CHARACTERIZATION OF ACID PROTEINASES OF PORCINE OVARIES AND ANALYSIS OF THEIR EXPRESSION

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Proteinases involve in a variety of important biological events in the mammalian ovary and play an important role in ovarian folliculogenesis and luteolysis. Insulin-like growth factor (IGF) system is thought to play a key role in ovarian functions. IGF-binding proteins (IGFBPs) present within the ovary can bind with IGFs, and block the biological action of the IGFs. Aspartic proteinase, Cathepsin D has been suggested for playing a role in ovarian functions, by altering the structure of IGFBP-3 and therefore reducing binding ability of IGFBP-3 to IGF. Purification of acid proteinases is necessary to analyze their effect on regulation of IGF function. Therefore one objective of the study was to purify acid proteinases from porcine ovaries.

The ovarian cycle is characterized by repeated pattern of cellular proliferation and differentiation that accompany follicular development as well as the formation and regression of corpus luteum (CL). The corpus luteum is a temporary endocrine gland that secretes progesterone to support pregnancy. Luteolysis is the process by which corpus luteum loses its capacity to synthesize and secrete progesterone. Analysis of expression of acid proteinases in different sizes of ovary, in follicular fluid (FF) from large follicles, in corpus luteum of mid luteal phase and different stages present in luteal phase of the porcine ovarian cycle was the other objective of this study.

Partial purification of acid proteinases was performed according to the method described earlier with some modifications, with DEAE cellulose chromatography, ammonium

sulphate precipitation, and Sephacryl S-200 chromatography. Enzymatic properties of ovarian fluid extract such as optimum pH and thermal stability were analyzed. Proteins obtained at each purification step were analyzed by SDS PAGE. Acid proteinases were purified from ovarian fluid extract, with the specific activity increment of 0.653 U/mg to 3.638 U/mg with a 5.6 fold of purification having 55.66% yield. Optimum pH was 3.00. Purified acid proteinases will be used for future studies in understanding their role in ovary.

Analysis of expression of acid proteinases was performed in ovaries of different sizes by extracting and determining proteinase activity and protein concentration. On average a 48.8 fold increase in the acid protienase activity and almost 8.0 fold increase in the specific activity were observed in large ovaries compared to that of small ovaries. The increase of the acid proteinase activity and the specific activity was significantly high in the larger ovaries ($p<0.000$ for both).

Similarly, expression of acid proteinases in both CL and FF of large follicles was analyzed. Specific activity of acid proteinases in CL was approximately 18 fold higher than that of FF. CL showed a significantly high acid proteinase activity ($p<0.000$) and specific activity ($p<0.000$) compared to activity and specific activity in FF of large follicles.

Statistically significant differences of the acid proteinase activity and specific activity were found among four different stages of luteal phase. Lowest specific activity was seen in corpus haemorrhagicum (CH) which is the first stage of luteal phase. There was approximately 5 fold increase of specific activity towards mid luteal phase compared to that of CH. A further increase of approximately 16 fold was seen in the early regression phase (ECA) compared to that of CH. Hence, highest activity and the specific activity were observed in ECA. Towards the later part of regression there was a significant decline ($p<0.001$) of specific activity compared to that of ECA.

These results strongly suggest that the expression of acid proteinases in the ovary changes at different stages of the ovarian cycle. Hence, acid proteinases are highly likely to play an important role in regulating ovarian functions and most probably the luteal function and having regular cycles. Further studies are necessary to clarify the function of acid proteinases of the luteal phase.