Proteomic analysis of ejaculated and epididymal sperm associated with freezability in wild small ruminants

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Introduction: The sperm proteome is known to affect cell cryoresistance and is reported to differ between epididymal and ejaculated sperm in small ruminants (Li *et al.*, 2016; Pini *et al.*, 2016). However, studies aiming at identifying proteins involved on sperm freezing-tolerance are scarce.

Objective: The aim of this study was to investigate the association between the freezing capacity and the proteome of ejaculated and epididymal sperm of the Iberian ibex (*Capra pyrenaica*), Mouflon (*Ovis musimon*) and Chamois (*Rupicapra pyrenaica*).

Methods

Ejaculated sperm | Epididymal sperm

→ Sperm cryopreservation

Sperm freezability analysis

Iberian ibex: Ejaculated n=22 Epididymal n=6
Chamois: Ejaculated n=6 Epididymal n=13
Mouflon: Ejaculated n=25 Epididymal n=12

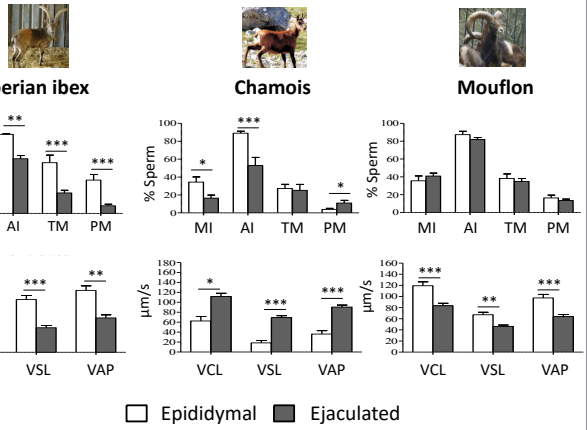
Sperm proteome analysis

by liquid chromatography - mass spectrometry (LC-MS)

Iberian ibex: Ejaculated n=6 Epididymal n=6
Chamois: Ejaculated n=6 Epididymal n=23
Mouflon: Ejaculated n=12 Epididymal n=16

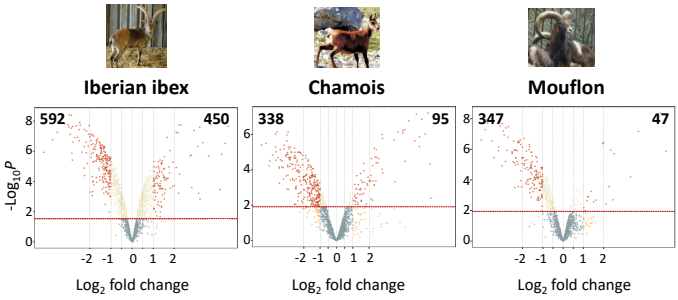
Results

Post-thaw sperm quality parameters



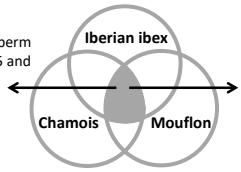
MI: membrane integrity; AI: acrosome integrity; TM: total motility; PM: progressive motility.
VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity.
Error bars represent SEM; t-test; *=$P < 0.05$; **=$P < 0.001$; ***=$P < 0.0001$

Quantitative differences between Ejaculated/Epididymal sperm proteins



Statistical analysis was done by the moderated t-test of the R package limma. Horizontal red line in the volcano plots represents adjusted *P* value 0.05. Numbers indicate differentially expressed proteins.

76 proteins more abundant in epididymal than in ejaculated sperm across species (adjusted $P < 0.05$ and abs($\log_2$ (fold-change)>1).



3 proteins more abundant in ejaculated than in epididymal sperm across species (adjusted $P < 0.05$ and abs($\log_2$ (fold-change)>1).

Sperm freezability is higher in epididymal than in ejaculated sperm

Quantitative differences between epididymal and ejaculated sperm proteome

Proteins associated with sperm freezability across the three species of study
adjusted $P < 0.05$ and abs($\log_2$ (fold-change)>1

76 proteins more abundant in Epididymal sperm (high sperm freezability) e.g.:

- Lactoferrin (LTF): increases cell defense response (González-Chávez *et al.*, 2009)
- Superoxide dismutase (SOD1): antioxidant activity (Marti *et al.*, 2008)
- Inositol-3-phosphate synthase q (ISYNA1): regulates changes in osmolarity (Chauvin *et al.*, 2004)
- T-complex protein 1 (CCT8) and Cullin-3 (CUL3): increases ram sperm freezability (Pini *et al.*, 2016)

3 proteins more abundant in Ejaculated sperm (low sperm freezability)

- EGF-like repeat and discoidin I-like domain-containing protein 3 (EDIL3): conferred by seminal plasma (Pini *et al.*, 2016)
- Seminal plasma protein PDC-109-like (LOC1021828): membrane-destabilizing activities (Kumar *et al.*, 2018)
- C-type natriuretic peptide precursor (NPPC)

Conclusions: Besides updating the sperm proteome of small ruminants, this study revealed differences of cryoresistance between epididymal and ejaculated sperm of three mountain ungulates contributing to identification of candidate markers of sperm freezability.

Acknowledgments: Supported by European Union H2020 Marie Sklodowska-Curie Action REPBIOTECH 675526 and the Spanish "Ministerio de Economía, Industria y Competitividad" (MINECO AGL2014-52081-R and AGL2017-85753-R).

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