Assessment of Myeloperoxidase activity in raw equine fresh semen

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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)

Material and method
Animals: 3 stallions, 5 collections, every other day

Experimental design:
• Samples containing 100x10⁶ spz of raw semen for assays
• Centrifugation
  • Samples containing 500x10⁶ spz
  • Dilution 1v semen/3v PBS
  • Cushion medium centrifugation (1000xg, 20 minutes)
  • 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

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] = ± 500 000 x 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: