

Effects of Epidermal Growth Factor (EGF) on Meiotic Maturation and Pronuclear Formation of Porcine Oocytes Produced *In Vitro*

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Numerous factors have an effect on the development of pig IVF embryos, and one of the most important might be the lower rate of nuclear maturation and high incidence of polyspermy. The objective of this study was to examine the effect of EGF on meiotic maturation and pronuclear (PN) formation of porcine oocytes.

Pre-pubertal gilt cumulus-oocyte-complexes (COCs) aspirated from 2~6 mm follicles of abattoir ovaries were matured in TCM199 containing 0.1 mg/ml cysteine, 0.5 μ g/ml FSH and LH, and EGF (0, 5, 10, 20, 40 ng/ml) for 22 hr at 39°C in a humidified atmosphere of 5% CO₂ in air. They were then cultured for an additional 22 hr without hormones. In Experiment 1, to examine the nuclear maturation at 44 hr of culture, the expanded cumulus cells were removed by vortexing for 1 min in 3 mg/ml hyaluronidase. The oocytes were fixed in acetic acid: methanol (1:3, v/v) at least for 48 hr and stained with 1% orcein solution for 5 min. Nuclear status was classified as germinal vesicle (GV), germinal vesicle breakdown (GVBD), prophase-metaphase I (PI-MI), and PII-MII under microscope. In Experiment 2, to investigate PN formation, oocytes were fertilized with Percoll-treated freshly ejaculated sperm (1×10^5 cells/ml) in mTBM with 0.3% BSA and 2 mM caffeine for 5 hr, and cultured in NCSU-23 medium with 0.4% BSA. At 6 hr of culture, the embryos were fixed in 3.7% formaldehyde for 48 hr and stained with 10 μ g/ml propidium iodide for 30 min. PN status was classified as no or one PN (unfertilized), 2 PN (normal

fertilized) and ≥ 3 PN (polyspermy). Differences between groups were analyzed using one-way ANOVA after arc-sine transformation of the proportional data. The rates of oocytes that had reached to PII-MII were significantly ($P < 0.05$) higher in all groups added EGF than that of non-treated group (67%), but it did not differ among the all added groups (86%, 85%, 79% and 81%, in 5, 10, 20 and 40 ng/ml EGF, respectively). No differences on the incidence of 2PN were observed in all treated groups (25%, 30%, 33%, 29% and 29%, in 0, 5, 10, 20 and 40 ng/ml EGF, respectively), however, in non-treated group, polyspermy tended to be increased (66% vs. 58%, 54%, 52% and 55%, 0 vs. 5, 10, 20, 40 ng/ml EGF, respectively).

These results suggest that EGF can be effectively used as an additive for enhancing oocyte maturation and reducing the incidence of polyspermy in pig.

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