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31 protocol optimization and evaluation of maturation promoting factor and mitogen-activated protein kinase activities in bovine cytoplasts obtained by chemical enucleation techniques.

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Abstract

Chemical enucleation using microtubule-depolymerizing drugs is an attractive procedure to simplify the enucleation process in nuclear transfer. The aim of this study was to optimize chemically assisted (CA) and chemically induced (CI) enucleation protocols using metaphase II (MII) and pre-activated bovine oocytes, respectively, and to evaluate the activity of maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK) in cytoplasts generated by these techniques. Initially, we determined the shortest effective treatment of MII and activated oocytes with 0.05εgmL(-1) demecolcine. Bovine oocytes in vitro matured (IVM) for 19h (MII) or activated artificially with 5εM ionomycin (5min) and 10εgmL(-1) cycloheximide (5h) after 26h IVM were treated with demecolcine and samples were collected at 0, 0.25, 0.5, 1.0, 1.5, and 2.0h of treatment. Oocytes were then stained with 10εgmL(-1) Hoechst 33342 and the protrusion or enucleation rates were determined. Next, we evaluated histone H1 and myelin basic protein (MBP) kinases, reflecting MPF and MAPK activities, respectively, in oocytes obtained from these treatments, and for that we used the method described by Kubelka et al. (2000 Biol. Reprod. 62, 292-302). Protrusion and enucleation rates were evaluated by the chi-squared (ϭ(2)) test, and MPF and MAPK activities were submitted to ANOVA and Tukey's test at 5% significance. For MII oocytes, effects of demecolcine were observed as early as 15min, with a significant difference (P<0.05) between control (12/112, 10.7%) and treated (33/114, 28.9%) groups in relation to protrusion rates. The largest number of protrusions was observed after 1.0h of treatment (control: 15/113, 13.3%(a); treated: 45/111, 40.5%(b)). In pre-activated oocytes, effects of demecolcine were also observed after 15min, and in both techniques there were no significant differences between groups treated with demecolcine for 1.0, 1.5, or 2.0h (CA: 40.5 to 52.5% of protrusion; CI: 35.2 to 46.7% of enucleation). In contrast to previous reports in which high concentrations of demecolcine for CA enucleation increased MPF activity, we observed no alterations in the activity of this factor at a demecolcine concentration of 0.05εgmL(-1). Activity of MAPK also did not differ significantly between the control and treated groups throughout evaluation. In the CI technique, a significant difference in MPF activity was observed after 0.5h (70.3%) and 2.0h of activation (39.1%), considering that the activity was 100% at the beginning of the evaluation. However, we observed no significant difference between the control and treated groups at any of the time points studied, as verified for MAPK activity. The exact effect of MPF on the nucleus in mammals is not well established. We believe that the use of low concentrations of demecolcine for short periods is less damaging to embryonic development and, until we have a better understanding of the effect of these kinases on the transferred nucleus, we recommend its use for chemical enucleation protocols in bovine.

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