MYELOPEROXIDASE ACTIVITY DECREASES IN EQUINE SEMEN FREEZING EXTENDERS

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**Myeloperoxidase (MPO)**

- Released after Neutrophil lysis or degranulation
- PMN granules: MPO, elastase, apoptotic factors
- Active enzyme: 64KDa subunits
- Inactive subunits: 86, 44 or 16KDa
- Pro-oxidant enzyme: HOCl
MPO IN SEMEN CRYOBIOLOGY

- Post-thaw total MPO concentration & motility associated
- MPO observed in/on Non-Sperm Cells present in semen
- Non-Sperm Cells: epithelial cells, cellular debris (PMN)
OBJECTIVES

- To compare MPO activity in equine sperm-rich pellet and post-thaw semen

- To compare activity of purified human MPO added:
  - In PBS
  - In equine freezing extender (INRA Freeze™)
MPO ACTIVITY ASSAYS

$\text{[MPO]}_{\text{active}}$ SIEFED = Specific Immunological Extraction Followed by Enzymatic Detection

<table>
<thead>
<tr>
<th>Samples</th>
<th>Timing</th>
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<tbody>
<tr>
<td>Equine semen (20 ejaculates)</td>
<td>Raw Sperm-Rich Pellet (100x10^6 spz)</td>
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<tr>
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<td>Post-thaw (100x10^6 spz)</td>
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<tr>
<td>500ng of pure human MPO in 5ml PBS</td>
<td>0h, 1h, 2h of cooling</td>
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<tr>
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<tr>
<td>500ng of pure human MPO in 5ml INRA FREEZE™</td>
<td>0h, 1h, 2h of cooling</td>
</tr>
<tr>
<td></td>
<td>Post-thaw</td>
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</tbody>
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Statistics: *Kruskal-Wallis* for median differences
Evolution of MPO activity during equine semen freezing

RESULTS

Active MPO concentration (AMC) (ng/ml)

- Raw Sperm-rich Pellet
- Post-thaw semen

7.79% of remaining activity

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Evolution of MPO activity in INRA FREEZE™ and in PBS

Overall: 23.93% of remaining activity
• MPO activity decreases in equine semen extender in this experiment
  ✓ MPO fixation on large proteins
  ✓ Previously seen in plasma

• MPO activity decrease is more important in presence of semen:
  ✓ Interaction with seminal plasma?
  ✓ Partial release during semen freezing procedures?
  ✓ Inactivation during freezing procedures?
THANK YOU FOR YOUR ATTENTION!

Questions?