

A flow-cytometric study of the effect of myeloperoxidase on stallion spermatozoal motility and structure

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Introduction

Myeloperoxidase (MPO):

= Pro-oxidant enzyme contained in and released by neutrophils

- Increased MPO concentrations observed in poor post-thaw quality semen in the equine¹

Aim of this study was to observe effect of active MPO addition in fresh purified semen on:

- Semen motility
- Membrane and acrosome integrity
- Mitochondrial potential and apoptosis induction



Material and methods

Animals: 1 Pony stallions and 2 draft stallions

Experimental design:

Semen collected 4 times

- Cushioned centrifugation in INRA96®
- Density Gradient centrifugation² (Bottom Layer, Nidacon®)
- Pellet rediluted (100x10⁶ spz/ml) in and incubated (2h):
 - Pure INRA96® (Blank)
 - 5ng/ml MPO solution in INRA96®
 - 50ng/ml MPO solution in INRA96®

Results

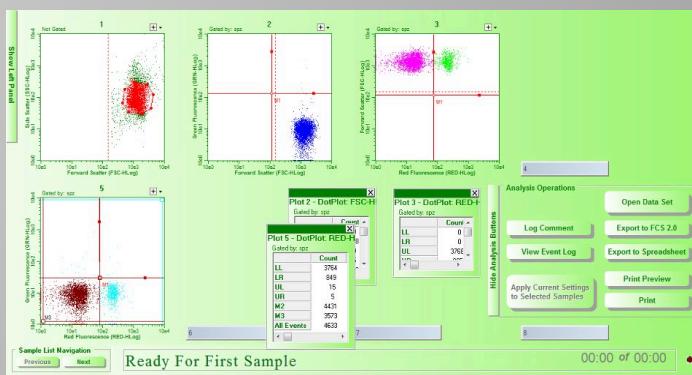
• No stallion effect observed after density gradient centrifugation

• Progressive Motility decreased by both MPO concentrations (p=0.001)

• No effect on Total Motility

• No effect of purified MPO addition in semen on:

- Membrane and acrosome integrity
- Mitochondrial potential
- Apoptosis induction



References:

¹Ponthier et al: Myeloperoxidase in equine semen: concentration and localization during freezing process. Journal of Equine Veterinary Science, 2012.

²Edmond et al: Effect of density-gradient centrifugation on quality and recovery rate of equine spermatozoa. Animal Reproduction Science, 2008.

³Baumber et al: The effect of reactive oxygen species on equine sperm motility, viability, acrosome integrity, mitochondrial potential and membrane lipid peroxidation. J of Andrology, 2000

Semen analysis:

Motility: CASA analysis

Flow cytometry: IMV Guava Easycyte Mini®

- Membrane and acrosome integrity: PI & PNA
- Mitochondrial potential and apoptosis: JC-1 & 7-AAD

Statistical methods:

• Kruskal-Wallis test was used to compare parameters obtained. Statistical significance established at p<0.05

Discussion

- Results observed with MPO addition in semen are similar to previous results using xanthine oxidase ROS production system³:
 - Decrease of progressive motility;
 - No effect on membrane and acrosome integrity;
 - No effect on mitochondrial potential or apoptosis.
- Effect of MPO and ROS on motility was not explained by lesions of membrane, acrosome or mitochondria.
- Further studies should determine if motility decrease due to MPO is not associated to lipoperoxidation, as previously observed with xanthine oxidase³.

Conclusions

- MPO addition induces progressive motility decrease without affecting mitochondrial potential, membrane and acrosome integrity
- Further studies should focus on other lesions induced by MPO on spermatozoa