

Partial Identification and Purification of Metalloproteinases in Equine Seminal Plasma

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The seminal fluid contains many proteinases deriving from distinct sources and some of these are responsible for modifying seminal plasma proteins or proteins on sperm surface. These modifications can help in fertilization and in sperm capacitation. However, there are some proteinases, as the matrix metalloproteinases with gelatinolytic activity, which are present in the seminal plasma of diverse mammals, but with functions and substrates unknown.

The aim of this work is to identify and purify partially, the matrix metalloproteinases (MMP) with gelatinolytic activity, as the MMP-2 and MMP-9, from equine seminal plasma.

The enzymatic activity of the gelatinases from equine seminal plasma was detected in gelatin zymographies. The proteases were partially purified from gels and then, the bands visualized in other new electrophoresis to verify molecular weights and activities. To verify the proteases effect on seminal plasma proteins we realized endogenous substrate proteolysis assays and these were visualized at PAGE and gelatin zymographies. The presence of MMP-9 proteinase was recognized by polyclonal antibodies.

Our results demonstrated that equine seminal plasma shows different gelatinolytic activities with distinct molecular weights, but when purified and visualized at new zymographies it seems like one main gelatinolytic band. It can be occurred due one binding between proteases and its endogenous substrates and, when the substrates are degraded, proteases can appear as one main molecular weight.

When detecting endogenous substrates at different times, it was verified one correlation between increasing gelatinolytic activities and proteolysis of plasmatic proteins with the molecular weights close to the proteases weights.

In assays using anti-MMP-9 different bands were positively marked. It can indicate the presence of protease fragments, even in regions where there is no gelatinolytic activity visualized.

Key words: Equine Seminal Plasma; Zymography; Proteinase; Matrix Metalloproteinase.