Expression of Genes Influencing Calcium Oscillation in Pig Oocytes

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Abstract – During fertilization in mammals, the sperm induces an oscillation in the intracellular free calcium (Ca^{2+}) concentration of the oocyte. In the present study we evaluated the expression of four genes believed to be involved in the regulation of Ca^{2+} signaling in pig oocytes.
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Keywords: Calcium oscillation / pig oocytes / gene expression

1. INTRODUCTION

After the release of Ca^{2+} from the endoplasmic reticulum a Ca^{2+} influx is generated across the plasma membrane. The Ca^{2+} influx is the result of an interaction between Orai and STIM (Stromal Interaction Molecule) proteins and leads to a temporary increase in cytoplasmic Ca^{2+} levels. In turn, SERCA (Sarco/Endoplasmic Reticulum Calcium ATPase) pumps on the surface of the endoplasmic reticulum pump Ca^{2+} back into the stores. In addition STX5 (Syntaxin 5) functions as the regulator of another Ca^{2+} release channel in the membrane of the endoplasmic reticulum (Figure 1).

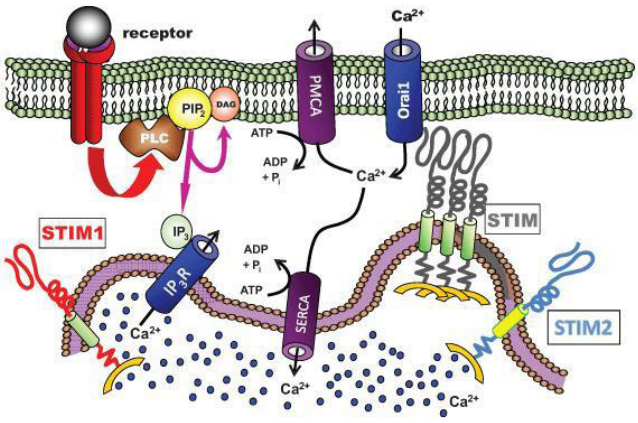

Figure 1: The mechanism of store-operated Ca2+entry

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2. MATERIALS STUDIED AND METHODS

The oocytes were aspirated from follicles of diameter 3-6 mm. Ovaries from the slaughter house were used.

The GV (Germinal Vesicle) stage oocytes (Fig 1B) were collected (93-70-80 oocytes per reactions) right after the aspiration, MI (meiotic arrest I) stage oocytes (92-70-80) after 22 hours of in vitro maturation (IVM) and MII phase oocytes (93-70-80) (Fig.1C) were taken after 44 hours of IVM.

Primers were designed for RT-PCR reaction (Table 1).

Table 1. Primers for the four observed genes

Primer	**Sequence**
ORAI2 F	5'-CACAACCTCAACTCGGTCAA-3'
ORAI2 R	5'-CTGCCAGGAAGAGCAGTGT-3'
SERCA2 F	5'-TCTGACTTTCGTTGGCTGTG-3'
SERCA2 R	5'-GATCATAATGACCCGGATGC-3'
STIM2 F	5'-ACCGGAGTCACAGACAGAAA-3'
STIM2 R	5'-CAATTATGAGGAGGGCGTGT-3'
STX5 F	5'-AGGATTTCGTGAGAGCCAAG-3'
STX5 R	5'-TTTGAAGTCATTGGACATGGAGG-3'

RNA isolation were carried out with the help of Dynabeads (Invitro Co.).

The cDNA retranscription and RT-PCR reactions were carried out with Bio-Rad reagents and Bio-Rad apparatus.

The gene expressions were compared to YWHAG gene (C094522) with Delta Delta Ct method.

For statistical analyses SAS program was used.

3. RESULTS

Figure 1 represents the relative Ct value differences of the four observed genes. The different letters (^{a,b}) marks the significant differences of the gene expressions.

After the statistical analysis significant ($p<0,05$) difference were found in the expression of the two observed genes (Orai2 and syntaxin 5) but there was no difference found in STIM2 and SRECA2 gene expressions. During maturation the expression of Orai2 and STX5 decreases (Figure 2).

The ratio of STIM and Orai proteins is important regarding to the Ca^{2+} influx. Comparing to the STIM level a reduced Orai level is needed for the calcium ion oscillation.

STX5 protein forms a complex with the polycistin-2 protein which inhibits the Ca^{2+} flow on the ER (endoplasmic reticulum) membrane so the level of STX5 is necessary for the sperm induced oscillation.

The SERCA pump is important in all developmental stages of oocytes and it participates in other cell communication processes, too.

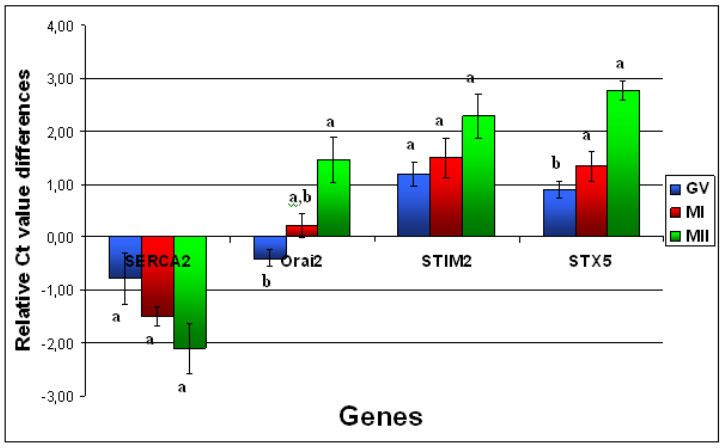

Figure 2: Difference of the relative Ct values of the observed genes in GV, MI and MII stage oocytes (a,b marks the significant differences)