MYELOPEROXIDASE ACTIVITY DECREASES IN EQUINE SEMEN FREEZING EXTENDERS

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Myeloperoxidase (MPO) is a pro-oxidant enzyme contained in and released by neutrophils, and associated with decreased post-thaw motility of equine semen. This study aimed to compare MPO activity in pure equine freezing extender, raw and post-thaw semen.

Active MPO Concentration (AMC) was measured with Specific Immunologic Extraction Followed by Enzymatic Detection in 20 ejaculates. Raw semen intra cellular AMC was determined in the supernatant after membrane lysis, each pellet containing 100x10⁶ spermatozoa. AMC was also assayed in supernatants of semen frozen following a conventional method using INRA Freeze[™] (IMV, France). Effect of freezing procedure on AMC was tested by experimentally adding 500ng of purified active MPO (Calbiochem, Germany) in 4 samples either with 5ml of PBS or INRA Freeze[™] before assay.

AMC was higher in sperm-rich pellet (0.306ng/ml) than in post-thaw semen (0.002ng/ml) (p=0.058). After experimental MPO addition, no activity variation was observed during the freezing procedure (after dilution, 1, 2 hours of cooling and post-thawing) within the same medium. Purified MPO activity was decreased in INRA Freeze[™] when compared to PBS at all timings of sampling (p=0.0286). When all samples were pooled, remaining activity in INRA Freeze[™] was 23.93±13.13%.

MPO fixation on large proteins contained in the extender experimentally reduces AMC, as previously observed in plasma. However, AMC decrease observed during semen freezing is more important than after experimental addition. That could be explained by a MPO interaction with seminal plasma, a partial MPO release or a MPO inactivation during equine semen freezing.