

MYELOPEROXIDASE ACTIVITY DECREASES IN EQUINE SEMEN FREEZING EXTENDERS

J. PONTHER, D.V.M., M. Sc., PH. D., DIPLOMATE ECAR (EQUINE)

T. FRANCK, M. Sc., PH. D.

A. NIESTEN

S. PARRILLA-HERNANDEZ, D.V.M, M. Sc.

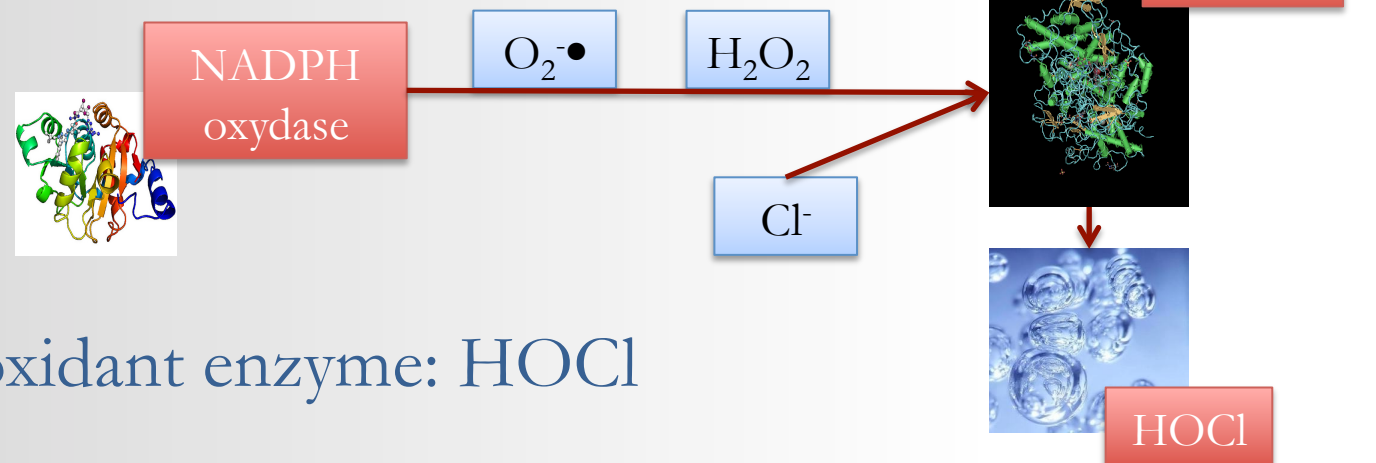
D. SERTEYN, D.V.M., M. Sc., PH. D., DIPLOMATE ECVAA

S. DELEUZE, D.V.M., M. Sc., PH. D., DIPLOMATE ECAR (EQUINE & CA)

University of Liège, Belgium – Equine Reproduction & CORD

MYELOPEROXIDASE (=MPO)

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



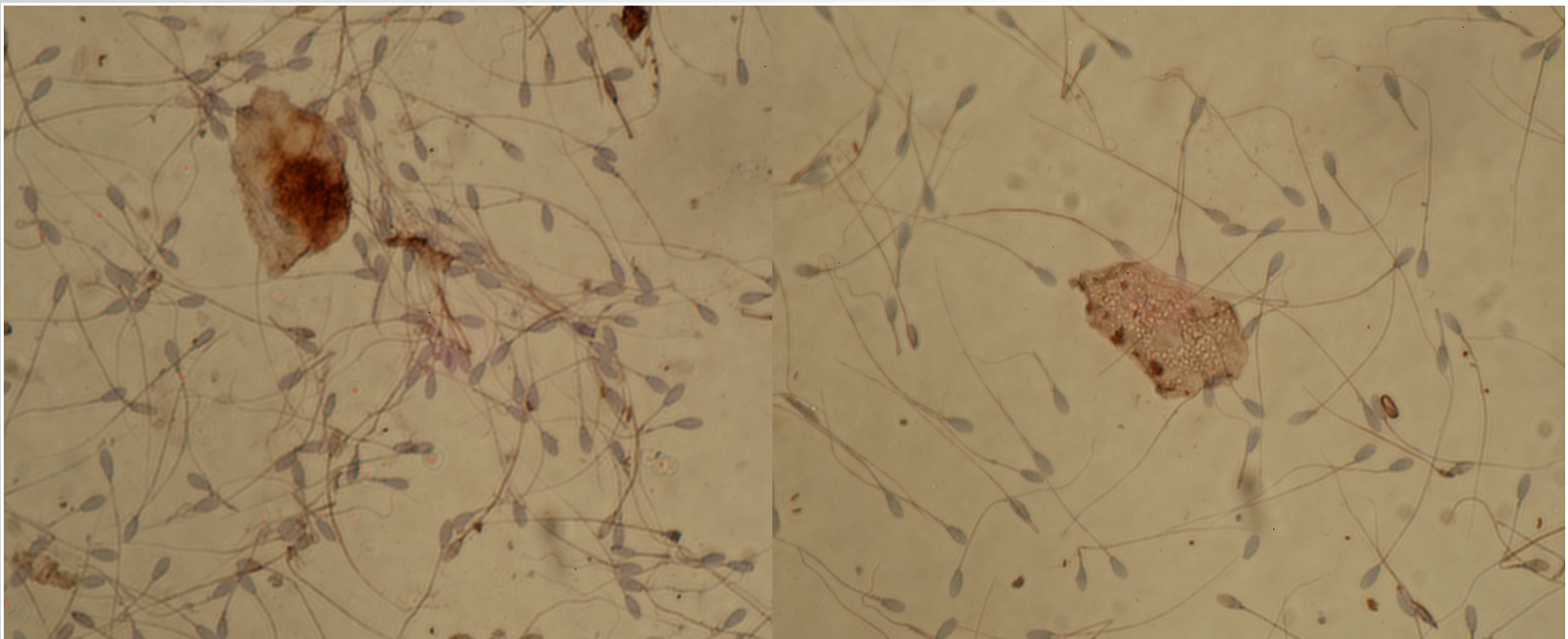
- Pro-oxidant enzyme: HOCl

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~~)

BACKGROUND



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



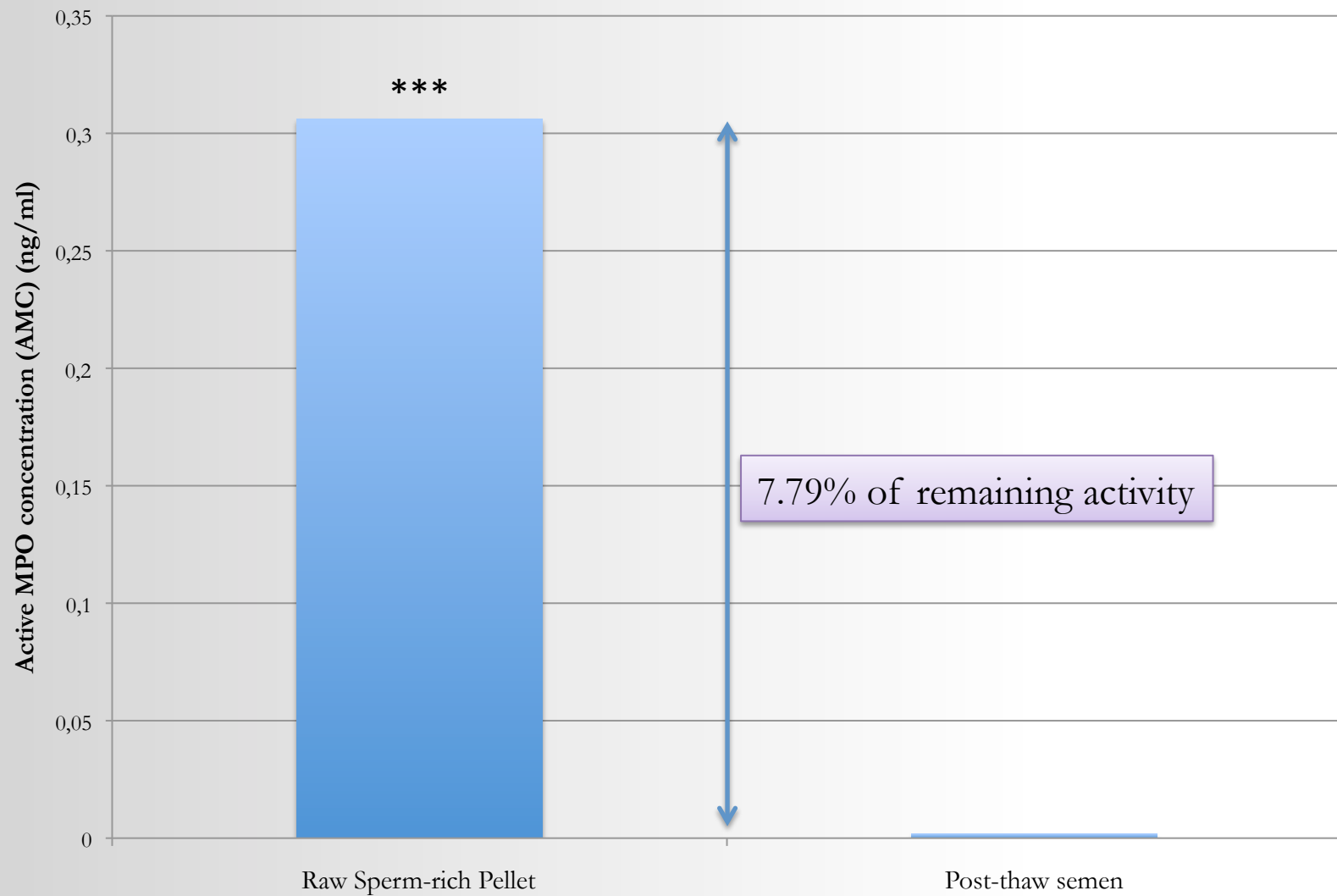
- $[MPO]_{\text{active}}$ SIEFED= Specific Immunological Extraction Followed by Enzymatic Detection

Samples	Timing
Equine semen (20 ejaculates)	Raw Sperm-Rich Pellet (100×10^6 spz) Post-thaw (100×10^6 spz)
500ng of pure human MPO in 5ml PBS	0h, 1h, 2h of cooling Post-thaw
500ng of pure human MPO in 5ml INRA FREEZE™	0h, 1h, 2h of cooling Post-thaw

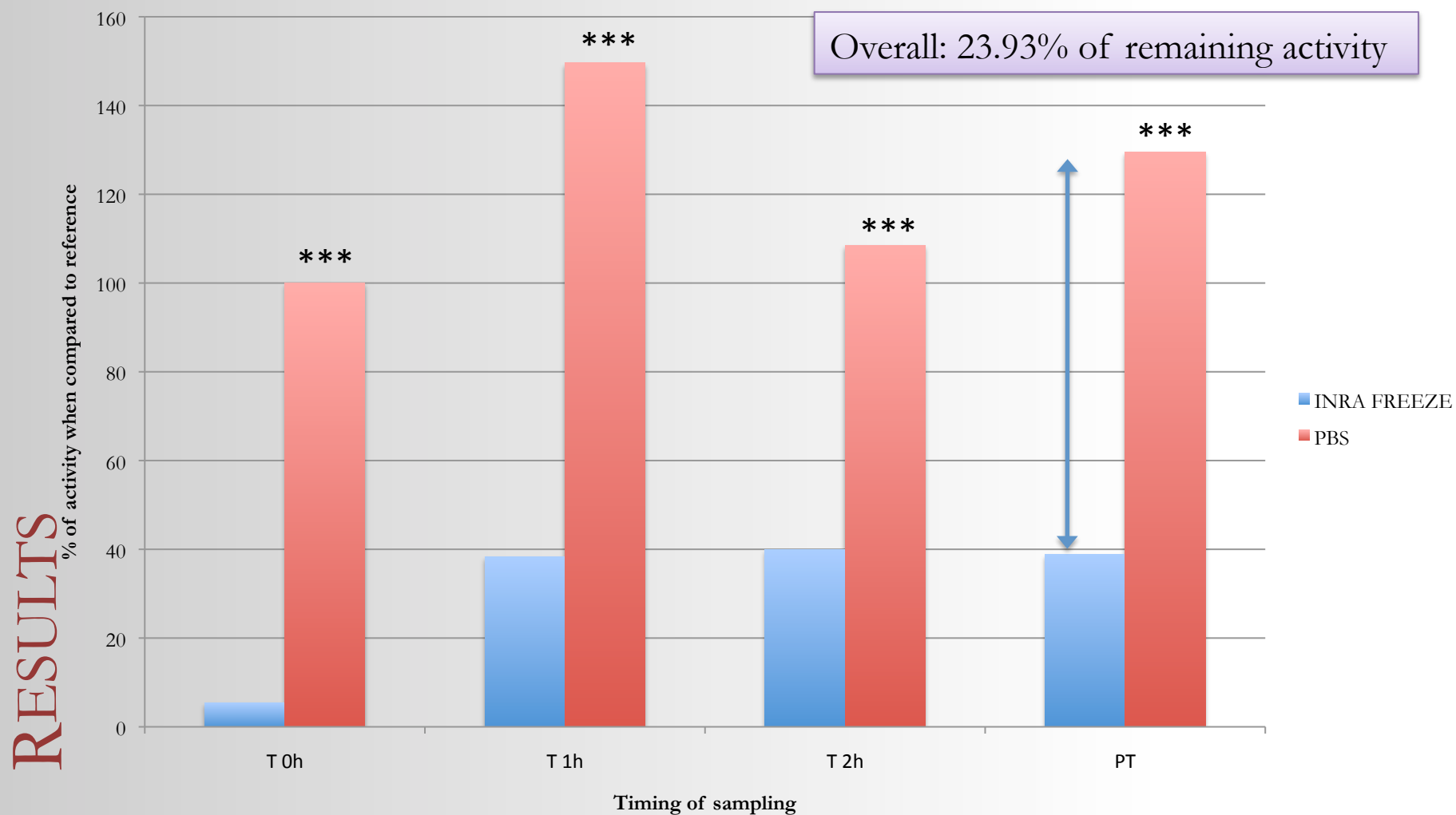
- Statistics: *Kruskal-Wallis* for median differences

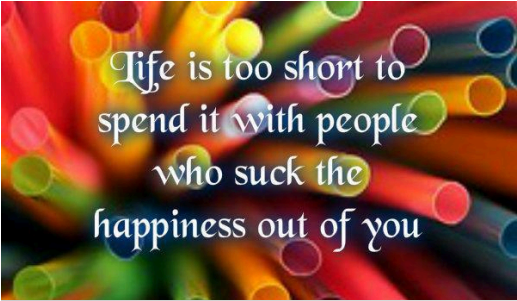
RESULTS

Evolution of MPO activity during equine semen freezing



Evolution of MPO activity in INRA FREEZE™ and in PBS





Life is too short to
spend it with people
who suck the
happiness out of you



- 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?

DISCUSSION

THANK YOU FOR YOUR ATTENTION!



Questions ?

