P-1 The Effect of Hypoxanthine on Glycosidase Activity and Acrosome Reaction of Frozen-Thawed Spermatozoa in Human

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Objectives: The human epididymis and its secretions actively promote sperm fertilizing capacity and provide protection for spermatozoa against harmful influences. Among epididymal secretions, glycosidases have been recently studied and associated with molecular changes on the sperm surface. On the other hand, hypoxanthine (HX) was identified as components of follicular fluid that brought about meiotic arrest when added to cultures of spontaneously maturing oocytes in numerous species. Although correlations have been reported between the ralationships of HX and oocyte maturation, the actions of HX for spermatozoa ability in vitro has not been elucidated. In this study, effect of HX on in vitro spermatozoa ability and glycosidase activity in frozen-thawed human spermatozoa were examined.

Materials and Methods: The standard medium used was TC-199 medium added 10% fetal bovine serum. The frozen-thawed spermatozoa were washed twice in TC-199 medium added various concentrations (0, 0.1, 0.5, 1 and 5 mM) of HX, and the functional state of the spermatozoa was assessed using the chlo-retracycline fluorescence assay. To determine the effect of HX on in vitro ability of spermatozoa prein-cubated, the sperm washed by centrifugation were incubated with and without HX for 1, 2, 3, 4 and 5 h. Glycosidase activity in spermatozoa and supernatant was measured by the liberation of p-nitrophenyl-derivatized substrates.

Results: When frozen-thawed spermatozoa were washed with different concentrations of HX, there were significantly ($p<0.05$) more acrosome-reacted in medium with 1 mM HX. In spermatozoa incubated for 1~5 h, however, HX treatment inhibited stimulation of acrosome reaction. On the other hand, the α-L-fucosidase, α-D-mannosidase, β-D-galactosidase and N-acetyl-β-D-glucosaminidase could be detected in spermatozoa just thawing and supernatant. Activity of all glycosidases were founded at high levels in spermatozoa treated without that than with HX. When spermatozoa were preincubated with and without HX, the activity of all glycosidase increased gradually with time prolonged of incubation despite of pre-sence of HX.

Conclusions: These results suggested that the presence of HX in frozen-thawed human spermatozoa has inhibitory effects in sperm function and the glycosidases activity in vitro.