P0326

Parthenogenetic Activation of Pig Oocytes Matured *In-Vitro* with Ethanol and Electrical Stimulus

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This study was carried out to investigate the effects of activation agents on parthenogenetic activation of pig oocytes matured *in vitro*. The medium used for oocyte maturation was tissue culture medium (TCM) 199 supplemented with 26.19 mM sodium bicarbonate, 0.9 mM sodium pyruvate, 10 μg/ml insulin, 2 μg/ml vitamin B_{12}, 25 mM Hepes, 10 μg/ml bovine apotransferrin, 150 μM cysteamine, 10 IU/ml PMSG, 10 IU/ml hCG, 10 ng/ml EGF, 0.4% BSA, 75 μg/ml sodium penicillin G, 50 μg/ml streptomycin sulfate and 10% pFF. After about 22 h of culture, oocytes were cultured without cysteamine and hormones for 22 h at 38.5℃, 5% CO_2 in air. Cumulus-free oocytes involving first polar body were activated by exposure to various concentrations of ethanol and exposure time of ethanol in Hepes-buffered NCSU23 medium. Also, oocytes were activated by electric pulse alone or combination with ethanol. For electrical activation, oocytes were rinsed twice in 0.3 M mannitol solution supplemented with 0.1 mM $CaCl_2$, 0.2 mM $MgCl_2$, 0.5 mM Hepes and 0.01% BSA, and transferred to a chamber consisting of two electrodes 1 mm apart which was overlaid with the same activation solution. Oocytes were activated with a single DC pulse of 1.3 kV/cm for 30 μsec. After activation treatments, oocytes were washed three times with Hepes-buffered NCSU23 medium and were washed twice with NCSU23 culture medium containing 0.4% BSA, and then cultured in 500 μℓ of the same medium for 20 h at 38.5℃, 5% CO_2 in air. The activation rates of oocytes were higher in 6, 7 and 8% ethanol concentrations compared with 0, 5, 9 and 10% ethanol concentrations. Significantly more oocytes (29.3~33.7%) were activated in the exposure for 8, 10, 12 and 15 min than those in the exposure for 0 and 5 min, but there was no difference due to exposure to 8% ethanol for 8 to 15 min. Electric pulse treatment followed by exposure to ethanol significantly improved the rate of oocyte activation (61.9%) compared with that of other 3 treatments. In conclusion, the optimal activation treatment of ethanol exposure alone for the *in-vitro* matured pig oocytes was 8% ethanol for 8 to 15 min. Electric pulse treatment followed by ethanol exposure significantly improved the rate of activation.

Key words) ***Parthenogenetic activation, Pig oocyte, Ethanol, Electrical stimulus***