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

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Oocytes maturation, characterized by germinal vesicle (GV) breakdown, formation of the first meiotic spindle, expulsion of the first polar body and arrest in metaphase of second meiotic division (MII), occurs in preovulatory follicles in response to the surge of gonadotropin and leads to an ovulated oocyte *in vivo*. However, meiotic resumption *in vitro* occurs spontaneously following removal of cumulus-oocytes complexes (COCs) from the follicle.

We had previously reported that in vitro maturation of porcine GV oocyte could be enhanced by co-culture with mammalian spermatozoa. 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 have the beneficial effects for in vitro maturation of porcine COCs.

COCs were collected by aspiration of 3~5 mm follicles from slaughterhouse ovaries. Spermatozoa were collected from reproductive tracts of slaughterhouse pigs. In the first experiment, groups of 10 to 15 COCs were cultured in 100 $\mu\ell$ drop of TCM 199 alone (control group) or TCM 199 containing $2.5\sim3\times10^6$ boar spermatozoa/ml treated with 1% Triton X-100 (TX group) or intact spermatozoa. In the second experiment, to investigate when the maturation-enhancing components of mammalian spermatozoa membrane are acquired, porcine COCs were co-cultured with $2.5\text{-}3\times10^6$ boar spermatozoa/ml from testes, caput, corpus and cauda epididymis. All oocytes were co-cultured with spermatozoa at 39℃ 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 Ⅰ, in control and TX group, the average maturation rate (oocytes reaching MⅡphase) was 12.1% and 16.3%, respectively. However, when porcine COCs were cultured in the TCM 199 containing intact spermatozoa, the rates of oocytes reached MⅡ stage were significantly ($P<0.01$) higher than those in control group or TX group. In Experiment Ⅱ, COCs cultured with various sources of intact spermatozoa had a significantly ($P<0.01$) higher percentage of MⅡ stage than those in control group or TX group, regardless of spermatozoa source. However, we could not observe that the rates of oocytes reached MⅡ stage according to various source of spermatozoa was significantly different.

The results demonstrate that (1) in vitro maturation of porcine oocytes can be enhanced by mammalian spermatozoa membrane, (2) the maturation enhancing component(s) of spermatozoa membrane acquire in testis, and (3) the biological effect of maturation enhancing component(s) does not lost during transportation of spermatozoa through epididymis.

Key words) ***In vitro Maturation, spermatozoa membrane***