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Growth Arrest Specific Gene 6 Improved *in vitro* Maturation of Oocytes and the Development Potential of Porcine Embryo after Parthenogenetic Activation

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LZ and LQ designed the research studies. LZ performed the experimental work, analyzed data and discussed the results and implications. LX provided help in data analysis. LX provided help in experiment. SD provided important support in the research. LZ and LS drafted the manuscript. SR helped in revision of the manuscript.

Key words
Porcine, Oocyte, *In vitro* mature, *Gas6*, Early embryonic

ABSTRACT

In this study, expression pattern-and function of growth arrest specific gene 6 (*Gas6*) during porcine oocyte maturation and early embryonic development were systemically investigated. The expression of *Gas6* in the process of follicular development was studied by immunohistochemical staining, immunofluorescence, QRT-PCR and western blot. Immunohistochemical staining results showed that Gas6 was expressed in the oocytes, especially the nucleus, from the primary to preovulatory stages. The further immunofluorescence results showed that *Gas6* was expressed thoughout the *in vitro* matured porcine oocytes, and the staining signal decreased in the first 12 h but then increased during the process of oocyte maturation. QRT-PCR analysis revealed that *Gas6* mRNA was continuously expressed during the process of oocyte maturation, disappeared after parthenogenetic activation, re-appeared and elevated in the blastocysts stage. The following western blot analysis revealed a similar expression pattern with QRT-PCR and immunofluorescence. In conclusion, the expression pattern of Gas6 gene is closely related to oocytes maturation and embryo development. *Gas6* may play a crucial role in the process of oocytes maturation and improve the development potential of early embryos by promoting the cytoplasmic maturation.

INTRODUCTION

Oocyte growth and maturation are long-lasting and necessary processes for fertilization and embryonic development (Fair, 2009; Li and Albert, 2013). Oocyte maturation involves in nuclear and cytoplasmic maturation, and amount of maternal mRNA, protein, and regulatory molecules were stored in oocytes to support completion of meiosis, fertilization, and early embryogenesis (Eppig and Wigglesworth, 2000; Coticchio *et al*., 2015). This process is mainly controlled by post-transcriptional regulatory mechanisms, such as RNA polyadenylation, localization, sorting, and masking, as well as protein phosphorylation (Gandolfi and Gandolfi, 2001). Therefore, functional analysis of certain gene(s) in the oogenesismay provide important information on the molecular regulatory mechanisms of oocyte nuclear and cytoplasmic maturation (Labrecque and Sirard, 2011).

The growth arrest specific (*Gas*) gene was first identified in cells under stressors such as nutrient deprivation or exposure to genotoxic agents (Manfioletti *et al*., 1993). Growth arrest-special gene 6 (*Gas6*) was previously isolated as a gene product inducible expressed in fibroblasts in response to serum starvation (Stenhoff *et al*., 2004). It has a structure similar to that of protein S, a vitamin K-dependent plasma protein with anticoagulant activityat 43% amino acid identity (Nagata *et al*., 1996). Numerous studies have indicated that *Gas6*-mediated signalling is implicated in cell survival, growth arrest, proliferation, differentiation, and other cell type-specific functions (Nakano *et al*., 1995; Goruppi *et al*., 1996; Li *et al*., 1996). Clinically, *Gas6* plays an important role in haematosis and thombosis (Manfioletti *et al*., 1993). In mice, *Gas6* is expressed in the gonads and supports primordial germ cell growth and survival in cultureand the regulation of spermatogenesis and apoptosis is also controlled by *Gas6* (Matsubara *et al*., 1996; Xiong *et al*., 2008). Previous studies haverevealed that *Gas6* is expressed in germinal vesicle (GV) oocytes, 8-cell embryo and the blastocyst (Fleming *et al*., 1997; Yoon *et al*., 2005). The absence of *Gas6* is associated with a deficit in embryonic

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erythoid precursor cells and *Gas6*$^{-/-}$ adult mice showed a platelet dysfunction (Angelillo Scherrer *et al*., 2008).

Cytoplasmic maturation, a poorly understood physiological process, supports the fertilization of the oocyte and early embryonic development (Farghaly *et al*., 2015). Previous studies found that *Gas6* downregulation impaired cytoplasmic maturation and pronuclear formation of oocytes in mice independent of maturation promoting factor (MPF) activity (Kim *et al*., 2011). This suggests that *Gas6* may have crucial function on oocytes maturation and early embryonic development. To date, efforts to elucidate the function of *Gas6* and receptor signalling have focused mainly on thombosis and spermatogenesis, while few studies on oocytes maturation and embryos development. Thus, the objective of the present study was to explore the role and expression pattern of *Gas6* in porcine oocytes and preimplantation embryos.

MATERIALS AND METHODS

Reagents

Chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO), unless noted otherwise.

Immunohistochemical (IHC) studies

Porcine ovaries were obtained from a local abattoir. The ovaries of cyclic sows aged 5-8 months were selected for this study. We selected 10 ovaries from different cyclic sowsand fixed them overnight in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS; 0.01 M, pH 7.6; Sigma, Poole) and transferred to 70% (v/v) ethanol until processing. The fixed tissue was embedded in paraffin wax after dehydration.

Sections (7 µm thickness) were dewaxed and rehydrated in decreasing concentrations of alcohol (95, 90, 80 and 70%, respectively, and distilled water), followed by two washes of 5 min each in PBS. Antigen retrieval was performed by microwaving the sections in citrate buffer (0.01 M, pH 6.0) at 100°C for 10 min, and the sections were kept in the buffer for 60 min until cool. After washing in PBS, the sections were incubated in 3% hydrogen peroxide in water for 15 min. After washing in PBS, a combined avidin-biotin block was performed according to the manufacturer's instructions (Proteintech, Wuhan). In brief, avidin was added to the blocking buffer, which consisted of 5% (w/v) bovine serum albumin (BSA; Sigma) in PBS. The slides were incubated for 30 min and then washed in PBS. Biotin was added to the blocking buffer and the slides were treated for 15 min and washed in PBS. Rabbit polyclonal antibodies directed against Gas6 (ab136249, 1:250 dilutions; Abcam) were used in blocking buffer diluted at 1:250. Negative controls were obtained by replacing the primary antibody with pre-immune serum. Slides with serial sections were incubated with antibodies in a humidified chamber at 37°C overnight. Slides were washed twice for 5 min in 0.1% (v/v) Tween 20 in PBS (PBST) and incubated with goat anti-rabbit biotinylated antibody (Proteintech, Wuhan) diluted 1:300 in blocking buffer for 1 h at room temperature. The sections were washed twice in PBST for 5 min, incubated in ABC-HP (Proteintech, Wuhan) for 1 h at room temperature and washed again in PBST. Bound antibodies were visualized by incubation in HP-TAB (brown stain; Tiangen Biotech, Beijing); the sections were counterstained with haematoxylin (Beyotime, Shanghai), dehydrated though increasing concentrations of alcohol and mounted with cover slips. The slides were examined using an Olympus Provis microscope (Olympus Optical Co., London) and the images were captured with a Kodak DCS420 camera (Eastman Kodak, New York, USA) and assembled in Photoshop CS6 software (Adobe, San Jose, USA). Approximately 100 follicles from each ovary were examined in sections. Staining density and IOD (integrated optical density) was analysed by image-pro plus 6.0 software (Media Cybernetics, Washington, USA). As we focused on the function of Gas6 in oocytes maturation, only staining in oocytes was analysed.

Cell culture media (CCM)

The base media for *in vitro* maturation (IVM) of oocytes in this study was medium 199 (M-199) (Invitrogen, Grand Island, NY) and Porcine Zygote Medium (PZM)-3 (Yoshioka *et al*., 2002) for embryo culture. M-199 was supplemented with 10% (v/v) porcine follicular fluid, 0.6 mM cysteine, 0.91 mM pyruvate, 10 ng/ml epidermal growth factor, 75 mg/ml kanamycin, and 1 mg/ml insulin (PM). The *in vitro* culture medium for embryo development was PZM-3, which was modified by adding 2.77 mM myo-inositol, 0.34 mM tri-sodium citrate, and 10 µM β-mercaptoethanol (You *et al*., 2012).

Oocyte collection and IVM

Porcine ovaries were obtained from a local abattoir and transported to the laboratory at 29-31°C. Cumulus-oocyte complexes (COCs) were aspirated from superficial follicles (3-6mm in diameter) in the ovaries using an 18-gauge needle and a 10-ml syringe. Only COCs with uniform cytoplasm and at least three layers of compact cumulus cells were selected for maturation and further studies. COCs were placed into Petri dishes containing 150µL of IVM medium with 10 IU/ml equine chorionic gonadotropin (eCG) and 10 IU/ml human chorionic gonadotropin (hCG) (Intervet International BV, Boxmeer, Holland). The COCs were cultured at 39 °Cin 5% CO_2 at maximum humidity. After 22 h in the maturation culture, the COCs were washed three times in fresh hormone-free IVM medium, and then

cultured in hormone-free IVM medium for an additional 20 hs for parthenogenetic activation (PA). The oocytes *in vitro* matured to GVBD (1-12 h), MI (12-24 h), post-MI (24-36 h) and MII (36-42 h) were collected to check the expression of *Gas6* mRNA and protein based on the previous study in our lab (Shuai, 2015).

After 42 h of IVM, cumulus-cell-free oocytes were incubated for 15 min in PBS containing 2 μg/mL Hoechst 33342. Following incubation, the oocytes were washed twice with fresh PBS medium. An epifluorescence microscope (TE300; Nikon, Tokyo, Japan) was used to identify the polocyte.

Parthenogenetic activation (PA)

Parthenogenetic activation was performed according to the previous study (Liu *et al*., 2015). In brief, after IVM, oocytes were denuded of cumulus cells by repeated pipetting with 0.1% hyaluronidase in cell culture medium (CCM) on a warm stage at 39℃. Denuded oocytes were washed 3 times in CCM. Cumulus free oocytes showing first polar body were selected and activated using a cell fusion generator with thee pulses of 80 V/ mm direct current (DC) for 50 msec.

Post-activation treatment and embryo culture

Following electrical activation, the PA embryos were washed three times in fresh *in vitro* culture medium (PZM-3), and 35 μL *in vitro* culture droplets were transferred under mineral oil and cultured at 39°C in 5% CO_2 at maximum humidity for 7 days. Cleavage and blastocyst formation were evaluated on day 2 and 7, with the day of PA designated as day 0. The total blastocyst cell count was obtained using Hoechst 33342 staining under an epifluorescence microscope. After incubation, oocytes were washed twice with fresh PBS medium, aliquoted into 10 μL droplets, and observed for fluorescence using an epifluorescence microscope (TE-300; Nikon) with a UV filter (370 nm). Fluorescent images were recorded and saved in TIFF format.

Immunofluorescence staining

Denuded oocytes and COCs were placed in 4% paraformaldehyde and fixed for 40 min at room temperature. Fixed oocytes were washed three times in PBS for 5 min each and were blocked with 1% BSA for 1 h and incubated with rabbit monoclonal anti-Gas6 antibody (ab136249, 1:250 dilutions; Abcam) at 4 °C overnight. Oocytes incubated with PBS containing 0.05% BSA were used as negative control. After washing, oocytes were incubated with FITC-conjugated anti-rabbit IgG (SA00003-3, 1:200, Proteintech, Wuhan) for 1 h at room temperature, and DNA was counterstained with propidium iodide. All of the experiments were repeated more than three times.

The occurrence of *Gas6* immunofluorescence in pig oocytes was determined by confocal scanning laser microscopy. The analysis of oocyte immunofluorescence by scanning laser confocal microscopy enabled the precise detection of unique distributions of *Gas6* because individual cells could be examined in sections and all portions of the oocytes could be studied in isolation. Oocytes were scanned at the same laser intensity and photomultiplier settings as far as possible. However, in some instances, such as the COCs and GCs, scans were performed at lower signal amplification in order to view cell-specific immunofluorescence in finer detail. For some images, such as the denuded oocytes, higher settings were used to accentuate the fluorescent signal in order to emphasize the differential distribution. Fluorescence unit was calculated and analysed with image-pro plus 6.0 software (Media Cybernetics, Washington, USA).

cDNA synthesis

Total RNA was extracted from COCs, oocytes and embryos (5 oocytes or embryos per tube, 3 repeats) using the RNeasy Micro Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions and residual genomic DNA was removed by using RNase Free DNase (Qiagen) treatment for 15 min (Lee *et al*., 2015). Messenger RNA from GV stage COCs, GVBD stage COCs, MI stage COCs, post-MI stage COCs, MII stage COCs, denuded germinal vesicle stage oocytes, denuded MII stage oocytes (as confirmed by the presence of one polar body), 2-cell stage embryos, 4-cell stage embryos, 8-cell stage embryos and blastocysts were reverse transcribed to cDNA using the SuperScript® II Reverse Transcriptase kit (Invitrogen, Life Technologies), according to the manufacturer's instructions. In brief, the reverse transcription system contains a 20-μL mixture as follows: 8 μL of mRNA, 1 μL of DNase Ⅰ, 1.3 μL of DNase I Buffer,1 μL of EDTA, l μL of dNTP, 2 μL of random primer,4 μL of 5×FS Buffer,0.5 μL of RNase Inhibitor,2 μL of DTT, and 0.3 μL of FS RT (reverse transcriptase).

Gene expression analysis by QRT-PCR

Preceding QRT-PCR amplification, primers for *Gas6* (GenBank: KC526197.1) and 18S were designed using Oligo 6.0 software (Table I). Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Gene transcripts were quantified using SYBR Green supermix (4913914001, Roche) and fluorescence data were acquired using a fluorescence ration PCR instrument (ABI 7500, Applied Biosystems, Foster City, CA, USA). In each run, 1 μL of cDNA was used as the template for

the amplification reaction and 1 µL of ddH_2O was used as negative control. The sample was added to 19 µL of reaction mixture, containing 8 µL of ddH_2O, 10 µL of Faststart Universal SYBR Green Master (ROX), and 0.5 uM aliquots of both forward and reverse primers. The thermal cycling profile started with a 3-min dwell temperature of 50°C, followed by 40 cycles of 30 sec at 94°C, 30 secs at the primer specific annealing temperature (60°C), 30 secs at 72°C, and a final step during which fluorescence was acquired. After 40 cycles, the program continued with a post-dwell of 1 min at 94°C. Finally, a melt curve was generated by temperature increments of 0.1°C starting from 60 to 100°C, with fluorescence acquisition after each step. Each sample was repeated 4 times, and the relative gene expression was calculated using the $2^{-\triangle\triangle Ct}$ method with 18s as the reference gene (Lee *et al*., 2015) and the expression ratio was calculated against Gas6 expression in the GV oocyte.

Table I. Primer sequences used in this study.

Gene	Primer sequences (5' to 3')	Product(bp)
Gas6	F: CTACTCCTGCCTGTGTGACG	188
	R: GGATGTCCTCACAGGTGCTC	
18S	F: GATGGGCGGCGGAAAATTG	107
	R: TCCTCAACACCACATGAGCA	

Western blot analysis

Protein extracts (150 oocytes or 150 embryos per lane, 3 repeats) were separated using 10% SDS-PAGE and transferred onto a 0.2µm nitrocellulose membrane (Pall, New York, US). The membrane was blocked for 1 h in TBST containing 5% non-fat dry milk. The blocked membranes were then incubated with rabbit polyclonal anti-gas6 antibody (ab136249, 1:1000, Abcam) in TBST. After incubation, membranes were incubated with horseradish-peroxidase-conjugated anti-rabbit IgG (SA00003-3, 1:2000, Proteintech, Wuhan) in TBST for 1 h at room temperature. After each step, the membranes were washed several times with TBST, and bound antibody was detected using an enhanced chemiluminescence detection system (Pierce ECL Western Blotting Substrate, Thermo).

Statistical analysis

All of the data we obtained in this study were analyzed using one-way ANOVA followed by Duncan's Multiple Comparison Tests as implemented in the SPSS statistical software package (IBM, Chicago, USA).

RESULTS

Immunohistochemistry (IHC)

The expression of *Gas6* in porcine ovarian tissue was firstly studied by immunohistochemical staining. Approximately 100 follicles from each ovary were examined in sections. Strong immunostaining for *Gas6* was observed in the oocytes, especially the nucleus, from the primary to preovulatory stages, with no staining in the negative control (Fig. 1A). Staining was also observed in zonapellucida and most granulosa layers of follicles, except the primordial follicles (Fig. 1A). The staining density (only staining in oocytes was analysed) was statistical analysed by IPP 6.0 software and results showed that the staining of Gas6 lessened from primary follicles to secondary follicles, while gradually enhanced following the growth of the follicles (Fig. 1B).

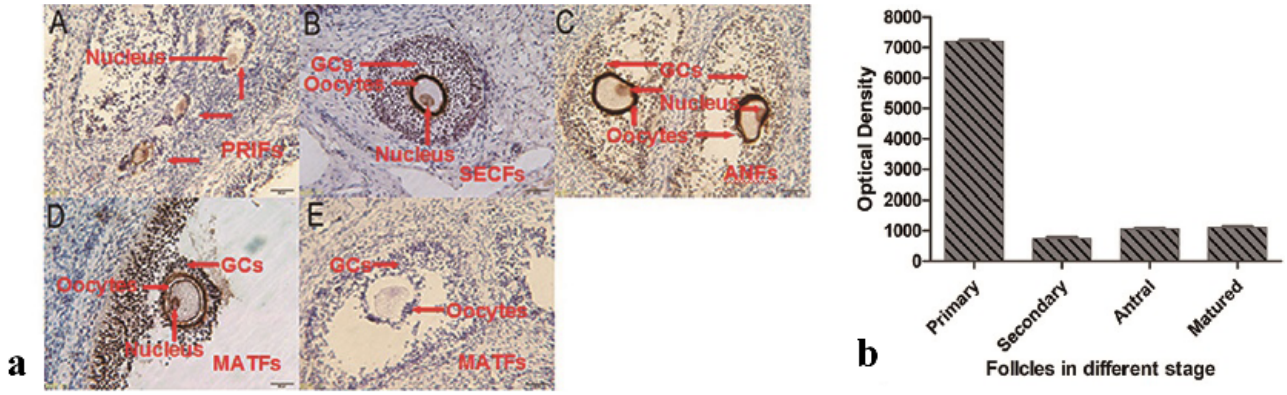

Fig. 1. Immunohistochemistry analysis of Gas6 in the ovaries of porcine. **a)** Gas6 protein were staining brown by immunostaining using anti-Gas6 antibody (A, B, C and D), pre-immune serum instead of anti-Gas6 antibody was used as negative control (E). PRIFs, primary follicles; SECFs, secondary follicles; ANFs, antral follicles; MATFs, mature follicles. Scale bars=100 µm. **b)** The optical density of oocytes in different stage was analyzed by image-pro plus 6.0 software. The results are presented as mean ± SEM. GCs, granule cells.

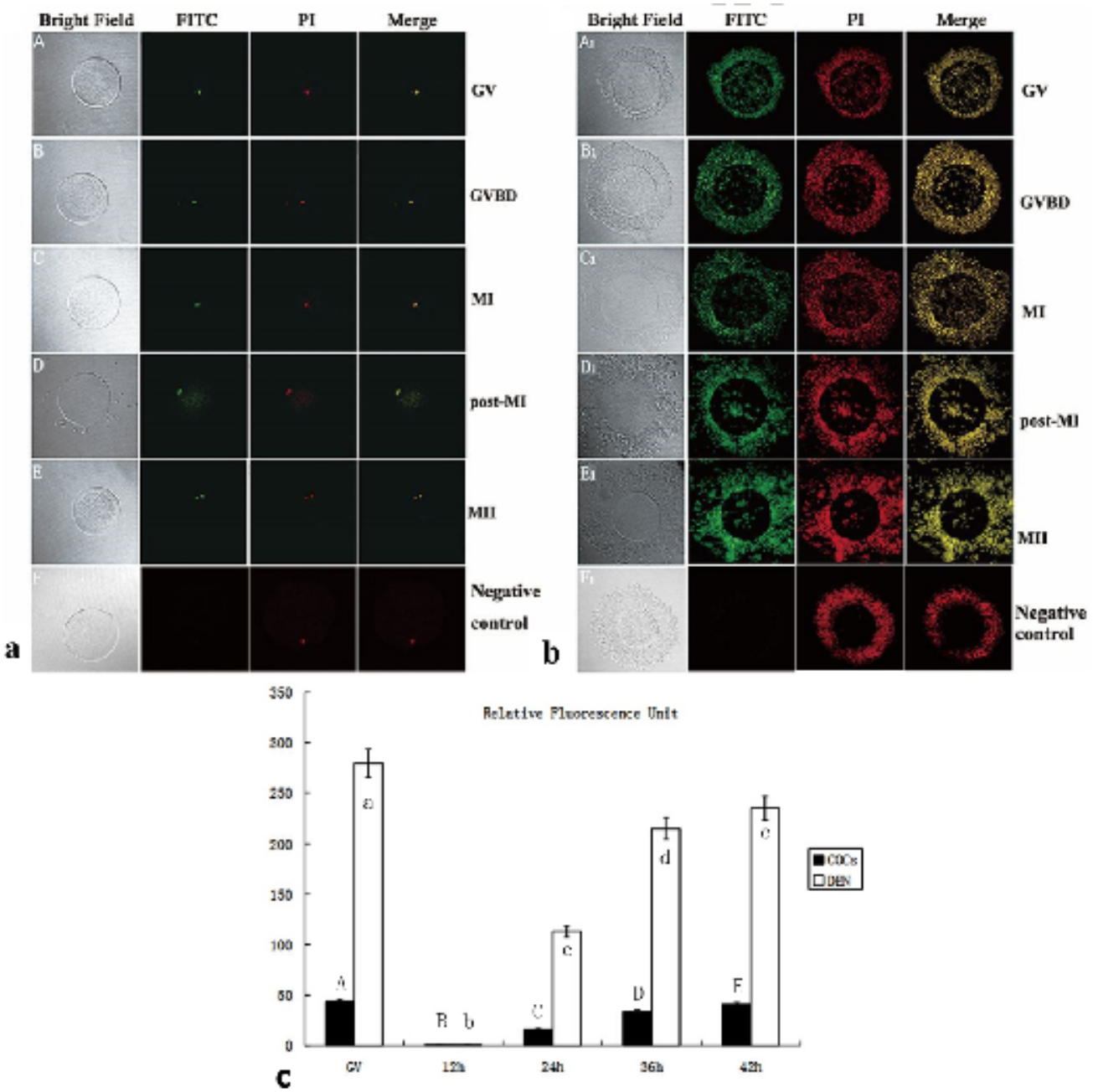

Fig. 2. Immunofluorescence analysis of the Gas6 in oocytes *in vitro* maturing. **a** and **b**, Identification of Gas6 in oocytes and COCs. The green staining indicates a FITC signal and the red stain indicates counterstaining with propidium iodide (PI). C, Relative fluorescence unit during the oocyte maturation was analyzed by image-pro plus 6.0 software. COCs, Cumulus-oocyte complexes. GV, germinal vesicle; GVBD, germinal vesicle broken down; MI, metaphase I; MII, metaphase II; COCs, cumulus-oocyte complexes; DEN, denuded oocytes. The results are presented as mean ± SEM.

Immunofluorescence staining

The expression of *Gas6* in *in vitro* matured oocytes was analysed by using Immunofluorescence staining. Immunofluorescence images of the oocytes stained with anti-Gas6 antibodies are presented in Figure 2. Analysis of oocytes stained with anti-Gas6 antibody demonstrated that *Gas6* was expressed persistently throughout the *in vitro* maturing of the oocytes. No expression was observed in the negative control. Specifically, comparing the immunofluorescence staining of the denuded oocytes and

COCs showed that *Gas6* localized in the nucleus of the oocytes andcumulus cells (Fig. 2A, B), which is consistent with the immunohistochemical localization results in follicles. Further analysis of the relative fluorescent signal demonstrated the expression pattern of the Gas6 protein in the COCs and denuded oocytes, which decreased to the bottom in the first 12 h, and then gradually increasing following the maturation of the oocytes (Fig. 2C).

Gas6 mRNA expression pattern in oocytes and embryos

The expression pattern of *Gas6* mRNA during oocytes maturation and preimplantation embryo development was determined by QRT-PCR. In each reaction, the absence of nonspecific amplification was confirmed by checking the melting curve of the *Gas6* and *18s* gene with a single peak. During oocyte maturation, the expression of *Gas6* decreased gradually in the first 12 h, while increased 5-folds in the following 12 h and remained in the MII stage (Fig. 3A), which is in close agreement with the immunofluorescence results above. In the pre-implanted embryos, the *Gas6* transcript was rapidly decreased to an undetectable level from MII oocytes to 2-cell and 4-cell stage, while recovered at 8-cell stage embryos and increased in the following development (Fig. 3B).

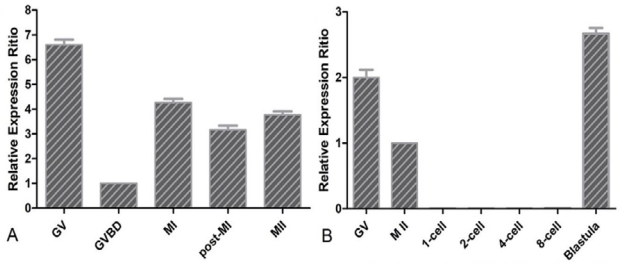

Fig. 3. QRT-PCR analysis of Gas6 in the process of oocytes maturation (A) and embryo development (B). The relative gene expression was calculated using the $2^{-\triangle\triangle Ct}$ method with 18S as the reference gene, and the expression ratio was calculated against Gas6 expression in the GV oocyte. Experiments were repeated 4 times, and data were presented as mean ± SEM.

Western blot analysis

To confirm the expression of *Gas6* protein, we further performed western blot analysis for samples of denuded oocytes, COCs and early embryos. A high level of expression of *Gas6* was observed in COCs matured for 42 h compared to the cumulus-cell-free oocytes and pre-maturation samples. No specific hybridization bands were found in the protein samples of embryos until the blastocyst stage. These data suggest that the expression of *Gas6* was constitutively throughout oocyte maturation and silenced in the early development of embryo, which is consistent with the results of QRT-PCR. The peak expression also appeared at the blastocyst stage (Fig. 4).

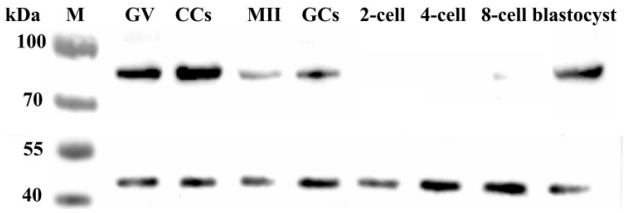

Fig. 4. Western blot analysis of Gas6 expressed in the oocytes and early embryos. GV, Germinal vesicle; CCS, Cumulus oocyte complexes; MII, Metaphase II stage oocytes; GCS, Granulosa cells.

DISCUSSION

First time we reported that the Gas6 is continuously expressed in the process of porcine follicular development and oocyte maturation. The expression of Gas6 was usually decreased in the first 12 h maturation of oocytes and recovered subsequently and was closely related to porcine oocytes maturation and embryo development.

Gas6 is an important participant in cell survival, growth arrest, proliferation and differentiation, and maternal effect gene that is essential for cytoplasmic maturation of oocytes and early embryogenesis. It was suggested that *Gas6* contributes in many aspects for gonad development (Matsubara *et al*., 1996), and numerous studies have indicated that the *Gas6* gene is involved in the regulation of early embryonic development (Hafizi and Dahlbäck, 2006; Kim *et al*., 2011). Cytoplasmic maturation is critical for embryonic development and is directly related to the normal activation of zygote genome, especially for the genome reprogramming of nuclear transfer embryonic. Multiple studies have focused on genes that affect the cytoplasmic maturation, and Gas6 has been discussed as a candidate gene. *Gas6* is expressed in germinal ridge and primordial germ cells and the developing ovary of mice (Keoung-Hwa Kim *et al*., 2008). When it comes to porcine, we found that Gas6 is continuously expressed during oocyte maturation both in the follicle and IVM medium, and the expression were mainly located in the nucleus, cumulus cells and zona pellucida. Further analysis of the express pattern indicated that the expression was reduced before the GVBD stage (in the first 12 h), while increased again in the following maturation and maintained to the MII stage. The expression of *Gas6* is closely related and plays important role in process of oocytes maturation. Supplement of Gas6 protein in the process of oocytes maturation did not significantly affect the polar body rate at MII stage, indicating that Gas6 is not essential for the

progression of nuclear maturation in porcine oocytes. While tracking the embryonic development potential of the oocytes, it was found that the addition of Gas6 protein significantly increased the cleavage rate, blastocyst rate and blastocyst cell number. Therefore, we believe that *Gas6* improves the embryonic development potential of the oocytes by promoting the cytoplasmic maturation during the *in vitro* maturation.

The expression of *Gas6* was located in the nucleus of the oocytes, while regulated the cytoplasm maturation of the oocytes becomes an interesting problem. Analysis suggested that, the cytoplasmic maturation begins mainly after GVBD, at which time the typical components of the nucleus, such as nuclear membrane and nucleolus dissolved, breaking the physical separation between the nucleus and the cytoplasm. Meanwhile, most of the organelles are located in the middle of the oocytes around the nucleus and facilitated the interaction between the nucleus and the cytoplasm. It is well known that oocyte maturation is regulated mainly by the activities of MPF and MAPK (Palmer and Nebreda, 2000; Kotani and Yamashita, 2002). Previous studies have demonstrated that downregulation of *Gas6* expression dramatically decreased the activities of MPF. Further study showed that oocytes rescued of the MPF activity with *Gas6*-silenced still failed to undergo PN formation despite sperm penetration into the cytoplasm, indicating that *Gas6* is directly involved in the regulation of cytoplasmic maturation, independent of MPF activity (Kim *et al*., 2011). This study systematically investigated the role of *Gas6* in the cytoplasmic maturation of oocytes but did not explain how *Gas6* regulates cytoplasmic maturation of oocytes independent of MPF activity. Combined with our findings, we speculate that *Gas6* acts directly or indirectly as a downstream receptor of MPF which can activate the activity of MPF. The silence of *Gas6* may reduce the downstream receptor and block the MPF pathway, which made the active MPF out of working, and the superfluous MPF activity was degraded by degradation of the cyclin B1 and increasing of the phosphorylation of Tyr15 in $p34^{cdc2}$. Therefore, the supplement of Gas6 protein in the oocytes may improve the downstream receptor of MPF and promote the MPF pathway, and result in better cytoplasmic maturation of the oocytes.

CONCLUSION

We report the expression of *Gas6* in the process of follicular development and *in vitro* maturation porcine of oocyte and analysed the gene regulation mode of *Gas6* in the process of oocyte maturation and embryonic development. It is generally accepted that a complex equilibrium of interactions links oocyte maturation and the culture media, which plays an active role by focusing growth factors, proteases, and protease inhibitors close to reproduction. We speculate that *Gas6* may be one of the key factors involved in the regulation of downstream receptors of MPF and may directly or indirectly affect the MPF pathway. This work provides a reference for further exploration of the regulatory mechanism of *Gas6* in the process of oocyte maturation and early embryonic development.

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Conflict of interest

The authors have declared no conflict of interest.

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