A-26: Partial purification and characterization of acid proteinases from porcine ovarian follicular fluid

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Cathepsin D like acid proteinase is suggested to be regulating insulin-like growth factor binding protein-3 which modulates the bioactivity of insulin-like growth factor (IGF). IGF has a stimulatory role on ovarian folliculogenesis. Presence of IGFBP-3 in porcine ovarian follicular fluid and its inhibitory action in folliculogenesis is identified. However studies on acid proteinases of ovary have not been reported. Aim of present study is purification of acid proteinases in crude extract of porcine ovarian follicular fluid (OFF) for characterization.

OFF was subjected to $(NH_4)_2SO_4$, precipitation, ion exchange chromatography, gel filtration and pepstatin inhibition. Maximum recovery of 94.7% acid proteinase activity was observed with 70% $(NH_4,)_2,SO_4$, saturation. Significant amount of binding of acid proteinase was observed to DEAE-52 cellulose at pH 8,8.5,9 and to CM cellulose at pH 4.5 with highest recovery at elution in DEAE-52 cellulose at pH 8.5 Acid proteinase activity of crude extract and both bound and unbound fractions to DEAE-52 cellulose were inhibited 100% by 1μM pepstatin. Bound fraction was eluted at 0.2M NaC1 as a single peak.

These results suggest the presence of 2 types of aspartic proteinases with different charges at pH 8.5 in OFF. Molecular weight of the bound proteinase is estimated to be approximately 40 kd based on results of gel filtration.