REGULATORY PROCESSES OF PORCINE OOCYTES ACTIVATION AND MATURATION

Regulační procesy aktivace a zrání prasečích oocytů

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Souhrn, klíčová slova

Naše studie se zabývaly regulačními procesy meiotického in vitro zrání prasečích oocytů a mechanismy jejich partenogenetické aktivace. Byly studovány účinky inhibitorů syntézy proteinů na partenogenetickou aktivaci oocytů, úloha vápníku při řízení meotického zrání ooytů, ulrastrukturální lokalizace jeho buněčných depozit a exprese protein kinázy C a syntázy oxidu dusnatého v průběhu meiotického zrání oocytů.

oocyt, prase, meiotické zrání, aktivace, vápník, protein kináza C, syntáza oxidu dusnatého

Summary, keywords

The aims of the studies were to investigate the regulatory processes contributing to pig oocyte maturation in vitro and the mechanisms involved in parthenogenetic activation of pig oocytes. The effect of protein synthesis inhibitors on parthenogenetic oocyte activation, role of calcium in oocyte meiotic maturation, ultrastructural localization of calcium deposits in oocyte, protein kinase C and nitric oxide syntase expression during oocyte maturation were investigated.

oocyt, pig, meiotic maturation, parthenogenetic activation, calcium, protein kinase C, nitric oxide synthase

Introduction – Úvod

The mechanisms regulating the maturation and activation of oocytes and the transition from metaphase II to interphase of the first embryonic cell cycle are not yet fully understood. The aim of the study was to investigate the regulatory processes contributing to pig oocyte maturation in vitro and the mechanisms involved in parthenogenetic activation of pig oocytes.

Methods - Metody

Porcine oocytes were obtained by aspiration from porcine ovaries and then cultivated *in vitro* in modified M199 medium at 39^0C in humidified 95% air 5% CO_2. In order to determine the effect of protein kinase inhibitors, the oocytes were cultured in vitro for 48 h and then exposed to calcium ionophore A23187 and cultured with cycloheximide or 6-dimethyl aminopurine. Proteins were detected by Western Blot using monoclonal antibodies and visualized with ECL detection system. Ultrastructural localization of intracellular calcium deposits was detected by combined oxalate-pyroantimonate method and examined by electron mikroscopy and picture analysis LUCIA.

Results - discussion – Výsledky - diskuse

Effect of protein synthesis inhibitors on parthenogenetic oocyte activation. Calcium ionophore A23187 alone is unable to induce all processes necessary for full-valued activation of the pig oocyte. Combined treatment of *in vitro* matured pig oocytes with calcium a ionophore A 23187 and protein synthesis inhibitors cycloheximide or 6-dimethyl aminopurine increases the activation rate of oocytes (Jílek et al., 2001, 2002).

Role of calcium in meiotic maturation of oocyte. Calcium ions play an important role in the regulation of meiotic maturation in mammalian oocyte. Mycotoxin cyclopiazonic acid (CPA), an inhibitor of calcium-dependent ATPases, mobilises intracellular calcium deposits in growing oocytes through inositol triphosphate receptors. CPA treatment significantly increased the ratio of growing oocytes that were able to overcome the methaphase I block spontaneously to complete their maturation at the methaphase II stage via calmodulin-dependent way (Petr et al., 1999).

Ultrastuctural localization of calcium deposits. Immature, follicle-enclosed pig oocyte contains numerous calcium deposits. Calcium deposits were observed within vacuoles, mitochondria, on the surface of yolk granules as well as in the caryoplasm, but they are absent from the endoplasmic reticulum. After isolation of the oocyte from the follicle, intracellular calcium deposits are immediately depleted. Replenishment of these calcium deposits started in the oocyte during the first 8 h of *in vitro* culture and continued through further culture of the oocytes (Petr et al., 2001).

Protein kinase C expression. Protein kinase C (PKC) is a key enzyme in cell signaling transduction. PKC represents a family of more than 11 phospholipids-dependent ser/thr kinases. PKC δ (from the group of novel PKCs) was only detected in different stages of meiotic maturation of porcine oocytes. These observations suggested that calcium-independent isoform plays a pivotal role in regulation of porcine oocyte maturation.

Nitric oxide synthase expression. Nitric oxide synthase (NOS) generates nitric oxide from L-arginine. Nitric oxide has been shown to be involved in regulation of meiotic maturation and oocyte activation. Three NOS isoforms (eNOS, iNOS and nNOS) have been identified in cells. eNOS and iNOS isoforms were detected in different stages of meiotic maturation of porcine oocytes. nNOS was not detected in any stage of meiotic maturation of oocytes.

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