



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2015_IJBcRR_16236
Title of the Manuscript:	Presence of multiple aspartic proteinases in porcine ovary
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Authors show in Figures 3 the electrophoretic analysis of fractions with aspartic proteinase activity partially purified under denaturing conditions. In order to better characterize the proteins partially purified in the unbound and bound fractions Molecular weight standards should be included. Besides, some aspects need to be mention in methods and discussion sections: were fractions run in the same gel? The amount of proteins loaded? In U fractions a major band is visualized, does it correspond to the third band (low molecular weight) observed in B-S fraction? Which one of these bands corresponds to the 40kDa molecule? In figure 4, it seems that U fraction (DEAE unbound fraction) is missing; and it is not clear when authors say "that based on the band positions observed with PAGE, there should be at least three different activities", are they pointing them just on the U-S fraction? it is difficult to detect if the reaction corresponds to one of more than one band, what is the molecular weight of these bands? Discuss the fact the in each fraction tested for aspartic proteinase activity, different activities are visualized. To better demonstrate the aspartic proteinase activity in these fractions, it is recommended the use of specific protease inhibitors such as pepstatin A. The aspartic proteinases detected in the different fractions were not identified but it is well known as it is stated in the discussion section that cathepsin D plays an important role during the luteal regression. To improve the present study, discuss about the molecular weight of	Figure 3-A: Fractions were loaded on two gels with additional samples. MW standards were loaded on each gel. Panel B with silver stain was added with MW markers. In these gels (Fig 3) 25 µl/ lane were loaded with a protein quantity varying from ~2.5 to 60 µg. In all lanes the major band seen was around 66 KDa. Coomassie blue staining was done only to see the extent of impurities. Proteinases identified seem to be minor proteins which are unlikely to see with Coomassie blue (Fig 3-A). After fractionating Pepstatin Sepharose (not included in the original manuscript) we had to do silver staining to visualise the protein bands [Added to the manuscript (Fig 3-B)]. Unbound fraction was diluted as it came out in a large volume during column washing. Therefore we had to concentrate U fraction using ammonium sulphate and dialyse to remove the salt, before loading to the gel. This concentrated fraction was CU. "Based on the band positions observed with PAGE, there should be at least three different APs or isoforms present in porcine ovary"- This



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	cathepsin D and if any of the aspartic proteinase activities detected in the different fractions correlates with this molecular weight. It could also be analysed by western blot if the aspartic proteinases present in the different fractions obtained are recognized by antibodies against cathepsin D.	statement is based on the overall result seen in Figure 4 in which all different fractions were originated from porcine ovary. Based on native PAGE, MW cannot be determined. However, based on gel filtration, both unbound and bound APs had a MW of approximately 40 KDa. MW of human cathepsin D Procathepsin D is 52 kDa. Single chain cathepsin D is 44 kDa. Double chain form has 31 and 14 kDa peptides.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments	For publication, manuscript should be improved attending the present observations.	Improved (highlighted the changes).