AN ANALYSIS OF ASPARTIC PEPTIDASES EXPRESSED BY TROPHOBLASTS AND PLACENTA OF EVEN-TOED UNGULATES

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ABSTRACT

The Pregnancy Associated Glycoproteins (PAGs) represent a multigene family of trophoblast expressed proteins, found exclusively in the placenta of even-toed mammals such as ruminants (cattle, sheep), pig, etc. In ruminants, the PAGs can be classified into *ancient* and *modern* PAGs based on their coding sequence. In addition, there are also differences in purported enzymatic activity as well as transcriptional regulation of expression. Many of the modern PAGs have accumulated mutations in and around the catalytic center, and some of those that incurred mutations in the two catalytic aspartates are predicted to be proteolytically inactive. In contrast, most of the ancient PAGs of ruminants and swine, have all the hallmarks of typical aspartic peptidases (APs). From the analysis of cattle genome, we found that there are 18 distinct PAG genes and 14 pseudogenes. Based on our preliminary analysis of the proximal promoter regions [500 base pairs (bp) upstream of the translational start point] of PAG genes, we found that there are pockets of conserved transcription factor binding sites that are different between ancient and modern PAGs. These differences likely influence the observed differences in expression between ancient and modern boPAGs. We gathered evidence by Real-time PCR and global analysis of expressed ESTs that confirm that, boPAG-2 is the most abundant of all boPAGs. We identified boPAG-2 and its closest paralog boPAG-12, as well as poPAG-2 the ancient PAG found in pigs, as the candidates for investigation of proteolytic activity. From our experiments we found that, boPAGs -2 and -12 and poPAG-2 are proteases with optimal activity under acidic pH conditions. We also illustrated differences in proteolytic activity towards substrates, and in their relative affinity towards an AP inhibitor (pepstatin A). We found that, in comparison to the two bovine paralogs, boPAGs -2 and -12, poPAG-2 was found to be a more robust enzyme. Finally, we demonstrated that APs secreted by embryos such as PAGs can be objectively measured in the medium conditioned by the culture of porcine embryos either individually or in pools for variable lengths of time in acidic conditions. We also observed that such activity seemed to correlate with stage and quality of embryos (assessed morphologically) *in vitro*. We, therefore, believe that this proteolytic activity potentially could serve as a marker for developmental competence of the embryos.