Ganglioside GD1a promotes oocyte maturation, furthers preimplantation development, and increases blastocyst quality in pigs

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Abstract. Gangliosides are key lipid molecules required for the regulation of cellular processes such as proliferation, differentiation, and cell signaling, including signaling of epidermal growth factor receptor (EGFR). Epidermal growth factor (EGF) has long been considered a potential regulator of meiotic and cytoplasmic maturation in mammalian oocytes. However, there is no report on the direct effect of ganglioside GD1a in porcine oocyte maturation. In this study, we first investigated a functional link between GD1a and meiotic maturation during in vitro maturation (IVM) of porcine embryos. Moreover, we confirmed the effect of exogenous GD1a treatment on blastocyst development, quality, and fertilization rate in early embryonic development. First, we observed that the protein level of ST3GAL2, a GD1a synthesizing enzyme, significantly increased (P < 0.01) in cumulus-oocyte-complexes (COCs) during IVM progress. The proportion of arrested germinal vesicles (GV) increased in oocytes treated with EGF+GD1a (41.6 ± 1.5%) at the IVM I stage. Upon completion of meiotic maturation, the proportion of metaphase II (M II) was significantly higher (P < 0.05) in the EGF+GD1a (89.9 ± 3.6%) treated group. After IVF, the percentage of penetrated oocytes was significantly higher (P < 0.05) in the EGF+GD1a (89.1 ± 2.3%) treated group than in the control group. Furthermore, exogenous GD1a treatment improved the developmental competence and quality of blastocysts during preimplantation embryo development stage. These results suggest that ganglioside GD1a may play an important role in IVM mechanisms of porcine maturation capacity. Furthermore, our findings will be helpful for better promoting the embryo development and blastocyst quality in pigs.

Key words: Ganglioside GD1a, Meiotic maturation, Preimplantation development, Pigs

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in EGFR and EGF secretion from COCs regulate oocyte meiotic maturation during in vitro maturation (IVM) [14].

Ganglioside GD1α is specifically formed by the addition of sialic acid to ganglioside GM1α by the synthesizing enzyme ST3 β-galactoside α-2, 3-sialyltransferase 2 (ST3GAL2) [15]. GD1α promotes proliferation of normal human dermal fibroblasts and differentiation of osteoblasts by activating EGFR signaling pathways [3, 16]. Moreover, GD1α as membrane component is also important in cellular signaling pathways required for oocyte maturation. According to a recent study, GD1α has been found to be expressed in interstitial cells during ovarian maturation in mice [17]. Moreover, exogenous GD1α treatment enhances EGFR activation and ligand binding [16]. However, there has been no investigation to date on the direct role and effects of GD1α expression in oocyte meiotic maturation during in vitro maturation of porcine oocytes and COCs.

Regulation of ganglioside GD1α may play a fundamental role in oocyte maturation similar to the role of EGF in cumulus cells of COCs. Therefore, we designed present studies to determine whether the addition of ganglioside GD1α into maturation medium might regulate oocyte maturation until IVM II like EGF. In addition, the effect of exogenous GD1α on matured oocytes was investigated by measuring fertilization rate and subsequent embryonic development parameters. Furthermore, the present study was initiated to suggest a functional link between ganglioside GD1α and oocyte maturation in porcine oocytes during IVM progression.

Materials and Methods

Chemicals reagents

Unless otherwise stated, all chemicals reagents used in this study were purchased from Sigma Chemical (St. Louis, MO, USA).

In vitro maturation (IVM)

Porcine ovaries were obtained from non-pregnant sows at a local abattoir and were transported to the laboratory in 0.9% saline supplemented with 75 μg/ml potassium penicillin G at approximately 30–35°C. Immature cumulus-oocyte complexes (COCs) were then aspirated from 3- to 6-mm follicles using a disposable 10-ml syringe with an 18-gauge needle. Undamaged COCs with similar quality cytoplasm and the surrounding cumulus cells were collected by mouth pipetting, then washed three times in Tyrode's lactate-N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (TL-HEPES) medium. Next, 50–60 immature COCs were matured in 500 μl of 2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (TL-HEPES) medium. During the maturation periods, GD1α was added three times and then further cultured in PMSG and hCG-free maturation medium [19]. After culturing for 22 h, COCs were washed three times by centrifugation in PBS supplemented with 1 mg/ml BSA (w/v), 100 mg/ml penicillin G, and 75 mg/ml streptomycin sulfate. At the end of the washing period, spermatozoa were resuspended in mTBM at pH 7.8. Oocytes were subsequently washed three times in mTBM, and then placed into 48 μl of mTBM under mineral oil. Next, 2 μl of diluted spermatozoa were added to a 48 μl drop of medium containing 15–20 oocytes to give a final concentration of 1.5 × 10^6 sperm/ml. Finally, oocytes were co-incubated with spermatozoa for 6 h at 38.5°C under 5% CO_2_. Next, embryos were cultured in 50 μl drops of PZM-3 medium with 3 mg/ml BSA at 38.5°C under 5% CO_2_. After 48 h of culture, 25–30 cleaved embryos were further cultured in 50 μl drops of PZM-3 medium supplemented with 3 mg/ml BSA at 38.5°C under 5% CO_2_. Western blot analysis

After each IVM and IVF experiment, a representative sample was denuded by gently pipetting in 0.1% hyaluronidase (w/v) and then washing in PBS containing 0.1% polyvinyl alcohol (PVA, w/v). Each sample was mounted on microscope slides. The samples were then fixed for 3 days in acetic acid:ethanol (1:3, v/v) solution and stained with 0.1% acetic orcein (v/v) solution for 5 min. The samples were de-stained in glycerol:acetic acid:water (1:1:3, v/v/v) solution, after which the meiotic stage was evaluated microscopically (Leica, Solms, Germany).

Assessment of meiotic maturation and pronucleus formation

At the end of each IVM and IVF experiment, a representative sample was denuded by gently pipetting in 0.1% hyaluronidase (w/v) and then washing in PBS containing 0.1% polyvinyl alcohol (PVA, w/v). Each sample was mounted on microscope slides. The samples were then fixed for 3 days in acetic acid:ethanol (1:3, v/v) solution and stained with 0.1% acetic orcein (v/v) solution for 5 min. The samples were de-stained in glycerol:acetic acid:water (1:1:3, v/v/v) solution, after which the meiotic stage was evaluated microscopically (Leica, Solms, Germany).

In vitro fertilization (IVF) and culture (IVC)

In vitro fertilization of porcine oocytes was performed as described by Abydeera and Day [20]. The IVF medium, modified Tris-buffered medium (mTBM), consisted of 113.1 mM NaCl, 3 mM KCl, 7.5 mM CaCl_2, 5 mM sodium pyruvate, 11 mM glucose, 20 mM Tris, 2.5 mM caffeine sodium benzoate and 1 mg/ml BSA. Fresh semen was kindly supplied once a week by AI (Darby Porcine AI Center, Anseong, Korea) and kept at 17°C for 5 days. Semen was washed three times by centrifugation in PBS supplemented with 1 mg/ml BSA (w/v), 100 mg/ml penicillin G, and 75 mg/ml streptomycin sulfate. At the end of the washing period, spermatozoa were resuspended in mTBM at pH 7.8. Oocytes were subsequently washed three times in mTBM, and then placed into 48 μl of mTBM under mineral oil. Next, 2 μl of diluted spermatozoa were added to a 48 μl drop of medium containing 15–20 oocytes to give a final concentration of 1.5 × 10^6 sperm/ml. Finally, oocytes were co-incubated with spermatozoa for 6 h at 38.5°C under 5% CO_2_. Next, embryos were cultured in 50 μl drops of PZM-3 medium with 3 mg/ml BSA at 38.5°C under 5% CO_2_. After 48 h of culture, 25–30 cleaved embryos were further cultured in 50 μl drops of PZM-3 medium supplemented with 3 mg/ml BSA at 38.5°C under 5% CO_2 for 4 days. Blastocyst formation was evaluated after 6 day of culture.

Assessment of apoptosis in blastocysts

Apoptotic blastocysts were detected using an In Situ Cell Death Kit.
Detection Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer’s instructions. On day 6, the blastocysts from IVF were washed with 0.1% PVA in PBS and then fixed in 4% PFA in PBS for 1 h at room temperature. Next, blastocysts were permeabilized using 0.1% (v/v) Triton X-100 for 30 min at 4°C. The fixed embryos were incubated in TUNEL reaction medium for 1 h at 38.5°C, then washed and mounted on slides. Whole-mount embryos were examined under an epifluorescence microscope (Olympus, Tokyo, Japan) following TUNEL assay and DAPI staining, and the number of apoptotic nuclei and total number of nuclei were counted.

**Statistical analysis**

All percentage data obtained in this study are presented as the mean ± standard deviation (SD). Moreover, Western blot experiments were performed in triplicate and all values were presented as the mean ± standard error of the mean (SEM). The results were analyzed using either a one-way ANOVA followed by Bonferroni’s Multiple Comparison Test or by performing a t-test. All data were analyzed using the GraphPad Prism 5.0 software package (San Diego, CA, USA). Differences were considered significant at *P < 0.05, ** < 0.01, and *** < 0.001.

**Results**

**GD1a synthesizing enzyme ST3GAL2 protein levels in DOs and COCs during IVM**

Many studies have reported that the expression of ganglioside GD1a can be accurately estimated by measuring the expression of its synthesizing enzyme ST3GAL2 [21, 22]. Therefore, we first measured the protein level of ST3GAL2 as a proxy for expression of GD1a in denuded oocytes (DOs) and cumulus cell oocytes (COCs) during the IVM process (IVM I; 22 h, IVM II; 44 h) by western blotting. As shown in Fig. 1, the expression of ST3GAL2 protein level was dramatically higher (P < 0.05) in COCs of IVM II (44 h) than in COCs of IVM I (22 h). ST3GAL2 protein was only detected in COCs, and not in DOs. This result demonstrated that the GD1a synthesizing enzyme ST3GAL2 was only expressed in COCs of IVM II (44 h). Based on this result, we determined that COCs of IVM II (44 h) could be used for the subsequent experiments using GD1a treatment.

**Effects of GD1a treatment during IVM on oocyte maturation**

First, to determine the proper concentration of GD1a treatment for COCs in IVM II, we evaluated aspects of oocyte maturation after treatment with various concentrations of GD1a. Porcine oocytes were cultured with various concentrations (1 μM, 10 μM and 20 μM) of GD1a for 44 h in EGF-free medium. As shown in Fig. 2, the proportion of the metaphase II (M II) stage oocytes was significantly higher (P < 0.05) in the 10 μM GD1a treated group (79.7 ± 3.0%) than in the control (57.2 ± 1.3%).

Next, to confirm the effects of GD1a on oocyte maturation in vitro, we measured the meiotic maturation of porcine oocytes after exogenous GD1a treatment. Therefore, we investigated the proportion of meiotic maturation after GD1a and/or EGF treatment for M I or M II phase. As shown in Fig. 3, we confirmed that the number of oocytes that reached metaphase I (M I) was significantly higher (P < 0.05) in
of EGF and/or GD1a supplementation on fertilization parameters, developmental competence, and apoptotic patterns of blastocysts

To investigate the effects of exogenous GD1a treatment on blastocyst development and fertilization rate after in vitro fertilization (IVF), we examined fertilization parameters, developmental competence, and apoptotic patterns of porcine blastocysts.

As shown in Fig. 4B, the proportion of penetration in oocytes of the EGF+GD1a treated group (89.1 ± 2.3%) was significantly higher (P < 0.05) than that of the EGF treated group (80.8 ± 0.9%), the GD1a treated group (82.6 ± 6.8%), and the control group (69.4 ± 2.2%) (Fig. 4A). Moreover, the percentage of polyspermic oocytes was significantly decreased (P < 0.05) in the EGF+GD1a treated group (41.5 ± 1.5%) relative to the EGF treated group (47.7 ± 2.9%), the GD1a treated group (45.7 ± 0.4%), and the control group (45.2 ± 0.4%) (Fig. 4C).

We measured the rate of development to blastocyst formation in IVF-derived porcine embryos from matured oocytes grown in maturation medium supplemented with EGF and/or GD1a. Treatment with EGF+GD1a resulted in development rates that were faster (47.5 ± 5.0%) (P < 0.05) than those of the matured control (25.5 ± 2.4%), treatment with EGF alone (33.0 ± 1.4%), and treatment with GD1a alone (33.7 ± 2.1%) (Fig. 5). Although no significant differences in cleavage were detected between groups under different maturation conditions, we confirmed that GD1a increases preimplantation developmental ability via stimulation of meiotic maturation of porcine oocytes.

Apoptosis was measured using the TUNEL assay (Fig. 6). The number of total nuclei was significantly higher (P < 0.05) in blastocysts derived from EGF+GD1a treated embryos (50.6 ± 10.4) than in the control group (34.7 ± 11.0). In addition, the number of TUNEL-positive nuclei was significantly lower (P < 0.05) in blastocysts
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derived from EGF+GD1a treated embryos (1.2 ± 1.0) than in the control (2.0 ± 1.3). Moreover, we confirmed that the percentage of TUNEL-positive cells (apoptotic cells) gradually decreased in groups treated with GD1a alone, EGF alone, and EGF+GD1a. These results indicate that addition of GD1a to porcine oocytes yields dramatic improvements in oocyte quality as well as in the rates of blastocysts development. Taken together, the data suggests exogenous GD1a treatment positively affects oocyte maturation by increasing fertilization rate, increasing developmental competence, and decreasing apoptosis of blastocysts.

Discussion

In the present study, we first identified that the expression of ganglioside GD1a synthesizing enzyme ST3GAL2 only increased in cumulus cell oocyte complexes (COCs) during IVM progress. Moreover, we confirmed that addition of GD1a to IVM medium improved meiotic maturation, the fertilization parameters, and preimplantation development of porcine embryos.

According to recent studies, changes in ganglioside and ganglioside synthesizing enzyme expression patterns in blastocysts can have profound impacts on embryo development and competence [23, 24]. As shown in Fig. 1, we measured the expression of ganglioside GD1a synthesizing enzyme ST3GAL2 in COCs during IVM progression. Ganglioside GD1a is specifically formed by the addition of sialic acid to GM1a by ST3GAL2. Therefore, we estimated the expression of GD1a by measuring ST3GAL2 expression.

Gangliosides modulate various signal transduction molecules,
including PDGFR, EGFR, insulin receptor, and nerve growth factor, in both extracellular and intracellular molecular interactions [3]. GD1a was previously found to be expressed in interstitial cells, theca cells, and oocytes during ovarian maturation in mice [17]. Additionally, the inhibition of GD1a synthesis suppresses the differentiation of human mesenchymal stem cells into osteoblasts [15]. In addition, exogenous ganglioside GD1a enhances EGF receptor ligand binding, dimerization, and signaling activation [16]. Therefore, we speculated that ganglioside GD1a was involved in development of COCs during IVM, through a mechanism similar to EGF-regulated EGFR signaling.

Consequently, we identified the effects of exogenous GD1a treatment on meiotic maturation of porcine oocytes. Exogenous GD1a at 20 μM was previously found to induce phosphorylation of EGFR in normal human dermal fibroblast cells [16]; therefore, the present study was conducted to determine the optimum concentration of GD1a in porcine oocytes by monitoring meiotic maturation to the metaphase II (M II) stage. A significantly higher proportion of oocytes reached M II in the 10 μM GD1a treated group than in the control group in our present study (Fig. 2). GD1a was previously shown to enhance EGF-induced EGFR phosphorylation, while the inhibition of ganglioside GD1a synthesis significantly suppressed the phosphorylation of EGFR [15, 16]. In the present study, the effects of GD1a and/or EGF treatment on nuclear maturation during IVM periods of porcine oocytes were investigated. The proportion of GV arrested oocytes was significantly higher in the EGF alone, GD1a alone, and EGF+GD1a treatment groups than the untreated control group. Additionally, the proportion of M II stage oocytes increased gradually in the EGF alone, GD1a alone, and EGF+GD1a treatment groups relative to the untreated control group (Fig. 3). Taken together, these results suggest that EGF plays an important role in porcine oocyte maturation, and that GD1a may affect EGF-mediated porcine oocyte activation. The proportion of M II stage oocytes is likely to have been enhanced by the interaction of EGF and GD1a. These findings suggest that addition of exogenous GD1a may help maintain EGF-induced activation of EGFR signaling.

Polyspermy is a common phenomenon in pigs, and polyspermic fertilization occurs more frequently than in other species under diverse experimental conditions [25]. Polyspermic fertilization has been a perennial problem impacting porcine IVF systems; therefore, this study was conducted to confirm potential oocyte deficiencies and improve embryo conditions after performing IVF [26]. We found that GD1a treatment during the IVM process enhanced oocyte maturation rate and there were more penetrated oocytes after IVF, but the number of polyspermic oocytes decreased (Fig. 4), and blastocyst formation rates increased after IVC (Fig. 5). Previous reports demonstrated that regulating oocyte maturation with dbcAMP treatment was associated with increased blastocyst formation rates as well as monospermic fertilization via in vitro culture of porcine embryos [27, 28]. Therefore, our findings indicate that GD1a treatment is essential for preserving the improvement in oocyte quality and monospermic fertilization achieved during the in vitro maturation process of porcine oocytes. Furthermore, the quality of matured oocytes affects the developmental competence and viability of porcine embryos after IVF.
Apoptosis has received increasing attention in the IVF research field because of its potential role in the cellular responses to stress, suboptimal developmental conditions, and early embryonic loss [29]. Overall, data suggests that the composition of a maturation medium has significant effects on apoptosis in blastocyst-stage embryos, and an optimal medium can lead to an overall decrease in susceptibility to embryo apoptosis. As expected, the number of apoptotic nuclei was dramatically lower in blastocysts of the EGF+GD1a treated group than in the untreated control. In addition, the total cell numbers were significantly higher in blastocysts treated with EGF+GD1a than in untreated controls (Fig. 6). Our results showed that GD1a promotes effective and high-quality blastocyst development. In future studies, we plan to investigate the effect of GD1a treatment on EGFR-induced downstream signaling pathways during IVM of porcine oocytes.

In summary, the present study provides the first evidence associating exogenous GD1a treatment with the promotion of porcine oocyte meiotic maturation during IVM. Exogenous GD1a played a critical role in meiotic progression in porcine oocytes, which influenced fertilization status and subsequent preimplantation development. Furthermore, we found that the embryonic qualities and developmental potential, including structural integrity and apoptotic patterns, of porcine IVF embryos can be determined based on the maturation conditions in the presence or absence of GD1a and early embryo development processes in porcine. Our results will be beneficial to achieving a better understanding of the relationship between gangliosides and meiotic maturation during porcine in vitro maturation and early embryo development processes.

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References

1. Mirkin BL, Clark SH, Zhang C. Inhibition of human neuroblastoma cell proliferation and EGF receptor phosphorylation by gangliosides GM1, GM3, GD1A and GT1B. Cell Prolif 2002; 35: 105–115. [Medline] [CrossRef]

2. Kwak DH, Seo BB, Chang KT, Cho YK. Roles of gangliosides in mouse embryogenesis and embryonic stem cell differentiation. Exp Mol Med 2011; 43: 379–388. [Medline] [CrossRef]

3. Kim SM, Jung JU, Ryu JS, Jin JW, Yang HJ, Ko K, Yoo HK, Jung KY, Choo YK. Effects of gangliosides on the differentiation of human mesenchymal stem cells into osteoblasts by modulating epididymal growth factor receptors. Biochem Biophys Res Commun 2008; 371: 866–871. [Medline] [CrossRef]

4. Xu Y, Tan LJ, Grachtchouk V, Voorhees JJ, Fisher GJ. Receptor-type protein-tyrosine phosphatase-kappa regulates epididymal growth factor receptor function. J Biol Chem 2005; 280: 42694–42700. [Medline] [CrossRef]

5. Scalfrett M, Baselga J. The epididymal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res 2006; 12: 5268–5272. [Medline] [CrossRef]

6. Uhm SJ, Gupta MK, Yang HJ, Chung HJ, Min TS, Lee HT. Epididymal growth factor can be used in lieu of follicle-stimulating hormone for nuclear maturation of porcine oocytes in vitro. Theriogenology 2010; 73: 1024–1036. [Medline] [CrossRef]

7. Illera MJ, Lorenzo PL, Illera JC, Petters RM. Developmental competence of immature pig oocytes under the influence of EGF, IGF-I, follicular fluid and gonadotropins during IVF-IVF processes. Int J Dev Biol 1998; 42: 1169–1172. [Medline] [CrossRef]

8. Singh B, Meng L, Rutledge JM, Armstrong DT. Effects of epididymal growth factor and follicle-stimulating hormone during in vitro maturation on cytoplasmic maturation of porcine oocytes. Mol Reprod Dev 1997; 46: 401–407. [Medline] [CrossRef]

9. Ben-Yosef D, Galliani D, Dekel N, Shabtai R. Rat oocytes induced to mature by epididymal growth factor are successfully fertilized. Mol Cell Endocrinol 1992; 88: 135–141. [Medline] [CrossRef]

10. De La Fuente R, O’Brien MJ, Eppig JJ. Epididymal growth factor enhances preimplantation developmental competence of maturing mouse oocytes. Hum Reprod 1999; 14: 3060–3068. [Medline] [CrossRef]

11. Goud PT, Gaud AP, Qian C, Laverge H, Van der Elst J, De Sutter P, Dhaout M. In-vitro maturation of human germinal vesicle stage oocytes: role of cumulus cells and epididymal growth factor in the culture medium. Hum Reprod 1998; 13: 1638–1644. [Medline] [CrossRef]

12. Louergar P, Carolan C, Van Langendonck A, Donnay I, Khatri H, Mermillod P. Role of epididymal growth factor in bovine oocyte maturation and preimplantation embryo development in vitro. Biol Reprod 1996; 54: 1420–1429. [Medline] [CrossRef]

13. Galli L, Boulesteix C, Ruffini S, Germann G. EGF-induced EGF-receptor and MAP kinase phosphorylation in goat cumulus cells during in vitro maturation. Mol Reprod Dev 2005; 71: 489–494. [Medline] [CrossRef]

14. Galli L, Chene N, Dahirel M, Ruffini S, Boulesteix C. Expression of epididymal growth factor receptor in the goat cumulus-oocyte complex. Mol Reprod Dev 2004; 67: 439–445. [Medline] [CrossRef]

15. Yang HJ, Jung KY, Kwak DH, Lee SH, Ryu JS, Kim JS, Chang KT, Lee JW, Choo YK. Inhibition of ganglioside GD1a synthesis suppresses the differentiation of human mesenchymal stem cells into osteoblasts. Dev Growth Differ 2011; 53: 323–332. [Medline] [CrossRef]

16. Liu V, Li R, Ladsich S. Exogenous ganglioside GD1a enhances epididymal growth factor receptor binding and dimerization. J Biol Chem 2004; 279: 36481–36489. [Medline] [CrossRef]

17. Choo YK, Chiba K, Tai T, Ogiso M, Hoshi M. Differential distribution of gangliosides in adult rat ovary during the oestrous cycle. Glycoconjugate J 1995; 5: 299–309. [Medline] [CrossRef]

18. Funahashi H, Cantley TC, Stumpf TT, Torielle SL, Day BN. In vitro development of in vitro-matured porcine oocytes following chemical activation or in vitro fertilization. Biol Reprod 1994; 50: 1072–1077. [Medline] [CrossRef]

19. Petters RM, Wells KD. Culture of pig embryos. J Reprod Fertil Suppl 1993; 48: 61–73. [Medline] [CrossRef]

20. Abydehere L, Day BN. Fertilization and subsequent development in vitro of pig oocytes inseminated in a modified tris-buffered medium with frozen-thawed ejaculated spermatzoa. Biol Reprod 1997; 57: 729–734. [Medline] [CrossRef]

21. Nomura M, Shimbo T, Miyamoto Y, Fukuzawa M, Kanesa Y. 13-Cis retinoic acid can enhance the antitumor activity of non-replicating Sendai virus particle against neuroblastoma. Cancer Sci 2013; 104: 238–244. [Medline] [CrossRef]

22. Wang L, Wang Y, Sato T, Yamagata S, Yamagata T. Ganglioside GD1a suppresses TNA-Alpha expression via PI3K at the transcriptional level in mouse oocyte-derived FBJ cells. Biochem Biophys Res Commun 2008; 371: 230–235. [Medline] [CrossRef]

23. Ju EJ, Kwak DH, Lee DH, Kim SM, Kim JS, Kim SM, Choi HG, Jung KY, Lee SU, Do SI, Park YI, Choo YK. Pathophysiological implication of ganglioside GM3 in early mouse embryonic development through apoptosis. Arch Pharm Res 2005; 28: 1057–1064. [Medline] [CrossRef]

24. Koo JY, Kwak DH, Lee KH, Kim SM, Hwang SB, Hwang JB, Han JS, Jin JW, Hyun JS, Khim JS, Kwon HJ, Nam SY, Kwak DH, Park YI, Koo DB, Choo YK. Expression of ganglioside GT1b in mouse embryos at different developmental stages after cryopreservation. Arch Pharm Res 2008; 31: 88–95. [Medline] [CrossRef]

25. Suzuki H, Saito Y, Kagawa N, Yang X. In vitro fertilization and polyspermy in the pig: factors affecting fertilization rates and cytoskeletal reorganization of the oocyte. Microsc Res Tech 2003; 61: 327–334. [Medline] [CrossRef]

26. Gergen CG. The evolution of porcine embryo in vitro production. Theriogenology 2014; 81: 24–37. [Medline] [CrossRef]

27. Sonfai T, Kikuchi K, Onishi A, Iwamoto M, Fuchimoto D, Papp AB, Sato E, Nagai T. Meiotic arrest maintained by CAMP during the initiation of maturation enhances meiotic potential and developmental competence and reduces polyspermy of IVM/IVF porcine oocytes. Zygote 2003; 11: 199–206. [Medline] [CrossRef]

28. Kim JS, Cho YS, Song BS, Wee G, Park JS, Choo YK, Yu K, Lee KK, Han YM, Koo DB. Exogenous dibutyryl cAMP affects meiotic maturation via protein kinase A activation; it stimulates further embryonic development including blastocyst quality in pigs. Theriogenology 2008; 69: 290–301. [Medline] [CrossRef]

29. Betts DH, King WA. Genetic regulation of embryo death and senescence. Theriogenology 2001; 55: 171–191. [Medline] [CrossRef]