

## The Use of CDC<sub>2</sub> Kinase Inhibitor as a Potent Activation in Reconstituted Bovine Embryos with Primordial Germ Cells

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The success for nuclear transplantation and ICSI mainly depends on the potential of oocyte activation, which mainly caused to prevent the re-accumulation of maturation promoting factor. This study, therefore, was to compare the effect of combination of ionomycin with an histone kinase inhibitor (dimethylaminopurine, DMAP) or cdc2 kinase inhibitor (sodium pyrophosphate, SPP) on oocyte activation, and to evaluate the efficiency of primordial germ cells (PGCs) as a karyoplast of nuclear transfer in bovine.

After 24-h culture of cumulus-oocyte-complexes, the expanded cumulus cells were removed by vortexing for 2 min in 3% sodium citrate solution. Oocytes suitable for activation and reconstitution (with a first polar body and dense cytoplasm) were selected. For activation treatment, oocytes exposed to 5 M ionomycin for 5 min (group 1), and followed by 1.9 mM DMAP for 3 h (group 2) or followed by 1.9 mM SPP for 3 h (group 3). Differences among groups were analyzed using one-way ANOVA after arc-sine transformation of proportional data.

In Experiment 1, activation effects in the three treatments and the control group, as judged by the extrusion of the second polar body and formation of a pronucleus (PN), were assessed. All three treatments led to a high activation rate (90% to 95%), with no significant differences among the groups, but few in the control activated. However, the extrusion of the second polar body and the rate of PN formation differed remarkably among treatment. In group 1 and 3, ~95% of the oocytes had extruded the second polar body, but one PN had formed in a higher proportion of oocytes in group 3 than in group 1 (90% vs. 5%). In Experiment 2, oocytes in the three treatments and the control group were compared for their rates of cleavage, and of development to the blastocyst stage.

The rates of cleavage and development into blastocysts in group 1 was significantly lower than those of groups 2 and 3 (8% and 0% vs. 51% and 11%,

and 45% and 6%, respectively,  $P < 0.05$ ). Finally, in Experiment 3, reconstituted oocytes being injected a PGC pre-treated with 1% sodium citrate were activated by groups 1, 2 and 3 to compare for their rates of cleavage and development to the blastocyst stage, and the ploidy of blastocysts. The data for this similar to Experiment 2. Chromosome analysis showed that most blastocysts (83%, 5/6) in group 3 were diploid, whereas 30% (3/10) in group 2.

We conclude that use of sodium pyrophosphate, as a cdc2 kinase inhibitor, greatly decreases the incidence of chromosomal abnormalities, and suitable for nuclear transplantation in cattle.

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