

Effect of Sucrose and Polybrene on the Gene Transfer into Porcine Oocytes using Retroviral Vector

Kim Kang Sig, Kim Teoan, Ju Jin Young, Kim Kwang Sung,
Lee Hoon Taek and Chung Kil Saeng

Animal Resource Research Center, Konkuk University, Seoul

In vitro matured porcine oocytes have very small volume of perivitellinespace (PVS). In these respect, the effects of sucrose and polybrene on the efficiency of gene transfer were investigated. As a gene (hGH) transfer vehicle, Vesicular stomatitis virus glycoprotein pseudotyped retroviral vector (VSV-G) was used. Sucrose treatment have no detrimental effect on the rates of cleavage and following development and induced the enlargement of PVS resulting the efficient introduction of retroviral vector stocks into PVS. Integration rates of retrovirus in 0.5, 1, 2, 3% sucrose treatment group were higher than that of the control group (39.3, 43.3, 35.7, 40.7% vs 8.3%), respectively. In addition, we observed that sucrose pretreatment during injection procedure significantly reduce the frequency of polyspermy. In general, polybrene is a polycation essential for retrovirus transduction. The groups with the addition of 0, 0.5, 5 µg/ml polybrene exhibited a significant beneficial effect on integration rates compared to the rates of the control group (56.5, 50.0, 57.1% vs 34.6%), respectively. But, when the oocytes were co-injected with retrovirus and 50µg/ml polybrene, the rates of cleavage and blastocyst development were 43.3 and 4.6%, respectively. These rates were lower than those of the control group (70.0 and 17.3%). In conclusion, sucrose pretreatment have increased efficiency of retroviral mediated gene transfer in porcine oocytes with no damage on IVF and embryo development. In addition, sucrose pretreatment was beneficial in polyspermy inhibition. Presence of polybrene during microinjection showed a beneficial effect on the gene transfer in porcine oocytes with the low concentration. And these results will provide a useful tool for production of transgenic pigs by retroviral mediated gene transfer.

Key words) *Transgenic porcine, Retrovirus, Sucrose, Polybrene*