

# **Proteomic analysis of seminal plasma from stallions (Mangalarga Marchador) influenced by seasonality**

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The horses are seasonal polyestral animals, and the reproductive activity is primarily regulated by the photoperiod. The circannual differences affect also the composition and content of seminal plasma, including proteins in the plasma that interact with the surface of the spermatozoa and modify the characteristics of their membrane. Proteins in seminal plasma have been shown as involved in stages of the fertilization process as the establishment of sperm reserves in the oviduct, the modulation of the sperm capacitation, and the interaction between gametes. Proteomics of the seminal plasma from stallions using two-dimensional electrophoresis (2-DE), mass spectrometry and bioinformatics analysis may contribute to the understanding of biochemical factors involved in fertility and semen quality. The aim of this study was to evaluate the total proteome of semen from stallions in reproductive age, and detect changes in the protein profile between seasons, aiming to evaluate the physiological consequences for equine reproduction process. Semen samples were harvested, fractions were reserved for andrological analysis, and sperm were removed, recovering the seminal plasma by centrifugation. For the 2-DE first dimension, the seminal plasma samples were subjected to the isoelectric focusing using IPG strips (24-cm) with pH gradient from 3 to 10 (EttanIPGphor System 3, GE Healthcare, USA). For the second dimension, proteins were separated in SDS-PAGE gel 14.5% T and stained with coomassie blue. Gels were scanned and analyzed using Image Master 2D Platinum 7.0 software (GE Healthcare, USA). The spots of all proteins from gels were manually localized and excised, proteins were reduced, alkylated and trypsinized, following sample analysis in a MALDI-TOF/TOF (Ultraflex III - Bruker Daltonics) mass spectrometer (by MS and MS/MS). We found a set of proteins showing differential abundance in the different seasons. The samples were identified using the MASCOT DAEMON v.2.0 and Peaks Studio 7.5 softwares, and the UniProt, SwissProt, Equidae databases. The data are being analyzed with the aid of bioinformatics tools associated with ExPASy platform and String. Our results indicated the presence of a group of kallikreins in the seminal plasma in breeding season. It is expected the identification of metabolic pathways and biological events that will allow to suggest procedures for the improvement of equine reproduction process. (Support: FAPEMIG, CNPq, CAPES, FINEP, NuBioMol, BIOAGRO).