COMPARISON OF ACID PROTEINASE ACTIVITY IN PORCINE OVARIES COLLECTED BEFORE AND AFTER ATTAINING ESTROUS CYCLING

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Introduction

Proteinases are critical for a number of essential biological processes. Precisely regulated proteolysis is indispensable for maintaining tissue remodeling and modulating growth factor actions by regulating their bioavailability. Female reproductive tract is unique, in that rapid and extensive tissue remodeling is required throughout each estrus cycle. In the mammalian ovary, tightly regulated proteolytic remodeling is required for follicular growth, ovulation, as well as for formation and regression of corpora lutea, in order to regulate the cycles. Although several studies have revealed the importance of groups of proteinases belonging to serine, cystein and matrix metallo proteinases on ovarian functions (Miyakosh *et al.*, 2006), investigations on acid proteinases are scarce. Previously we have purified and characterized two acid proteinases from porcine ovaries (Perera *et al.*, 1998). Those acid proteinases were identified as aspartic proteinases. The objective of this study was to analyze whether the total acid proteinase (proteinases with acidic pH optima) activity in the ovaries differs in the absence or presence of cycling features.

Materials and Methods

Ovaries from pigs of 6-8 months old were obtained from a private abattoir. Ovaries were brought to the laboratory on ice, and stored at -80°C until tested. Twenty large ovaries with corpora lutea (CL) and different stages of the follicles (6.53-7.97 g), and twenty small ovaries without CL (1.18- 1.83 g) were selected for the study. Each ovary was weighed separately and ovarian fluid extracted in 0.02 M phosphate buffer saline at pH 7.5 (2 ml/ g of ovary). Extraction was carried out at 4°C, using the method described by Hambata, *et al* (1994). Acid proteinase activity was determined using the method of Moriyama and Takahashi (1978) by using denatured haemoglobin as the substrate at pH 3.0. Two tests (T) and two blanks (B) were carried out for each sample. Test was prepared with extract, buffer (pH 3.0) and the substrate and incubated at 37°C for 45 minutes. Reaction was terminated by adding 5% (w/v) trichloroacetic acid. The only difference in the blank was that the extract was added to the tube after the addition of trichloroacetic acid. The reaction mixture was then centrifuged and the absorbance of the supernatant was measured at 280 nm. An increase in absorbance of 1.0 at 280 nm per hour per ml of sample was defined as one unit (U) of proteolytic activity.

Table 1. Activity and the specific activity of acid proteinases in the ovaries

Ovary type	Weight (g) Mean ± SD	Activity (U/ ml) Mean ± SD	Total Protein (µg/ ml) Mean ± SD	Specific activity (U/ µg) Mean ± SD
Small Ovary	1.50 ± 0.20	6.40 ± 0.32	12.92 ± 2.88	0.53 ± 0.18
Large Ovary	7.14 ± 0.45	65.70 ± 14.54	15.45 ± 0.31	4.26 ± 0.97

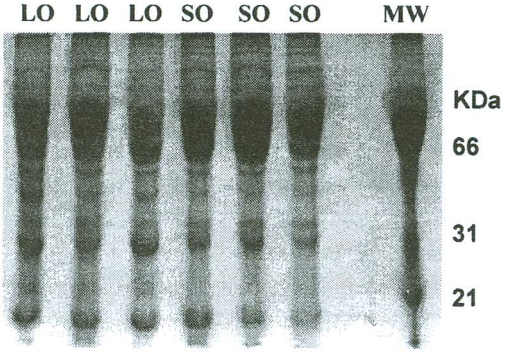

Figure 1. Pattern of protein bands observed with SDS PAGE of ovarian extracts of large (LO) and small (SO) ovaries of pigs

Specific activity was expressed as the activity per mg of total protein (U/mg). Total protein concentration was determined by Bradford method (1976).

SDS PAGE was used to analyse the gross pattern of proteins in the extracts obtained from the two groups of ovaries. Five µl of ovarian extract was loaded in each well. Significance of the differences in activity and specific activity between the two groups were determined using T- Test (Statistical package-Minitab, version-30), Data are presented as mean ± SD.

Results

Table 1 shows a summary of the results obtained with the measurement of proteinases.

On an average a 10 fold increase in the acid proteinase activity and almost 8.0 fold increase in the specific activity were observed in large ovaries compared to those of small ovaries. The increase was significant ($p<0.000$ for both parameters).

Gross pattern and the concentrations of proteins in the two groups observed with SDS PAGE were very similar, even though the proteinase activity was markedly different (Figure 1).

Discussion

Pigs were around peripubertal age at slaughter. Gross anatomical differences were observed in the ovarian structures in the two groups in this study. Large ovaries consisted of various stages of CL and developing follicles. Small ovaries did not consist of CL or large follicles even though small follicles were present. According to the anatomical features, large ovaries are from pigs

that have attained estrous cycling and the small ovaries seem to be from gilts that have not attained cycling.

Based on our observations, we postulate that the acid proteinase activity increases in the ovary, after attaining estrous cycling. This increase may be due to higher expression or activation of acid proteinases in CL or the larger follicles or both.

Conclusion

The activity and the specific activity of acid proteinases were significantly higher in large ovaries compared to that of small non-cycling ovaries. Acid proteinases are likely to play an important role in regulating ovarian cyclic functions. Further studies are in progress to analyze the activity of acid proteinases in different stages of the ovarian cycle.

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