EFFECT OF CULTURE MEDIUM SERUM-DERIVED UROKINASE-TYPE PLASMINOGEN ACTIVATOR ON THE OOCYTE IN VITRO MATURATION

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BACKGROUND: Bidirectional intercellular communication between oocytes and their surrounding cumulus cells is important for the oocyte maturation. Supplementation of urokinase-type plasminogen activator (uPA) to the oocyte in vitro maturation (IVM) medium caused earlier and more extensive expansion of cumulus cells and oocyte maturation. uPA expression is increased after IVM 16 h onwards. How cumulus cell-expressed or culture medium serum-derived uPA affects cumulus expansion and oocyte maturation, and whether cumulus cell dissociation is associated with uPA activity remain to be determined.
OBJECTIVE: To determine whether intrinsic uPA activity from cumulus-oocyte complex (COC) or exogenous uPA activity from the serum can improve cumulus expansion and oocyte maturation and cumulus dissociation from the oocytes.
MATERIALS AND METHODS: Compact COCs isolated from pregnant mare serum gonadotropin (PMSG)-primed ovaries that contained a germinal vesicle nucleus were cultured in MEMα medium supplemented with 10% fetal bovine serum, follicle-stimulating hormone, and epidermal growth factor (the IVM medium) in a humidified 5% CO_2 atmosphere at 37°C for 16 h for full cumulus expansion and oocyte maturation. Cumulus dissociation was obvious after 20 h of culture. Two uPA specific inhibitor, 4-chlorophenylguanidine hydrochloride (4cgh) and UK122, were added before IVM or 16 h after IVM. COCs ovulated into the oviduct were cultured in MEMα medium supplemented with or without 4cgh for 8 h. siRNA against uPA and a non-targeting negative control siRNA were preincubated with COCs isolated from PMSG-primed ovaries in MEMα medium supplemented with 10 mM milrinone for 24 h and then were transferred to IVM medium.
RESULTS: Cumulus expansion and oocyte maturation were does-dependently inhibited by the two uPA specific inhibitors, 4cgh and UK122. The inhibition of cumulus expansion by 4cgh was first detected at 8 h after IVM. Increasing medium serum levels reversed the suppression of 4cgh in a dose dependent manner. Down-regulation of uPA expression in cumulus cells using siRNA showed no effects on cumulus expansion and the subsequent oocyte maturation. Cumulus cells surrounding the oocyte were disassembled after IVM for 22 h; however, the dissociation was inhibited by adding 4cgh at 16 h after IVM. Similarly, the COC size largely become smaller 8 h after ovulation compared to the size just at ovulation, but the shrinkage was remarkably reduced by the addition of 4cgh.
CONCLUSIONS: The uPA activity, involved in oocyte in vitro maturation, was primarily contributed by the serum supplementation to the IVM medium, but not derived from the expression of cumulus cells. The uPA activity is also involved in the dissociation of cumulus cells from murine oocytes. The investigation of the components of IVM medium may be helpful for a better understanding of the mechanisms underlying the oocyte in vitro maturation.