



SDI FINAL EVALUATION FORM 1.1

PART 1:

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 Article:	Original Research Article

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
In the new version of the manuscript, I verified that the authors applied some of my previous suggestions. However, some of the recommended experiments were not adequately performed and, for this reason, the data presented in the manuscript are inconsistent and poor to affirm that the isolated proteins are aspartic proteinase. The authors add in the manuscript a new chromatography experiment (Pepstatin Sepharose) as one of purification step and comments about inhibition assays in abstract and methodology. Generally, the affinity chromatography in the first step in a strategy of purification. I have difficulty to find the data of inhibition in the manuscript, or these data were not showed. In addition, the manuscript maintains several problems in the figures as: (i) legends difficulty to unstaring, because of acronyms; (ii) labels of molecular standard proteins.	Based on having a pH optima of the target proteinases at pH 3.00 (No significant activity < pH 2.0 and pH > 5.00), complete inhibition of proteolytic activity in presence of Pepstatin A, absence of any inhibition in presence of PMSF and soy bean trypsin inhibitor and ability of purifying the proteinases using pepstatin sepharose, we believe that there is sufficient data to suggest that the proteinases studied are aspartic proteinases. However, if the reviewer think the evidence is not sufficient we can change "aspartate proteinase" as "acid proteinase" All new changes done in the manuscript are highlighted in blue and changes made in first revision remain highlighted in yellow. Line 168- 170- added following AP activity was inhibited 100% after pre incubating the AP fractions and 1 M formic buffer (pH 3.0) with 1 µM Pepstatin A for 15 min. There was no inhibition of AP activity when pre incubated AP fractions with 1 mM Phenylmethylsulphonyl fluoride and 1 µM Soy bean trypsin inhibitor. Aim of using affinity chromatography after two other chromatography methods was to remove bulk impurities from large volume of crude ovarian extract using larger columns at a lower cost and then to achieve high level of purity with more refined fractionation. Acronyms were removed throughout the manuscript and numbered the lanes of gels. Captions of Table 1, Fig 2 and 3 were changed accordingly.