

Biotechnologiczne, genetyczne oraz epigenetyczne aspekty sztucznej aktywacji rekonstruowanych oocytów w klonowaniu somatycznym świń

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Artificial activation of porcine oocytes in somatic cell cloning procedure - biotechnological, genetic and epigenetic aspects

Summary

In the somatic cell cloning of pigs, nuclear transfer-derived oocytes are artificially stimulated with the use one of the three experimental protocols: 1) electrical, chemical or physicochemical delayed activation (i.e., post-activation); 2) simultaneous fusion and electrical activation (SF-EA) or simultaneous electrofusion and physicochemical activation, as well as 3) chemical sequential (combined) electrical and chemical activation. In the first activation protocol, somatic cell nuclei at G0/G1 or G2/M stages are introduced into enucleated Metaphase II oocytes (ooplasts), which are activated 30 minutes to several hours after nuclear transfer. In the second activation protocol, somatic cell nuclei at G1 or G0 stage are introduced into non-activated Metaphase II ooplasts and simultaneously obtained clonal nuclear-cytoplasmic hybrids are activated. In turn, the third activation protocol includes the SF-EA followed by an additional treatment of the reconstituted oocytes with chemical factors, which is initiated after a 1.5-2-h delay. The concentration of calcium cations in the fusion/activation medium affects not only the transition from meiotic to mitotic control of cell cycle of clonal cybrids, but also the degree of ploidy of reconstructed zygotes as a result of both emission of second polar body and formation of pseudopronucleus/pseudopronuclei. The artificial stimulation of reconstituted oocytes also determines the processes of architectural remodeling and epigenetic reprogramming of donor cell nuclei in nuclear-transferred embryos. Moreover, the transcriptional and translational activity of genes (eg *Oct-3/Oct-4*) that are crucial for preimplantation development of porcine cloned embryos is dependent on physicochemical parameters of calcium oscillations induced by activation of clonal nuclear-cytoplasmic hybrids.

Key words:

porcine cloned embryo, clonal cybrid, activation, Ca²⁺ ion, calcium oscillations, *Oct-3/Oct-4* gene expression, NEBD, PCC, NS, pseudopronucleus, donor nuclear chromatin, architectural remodeling, donor genomic DNA, epigenetic reprogramming.

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