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Effect of Mammalian Spermatozoa on In Vitro Maturation of Porcine Germinal Vesicle Oocyte in Chemically Defined Medium

Sung-Ryoung Kang and Byung Ki Kim

Department of Biology, Dong-Eui University

We had previously reported that in vitro maturation of porcine germinal vesicle oocyte could be enhanced by co-culture with mammalian spermatozoa, regardless of mammalian species. Its effects on oocytes maturation has, however, not been studied in detail. The present study was performed to test the hypothesis that the membrane of mammalian spermatozoa has the beneficial effect for in vitro maturation of porcine cumulus oocyte complex (COC). COCs were collected by aspiration of 3-5 mm follicles from slaughterhouse ovaries. Spermatozoa were collected from reproductive tract of slaughterhouse pigs. In the first experiment, groups of 10 to 15 COCs were cultured in 100μl drop of TCM 199 alone (control group) or TCM 199 containing 2-3$\times 10^6$ boar spermatozoa/ml treated with 1% Triton X-100 (Triton treat group) or intact spermatozoa. In the second experiment, to evaluate when the maturation-enhancing components of mammalian spermatozoa membrane were acquired, porcine COCs were co-cultured with 2-3¡¿106 boar spermatozoa/ml from testes, caput, corpus and cauda epididymis. All oocytes were co-cultured with spermatozoa at 39° C in atmosphere of 5% CO_2 in air for 24 h. For another 24 h of culture oocytes were re-incubated with TCM 199 alone. After 48 h of culture, oocytes were fixed in acetic alcohol and stained with 1% orcein in 45% acetic acid and examined under phase contrast microscopy for maturation status.

In Experiment 1, in control group and Triton treat group, the average maturation rate (oocytes reaching M-II phase) was 15.1 % and 16.4%, respectively. However, when porcine GV oocytes were cultured in the TCM 199 containing intact spermatozoa, the rates of oocytes reached M-II stage were 44.8%. In Experiment 2, COCs cultured with the intact spermatozoa from various regions of reproductive tract had a significantly ($P<0.01$) higher percentage of M-II stage than those in control group. However, we could not observe that the rates of oocytes reached M-II stage according to region of spermatozoa was significantly different. The results demonstrate that (1) in vitro maturation of porcine oocytes can be enhanced by mammalian spermatozoa, (2) spermatozoa membrane has the component(s) enhancing in vitro maturation, (3) the maturation enhancing component(s) of membrane of spermatozoa acquire in testis, and (4) the biological effect of the component(s) may be conserved during transportation of spermatozoa through epididymis.