

Introduction

Myeloperoxidase (MPO):

= Pro-oxidant enzyme contained in and released by neutrophils

- High MPO observed in poor post-thaw quality semen in the equine¹
- MPO activity decreased in commercial equine extenders¹

Aim of this study : MPO concentration and activity in:

- Non-extended raw semen
- Supernatant of centrifuged semen (diluted in crystalloid solution)



MPO immunostained non-sperm cell

Material and method

Animals: 3 stallions, 5 collections, every other day

Experimental design:

- Samples containing 100×10^6 spz of raw semen for assays
- Centrifugation
 - Samples containing 500×10^6 spz
 - Dilution 1v semen/ 3v PBS
 - Cushion medium centrifugation (1000xg, 20minutes)
 - Supernatant used for assays

Semen analysis:

Semen concentration: Nucleo Counter Sp100™

Total MPO concentration: ELISA Assay¹

Active MPO concentration: SIEFED Assay¹ (specific immunological extraction followed by enzymatic detection)

Statistical methods:

- Kruskal-Wallis test for median comparisons
- Spearman test for correlations between parameters
- Statistical significance established at $p < 0.05$

Results

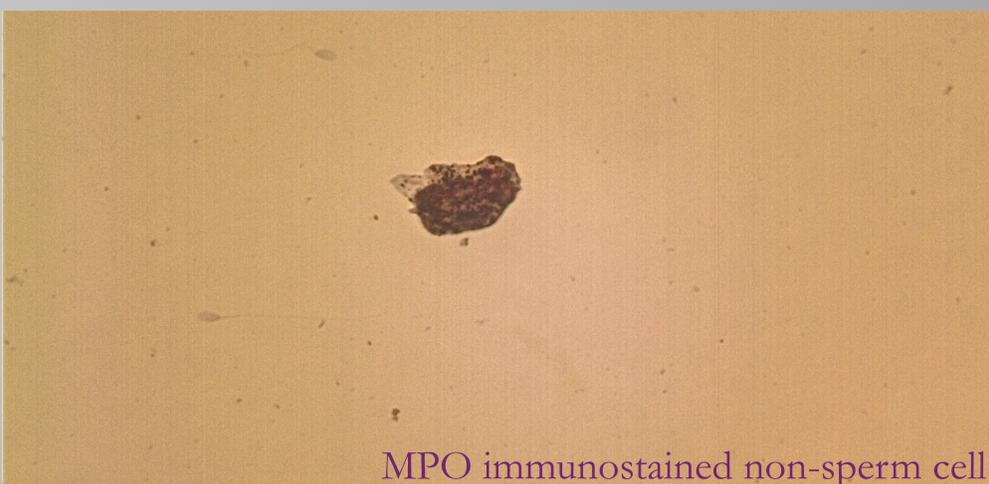
Raw semen:

- Median Total [MPO] = 580500ng/mL
- Median Active [MPO] = 1.098ng/mL
- High correlation between Total and Active [MPO] ($r=0.7096$, $p=0.0030$)

Supernatant:

- Median Total [MPO] = 107500ng/mL
- Median Active [MPO] = 0.236ng/mL
- No correlation between Total and Active [MPO] ($r=0.2121$, $p=0.4479$)

Difference between Total [MPO] in Raw semen and Supernatant (corrected for dilution) $p < 0,05$



MPO immunostained non-sperm cell

Discussion

Higher Total [MPO] in Raw semen than in Supernatant:

- Cellular release of total MPO in raw semen during cold-chock (supernatant value below)

Higher Active [MPO] in Raw semen than in Supernatant:

- Cellular release of active MPO in raw semen during cold-chock (supernatant value below)

No large cellular debris or proteins inside medium:

- Assessment of activity
- Total [MPO] = $\pm 500\ 000 \times$ Active [MPO] in both samples

Conclusions

Confirmation of Total and Active MPO release in semen:

- by cellular part of the ejaculate
- during procedures inducing cold-chock

References:

- ¹Ponthier J. et al: Concentration, activity and biochemical characterization of myeloperoxidase in fresh and post-thaw equine semen and their implication on freezability. *Reprod Dom Anim*, 2014, 49(2): 285-91.