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Existence or Unexistence of Plasminogen in Porcine Oocytes Frozen-Thawed and Fresh during *In Vitro* Maturation by Open Pulled Straw Method

J. B. Chen, S. J. Sa, H. T. Cheong, B. K. Yang and C. K. Park

College of Animal Resources Science, Kangwon National University

Plasminogen activators (PAs) are serine proteases, known to be secreted by a large number of cell types, that convert plasminogen to plasmin. PAs are classified into two groups on the basis of molecular mass: urokinase-type PA (uPA), which is secreted as an inactive single-chain molecule of 31-54 kDa, and tissue-type PA (tPA), which is secreted in an active form with a molecular mass of around 70 kDa. PAs play roles not only in fibrinolysis but also in various reproductive processes including ovulation and implantation. We examined whether plasminogen activators (PAs) are produced by porcine fresh or frozen-thawed cumulus-oocytes complexes (COCs) and cumulus cell free-oocytes. Cumulus cell-enclosed oocytes were collected from 2~7mm antral follicles and cultured in NCSU-23 medium with 10%(v/v) porcine follicular fluid, 0.57mM L-cystein, 10 IU/㎖ hCG, eCG, 10ng/㎖ EGF with 38.5℃, 5% CO_2 in air for 22~24 hours or 44~48 hours. COCs were frozen by open pulled straw(OPS) method. Cumulus cells were removed by repeated passage-through a fine pipette in maturation medium. PA activities in porcine COCs and denuded-oocytes were quantified using SDS-PAGE, casein-agar zymography, and densitometry. In fresh or frozen-thawed COCs and oocytes for 0 hour cultured, no activity of PAs was detected. However, at 24 hours of culture uPA was detected in COCs and denuded-oocytes. At the frozen-thawed COCs and oocytes cultured for 24 hours, no PAs were observed. After COCs were cultured for 48 hours, tPA, uPA, and tPA-PAI were observed in COCs only. At the frozen-thawed COCs and oocytes cultured for 48 hours, no PAs were observed. This work was supported by grant NO. R01-2003-000-10500-0 from the Basic Reseach Program of the Korea Science & Engineering Foundation.

Key words) *Frozen-thawed oocytes, Pigs, Plasminogen activators, tPA-PA inhibitor*