UAB Universitat Autònoma de Barcelona

Impact of enzymatic antioxidants from seminal plasma on DNA fragmentation and lipid peroxidation in frozen-thawed horse semen.

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February 2024 - Final Degree Project
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Introduction

- The manipulation of ejaculates increases reactive oxygen species (ROS) and may cause oxidative stress (OS). Small amounts of ROS are necessary for proper sperm function but high levels cause a problem.
- Seminal plasma (SP) plays an important role because it contains antioxidants that help combat some of the oxidative stress faced by spermatozoa during freeze-thawing.
- The present study aimed to determine if the enzymatic antioxidants in horse SP (superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and paraoxonase type 1 (PON1)), are related to DNA fragmentation and membrane LPO of frozen-thawed horse spermatozoa.

Material and Methods

20 ejaculates from 20 individuals between 5 i 15 years old

→ Obtaining SP → **Assessment of antioxidant activity levels of SP**
- **SOD**
- **CAT**
- **GPX**
- **PON1**

→ 1:5 dilution with Kenney Extender → Cryopreservation → Thawing of 0,5mL straws at 38ºC x 30s

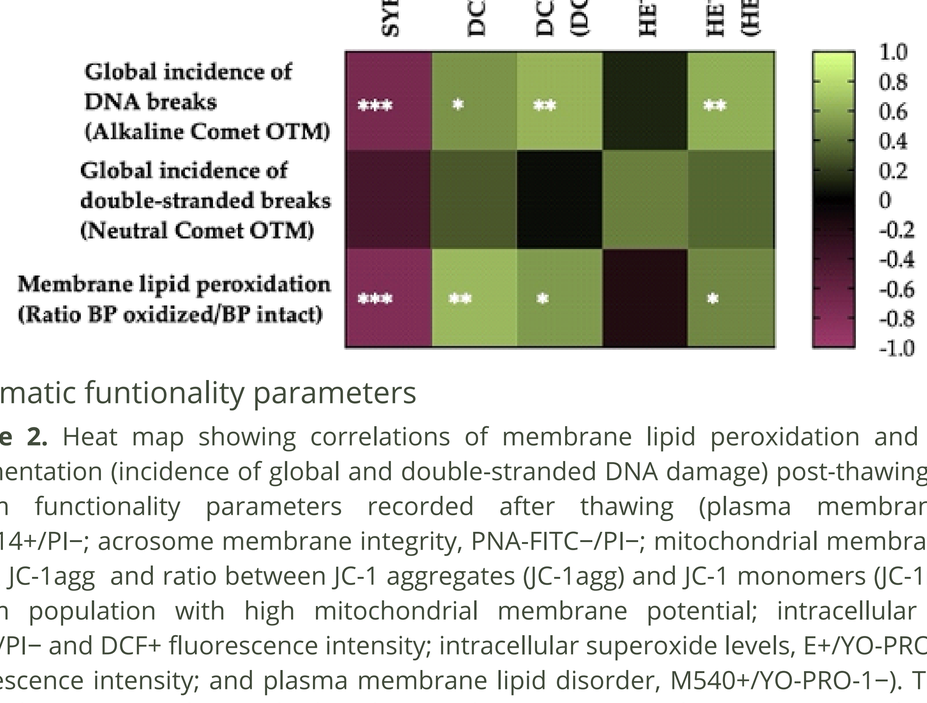
- Spermatic motility: **CASA**
- Sperm functionality analysis **flow cytometry**
- DNA fragmentation assessment: **Comet**

→
- **GFE**
- **PFE**

Results and Discussion

Spermatic motility parameters

Figure 1. Heat map showing the correlations of membrane lipid peroxidation and sperm DNA fragmentation (incidence of global and double-stranded DNA damage) post-thawing (n=20), with horse sperm motility parameters after thawing (total motility, TM; progressive motility, PM; curvilinear velocity, VCL; straight line velocity, VSL; average path velocity, VAP; linearity coefficient, LIN; straightness coefficient, STR; wobble coefficient, WOB; amplitude of lateral head displacement, ALH; and beat-cross frequency, BCF). The colors on the scale (1 to −1) indicate whether the correlation is positive (lime) or negative (burgundy). (**) p ≤ 0.01; (***) p ≤ 0.001.



Spermatic funtionality parameters

Figure 2. Heat map showing correlations of membrane lipid peroxidation and sperm DNA fragmentation (incidence of global and double-stranded DNA damage) post-thawing (n=20), with sperm functionality parameters recorded after thawing (plasma membrane integrity, SYBR14+/PI−; acrosome membrane integrity, PNA-FITC−/PI−; mitochondrial membrane potential, MMP, JC-1agg and ratio between JC-1 aggregates (JC-1agg) and JC-1 monomers (JC-1mon) for the sperm population with high mitochondrial membrane potential; intracellular ROS levels, DCF+/PI− and DCF+ fluorescence intensity; intracellular superoxide levels, E+/YO-PRO-1− and HE+ fluorescence intensity; and plasma membrane lipid disorder, M540+/YO-PRO-1−). The colors on the scale (1 to −1) indicate whether the correlation is positive (lime) or negative (burgundy). (**) p≤ 0.01; (***) p ≤ 0.001

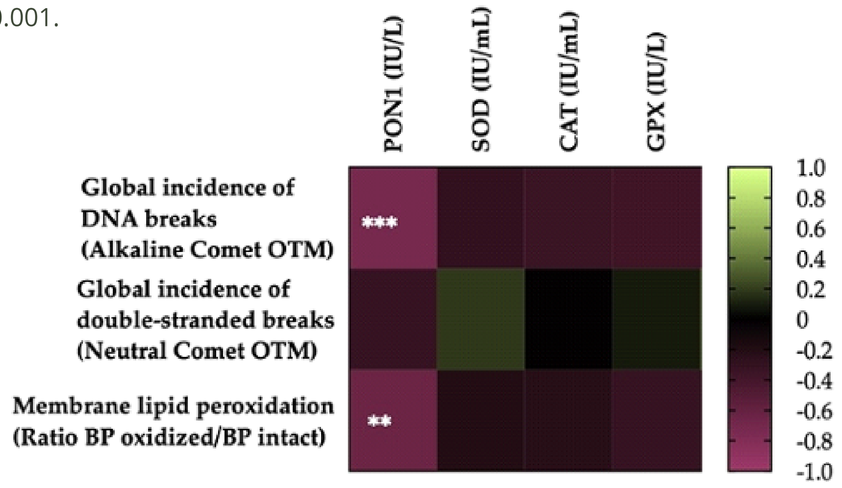


Figure 3. Heat map showing correlations of membrane lipid peroxidation and sperm DNA fragmentation (incidence of global and double-stranded DNA damage) post-thawing (n=20), with activity levels of the enzymatic (paraoxonase type 1, PON1; superoxide dismutase, SOD; catalase, CAT; and glutathione peroxidase, GPX) in the seminal plasma of horse ejaculates. The colors on the scale (1 to −1) indicate whether the correlation is positive (lime) or negative (burgundy). (**) p ≤ 0.01; (***) p ≤ 0.001.

Conclusion

There were differences in membrane LPO and the global incidence of DNA strand breaks when the frozen-thawed sperm of horse ejaculates of different cryotolerance (GFE and PFE) were compared.

LPO and the incidence of global DNA breaks in frozen-thawed sperm were found to be positively correlated with ROS levels.

The differences observed in the LPO and DNA fragmentation of frozen-thawed spermatozoa from different stallions/eyaculates is influenced by the antioxidant activity of PON1 present in the SP.