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The Use of Bull Round Spermatids for Producing Reconstructed Embryos

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Recently, sperm has been used as a vector to carry exogenous genes for the production of transgenic animals. However, the success in cattle is low, due to deficiencies in oocyte activation and sperm decondensation caused by high disulphide bond (S=S) content in mature sperm. This study was carried out to develop an effective method for producing transgenic animals with round spermatids (RS). Two methods of embryo production - electric fusion (EC) or intracytoplasmic injection (IC) and three activation treatments were compared. RS were isolated from bull testes by Percoll density gradients (20, 35, 40, 45 and 90%). Fusion between ooplast and RS was performed with a single DC electric pulse (1.0 KV/cm, 45 sec) in 0.28 M mannitol solution supplemented with 100 M CaCl2 and 100 M $MgCl_2$. MII oocytes were divided into three activation groups for EC and IC methods, respectively. In Group 1, oocytes were activated with ionomycin (5 M, 5 min) prior to EC/IC. In Group 2, oocytes were activated with ionomycin (5 M, 5 min) before EC/IC. In Group 3, oocytes were activated twice with ionomycin, before and after EC/IC. All eggs were then incubated in cycloheximide (10 g/mL) for 5 h and cultured in CR1aa medium to day 8. The rates of cleavage and blastocyst development were evaluated on day 2 and 8, respectively. In IC, cleavage rates were significantly higher ($P<0.05$) in Group 3 (99/123, 80.5%) compared to Groups 1 and 2 (31/68, 45.6% and 45/70, 64.3%, respectively). Blastocyst development rates were also significantly higher ($P<0.05$) in Group 3 (15/123, 12.2%) compared to Group 2 (1/70, 1.4%), but rates did not differ compared to Group 1 (4/68, 5.9%). Whereas, in EC, the cleavage rates in all three activation treatment groups remained low (~ 43-45%), and no blastocysts developed. These results suggest that intracytoplasmic RS injection combined with repeated ionomycin activation is more efficient than electric fusion for producing developmentally competent embryos. [Spported by High Technology Development Proje

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