

## Assessment of *zona pellucida* glycoprotein and integrin transcript contents in porcine oocytes

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### SUMMARY

Using reverse transcription and real-time quantitative PCR analysis we evaluated the transcript levels of integrins ( $\alpha$ L,  $\alpha$ M,  $\beta$ 1, and  $\beta$ 6), CD9 and CD18 antigens as well as *zona pellucida* glycoproteins (pZP1, pZP2, pZP3 and pZP3 $\alpha$ ) in oocytes isolated from puberal gilts (n=20) and multiparous sows (n=20). We found significantly ( $p < 0.05$ ) higher transcript contents of  $\alpha$ L,  $\alpha$ M,  $\beta$ 1, and  $\beta$ 6 integrins, CD9 antigen, and pZP2 and pZP3 in puberal gilt oocytes compared to multiparous sow oocytes. Our results suggest that a decrease in the level of oocyte transcripts encoding essential proteins involved in oocyte fertilization may be associated with increased porcine female age. *Reproductive Biology* 2009, **9**, 1: 71-78.

**Key words:** sperm-oocyte interaction molecules, fertilization, pig

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## INTRODUCTION

Fertilization is a highly complex process involving many proteins regulating species-specific gamete recognition, adhesion and fusion [3]. Several lines of evidence suggest the significant role of integrins in sperm-oocyte interaction during fertilization [5, 17]. CD9 and CD18 antigens present in oocyte cell membrane were demonstrated to contribute to oocyte fertilization [8, 12]. Moreover, the porcine *ZPs* genes encode pZP1, pZP2, pZP3 and pZP3 $\alpha$  (pZP4) glycoproteins [8, 11] and pZP3 and pZP2 were found to bind to sperm membrane proteins before and after acrosome reaction, respectively [4]. It has been indicated that the age of female donors plays a significant role in reproductive, developmental and meiotic competence of oocytes [1, 14, 15]. In the current paper, the number of mRNA copies of several integrins ( $\alpha$ L,  $\alpha$ M,  $\beta$ 1,  $\beta$ 6), CD9 and CD18 antigens as well as pZP proteins (pZP1, pZP2, pZP3, pZP3 $\alpha$ ) was evaluated in oocytes isolated from puberal gilts and multiparous sows.

## MATERIALS AND METHODS

This study was conducted on puberal Polish Landrace $\times$ Polish Large White gilts ( $n=20$ , 100 kg bw, 170 days old) and multiparous sows ( $n=20$ , 230 kg bw, 4 years old) exhibiting 3-5 pregnancies with a mean litter of 11 piglets. The experiments were approved by the local Ethics Committee. The gilts were euthanized by electrical shock and exsanguined. The ovaries were collected and transported to the laboratory in a 0.9% NaCl solution at 38°C [7]. Cumulus-oocyte complexes (COCs) were collected from antral follicles (diameter > 5 mm) which are able to reach the metaphase II stage [6]. The COCs were washed two times in modified Dulbecco PBS (mDPBS; Sigma, USA; [7]). Oocytes were treated with 13  $\mu$ M BCB (brilliant cresyl blue; Sigma, USA) diluted in mDPBS at 38.5°C, 5% CO<sub>2</sub> in air for 90 min [10]. The oocytes were examined under an inverted microscope and classified as BCB<sup>+</sup> or BCB<sup>-</sup>. The BCB<sup>+</sup> oocytes had finished their growth phase and were more likely than the BCB<sup>-</sup> oocytes to reach the adequate level of devel-

opmental competence. To separate oocytes from granulosa cells, the BCB<sup>+</sup> COCs were incubated with hyaluronidase (Sigma, USA) for 2 min at 38°C. The cumulus-cell-free oocytes were used for RNA isolation. To ensure that cumulus cells did not contaminate the oocytes, we proved the absence of cytochrome P450 aromatase transcript by reverse transcription (RT) and real-time quantitative PCR (RQ-PCR).

Total RNA was isolated from 20 oocytes per sample (Qiagen GmbH, Germany; [7]). RNA samples were treated with DNase I, quantified and then reverse-transcribed into cDNA. For amplification, 2 µl of total (20 µl) cDNA solution was added to 18 µl of QuantiTect<sup>®</sup> SYBER<sup>®</sup> Green PCR Master Mix (QIAGEN GmbH, Germany) and primers (0.5 µM; tab. 1). Quantitative analysis of cDNA was performed by using the RQ-PCR SYBR Green I system Light Cycler (Roche Diagnostics GmbH, Germany). The quantity of transcript of  $\alpha$ L,  $\alpha$ M,  $\beta$ 1, and  $\beta$ 6 integrins, CD9 and CD18 antigens as well as pZP1, pZP2, pZP3 and pZP3 $\alpha$  in each sample was standardized using the internal standard glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The preparation of standard curves and verification of the RQ-PCR product was previously described [7]. Results were estimated using one-way analysis of variance (ANOVA) followed by Dunn's *post-hoc* test and  $p < 0.05$  was determined as the level of significance.

## RESULTS AND DISCUSSION

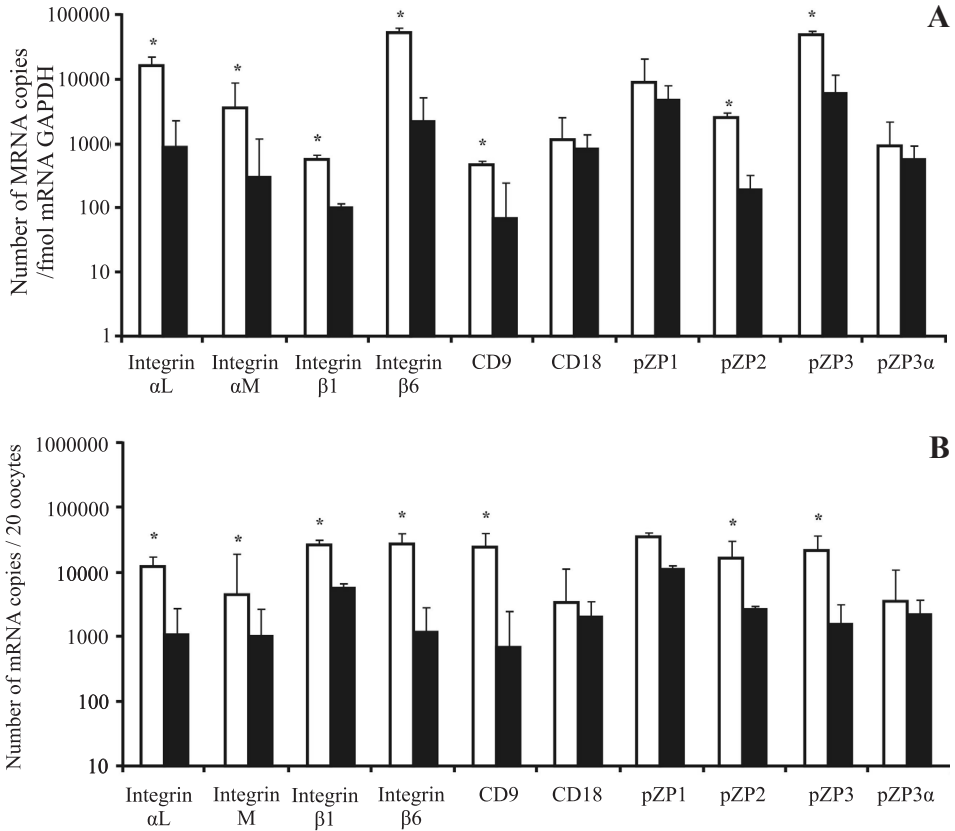
It has been reported that oocyte surface proteins, such as integrins, pZPs, CD9 and CD18 may play an essential role in the process of sperm-oocyte interaction and fusion [4]. Decreased expression of integrins and other molecules or modification of *zona pellucida* proteins resulted in improper interaction between male and female gametes and led to impaired oocyte fertilization [9, 13]. In addition, an application of monoclonal antibodies against the integrin alpha chain and CD9 in mice as well as modification or complete deficiency of ZP proteins in rats, resulted in inhibition of oocyte fertilization [2, 4, 8, 12, 13, 16].

Significantly ( $p < 0.05$ ) higher transcript contents of  $\alpha$ L,  $\alpha$ M,  $\beta$ 1, and  $\beta$ 6 integrins, CD9 antigen, and pZP2 and pZP3 were found in gilt oocytes compared

Table 1. Primer sequences used for RQ-PCR analysis

Transcript	Sequence (5'-3' direction)	Gene accession no.	Exons	Product size (bp)
Integrin $\beta$ 1	ATGCCCTACTTCTGCTCGATGT TCCCTTTGCTACGGTTGGTTA	NM213968	1, 2	133
Integrin $\beta$ 6	TAACGATGGGCTCTGTCA GGCTTGAGTCCCTCTGAA	LOC396672	2, 3	212
Integrin $\alpha$ L	ATCGTGATGAGGCTGTGTC GAGGCTTCCCATGCTGTTTC	DQ474234	1, 2	189
Integrin $\alpha$ M	TGCTGGGGAGGACTCACA CCCAAAGGATGCCCGAAC	U40072	4, 5	139
CD9	GGGCTGGCTCACTTCGCT CCAATGGCAAGGACCCGCA	AF525029	2, 3	193
CD18	GGTACCCCATCGACCTGT TTGACGAAGGAAGCACCG	NM213908	5, 6	173
pZP1	AGAGGAGACAGTGGGAGAC AAGAGGGTCCACCACAGAG	S74651	1, 2	219
pZP2	CCAGGTATTGTCACTTGCC CGCACTCTTTTGGTACAGG	NM213848	2, 3	185
pZP3	GCTGGAGGTTCTTCGTCTG TACGGTGGGTGGCTTTGAG	NM213893	4, 5	113
pZP3 $\alpha$	TGGCTCTGCTTCCCGCTGT GAGTTGCTGTGTCCTGGCT	NM214045	6, 7	136
CYP 19	GTCCTTTTTGGCAGCATTTG CAGAAAATAGCCAGGACCT	U92246	2, 3	102
GAPDH	CTGCACCACCAACTGCTTT TTCTGGGTGGCAGTGATG	AF069649	7, 8	105

to oocytes isolated from multiparous sows (fig. 1A and B). However, we did not observe any significant differences in the numbers of mRNA copies of CD18, pZP1, and pZP3 $\alpha$  between the oocytes of gilts and sows (fig. 1A). The differences in transcript number suggest reduced densities of corresponding proteins



*Figure 1.* Transcript levels of  $\alpha$ L,  $\alpha$ M,  $\beta$ 1, and  $\beta$ 6 integrins, CD9 and CD18 antigens as well as pZP1, pZP2, pZP3 and pZP3 $\alpha$  proteins in oocytes isolated from puberal gilts and multiparous sows. The transcript copies are presented per fmol of GAPDH mRNA (A) or per 20 cells (B). The porcine oocytes (n=20 from each animal) derived from twenty puberal gilts (white bars) and twenty multiparous sows (black bars) were used to isolate RNA. RNA was reverse-transcribed into cDNA and quantitative real-time PCR was used to evaluate the presence and quantity of the transcripts. Each sample was determined in triplicate from each of the same twenty sows and twenty gilts (\*p<0.05)

on oocyte surface in multiparous sows leading to lower fertilization ability. Changes in number and quality of oocytes obtained from gilts of varying ages have been described previously [1, 14]. Particularly the follicular development and meiotic and developmental competence of porcine oocytes were intensively investigated [1, 14]. Brussow et al. [1] observed an increased developmental competence of oocytes isolated from prepuberal and cycling gilts as compared to primiparous sows. Moreover, Ratky et al. [14] also found more uniform oocytes with expanded cumulus from prepuberal and puberal gilts than in multiparous sows. They also suggested the presence of a higher percentage of matured oocytes from prepuberal and puberal gilts than from multiparous sows [14]. Furthermore, it has been reported that previous lactation and nutrition quality were associated with reduced reproductive potential and fusibility of oocytes isolated from multiparous sows [18].

Our results suggest that lower contents of oocyte transcripts encoding essential molecules involved in oocyte fertilization can be linked to porcine age. However, we need to conduct more experiments to establish an association between levels of the investigated transcripts in oocytes and their fertilization ability in puberal gilts and multiparous sows.

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