It seems that restoration of the epigenetic modifications at the pronuclear or pseudopronuclear stage was sufficient to enhance the blastocyst formation rate. However, to overcome the developmental failure
of parthenogenetic embryos, further studies should solve the problem of how to regulate epigenetic modifications in a temporal and spatial manner.

Abbreviations

VPA (Valproic acid); acH3K9 (acetylated histone-H3 at lysine-9); acH4K5 (acetylated histone-H4 at lysine-5); H3K4me2 (dimethylation of histone-H3 lysine-4); H3K27me2 (dimethylation of histone-H3 lysine-27); IVF (in vitro fertilization); PZM-3 (porcine zygote medium-3); ROI (region of interested); IOD (integral optical density).

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Disclosure Statement

The authors have no conflict of interest to disclose.

References

1. Liu N, Enkemann SA, Liang P, Hersmus R, Zanazzi C, Huang J, Wu C, Chen Z, Looijenga LH, Keefe DL, Liu L: Genome-wide gene expression profiling reveals aberrant MAPK and Wnt signaling pathways associated with early parthenogenesis. J Mol Cell Biol 2010;2:333-344.
2. Nikaido I, Saito C, Mizuno Y, Meguro M, Bono H, Kadomura M, Kono T, Morris GA, Lyons PA, Oshimura M, Hayashizaki Y, Okazaki Y, Group RG, Members GSL: Discovery of imprinted transcripts in the mouse transcriptome using large-scale expression profiling. Genome Res 2003;13:1402-1409.
3. Huan Y, Wu Z, Zhang J, Zhu J, Liu Z, Song X: Epigenetic Modification Agents Improve Gene-Specific Methylation Reprogramming in Porcine Cloned Embryos. PLoS One 2015;10:e0129803.
4. Inoue K, Oikawa M, Kamimura S, Ogonuki N, Nakamura T, Nakano T, Abe K, Ogura A: Trichostatin A specifically improves the aberrant expression of transcription factor genes in embryos produced by somatic cell nuclear transfer. Sci Rep 2015;5:10127.
5. Kang JD, Li S, Lu Y, Wang W, Liang S, Liu X, Jin JX, Hong Y, Yan CG, Yin XJ: Valproic acid improved in vitro development of pig cloning embryos but did not improve survival of cloned pigs to adulthood. Theriogenology 2013;79:306-311, e301.
6. Huang YY, Tang XC, Xie WH, Zhou Y, Li D, Zhou Y, Zhu JG, Yuan T, Lai LX, Pang DX, Ouyang HS: Vitamin C enhances in vitro and in vivo development of porcine somatic cell nuclear transfer embryos. Biochem Biophys Res Commun 2011;411:397-401.
7. Zhou N, Cao Z, Wu R, Liu X, Tao J, Chen Z, Song D, Han F, Li Y, Fang F, Zhang X, Zhang Y: Dynamic changes of histone H3 lysine 27 acetylation in pre-implantational pig embryos derived from somatic cell nuclear transfer. Anim Reprod Sci 2014;148:153-163.
8. Huang YY, Tang XC, Xie WH, Zhou Y, Li D, Yao CG, Zhou Y, Zhu JG, Lai LX, Ouyang HS, Pang DX: Histone Deacetylase Inhibitor Significantly Improved the Cloning Efficiency of Porcine Somatic Cell Nuclear Transfer Embryos. Cell Reprogram 2011;13:513-520.
9. Huang Y, Ouyang H, Xie W, Chen X, Yao C, Han Y, Han X, Song Q, Pang D, Tang X: Moderate expression of Wnt signaling genes is essential for porcine parthenogenetic embryo development. Cell Signal 2013;25:778-785.
10. Zhu S, Wei W, Ding S: Chemical strategies for stem cell biology and regenerative medicine. Annu Rev Biomed Eng 2011;13:73-90.
11 Hu N, Wang C, Liang X, Yin L, Luo X, Liu B, Zhang H, Shui W, Nan G, Wang N, Wu N, Chen X, He Y, Wen S, Deng E, Zhang H, Liao Z, Lu H, Haydon RC, He TC, Huang W: Inhibition of histone deacetylases potentiates BMP9-induced osteogenic signaling in mouse mesenchymal stem cells. Cell Physiol Biochem 2013;32:486-498.

12 Huangfu D, Maehr R, Guo W, Eijikelenboom A, Snitow M, Chen AE, Melton DA: Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 2008;26:795-797.

13 Pfannkuche K, Hannes T, Khalil M, Noghabi MS, Morshed A, Hescheler J, Droge P: Induced pluripotent stem cells: a new approach for physiological research. Cell Physiol Biochem 2010;26:105-124.

14 Gao H, Bai H, Ao X, Sa R, Wang H, Wang Z, Yue Y, Yu H: The effect of valproic acid on bovine oocyte maturation and early embryonic development in vitro. Cytotechnology 2014;66:525-532.

15 Wittayarat M, Sato Y, Do LT, Morita Y, Chatdarong K, Techakumphu M, Taniguchi M, Otoi T: Histone deacetylase inhibitor improves the development and acetylation levels of cat-cow interspecies cloned embryos. Cell Reprogram 2013;15:301-308.

16 Wee G, Koo DB, Song BS, Kim JS, Kang MJ, Moon SJ, Kang YK, Lee KK, Han YM: Inheritable histone H4 acetylation of somatic chromatin in cloned embryos. J Biol Chem 2006;281:6048-6057.

17 Wang Z, Zhao T, Zhang P, Zhang S, Guan J, Ma X, Yin Y, Zhang J, Tang B, Li Z: Histone deacetylase 1 down-regulation on developmental capability and histone acetylation in bovine oocytes and parthenogenetic embryos. Reprod Domest Anim 2011;46:1022-1028.

18 Huang Y, Vasilatos SN, Boric L, Shaw PG, Davidson NE: Inhibitors of histone demethylation and histone deacetylation cooperate in regulating gene expression and inhibiting growth in human breast cancer cells. Breast Cancer Res Treat 2012;131:777-789.

19 Pham KT, Inoue Y, Vu BV, Nguyen HH, Nakayashiki T, Ikeda K, Nakayashiki H: MoSET1 (Histone H3K4 Methyltransferase in Magnaporthe oryzae) Regulates Global Gene Expression during Infection-Related Morphogenesis. PLoS Genet 2015;11:e1005385.

20 Foda BM, Singh U: Dimethylated H3K27 Is a Repressive Epigenetic Histone Mark in the Protist Entamoeba histolytica and Is Significantly Enriched in Genes Silenced via the RNAi Pathway. J Biol Chem 2015;290:21114-21130.