

The common techniques of enucleation are inexact and time-consuming. Nuclear DNA staining with Hoechst is frequently used, whereas the staining agent is toxic for the oocyte and has negative impact on its further development. Chemical enucleation is a method which facilitates the production of cytoplasts in higher amount and shorter time. Chemical enucleation has a potential use in the field of biotechnology, especially in nuclear transfer methods (therapeutical, reproductive cloning). The chemical enucleation was successfully used on several model animals: mouse, sheep, cattle. The technique is being continually improved on pigs, regarding their similarity to human where it should be consequently applied. It was discovered that same chemicals have different effect on oocytes of each species and therefore it is necessary to examine each species separately. In our study we use porcine oocytes for chemical enucleation. We determined demecolcine in concentration 0,4 $\mu\text{g/ml}$ and acting time 30 minutes as the most suitable for chemical enucleation. Furthermore we used cytochalasin B in concentration 7,5 $\mu\text{g/ml}$ and acting time 10 minutes. The protrusion rate correlates with quality of oocytes. In other experiment we focused on distribution of mitochondria after fusion of normal and enucleation oocyte. We found equal mitochondrial distribution in 13% of fusion oocytes after 12 hours cultivation and unequal distribution that was observed in 87%. We did not observe distinctive difference in distribution of mitochondria, in oocytes after electric activation. We stained cytoskeletal structures in oocytes after demecolcine and cytochalasin B treatment. Microtubules were disrupted and chromosomes were tightly condensed. Fibrillar actin were aggregated mostly in protrusion with chromosomes. 12 hours after oocyte fusion were microtubules practically restored. We used oocyte after treatment by demecolcine and cytochalasin B for SCNT and production blastocyst.