Abstract

The intracellular sperm injection (ICSI) technique is a very effective tool for the fertilization research. In the newly established laboratory at the Faculty of Science of the Charles University it was necessary to introduce this method and define the early developmental potential of fertilized oocytes. After fertilization oocytes were incubated to the blastocyst stage with a success comparable with other laboratories (17%).

The ubiquitin-proteasome system which plays a major role in a protein degradation within cells is involved in a regulatory mechanism of sperm maturation. It is also responsible for a penetration of a vitelline membrane. In these processes ubiquitin residues are localized extracellulary. High level of sperm ubiquitination correlates with their low quality. Hypotetically it can be expected that the ubiqutination of impaired sperm cells can be used as a negative marker for their recognition and degradation by 26S proteasome complex localized. Experiments in this diploma thesis were designed based on the hypothesis that the executive part of the selective mechanism is the 26S proteasome. Therefore the effect of MG132 peptide inhibition of the 20S proteasome on the pronuclei formation and subsequent early embryonic development after ICSI was studied.

Inhibition of 20S proteasome had no significant effect on initiation of sperm decondenzation. On the other hand, significant difference was observed during formation of pronuclei. In the presence of pronuclei were formed only in 17% and 9% respectively if compared with fertilized oocytes cultivated in a medium without MG132 (58%, 71%). The early embryonic development to the blastocyst stage was improved about 13% (p=0,099) in fertilized oocytes incubated with MG132 for the first 18 hours after fertilization in comparison to the control group. This result suggests that the inhibition of 26S proteasome complex during the period of pronuclei formation could be the reason why even low quality, surface ubiquitinated sperms are not recognized by this complex and therefore able to participate on the formation of viable embryos at least to the blastocyst stage.

Keywords

Domestic pig, ubiquitin, proteasomal complex, MG132, ICSI, fertilization success, early embryonic development