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PRESENCE OF MULTIPLE ASPARTIC PROTEINASES IN PORCINE OVARIAN EXTRACTS

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Tightly regulated proteolytic activity is vital for ovarian functions such as follicular growth, ovulation, luteal formation and regression, and hence to have regular cycles. Even though several studies reveal the importance of proteinases belonging to different classes on ovarian functions, investigations carried out on aspartic proteinases (AP) are scarce. Previously we have shown AP activity at different stages of the ovarian cycle in pigs. The objective of the present study was to determine whether there are multiple APs present in porcine ovaries.

Porcine ovaries (n= 50) from pigs aged 6-8 month age at various stages of the cycle were obtained from John Keells abattoir, JaEla. Ovarian extract (OE) was prepared by homogenization in phosphate buffered saline (pH 7.5), using the whole ovary at 4°C. OE was subjected to partial purification using DEAE-52 anion exchange chromatography at pH 8.5, followed by Sephacryl S-200 gel permeation chromatography. AP specific activity (U/mg) of the OE and the chromatography fractions was determined at pH 3.0 using haemoglobin as the substrate. OE and the fractions with peak AP activity were analyzed using polyacrylamide gel electrophoresis (PAGE). Native conformation of the proteins was maintained until the staining step in order to preserve the AP activity. At the end of electrophoresis, gel was prepared in order to react with haemoglobin at pH 3.0 for 1 h at 37°C. After incubation, gel was stained and destained overnight.

DEAE-52 chromatography resulted in two different AP fractions, DEAE- unbound (DE-U) and bound (DE-B). Sephacryl S-200 chromatography of both DE-U and DE-B fractions resulted in one AP fraction each namely, DE-U-S and DE-B-S respectively, with approximate molecular weight of 40 kDa (based on the results of the molecular weight markers). Specific activity of AP in OE, DE-U, DE-B, DE-U-S and DE-B-S were 1, 38, 4.5, 160 and 25 U/ mg respectively. PAGE separated APs into several bands and were observed as clear bands in a bluish black background after the detection procedure. DE-U and DE-B fractions showed three and two separate bands respectively corresponding to AP activity.

At pH 8.5, DE-B AP fraction seems to bear a net negative charge. DE-U seems to show a comparatively poor affinity to DEAE-52. Based on band positions observed at PAGE, there should be at least three different APs or isoforms with different net charges that are present in porcine ovaries. Further studies are necessary to identify the role of these enzymes.

The results of this study show evidence for the presence of multiple aspartic proteinases in porcine ovaries.