Abstract

The PhD thesis is focused on the effect of porcine sperm cell extracellular ubiquitination on early embryonic development up to the blastocyst stage after ICSI. In addition, it also presents a potential improvement of the technique of *in vitro* fertilization using oocyte incubation with ion channels regulators.

To address these aims, we established an entirely novel methodology for sperm cell sorting using flow cytometry and subsequent cryopreservation. We determined the conditions for successful sperm cell sorting based on extracellular ubiquitination rate providing highly specific selection as well as sufficient numbers of viable sperms for fertilization using the ICSI method. Concerning the following cryopreservation, established methods were optimized to enable freezing of a minimal sperm cell suspension volume with low cell numbers.

The performed experiments showed a direct relationship between the rate of extracellular ubiquitination and the capability of sperms to give rise to a properly developing embryo. Highly ubiquitinated sperm cells were less successful regarding the embryonic development to the blastocyst stage if compared with the lowly ubiquitinated group (6,2 % vs. 16,7 %, $P<0,001$). Interestingly, the rate of extracellular ubiquitination showed no effect on the pronuclear formation and the first cleavages prior to embryonic genome activation.

We hypothesized that there may exist a negative selection mechanism responding to the extracellular sperm ubiquitination. This hypothesis was further supported by the monitoring of the impact of ubiquitin masking using a specific antibody. The success rate of both fertilization and development to the blastocyst stage was significantly improved after the sperm cell incubation with the anti-ubiquitin antibody.

Incubation with K^+ a Ca^{2+} ion channel regulators enabled the suppression of ageing in metaphase II oocytes. This effect was previously observed after the application of NO and H_2S gasotransmitters. Using specific regulators, we were able to inhibit or enhance this protective effect and describe the probable mechanism by which the gasotransmitters function in matured oocytes.

Keywords

Pig, sperms, oocytes, ICSI, ubiquitin, ion channels, gasotransmitters