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The dynamic pattern of *PLIN3* in pig oocytes and cumulus cells during *in vitro* maturation

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Summary

Lipid droplets (LDs) are the main energy resource for porcine preimplantation embryonic development. *PLIN3* has been implicated in LD formation and regulation. Therefore, this study aimed to detect the dynamic pattern of *PLIN3* in pig oocytes and cumulus cells (CC) during *in vitro* maturation (IVM), and to determine the relationship between *PLIN3* and LD content. IVM with cumulus-enclosed oocytes (CEO), cumulus-denuded oocytes (DO) and the CCs denuded from the corresponding oocytes (DCC) was performed in porcine follicular fluid (PFF) or PFF-free optimized medium. DO and the DCC were cultured together under the same conditions as described above, while the DO was named DTO and the DCC was named DTCC in this group. Firstly, our results revealed LDs distributed widely in oocytes and CC, while the *PLIN3* protein coated these LDs and spread out ubiquitously in the cytoplasm. Secondly, not only the mRNA level but also at protein level of *PLIN3* in immature naked oocytes (IO) was higher than that in matured CEO, DO and DTO. Although *PLIN3* was expressed at lower levels in CC from immature oocytes (ICC), the protein level of *PLIN3* was comparably higher in the ECC and DCC groups. The triglyceride (TG) content in CEO and DO was significantly less abundant compared with that in IO. Therefore, our results indicated that co-culturing of oocytes and CC might affect *PLIN3* expression levels in CC but not in oocytes. Lipid accumulation in pig oocytes during maturation might be affected by *PLIN3* cross-talk between oocytes and CC.

Keywords: Cumulus cells, Lipid droplet, Oocytes, Pig, *PLIN3*

Introduction

Endogenous lipids are found in the form of lipid droplets (LDs) in mammalian oocytes, in which triglycerides (TGs) are mainly stored and represent an important source of energy during oocyte development (Sturmey & Leese, 2003; Ferguson & Leese, 2006; Sturmey *et al*., 2006). LDs are also known as the temporary storage area and transit depot of some important proteins (Greenberg *et al*., 1991; Brasaemle *et al*., 1997; Straub, 2015). Although investigations into LD metabolism in different cells have been carried out in various studies (Kim *et al*., 2001; Murphy, 2001; Dichlberger *et al*., 2011), very limited information is available for pig oocytes. There are significant differences in common cells such as oocytes floating in the oviduct and uterus, for which their energy is provided mainly by fatty acid (FA) oxidation (Homa *et al*., 1986). Most of these FAs originate from TGs that are stored during *in vitro* maturation (IVM). Therefore, it is very important to investigate accumulation of LDs in oocytes during IVM.

LDs consist of a neutral lipid core that is surrounded by proteins involved in the formation and trafficking of LDs (Greenberg *et al*., 1991; Brasaemle *et al*., 1997; Kim *et al*., 2001). The main proteins known to regulate lipid metabolism are members of the PAT protein family, which include PLIN1–5 [perilipin, adipose differentiation-related protein (ADRP), tail-interacting protein of 47 kDa (TIP47), S3-12, and OXPAT] (Bickel *et al*., 2009). *PLIN3* is expressed in

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almost all tissues. Initially, *PLIN3* was identified as a protein of 47 kDa that bound to the cytoplasmic domain of cation-dependent and cation-independent mannose 6-phosphate receptors (Diaz & Pfeffer, 1998). *PLIN3* plays an important role in regulating lipolysis and TG storage in the adipose tissue (Covington *et al*., 2015), and can influence neutral lipid formation and accumulation significantly by binding and transporting free fatty acids (FFAs) from the cytoplasm to LDs (Wolins *et al*., 2005; Camera *et al*., 2014). Therefore a role for *PLIN3* in oocyte lipolysis regulation may be expected during the maturation process (Prates *et al*., 2014). *PLIN3* is found in nascent LDs when the high lipid level environment in adipocytes and other cell types is challenged (Schweiger & Zechner, 2015). However, limited knowledge of the function of *PLIN3* in oocytes has been apparent to date.

Cumulus cells (CC) can contact the oocyte directly via functional gap junctions through the surrounding zona pellucida (Buccione *et al*., 1990). Oocyte maturation can be impaired if the CCs are lost prematurely (Thompson *et al*., 2007). Cumulus cells can be considered as a barrier between the oocyte cytosol and the follicular fluid (Lolicato *et al*., 2015) and may influence oocytes by influencing the consumption of nutrient storage; it can regulate local FA synthesis and lipolysis to provide energy for maturation (Auclair *et al*., 2013). Several studies have shown that intracellular lipid contents in oocytes after IVM are decreased compared with those in immature naked oocytes (IO) (Ferguson & Leese, 1999; Kim *et al*., 2001; Auclair *et al*., 2013). The expression pattern of *PLIN3* in oocyte and early embryos in cattle suggests that these transcripts might be involved in the process of LD formation and accumulation (Sastre *et al*., 2014). Therefore, it is important to study the expression of *PLIN3* in pig oocytes with or without CC.

Materials and methods

Samples collection and *in vitro* maturation

Ovaries were collected from prepubertal gilts at a local abattoir and were transported to the laboratory in 0.9% (w/v) NaCl at 35°C within 5 h. Briefly, cumulus–oocyte complexes (COCs) were aspirated from 2–6 mm follicles using an 18-gauge needle connected to a 20-ml disposable syringe. COCs with several compact layers CC were selected and washed three times in HEPES-buffered Tissue Culture Medium 199 (TCM-199) plus 0.8-mM L-glutamine and 2% (v/v) cattle serum (CS). Part of the oocyte was stripped off the CC by repeated pipetting in 1 mg/ml hyaluronidase. Completely naked morphologically undamaged oocytes and the CC denuded from the corresponding oocytes (DCC) were chosen. Subsequently, groups of either 60 intact cumulus-enclosed oocytes (CEO) or cumulus-denuded oocytes (DO) and the corresponding DCC were subjected to IVM in 4-well dishes (NUNC, Roskilde, Denmark) with 400 ml TCM-199 supplemented with 10% (v/v) CS, 10% (v/v) porcine follicular fluid (PFF), 0.8-mM L-glutamine, 75 μg/ml potassium penicillin G, 50 μg/ml streptomycin sulfate, 15 IU/ml serum gonadotrophin, and 15 IU/ml hCG, at 38.5°C for 42 to 44 h in a 5% CO_2 and 20% O_2 atmosphere at maximum humidity. In another group, DO and the DCC were cultured together in 4-well dishes (NUNC, Roskilde, Denmark) under the same conditions as above, DO were named DTO and the DCC named DTCC. To evaluate the possible effect of PFF on oocyte maturation, oocytes from the CEO, DO and DTO groups were respectively cultured in 4-well dishes (NUNC, Roskilde, Denmark) under the same *in vitro* maturation situations as described above, but without PFF. The oocytes and CC in each group were collected and their descriptions are listed in Table 1. The oocytes in each group are shown in Fig. 1.

Immunofluorescence

Briefly, oocytes were fixed with 4% paraformaldehyde at room temperature (RT) for 30 min. After thorough washing three times, they were then permeabilized with 0.5% Triton X-100 for 1 h and were incubated in blocking buffer (1% BSA in PBS) at RT for 1 h to reduce the non-specific binding of the secondary antibodies. Samples were then incubated with primary antibodies (1:200 dilution in 1% BSA, overnight, 4°C) against *PLIN3* of rabbit anti-*PLIN3* (NB110–40764, Novus Biologicals, CA, USA). Subsequently, samples were washed three times in phosphate-buffered solution (PBS), and then incubated for 1 h at RT with anti-rabbit secondary antibody (1:100 dilution in 1% BSA). After being washed three times in PBS at RT, LDs were stained with BODIPY for 1 h at 37°C Finally, after being washed three times, samples were stained with 10 μg/ml Hoechst 33342 (Molecular Probes, Eugene, OR, USA) for 10 min. All oocytes were mounted on slides with glycerol and were observed using a scanning microscope (Zeiss LSM 700 META).

RNA isolation and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Firstly, a pool of 60 oocytes and the CC which were stripped from these 60 oocytes were collected in different groups, as described above. Subsequently, RNA were extracted from the oocytes and CC using the Dynabeads® mRNA DIRECT™ Kit (Ambion, Invitrogen Life Technologies) following the manufacturer's protocol. The total extracted RNA was loaded into the cDNA reaction using the PrimeScript® RT Master

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Table 1 Definition of study groups and abbreviations used in this study

Abbreviations	Definition
ADRP	Adipose differentiation-related protein
BSA	Bovine serum albumin
cDNA	DNA complementary to RNA
CC	Cumulus cell
CEO	Mature cumulus-enclosed oocyte
COC	Cumulus–oocyte complex
CS	Cattle serum
CTR	Control
DCC	CC stripped off from immature COCs were cultured independently *in vitro* for 44 h
DO	Mature cumulus-denuded oocytes
DTCC	CC stripped off from immature COCs were cultured together with IO *in vitro* for 44 h
DTO	Mature DO cultured together with CC
ECC	CC stripped off from mature cumulus-enclosed oocytes
FA	Fatty acid
FFA	Free fatty acid
ICC	CC stripped off from immature COCs
IO	Immature naked oocyte
IVM	*In vitro* maturation
LDs	Lipid droplets
OD	Optical density
PBS	Phosphate-buffered solution
PFF	Porcine follicular fluid
qRT-PCR	Quantitative real-time reverse transcription polymerase chain reaction
RT	Room temperature
TBST	Tris-buffered saline containing 0.1% Tween 20
TCM-199	Tissue culture medium 199
TG	Triglyceride
TIP47	Tail-interacting protein of 47 kda

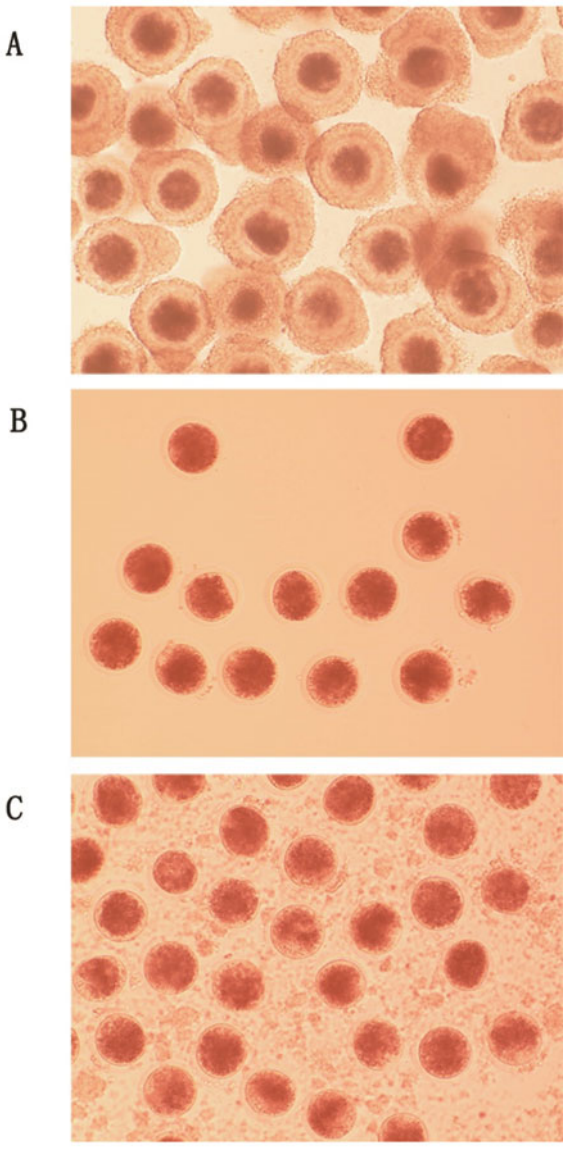

Figure 1 (*A*) Immature naked oocytes (IO) with several layers of cumulus cells (CC). The IO were cultured *in vitro* for 42 h and developed into mature cumulus-enclosed oocytes (CEO). (*B*) Oocytes that were stripped off CC in germinal vesicles (GV) and cultured *in vitro* for 42 h are the mature cumulus-denuded oocytes (DO). (*C*) DO and CC which were stripped from cumulus–oocyte complexes (COCs) in GV were cultured together *in vitro* for 42 h, the DO in this group are the mature DO cultured together with CC (DTO).

Mix (Perfect Real-Time) kit (TaKaRa Bio) following the instructions. We examined target genes (*PLIN3*) in porcine oocytes and CC using PCR. qRT-PCR was performed for the target genes (*PLIN3*) using a LightCycler 480 SYBR Green I Master (Roche, USA) using a standard protocol. The reaction mixture contained 10 μl 2× SYBR Green I master mix, 0.5 μl forward primer (10 μM), 0.5 μl reverse primer (10 μM), 2 μl each sample or standard, and 7 μl ddH2O. The optimized parameters for the thermal cycler were as follows: activation at 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min and 95°C for 30 s. The temperature was then gradually increased (0.5°C/s) to 95°C to generate the melting curve. For each group, qRT-PCR was performed in triplicate, and *18s* ribosomal RNA was used as an endogenous reference gene. As shown in Table 2, primers for gene (*PLIN3*) and reference gene (*18s*) were designed for qPCR using PRIMER 5 software (Primer-E Ltd, Plymouth, UK).

Western blot

A pool of 60 oocytes and the CC, which were stripped from these 60 oocytes, were collected in different groups, with biological replicates in every group. After

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Table 2 qRT-PCR primers of PLIN3 and reference genes in porcine oocytes

Gene name	Gene ID	Forward primer sequence	Reverse primer sequence	Product length (bp)
PLIN3	595103	GATCAGAGCTACTTCGTGCGTC	CTCCCTGCTTGACAGTTTCCATC	163
18s	100861538	CCCACGGAATCGAGAAAGAG	TTGACGGAAGGGCACCA	122

being washed in PBS three times, the CCs and the oocytes were lysed in RIPA buffer containing PMSF (Solarbio), and then these were incubated on ice for 10 min and boiled at 100°C for 10 min, then immediately were transferred to a freezer to be frozen at −20°C until use. The proteins samples were separated on a 12% SDS-PAGE and transferred to a polyvinylidene fluoride membrane. After being blocked with Tris-buffered saline (TBS) containing 0.1% (w/w) Tween 20 (TBST) and 5% (w/v) non-fat dried milk powder for 1.5 h at RT, the membranes were rinsed in TBST, and probed with an anti-*PLIN3* polyclonal antibody (1:500; Novus Biologicals, CA, USA) at 4°C overnight. After washing three times in TBST (10 min each), the membranes were incubated for 1.5 h with secondary anti-rabbit HRP-conjugated antibodies (1:2,000; Cell Signaling Technology, Beverly, MA, USA) at RT. Finally, the membranes were washed three times in TBST and then the specific proteins were visualized using chemiluminescence reagent (Millipore, Billerica, MA, USA).

Measurement of the TGs content of oocytes

TGs were measured using a commercial kit (Ambion, PPLYGEN Technologies) according to the manufacturer's instructions. A group of 90 oocytes was placed in lysate for 10 min at RT and then incubated at 70°C for 10 min. Biological replicates were made for every group. The lysates were centrifuged at low speed at room temperature for 5 min to collect the cells. The supernate was used to measure the TG content of the oocytes. The glycerine standard was diluted to 1000, 500, 250, 125, 62.5 and 31.23 μmol/l. The ratio of R1 and R2 reagents was 4:1. Subsequently, the lysate sample and the glycerine standard were reacted with the reagents mixture for 10 min at 37°C in a 96-well microplate. The optical density (OD) was measured using an ELISA reader at 550 nm wavelength.

Statistics

All analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) with one-way analysis of variance (ANOVA). Differences among treatments were examined using the Duncan multiple range test. Data were expressed as mean ± standard error of the mean (SEM). For all analyses, significance was set at a P-value < 0.05.

Results

LDs and PLIN3 distribution in pig oocytes and CC

As shown in Fig. 2*A*, there are numerous LDs in oocytes during IVM, and the LDs were larger in the IO and CEO groups compared with the DO groups. Most of the LDs in the IO and CEO groups were in the cytoplasm but were not around the nucleus. In the DO group the LDs were distributed evenly in the cytoplasm. In all these three groups of oocytes, *PLIN3* coated the LDs and co-localized with the LDs in the cytoplasm. In addition, the *PLIN3* protein was also observed in the cytoplasm around the nucleus where few LDs were observed. The distribution of LDs and *PLIN3* in CC is shown in Fig. 2*B*. The *PLIN3* was distributed in the cytoplasm of CCs, but the relative location within the LDs was not very clear. Some LDs were observed in the CC cytoplasm.

mRNA level of PLIN3 in pig oocytes and CC

The expression of *PLIN3* in CCs and oocytes in different groups was validated by real-time RT-PCR, as shown in Fig. 3. Firstly, the mRNA level of *PLIN3* in IO was higher than in the matured oocytes (IO vs. CEO, DO and DTO, $P < 0.05$), while *PLIN3* levels remained as that in matured oocytes of the CEO, DO and DTO groups. Secondly, the expression of *PLIN3* in the CCs from the immature oocytes (ICC) was the lowest level ($P < 0.05$), while the expression of *PLIN3* in the CCs from the IO but after *in vitro* culturing (DCC) was the highest level ($P < 0.05$). However, no significant difference was observed in ECC and DTCC groups ($P > 0.05$). Thirdly, the possible effect of PFF on *PLIN3* in oocytes was evaluated, as shown in Fig. 4. No significant difference was observed in the level of *PLIN3* mRNA of oocytes during the *in vitro* maturation with or without PFF.

The protein level of PLIN3 in pig oocytes and CC

As shown in Fig. 5, the level of PLIN3 protein was higher in IO compared with CEO and DO groups. However, the PLIN3 protein level in ECC and DCC groups was higher compared with ICC group, and no significantly difference at the PLIN3 protein level was observed in the DCC compared with ICC and ECC groups.

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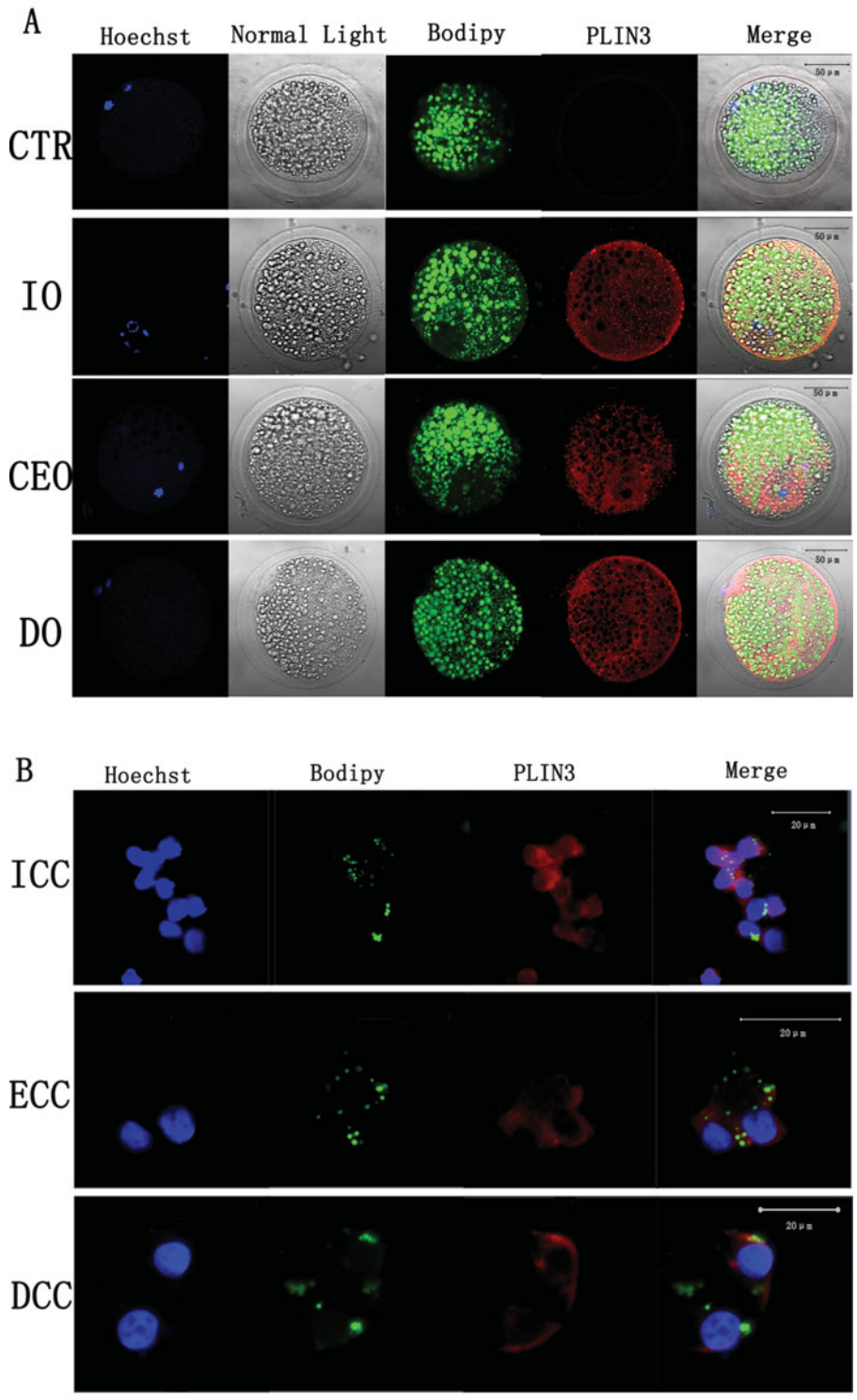

Figure 2 Lipid droplets (LDs) and PLIN3 protein analysis in pig oocytes and cumulus cells. (*A*) Distribution of LDs and PLIN3 protein in oocytes including immature naked oocytes (IO), mature cumulus-enclosed oocytes (CEO) and mature cumulus-denuded oocytes (DO). (*B*) Distribution of LDs and PLIN3 protein in cumulus cell (CCs) including CC stripped off from immature COCs (ICC), CC stripped off from mature cumulus-enclosed oocytes (ECC) and CC stripped off from immature COCs were cultured independently *in vitro* for 44 h (DCC). PLIN3 protein is shown in red. Lipid droplets stained with BODIPY are shown in green. The control (CTR) is the oocyte stained with BODIPY and second antibody for PLIN3. Images were visualized using a confocal laser scanning microscope with a ×40 magnification objective.

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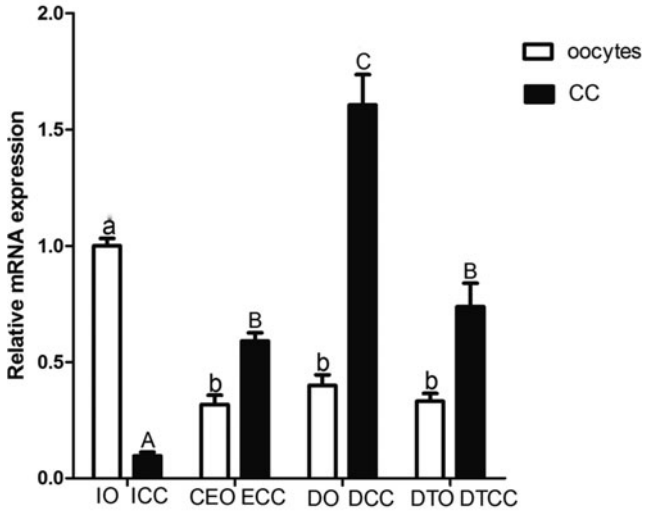

Figure 3 Relative mRNA level of *PLIN3* in pig oocytes [immature naked oocytes (IO), mature cumulus-enclosed oocytes (CEO), mature cumulus-denuded oocytes (DO) and mature DO cultured together with CC (DTO)] and cumulus cells [CC stripped off from immature COCs (ICC), CC stripped off from mature cumulus-enclosed oocytes (ECC), CC stripped off from immature COCs were cultured independently *in vitro* for 44 h (DCC) and CC stripped off from immature COCs were cultured together with IO *in vitro* for 44 h (DTCC)]. *18s* is the internal reference gene. Porcine follicular fluid (PFF) was added to the medium. ^{A,B,C,a,b,c}Different letters indicate significant differences within oocytes or CC, $P < 0.05$.

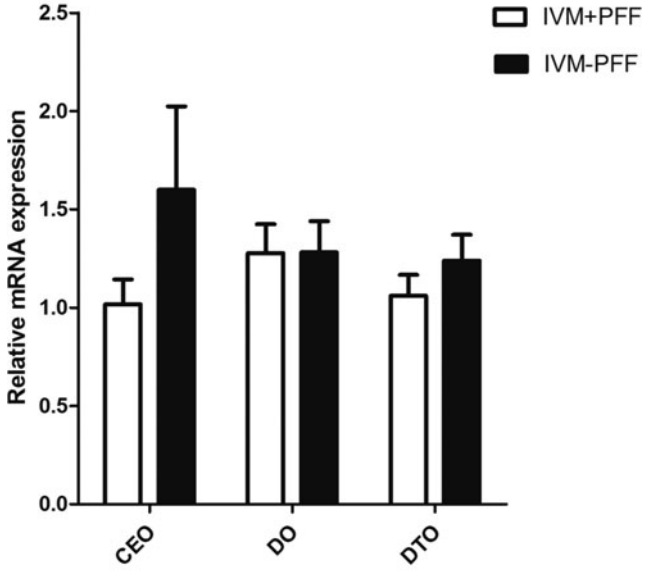

Figure 4 Relative mRNA level of *PLIN3* in pig matured oocytes including mature cumulus-enclosed oocytes (CEO), mature cumulus-denuded oocytes (DO) and mature DO cultured together with CC (DTO). *18s* is the internal reference gene. Oocytes were cultured in the medium with or without added porcine follicular fluid (PFF). (IVM + PFF, the medium for maturation *in vitro* had added PFF added; IVM-PFF, the medium for maturation *in vitro* did not have PFF added.)

TGs content in pig oocytes

As shown in Fig. 6, TGs in the oocytes were measured using a commercial kit. TG content in both CEO and DO was less than that in IO ($P < 0.05$, respectively), and no difference was observed in the CEO and DO groups.

Discussion

In different mammalian species, intracellular lipids are important sources of energy in the oocyte during maturation (McEvoy *et al*., 2000; Sturmey *et al*., 2006). Maturation, whether *in vitro* or *in vivo*, strongly affects oocyte ultrastructure compared with the immature state (Hyttel *et al*., 1986; de Loos *et al*., 1992), while lipids stored in oocytes undergo dramatic changes (Walther & Farese, 2009). Previous studies have demonstrated that, during maturation, numbers of LDs in bovine oocytes increased significantly (Aardema *et al*., 2011) and, in mouse oocytes, LDs underwent structural reorganization and aggregates centrally (Yang *et al*., 2010). Regardless of the LD origin, these cells are constantly changing their shape, volume, and location (Prates *et al*., 2013). In particular, LDs in pig oocytes exhibited a pronounced peripheral distribution pattern following maturation *in vitro* (Sturmey *et al*., 2006). COC composed of the surrounding CCs is a complete functional and dynamic unit playing a pivotal role in oocyte metabolism during maturation (Prates *et al*., 2014). The bidirectional exchanges of nutrients and regulatory molecules between oocyte and contiguous CC are crucial for oocytes competence acquisition and early embryonic development (Sutton *et al*., 2003; Gilchrist & Thompson, 2007; Ouandaogo *et al*., 2011). It is also likely that CCs directly influence oocyte TG and FA deposition, similar to their role in controlling oocyte cholesterol content (Su *et al*., 2008). In support of this, IVM of bovine oocytes in the absence of CCs results in decreased intracellular lipid stores, suggesting that the oocytes have less capacity for lipid storage or may more heavily utilize intracellular lipid stores for energy requirements in the absence of CC-supplied metabolites (Auclair *et al*., 2013). In bovines, CCs metabolize glucose to pyruvate and cystine to provide the oocyte with these energy sources (Geshi *et al*., 2000; Tatemoto *et al*., 2000; Tanghe *et al*., 2002). In the absence of CC, oocyte energy metabolism machinery is altered and likely partially compensates for the lack of nutrients by utilizing the oocyte's own storage, but the nuclear maturation rate is not affected by the CC (Auclair *et al*., 2013). A previous study observed that LD content of bovine oocytes decreased after IVM, more

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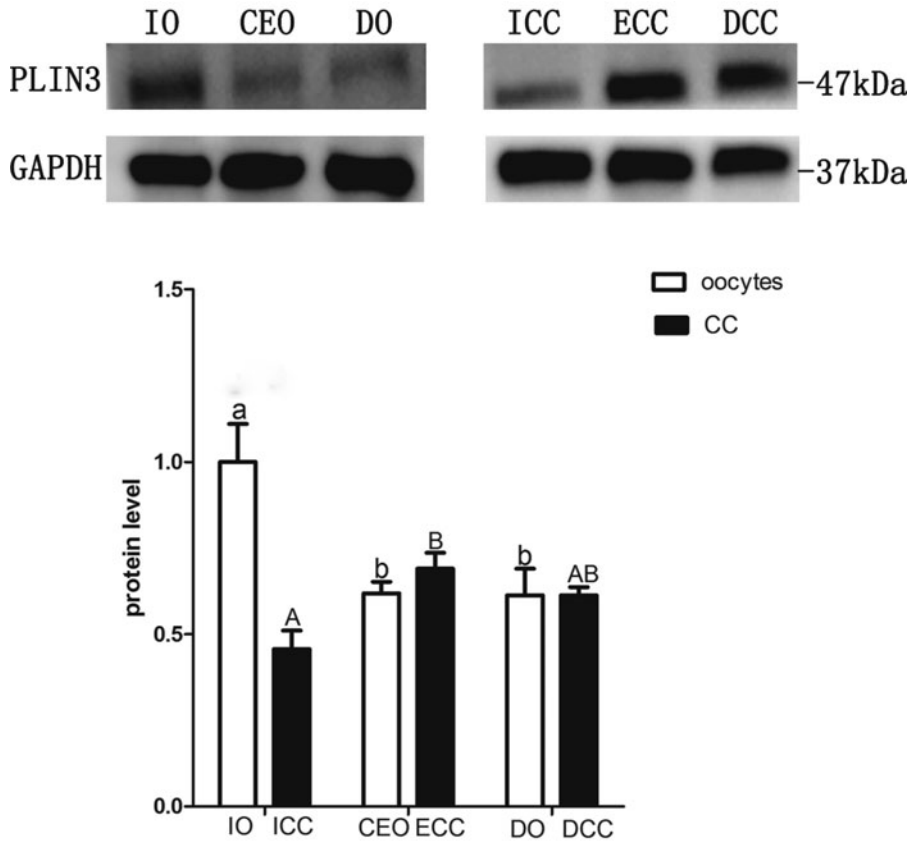

Figure 5 Western blots show the *PLIN3* protein level in oocytes and cumulus cells (CC). Porcine follicular fluid (PFF) was added to the medium. ^{A,B,a,b}Different letters indicate significant differences within oocytes or CC, $P < 0.05$.

being consumed in DO than in CEO groups (Auclair *et al.*, 2013). The decrease of LD abundance during IVM means that they are consumed by the oocytes as an energy source during maturation. However, their consumption rate seems to be influenced by CC. As shown in our study, the morphology of LDs in DO is different to that in CEO and IO groups. The morphological differences suggest that the LD distribution in oocytes was sensitive to the absence of CC, thus influencing oocyte morphological appearance and embryo development.

Pig oocytes have extremely high lipid levels compared with other animals (Loewenstein & Cohen, 1964) and lipids in pig oocytes are mainly in the form of LDs. The main proteins known to regulate LD metabolism are members of the PAT protein family. Previous studies have demonstrated that PLIN1 was originally and consistently found on the surface of the lipid droplet which is limited to adipocytes and steroidogenic cells (Greenberg *et al.*, 1991; Greenberg *et al.*, 1993; Blanchettemackie *et al.*, 1995). Unlike PLIN1 which has a tissue-restricted distribution, PLIN2 is more generalized to many other tissues (Brasaemle *et al.*, 1997). The reported function of PLIN2 was to protect TGs from hydrolysis by cellular lipases in non-adipocyte (Brasaemle *et al.*, 1997; Wolins *et al.*, 2001; Wolins *et al.*, 2005). However, PLIN2 is less competitive than PLIN1 against TG hydrolysis. In addition, although PLIN4 is expressed little, if at all, in brown adipose tissue (Wolins *et al.*, 2003), PLIN5 is highly expressed in brown but not white adipose tissue (Wolins *et al.*, 2006). PLIN3 and PLIN5 can form the emergence of new droplets that are stable cytosolic proteins when the droplet is quiescent (Wolins *et al.*, 2001). In addition, PLIN3 remains active for a longer time and was observed o be soluble in cytosol (Skinner *et al.*, 2009). PLIN3 existing in cytosol can promptly be recruited to be newly synthesized LDs, suggesting its involvement in protection and trafficking of nascent LDs. It has been reported that after several hours supplementation of long FAs for adipocyte culture, a gradient of TGs droplets is established, while PLIN3 was concentrated on the smallest peripheral droplets (Wolins *et al.*, 2006). A previous study has shown that *PLIN3* was downregulated in response to IVM in cattle (Sastre *et al.*, 2014), which is similar to our present study. The decreased level of PLIN3 protein in response to IVM might indicated that the protection of PLIN3 to

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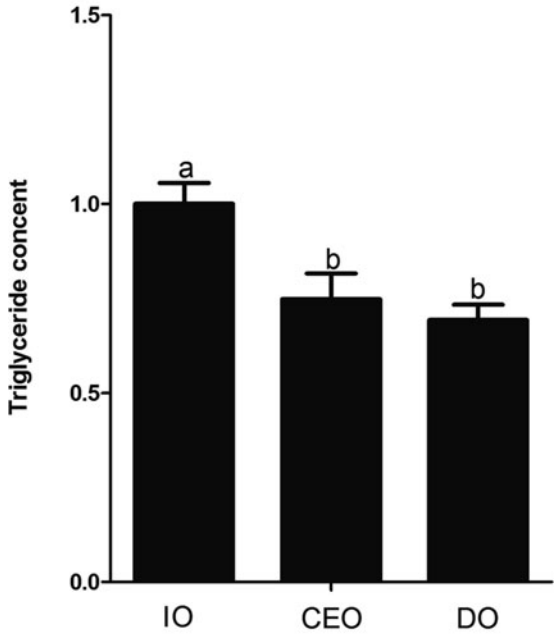

Figure 6 The content of triglycerides (TGs) in oocyte including immature naked oocytes (IO), mature cumulus-enclosed oocytes (CEO) and mature cumulus-denuded oocytes (DO). [a,b]Significant difference is indicated by the different letters with $P < 0.05$.

LDs would be also decreased at lipolysis during oocyte maturation. In DTCC and DTO oocytes, the original gap junctions between cumulus cells and oocytes were broken down when cocultured together to evaluate the environmental connection between oocytes and cumulus cells. Our results showed that co-culturing of oocytes and CC can affect *PLIN3* expression levels in CC but not in oocytes. Another study also showed the *PLIN3* is at high levels in typical LD-associated protein in sebaceous gland cell differentiation which was involved in lipid metabolism. Accumulation and storage of lipids in mammalian cells take place in LDs (Dahlhoff *et al*., 2015). Compared with the expression pattern of *PLIN3* in oocytes, the PLIN3 protein level increased more in ECC and DCC than ICC. The data showed that lipid metabolism in CC might differ from that in oocytes. Lipids in CC may be mainly synthesized during IVM, but may be mainly lipidolytic in oocytes during IVM. CCs from matured COCs or independently growth *in vitro* can access nutrient from the medium. The lower expression level of *PLIN3* in ICC showed that there is a small quantity of lipids used as energy for growth. PFF, reported to be important for oocyte maturation, has been added to the IVM medium (Bijttebier *et al*., 2008). However, no data were available on the effect of PFF on lipid. Therefore, IVM medium with or without PFF was investigated in the present study. Our results showed that PFF addition to the medium had no effect on *PLIN3* expression in oocytes.

The most abundant intracellular lipids stored within oocytes were shown to be the TGs, representing approximately 36 and 46% (w/w) of total FAs in cattle and pig, respectively (Homa *et al*., 1986; McEvoy *et al*., 2000). In HeLa cells, PLIN3 is recruited to LDs and is actively involved in organizing and expanding LDs, while siRNA knock-down of *PLIN3* prevented LD maturation and TG accumulation (Bulankina *et al*., 2009). It is remarkable that the use of lipids as an energy source occurs in oocytes undergoing maturation *in vitro*, hence lipase levels increase significantly during this period (Cetica *et al*., 2002; Ferguson & Leese, 2006). Therefore, the lipids in IOs should be enough to provide energy for oocyte maturation *in vitro*. In our study, the expression of *PLIN3* and TG content in IO were higher than that of matured oocytes. Our results also provided evidence that the TG content in oocytes was decreased in bovine, and in pig oocytes decreased by 13 ng during *in vitro* maturation (Sturmey & Leese, 2003). This fact is consistent with the decrease in TG content during IVM observed in the present study. These results indicate that *PLIN3* expression seems to play a role in the synthesis of TGs and acts in parallel with TG accumulation in oocytes. Further work would be needed to determine the precise mechanism of *PLIN3* in the lipid metabolism of oocytes during IVM.

In this work, the existence and distribution of *PLIN3* and LDs were firstly investigated in pig oocytes and CC during IVM. We noted that the dynamic pattern of *PLIN3* in pig oocytes and CCs during IVM, and the oocytes and CCs, occurred in different groups. Our results might indicate that co-culturing oocytes and CCs can affect *PLIN3* expression levels in CCs but not in oocytes. Furthermore, lipid accumulation in pig oocytes during maturation might be affected by PLIN3 cross-talk between oocytes and their cumulus cells.

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Conflict of interest

The authors have no conflicts of interest to declare.

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