

The morphology of porcine oocytes is associated with *zona pellucida* glycoprotein transcript contents

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SUMMARY

We hypothesized that oocyte morphology may be associated with the accumulation of specific mRNAs encoding proteins responsible for the gamete fertilization ability. Therefore, the aim of the study was to evaluate the transcript levels of porcine *zona pellucida* (pZP1, pZP2, pZP3 and pZP4) glycoproteins in oocytes classified by a four-grade morphological scale (I-IV) accounting for either a homogeneous cytoplasm and a complete cumulus oophorus (grade I) or a heterogenous cytoplasm and decreased number of cumulus layers in the other grades (II, III and IV). We observed a significant increase of all investigated pZP glycoprotein mRNAs in grade I oocytes as compared to other grades ($p < 0.05$). Our observations suggest that porcine oocyte morphology is associated with pZP transcript contents

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and may be related to an increased fertilization ability of higher quality oocytes. *Reproductive Biology* 2009 **9** 1: 79-85.

Key words: oocyte morphology, ZP glycoprotein, pig

INTRODUCTION

The factors affecting the quality and developmental potential of embryos include oocyte morphology, the ability of oocytes to resume meiosis, maturation competence, fertilization ability, sperm-oocyte interaction, and specific culture conditions during *in vitro* maturation (IVM) and *in vitro* fertilization (IVF; [4, 7, 13]). The proteins involved in the process of gamete fusion include *zona pellucida* glycoproteins (ZPs; [1, 5, 14]). The porcine *ZP* genes encode pZP1, pZP2, pZP3 and pZP3 α (pZP4) glycoproteins, among which pZP3 and pZP2 bind to the sperm cell membrane proteins before and after the acrosome reaction, respectively [3, 15]. The expression of the ZP glycoproteins determines the fertilization ability of the oocyte and the formation of zygote [1, 5, 14]. The morphological quality of the oocyte may be one of the factors affecting its developmental competence and ability to reach the blastocyst stage. However, the morphological oocyte analysis is inadequate to determine a gamet's developmental competence. Therefore, the aim of this study was to analyze the association between oocyte morphology and pZPs transcript contents in porcine oocytes collected from the ovaries of puberal gilts.

MATERIALS AND METHODS

The study was performed on crossbred Landrace puberal gilts (n=30, 95-120 kg bw, 140-180 days old). 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 (38°C). The collected cumulus-oocyte complexes (COCs) were cultured in NunclonTM Δ 4-well dishes in 500 μ l standard tissue

culture medium (TCM 199) with Earle's salts and *L*-glutamine (Gibco BRL Life Technologies; Grand Island, NY, USA; [16]). The wells were covered with a mineral oil overlay and cultured for 48 h (38°C, 5% CO₂ in air). After culture, the matured COCs were selected under a stereoscopic microscope, counted and morphologically evaluated with the use of the four-grade scale [9]. Grade I COCs were characterized by a homogeneous cytoplasm and a complete cumulus oophorus. Grade II COCs displayed a homogeneous cytoplasm and an incomplete but compact cumulus oophorus with more than five cell layers. Grade III COCs exhibited a heterogeneous cytoplasm and a greater-than-three-cell-layer cumulus oophorus. Grade IV oocytes were characterized by a strongly heterogeneous cytoplasm and either a partially or entirely absent cumulus.

The BCB (brilliant cresyl blue) test was used to differentiate immature and mature oocytes. The BCB test can be used for indirect measurement of oocyte growth and selection of competent gametes (BCB⁺) for *in vitro* embryo production [12]. The BCB⁻ oocytes were demonstrated to have a decreased developmental competence, penetrability and fertilization ability [12]. Thus, the BCB⁻ oocytes were not included in this study. In our experiment, the denuded oocytes were treated with 13 μM BCB (Sigma-Aldrich Co.; St. Louis, MO, USA) diluted in modified Dulbecco's phosphate buffered saline (DPBSm) at 38.5°C under 5% CO₂ in air for 90 min [8]. The percentage of the BCB⁺ oocytes after culture was 34%. The BCB⁺ oocytes were incubated (2 min, 38°C) with hyaluronidase (Sigma-Aldrich Co.; St. Louis, MO, USA) to separate cumulus and granulosa cells.

Total RNA was isolated from 40 denuded oocytes (from 30 puberal gilts) among which some were described as grade I (n=10), grade II (n=10), grade III (n=10), and grade IV (n=10) oocytes, using an RNeasy mini column Qiagen GmbH (Hilden, Germany; [6]). Real-time quantitative polymerase chain reaction (RQ-PCR) was conducted in a LightCycler real-time PCR detection system Roche Diagnostics GmbH, (Mannheim, Germany) using SYBR[®] Green I. The expression levels of specific oocyte mRNAs were calculated in relation to GAPDH, β-actin and 18S rRNA. Gene expressions were compared between grade I oocytes and each of the other oocyte grades (II, III, and IV) using Student's t test.

RESULTS AND DISCUSSION

It was recently demonstrated that grade I oocytes with a homogeneous ooplasm and compact cumulus cells are considered to be healthy and possesses an increased developmental potential and fertilization ability [9]. Therefore, we compared other grades of oocytes (II, III and IV) to grade I oocytes. These grades II-IV oocytes were described to display decreased developmental and fertilization potentials related to several morphology disturbances [9]. We observed a 2- and 4-fold increase in the pZP1 transcript level in grade I oocytes as compared to the grades III and IV oocytes, respectively ($p < 0.05$, fig. 1). A difference between grade I and grade II oocytes was not found ($p = 0.108$). We also noted higher pZP2 and pZP3 mRNA levels in grade I oocytes as compared to the

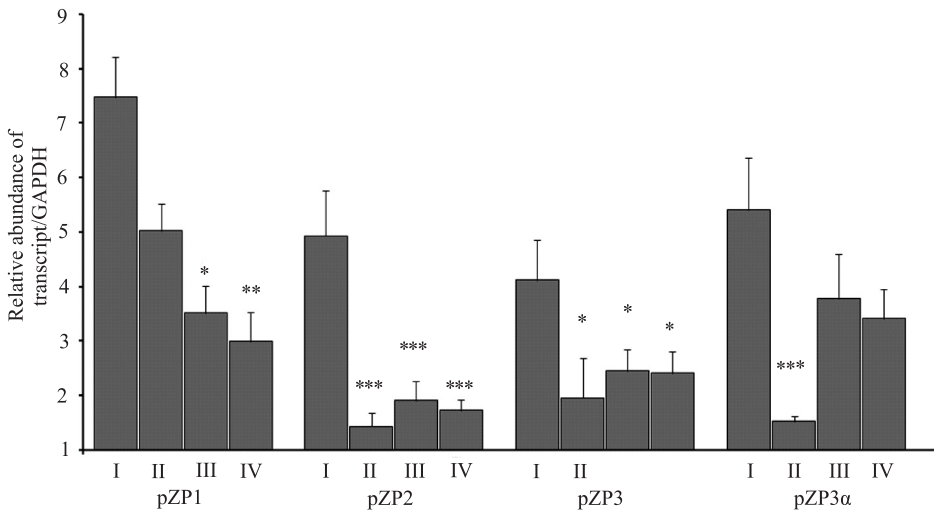


Figure 1. Transcript levels (mean \pm SEM) of porcine zona pellucida (pZP1, pZP2, pZP3 and pZP3 α) glycoproteins in morphologically different oocytes isolated from pubertal gilt ovaries. Total RNA was isolated from oocytes (grade I oocytes: $n = 10$; grade II oocytes: $n = 10$; grade III oocytes: $n = 10$; grade IV oocytes: $n = 10$) following *in vitro* maturation, denuding and brilliant cresyl blue testing. The RNA was reverse-transcribed into cDNA and real-time polymerase chain reaction was used to evaluate the pZP1, pZP2, pZP3, pZP3 α transcript levels. Asterisks represent statistical differences between the particular oocyte grade and grade I oocytes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

other grades ($p < 0.05$). In addition, pZP3 α transcript content was significantly higher in grade I oocytes than in grade II oocytes ($p < 0.05$, fig. 1).

Although it has been demonstrated that oocytes with compact cumulus cells and a homogeneous ooplasm are considered to be healthy, the complete characteristics of fully competent oocytes have not been established. An increased level of all pZP transcripts observed in grade I oocytes may reflect an increased fertilization ability of these oocytes. A lower level of pZP transcripts may be responsible for some structural and biochemical changes in the *zona pellucida* that occur during oocyte maturation [2, 10]. Our observations that the morphology of oocytes corresponds with pZP mRNA contents encoding glycoproteins confirm the recent findings that the morphology of the *zona pellucida* differs greatly between oocytes [1, 11]. We suggest that the grade I oocytes with a homogenous cytoplasm and a complete cumulus oophorus have an increased expression of genes of sperm-oocyte interaction molecules which may result in the best viability and fertilization ability of the oocytes.

Table 1. Primer sequences used for RQ-PCR analysis

Transcript	Sequence (5'-3' direction)	Gene accession no.	Exons	Product size (bp)
pZP1	AGAGGAGACAGTGGGAGAC AAGAGGGTCCACCACAGAG	S74651	1, 2	219
pZP2	CCAGGTATTGTCACCTGCC CGCACTCTTTTGGTACAGG	NM213848	2, 3	185
pZP3	GCTGGAGGTTCTTCGTCTG TACGGTGGGTGGCTTTGAG	NM213893	4, 5	113
pZP3 alpha	TGGCTCTGCTTCCGCTGT GAGTTGCTGTGTCCTGGCT	NM214045	6, 7	136
GAPDH	CTGCACCACCAACTGCTT TTCTGGGTGGCAGTGATG	AF069649	7, 8	105
β -actin	GGGAGATCGTGCGGGACAT CGTTGCCGATGGTGATGAC	DQ845171	4, 5	141
18S rRNA	GTGAAACTGCGAATGGCTC CCGTCGGCATGTATTAGCT	AB117609	1, 2	105

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