



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

General guideline for Peer Review process:

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	After reading this manuscript, I could observe that it is very simple in the used methods. I detected some incorrect statements based on the results, requiring complementary assays. It is my opinion that presented results show only that association of anion exchange chromatography with molecular exclusion chromatography was useful to isolate a protein fraction (40 kDa) with acid proteinases activity that hydrolyze denatured bovine haemoglobin. The manuscript presents many inappropriate appointments that needs to be clearer as title, summary and discussion. There are many examples in the manuscript that need rewrites, corrections and clarifications, as follows: (i) The introduction section is incomplete. It is vague to me why study aspartic proteinases of porcine ovaries. (iii) The used methods are no acceptable to the statement on the "Presence of multiple aspartic proteinases in porcine ovary", as is written in the title. The acid-denatured haemoglobin as substrate for aspartic proteinases is more acceptable when assayed inhibition test with specific inhibitor. The use of specific substrates is an adequate procedure to prove the classification of proteinases, and no were assayed here. (iv) There are many problems with electrophoresis performed: (A) staining with coomassie blue is not sensitive method to reveal purified proteins. The	(i) Introduction was modified (iii) We have used the specific inhibitor Pepstatin A (1 μM) for aspartic proteinases. The detected proteinase activity was 100% inhibited in presence of pepstatin. No inhibition occurred with PMSF and trypsin inhibitor. We could further purify the proteinases using Pepstatin Sepharose. (Added to the revised manuscript.) However specific substrates were not used. (iv) SDS-PAGE with Coomassie blue staining was done only to get some idea about the amount of contaminant proteins. After fractionating using Pepstatin Sepharose we had to do silver staining to detect the proteins. (Added to the revised manuscript.) (v) Figure 2 was replaced by a table. (vi) Please see the feedback for (iii) and (iv). (vii) An amendment was made (viii) Amended.



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	recommendation is silver staining. (B) I have difficulty to understand the presented results of substrate-SDS-AGE, since that no inhibition assay was performed here with specific inhibitor for aspartic protease activity. (v) The figure 2 is better as a table to show yield of obtained fractions. (vi) The results need a careful interpretation since there not was proved that the isolated proteins are aspartic proteinases. The statement "...Results show evidence for the presence of multiple aspartic proteinases in porcine ovary..." is incorrect since that control assays with inhibitors and substrate specifics were not performed. (vii) The discussion is poor, for example the statement ".....Together, these studies indicate that there is an AP-prolactin mediated mechanism governing the luteal regression...", is not related with the presented results and the manuscript not show result to prove this statement. (viii) All legends of the figures need rewriting since are incomplete e and no helper the reader understand the results. The author explains that ovaries from pigs were obtained from a private abattoir, during routine operations. However, there are no information of ethical committee approbation.	Ethical clearance was not sorted as the porcine ovaries were collected during routine Factory operations without any interference with the Factory procedures.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		