Reversible Effects of Exogenous GM3 on Meiotic Maturation and Cumulus Cells Expansion of Porcine Cumulus-oocyte Complexes

Jin-Woo Kim, Hyo-Jin Park, Jae-Min Jung, Seul-Gi Yang, Min-Ji Kim, In-Su Kim, Ho-Geun Jegal and Deog-Bon Koo[†]

Department of Biotechnology, College of Engineering, Daegu University, 201 Daegudae-ro, Jillyang, Gyeongsan, Gyeongbuk 38453, Republic of Korea

Abstract

Ganglioside GM3 is known as an inhibition factor of cell differentiation and proliferation via inhibition of epidermal growth factor receptor (EGFR) phosphorylation. Our previous study showed that the exogenous ganglioside GM3 reduced the meiotic maturation of porcine oocytes and induced apoptosis at 44 h of *in vitro* maturation (IVM). However, the role of ganglioside GM3 in the relationship between EGFR signaling and apoptosis during porcine oocyte maturation has not yet been studied. First, porcine cumulus-oocyte complexes (COCs) were cultured in the NCSU-23 medium with exogenous ganglioside GM3 according to maturation periods (non-treated, only IVM I: 0 - 22 h, only IVM II: 22 - 44 h and IVM I & II: 0 - 44 h). We confirmed that the proportion of germinal vesicle breakdown (GVBD) increased significantly in the IVM I treated group than in the control group. We also confirmed that the meiotic maturation until M II stage and polar body formation decreased significantly in the only IVM I treated group. Cumulus cell expansion and mRNA levels of the expansion-related factors (*HAS2*, *TNFAIP6* and *PTX3*) decreased significantly in the IVM I treated group than in the control group. Protein levels of EGFR, p-EGFR, ERK1/2, and p-ERK1/2 decreased significantly in the GM3-treated groups, during the IVM I period. In addition, cellular apoptosis, determined using TUNEL assay, and protein levels of Cleaved caspase 3, were increased significantly in the GM3-treated COCs during the IVM I period. Based on these results, ganglioside GM3 exposure of porcine COCs during the IVM I period reduced meiotic maturation and cumulus cell expansion via inhibition of EGFR activity in pigs.

Received : 3 December 2018

Revised : 13 December 2018

Accepted : 19 December 2018

Key Words : Ganglioside GM3, Oocytes maturation, EGFR, Apoptosis, Pig

[†] Correspondence: Deog-Bon Koo (ORCID: 0000-0001-7825-9598)

Tel: +82-53-850-6557, Fax: +82-53-850-6559

E-mail address: dbkoo@daegu.ac.kr

INTRODUCTION

Gangliosides, which are glycosphingolipids containing one or more sialic acid residues, exist in the plasma membrane of most mammalian cells (Julien, et al. 2013; Kim, et al. 2016). Gangliosides play a role in the regulation of various biological processes, such as differentiation, apoptosis, and signaling in animal cells (Huang, et al. 2013; Li, et al. 2015; Wang, et al. 2003). Gangliosides regulate the activity of tyrosine kinase receptors, such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR) in neuron and cancer cells (Julien, et al. 2013). Recent studies investigated the relevance of the relationship between gangliosides and early embryonic development in mice and pigs (Ju, et al. 2005; Kim, et al. 2016; Park, et al. 2017).

In vitro maturation (IVM) of oocytes is an important step for *in vitro* production (IVP) of embryos and blastocyst in pigs. During IVM progression, the relationship between oocytes and cumulus cells in cumulus-oocyte complexes (COCs) arranges the transfer of various nutrients and regulates receptor-related signaling, secretion functions, and gap junction signaling (Liu, et al. 2015). In addition, the activity of EGFR-mediated ERK1/2 induces cumulus expansion through the activation of cumulus cell expansion-related factors, such as *HAS2*, *TNFAIP6*, and *PTX3* during IVM of porcine oocytes (Meinecke and Krschek 2003; Sen and Caiazza 2013; Su, et al. 2003). In this process, EGFR-derived ERK1/2 signaling is known to play an important role in the cumulus cell expansion and meiotic maturation in oocytes of mammals, including mouse, human, porcine, and cattle oocytes (De La Fuente, et al. 1999; Goud, et al. 1998; Illera, et al. 1998; Rieger, et al. 1998).

Among the gangliosides, GM3 as a common precursor has the simplest structure and is the first product of the gangliosides synthesis process (Yoshikawa, et al. 2015). Ganglioside GM3 is well-known for the inhibition of EGFR-, FGFR-, and PDGFR-related signal pathways in neuron cells (Julien, et al. 2013). In the reproductive system, ganglioside GM3 is expressed in Sertoli cells, sperm, developmental follicle, zygote, and developmental embryos in mice (Kwak, et al. 2011). Also, ganglioside GM3 expressions increased in the apoptosis-induced embryos in pigs and mice (Chae, et al. 2015; Ju, et al. 2005). Moreover, according to our previous study, exogenous ganglioside GM3 induces cellular apoptosis by blocking cell proliferation by reducing PI3K/AKT signaling in porcine COCs during IVM (Park, et al. 2017). However, the effects of ganglioside GM3 in the relation of EGFR activity on meiotic maturation, polar body formation,

and cumulus cells expansion in porcine COCs during IVM periods (metaphase I; 22 h and metaphase II; 44 h) have not been reported.

Therefore, the aim of the present study was to investigate the changes in the meiotic maturation, cumulus cell expansion, and cumulus expansion factors via the EGFR-mediated ERK1/2 signaling activity according to ganglioside GM3 treatment periods (non-treated, only IVM I: 0 - 22 h, only IVM II: 22 - 44 h and IVM I & IVM II: 0 - 44 h) in porcine COCs (Graphical hypothesis, Figure 1).

MATERIALS AND METHODS

Chemicals

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

In vitro maturation (IVM)

Porcine ovaries were obtained from the local slaughterhouse and carried to laboratory using 30-35°C, 0.9% saline added with 75 µg/ml potassium penicillin G. Follicular fluid was aspirated between 3 - 6 mm follicles using 10 ml syringe with 18 gauge needle. After, immature cumulus-oocyte complexes (COCs) surrounded by cumulus cells were selected using mouth pipettes under the microscope. And then, COCs washed three times in tyrode's lactate-4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (TL-HEPES) medium. Selected COCs were matured in 4-well multi-dish (Nunc, Roskilde, Denmark) with 500 µl IVM I medium at 38.5°C, 5% CO₂ in air. The IVM medium was Carolina State University (NC SU) 23 medium with 0.57 mM cysteine, 10% follicular fluid, 10 ng/ml epidermal growth factor (EGF), 10 ng/ml β-mercaptoethanol, 10 IU/ml pregnant mare serum gonadotropin (PMSG) and 10 IU/ml human chorionic gonadotropin (hCG). After 22 h, COCs were washed three times with IVM II medium without PMSG and hCG and then cultured in IVM II medium for 22 h at 38.5°C, 5% CO₂ in air. Our previous study confirmed that meiotic maturation and cumulus cells expansion of porcine COCs was reduced in 10 µM GM3 during IVM (Park, et al. 2017). Therefore, all experiments were performed using 10 µM GM3. During the maturation periods, ganglioside GM3 was added to the maturation medium according to three periods of IVM such as IVM I (0 - 22 h), IVM II (22 - 44 h), IVM I & IVM II (0 - 44 h).

Figure 1. Schematics of the hypothesis and design for the effects of exogenous ganglioside GM3 on oocyte maturation and cumulus cell expansion in porcine COCs during 22 h and/or 44 h of IVM. We were divided into four groups of the exogenous ganglioside GM3; non-treated, IVM I (0 - 22 h), IVM II (22 - 44 h) and IVM I & IVM II (0 - 44 h). After 44 h of IVM, we investigated that the changes in meiotic maturation, cumulus cells expansion and polar body production via EGFR-mediated ERK1/2 signaling in matured COCs. Moreover, cellular apoptosis and Cleaved caspase 3 as apoptotic factor by GM3 treatment were observed using a TUNEL assay and Western blot analysis in matured COCs during IVM.

Assessment of meiotic maturation and polar body extrusion

At the end of IVM 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). Then checking for extrusion of first polar body. Next, each sample were mounted on microscope slide. The samples were then fixed for 2 - 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 cumulus cells expansion

We evaluated the cumulus cells expansion under a optical microscope (Leica, Solms, Germany) after 44 h maturation. Assessment of cumulus cells expansion was performed as described by Mlynarcikova et al (Mlynarcikova, et al. 2009).

The degree of cumulus cells expansion was measured according to a subjective scoring system from 0 to +4. No expansion (0), separation of only the outermost layer of cumulus cells (+1), further expansion involving the outer half of the cumulus cells (+2), further expansion up to, but not including, the corona radiate (+3) and complete expansion including the corona radiated cells (+4). Expanded COCs rated as +2 or higher were included in assessment.

RT-PCR

Total RNA was isolated from matured COCs using Tri-zol reagent (Invitrogen, Carlsbad, CA) and quantified using nano Q. Extracted RNA from each sample were synthesized to cDNA using the *AccuPower®* CycleScript RT PreMix (Bioneer Inc., Daejeon, Korea) according to the manufacturer's instructions. RT-PCR analysis was performed on a *AccuPower®* PCR PreMix in a 20 μ l reaction volume containing 1 μ l cDNA, 17 μ l distilled water,

Table 1. Primer sequencing used for RT-PCR

Gene	Genebank accession	primer	Sequence	T _m (°C)	Product size (base pairs)
<i>HAS2</i>	NM_214053.1	Forward	TGGCTGTACAATGCGATGTG	55	402
		Reverse	GGTGAAAATCACACCACCCA		
<i>PTX3</i>	NM_001244783.1	Forward	TCAGTGCCTGCATTTGGGTC	58	225
		Reverse	CTACATGCCCTTGTTTCAGAA		
<i>TNFAIP6</i>	NM_001159607.1	Forward	TCTTCCTGTGGGAAGAGGCT	55	337
		Reverse	TCGCTTCTGTTCAGACGGAC		
<i>GAPDH</i>	NM_001206359.1	Forward	GAAGGTCGGAGTGAACGGAT	55	527
		Reverse	CATGGACCGTGGTCATGAGT		

and 10 pmol each of forward and reverse primers (Table 1). The conditions for PCR were performed as follows: 95°C for 5 min, and 25 - 30 cycle of denaturation at 95°C for 30 sec, annealing at 55 and 58°C for 30 sec, extension at 72°C for 30 sec, and 72°C for 5 min. The products performed electrophoresis using 2% agarose gel and detected UV light.

Western blot analysis

COCs (20-25 per group) were placed into 2 µl of 0.1% PVA-PBS and were added to 16 µl PRO-PREP protein lysis buffer (iNtRON, Daejeon, Korea) and 4 µl of 5x laemmli buffer. Protein samples was denatured at 100°C for 10 min and then separated on a 10 - 12% polyacrylamide gel by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Hoefer, Holliston, MA, USA) for 3 h - 3 h 30 min at 15 mA, and transferred to a nitrocellulose membrane for 2 h at 400 mA. The membrane was blocked with 5% skim milk or 5 % BSA in Tris-buffered saline containing 1% Tween 20 (TBST) for overnight at 4°C. The membrane was incubated with the appropriate primary antibody; anti-ST3GAL5 (ab65541, Abcam), anti-EGFR (sc-03, Santa Cruz), anti-phospho-EGFR (sc-12351, Santa Cruz), anti-ERK1/2 (#9102, Cell Signaling), anti-phospho-ERK1/2 (#9101, Cell Signaling), anti-Cleaved caspase 3 (#9664, Cell Signaling) and anti-β-actin (sc-47778, Santa Cruz). Membranes were washed using TBST buffer and then incubated with a secondary antibody-HRE-conjugated anti- mouse/rabbit/goat IgG for 2 h room temperature. And then, the membranes were washed with TBST buffer. The signals were detected using the ECL kit. Band intensities were quantified with Image J software (NIH, MD, USA). All experiments were performed three times.

TUNEL assay

Apoptotic cumulus cells were detected using an *In Situ* Cell Death Detection Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The COCs from IVM II were washed with 0.1% PVA in PBS and then fixed in 4% PFA in PBS for 1 h at room temperature. Next, COCs were permeablized using 0.1% (v:v) Triton X-100 for 30 min at 4°C. The fixed COCs were incubated in TUNEL reaction medium for 1 h at 38.5°C, then washed and mounted on slides. Whole-mount COCs 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

Changes in meiotic maturation and cumulus cells expansion in porcine COCs according to GM3 (10 μ M) treatment time during IVM periods.

We investigated the meiotic maturation and cumulus cells expansion according to GM3 treatment time (Con; non-treated, only IVM I treated; 0 - 22 h, only IVM II treated; 22 - 44 h, IVM I & IVM II treated; 0 - 44 h) during porcine oocytes maturation. First, we confirmed that the meiotic maturation rate until metaphase II (M II) stage oocytes decreased significantly in the GM3-treated group for only IVM I periods (only IVM I treated group; $78.5 \pm 1.6\%$ vs Con; $89.8 \pm 5.6\%$; $p < 0.05$). Especially, the rate of germinal vesicle break-down stage (GVBD) oocytes significantly increased in the IVM I treated group ($10.9 \pm 5.5\%$) compared to that in the other groups (Table 2; Con: $0.8 \pm 1.8\%$ and IVM II: $2.8 \pm 2.9\%$; $p < 0.05$). In addition, the rate of polar body extrusion decreased significantly ($p < 0.01$) in the oocytes treated with GM3 for IVM I period (Figure 2A). Next, we confirmed that cumulus cells expansion and expressions of cumulus cells expansion factors (*HAS2*, *TNFAIP6*, and *PTX3*) were significantly decreased ($p < 0.001$) in the IVM I treated group compared to that in the control group (Figure 2B, C). In case of GM3 treatment for 0 - 44 h IVM, as expected, meiotic maturation, GV arrest, polar body formation, cumulus cell expansion, and oocyte maturation rate were higher than the other treated groups. These results demonstrated that ganglioside GM3 interfered with the porcine oocytes maturation and cumulus cells expansion at the time of early maturation.

Protein levels of ST3GAL5 and EGFR-ERK1/2 signaling in porcine COCs according to GM3 treatment time

To confirm the effects of exogenous ganglioside GM3 on EGFR-mediated ERK1/2 signaling in porcine COCs maturation, we investigated the protein expression of ST3GAL5, EGFR, p-ERGR, ERK1/2, and p-ERK1/2 in porcine COCs, according to GM3 treatment time. As shown in Figure 3, we confirmed that treatment with exogenous ganglioside GM3 during 22 h IVM decreased the expression of ST3GAL5 and EGFR downstream factors (EGFR and ERK1/2) protein levels in porcine COCs at 44 h of IVM. In particular, the expression of EGFR, p-EGFR, and p-ERK1/2 protein levels appeared lowest in matured COCs derived from IVM I (0 - 22 h) group ($p < 0.001$; compared to control group). Our results demonstrated that ganglioside GM3 inhibits the EGFR-derived ERK1/2 signaling pathway, regardless of treatment time. The expression of EGFR-mediated ERK1/2 signaling proteins in porcine COCs decreased in ganglioside GM3-treated groups during IVM I.

Changes of cellular apoptosis in porcine COCs according to GM3 treatment timing

To confirm the cellular apoptosis by exogenous GM3 treatment on porcine oocyte maturation, we performed the TUNEL assay and western blotting analysis for protein level of Cleaved caspase 3. As a result, the rate of positive apoptotic cells was significantly increased ($p < 0.01$) in the 22 h GM3 treatment group compared to that in the control group (Figure 4A). As shown in Figure 4B, we confirmed that treatment of exogenous ganglioside GM3 during 22 h IVM significantly increased ($p < 0.05$) the expression of Cleaved caspase 3 protein level in porcine COCs at 44 h. These

Table 2. Effects of exogenous GM3 according to treatment periods on meiotic maturation of porcine COCs

GM3 treatment (10 μ M) periods	No. of oocytes examined	% of oocytes (n)			
		GV	GVBD	M I	M II
Non-treated	156	-	0.8 ± 1.8 (1) ^a	9.4 ± 6.8 (14) ^a	89.8 ± 5.6 (141) ^a
0 - 22 h	140	1.4 ± 1.9 (2)	10.9 ± 5.5 (15) ^b	9.3 ± 3.2 (13) ^a	78.5 ± 1.6 (110) ^b
22 - 44 h	158	3.9 ± 2.9 (6)	2.8 ± 2.9 (4) ^a	9.8 ± 3.8 (15) ^a	83.6 ± 4.6 (133) ^b
0 - 44 h	143	2.1 ± 2.0 (3)	7.4 ± 3.4 (10) ^b	19.6 ± 2.5 (28) ^d	70.9 ± 5.3 (102) ^c

This experiment was replicated three times. Data are the mean $\pm$ SD. Different superscripts denote a significant difference compared with other groups ($p < 0.05$)

Figure 2. Effects of exogenous ganglioside GM3 on oocytes maturation and cumulus cell expansion in porcine COCs during 22 h and/or 44 h of IVM. (A) Polar body extrusion and (B) morphological of cumulus cells expansion in matured COCs according to different GM3 treatment periods. Scale bars = 100 μ m. Red asterisk is expanded COCs. (C) Expression of *HAS2*, *TNFAIP6* and *PTX3* in COCs cultured with GM3 treatment periods at IVM II. These mRNA levels were normalized to *GAPDH* expression as a control. Data in the bar graph represent the mean $\pm$ SEM of three independent experiments. Differences in non-treated group compared with other groups were considered significant at * $p < 0.05$, ** < 0.01 , and *** < 0.001 .

results demonstrated that treatment of exogenous ganglioside GM3 during early maturation periods induced apoptosis of porcine COCs.

DISCUSSION

In the present study, we first demonstrated the detailed effects of exogenous ganglioside GM3 on meiotic maturation, GV arrest, polar body formation, and cumulus cells expansion, according to the GM3 treatment periods (non-treated, IVM I, IVM II, and IVM I & IVM II) during porcine oocyte IVM. Our results showed that porcine oocyte

maturation was reduced by increasing GV stage arrest at 44 h IVM after 10 μ M GM3 treatment. In addition, the protein levels of EGFR, p-EGFR, ERK1/2, and p-ERK1/2 decreased in the GM3-treated group for IVM I only compared to the control group. Under GM3 treatment for 22 h of IVM, cellular apoptosis and apoptotic protein level (Cleaved caspase 3) in the matured COCs increased compared to the non-treated control. The meiotic maturation, cumulus cell expansion, and polar body formation in porcine COCs in the GM3-treated group for 22 h IVM decreased by reduction of the EGFR-derived ERK1/2 signal activity.

Gangliosides are found in the plasma membranes of mammalian cells and are particularly abundant in the neurons (Mirkin, et al.

Figure 3. Western blot analysis of ST3GAL5 and EGFR-derived ERK1/2 signaling in matured porcine COCs at 22 h and/or 44 h of IVM after GM3 10 μ M treatment. (A) Western blot analysis of the EGFR-derived ERK1/2 signaling (EGFR, p-EGFR, ERK1/2 and p-ERK1/2) factors in matured COCs after GM3 10 μ M treatment at 22 h and/or 44 h of IVM. The protein levels were normalized to that of β -actin as a control. (B) Histogram values of densitometry analysis were obtained using ImageJ software. Bar graph data represent the least-squares means $\pm$ SEM of three independent experiments. (* p < 0.05, ** p < 0.01, and *** p < 0.001 compared to non-treated group as control)

2002). Gangliosides are found at each embryonic developmental stage of mice, including follicular development, spermatogenesis, and embryogenesis. Ganglioside GM3 is expressed in primary follicles and Graafian follicles during the follicular growth in mouse ovaries (Kwak, et al. 2011). In diabetic mice, ganglioside GM3 decreased during preimplantation embryo development (Kwak, et al. 2003). A recent study reported that ganglioside GD1a improved oocytes maturation and preimplantation embryos development in porcine (Kim, et al. 2016). In contrast, ganglioside GM3 reduced meiotic maturation and cumulus cells expansion during porcine COCs maturation (Chae, et al. 2015; Park, et al. 2017). To confirm the effect of ganglioside GM3 on porcine oocytes maturation, we examined the ganglioside GM3 divided into four treatment periods groups. To confirm the changes in GM3 expression pattern, we also investigated the protein levels of ST3GAL5, a specific GM3-synthesizing enzyme, in matured porcine COCs. As a result, the expression of ST3GAL5 was

decreased regardless of ganglioside GM3 treatment time in porcine COCs (Figure 4). According to our previous study, the exogenous GM3 exposure for 44 h IVM in porcine oocyte induced apoptosis by reduction of cell proliferation signaling (Chae, et al. 2015). As shown in Figure 2 and Table 1, meiotic maturation, cumulus cells expansion, and mRNA levels of cumulus cells expansion factors (*HAS2*, *TNFAIP6*, and *PTX3*) in porcine COCs of the ganglioside GM3-treated group for IVM I (0 - 22 h) were significantly decreased. These results showed the negative effects of 10 μ M GM3 treatment for 22 h IVM, which causes damage similar to GM3 treatment for 44 h IVM, and reduces the meiotic maturation rate, cumulus expansion, and polar body formation of porcine COCs. Based on these results, we determined that ganglioside GM3 during the IVM I (22 h) period was involved in GV arrest via inhibition of EGFR-derived ERK1/2 signal on meiotic maturation and cumulus cells expansion in porcine COCs.

Figure 4. Changes in cellular apoptosis and apoptotic factor expression in matured COCs according to different GM3 treatment periods. (A) Representative TUNEL assay images and (B) western blots analysis of apoptosis factors (Cleaved caspase 3) in matured COCs according to different GM3 treatment periods. Scale bars = 100 μ m. White arrow is apoptotic cells. The protein levels were normalized to that of β -actin as a control. Data in the bar graph represent the mean $\pm$ SEM of three independent experiments. Differences in non-treated group compared with other groups were considered significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Activation of EGFR is important for the maturation of porcine COCs (Meinecke and Krischek 2003; Prochazka and Blaha 2015; Su, et al. 2003). In a previous study, ganglioside GM3 was found to inhibit the activation of EGFR in various cells (Julien, et al. 2013). The addition of GM3 to the culture medium inhibited the phosphorylation of EGFR in human epidermoid carcinoma cell line A431 (Bremer, et al. 1986). In human neuroblastoma cells, inhibition of EGFR phosphorylation and cell proliferation by GM3 treatment was reported (Mirkin, et al. 2002). Therefore, we investigated the relationship between EGFR signaling and ganglioside GM3 treatment periods in porcine COCs. Similar to the previous study, expressions of EGFR, p-EGFR, ERK1/2, and p-ERK1/2 proteins were significantly decreased ($p < 0.001$) in the GM3-treated group during IVM I period compared to the control group (Figure 3). These results suggest that GM3 treatment during IVM I showed reversible effects on porcine oocyte maturation by reduction of EGFR-related signaling molecules.

During IVM of porcine COCs, cumulus cells contribute to

the meiotic maturation of oocytes through the transfer of nutrients and signaling transduction (Liu, et al. 2015). According to previous studies, acrylamide reduced oocytes maturation via increasing of apoptotic cells in mouse COCs (Liu, et al. 2015), whereas, BMP15 reduced apoptotic cells in cumulus cells (Zhai, et al. 2013). A previous study also reported that ganglioside GM3 induced apoptosis and DNA damage during mouse oocytes maturation (Kwak, et al. 2003; Park, et al. 2017). Therefore, apoptosis is an important indicator used in the evaluation of COCs quality. As shown in Figure 4, the percentages of apoptotic cells and Cleaved caspase 3 were significantly increased in the ganglioside GM3 treatment group during IVM I period. Our result suggests that ganglioside GM3 induces apoptosis during IVM of porcine COCs.

In conclusion, we demonstrated that ganglioside GM3 inhibits oocyte maturation and cumulus cell expansion by reducing EGFR activity and inducing apoptosis (Graphical summary Figure 5). Especially, ganglioside GM3 treatment during the IVM I period

Graphical summary

Figure 5. Graphical summary; exogenous ganglioside GM3 treatment during IVM I (0 - 22 h) induced the decreasing meiotic maturation, polar body formation and cumulus cells expansion factors through the reduction of EGFR-related ERK1/2 activity and increasing of cellular apoptosis in porcine oocyte.

significantly inhibited meiotic maturation, cumulus cells expansion and EGFR-related signaling pathway. Also, ganglioside GM3 significantly increased apoptotic cell numbers and expression of apoptotic factor in porcine COCs. Therefore, the role of ganglioside GM3 in porcine oocyte maturation may be confirmed by the inhibition of ganglioside GM3.

ACKNOWLEDGMENT

This research was supported by a Daegu University Research Grant, 2016.

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