Endocrine and molecular basis of small ruminant sperm cell cryoresistance

Julián Santiago-Moreno

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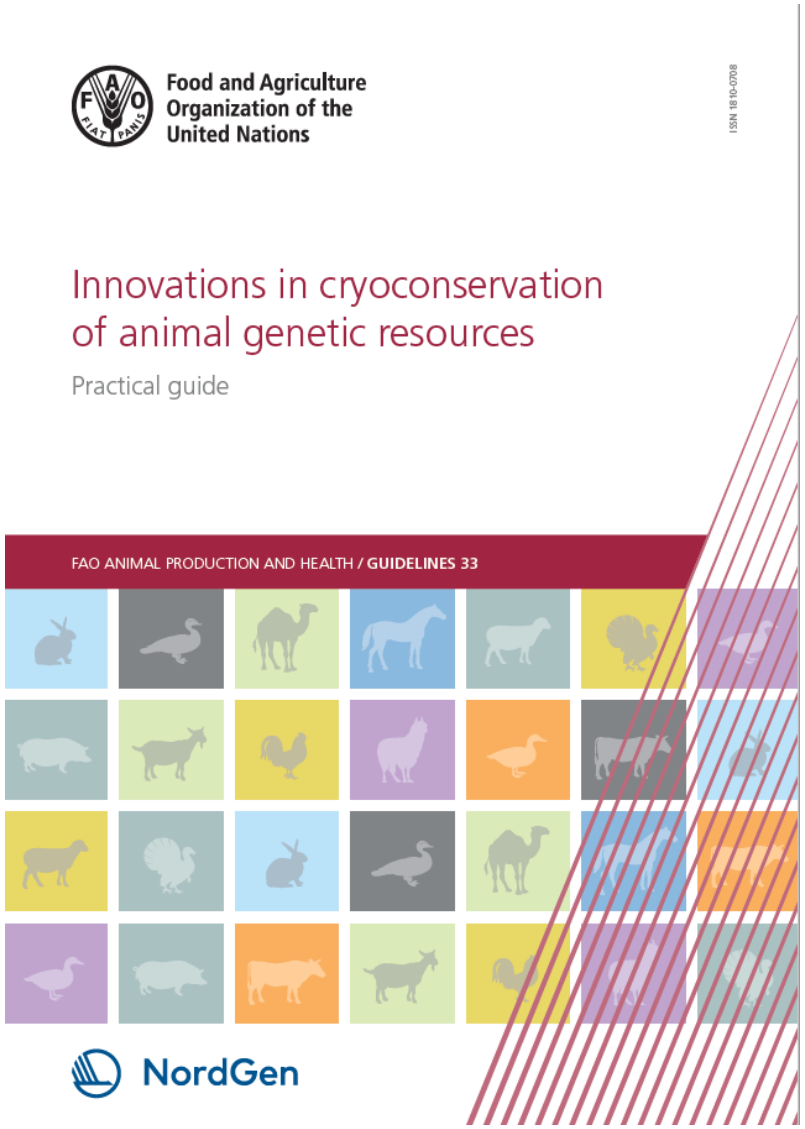
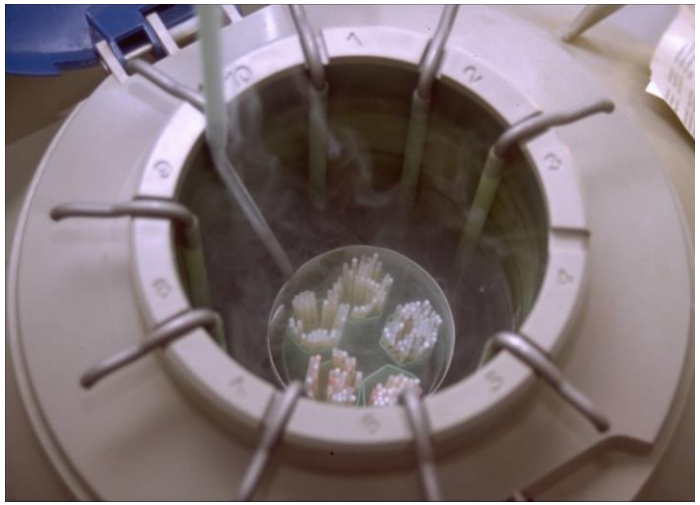
The 3rd CZU Prague hybrid seminar "Biotechnology in small ruminant reproduction: an international experience"



- Many ruminant breeds and species are threatened. Gene banks ensure the long-term survival of threatened species → PRIORITY

- Sperm cryopreservation: main tool for *ex situ* assisted reproduction programs.

- Artificial insemination with cryopreserved semen often provides poor fertility results in many small ruminants.



WHY USE SMALL WILD RUMINANT SPECIES AS AN ANIMAL MODEL?

- Domestication has reduced or even repressed the reproductive seasonality and the annual endocrine pattern in ruminant species. Thus, the ancestor wild species are an adequate model to study the role of endocrine status on sperm freezability.

- Many wild ruminant species are threatened by poaching, habitat loss, heterozygosity loss due to habitat fragmentation, and climatic change.

FACTORS INFLUENCING THE CELL RESPONSE TO THE CRYOPRESERVATION PROCESS

SAMPLE ORIGIN
(EJACULATED OR EPIDIDYMAL)

SPERM AND SEMINAL PLASMA
PROTEOME

ENDOCRINE STATUS

SPERM CRYORESISTANCE

SEASON OF SPERM COLLECTION

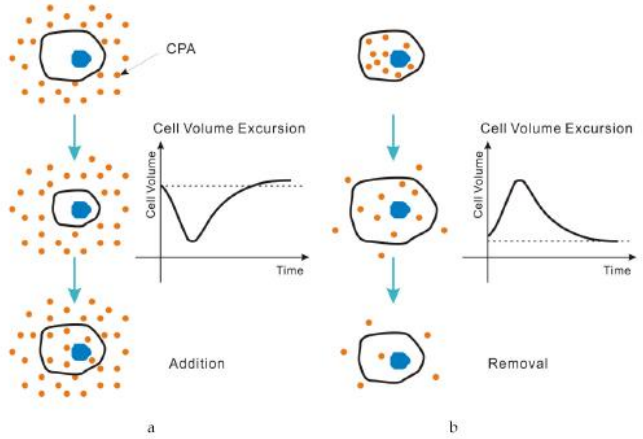


Fig. 1. Cell volume excursion during addition and removal of CPAs

Adapted from Gao et al 2012, Current Frontiers in Cryobiology

Cryoresistance ratio (CR) defined as **CR= (post-thaw value/fresh value) × 100**

SPERM PROTEOME AND CRYORESISTANCE

Cryobiology 99 (2021) 64–77

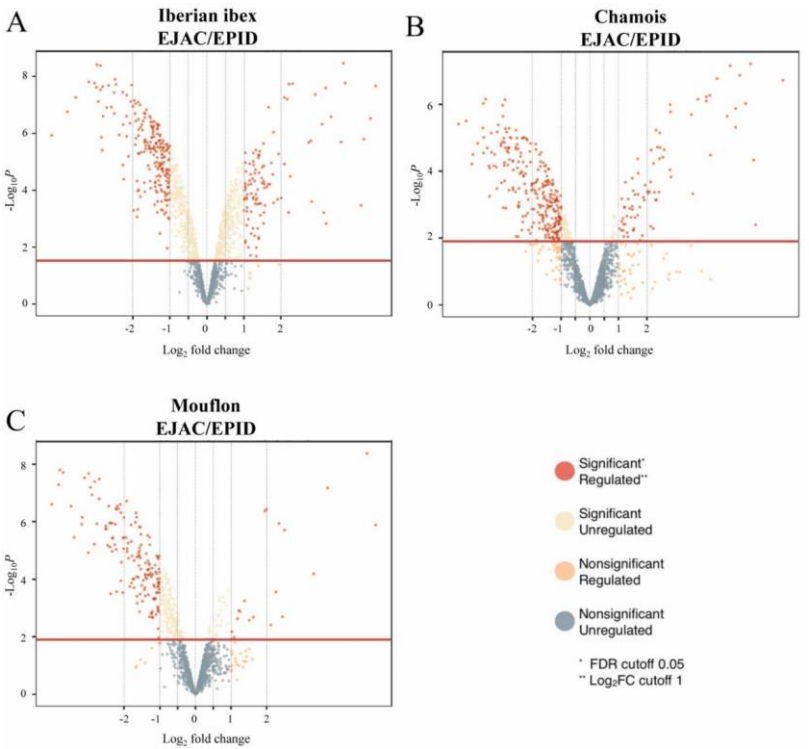
Contents lists available at ScienceDirect

Cryobiology

journal homepage: http://www.elsevier.com/locate/cryo

Differential proteome between ejaculate and epididymal sperm represents a key factor for sperm freezability in wild small ruminants

Lucía Martínez-Fresneda [a,b,f], Marc Sylvester [c], Farhad Shakeri [d,e], Andreas Bunes [d,e], Juan C. Del Pozo [g], Francisco A. García-Vázquez [f], Christiane Neuhoff [b], Dawit Tesfaye [h], Karl Schellander [b], Julian Santiago-Moreno [a,*]



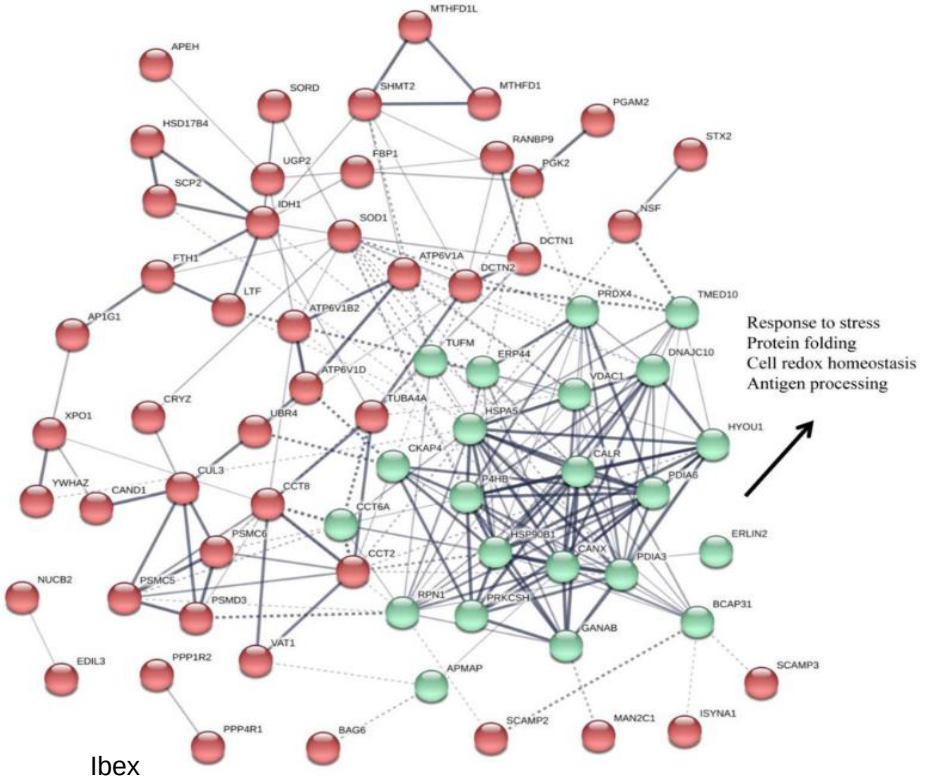
Proteins were differentially abundant according to the sperm origins.

83 proteins are more abundant in the epididymal sperm, whereas 4 proteins are more abundant in the ejaculated sperm.

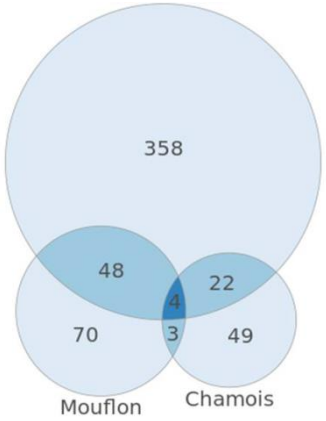
Epididymosomes, extracellular vesicles, and accessory sex glands, through seminal plasma, could contribute to the replacement and addition of proteins in sperm.

↓

Modification of sperm freezability



Ibex



Ibex

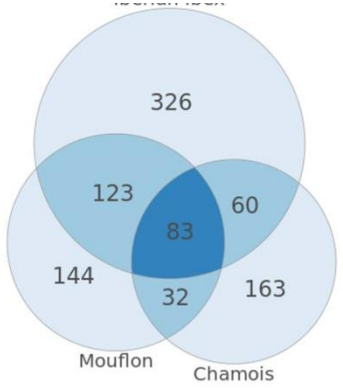


Table 2: Proteins found more abundant in epididymal than in ejaculated sperm in mouflon, ibex, and chamois, and that could be candidate markers of high sperm freezability in these species.

Gene symbol	Protein name
APEH	Acylamino-acidreleasing releasing enzyme
ATP6V1B2	V-type proton ATPase subunit B, brain isoform isoform
BAG6	Large proline-rich protein BAG6
CAND1	Cullin-associated NEDD8-dissociated protein 1
CCT2	T-complex protein 1 subunit beta
CCT6A	T-complex protein 1 subunit zeta
CCT8	T-complex protein 1 subunit theta
CLMN	Calmin
CUL3	Cullin-3
DCTN1	Synactin subunit 1
FBP1	Fructose-1,6-bisphosphatase 1
HIP1	Huntingtin-interacting protein 1
IDH1	Isocitrate dehydrogenase
ISYNA1	Inositol-3-phosphate synthase 1
MAN2C1	Alpha mannosidase 2C1
PGAM2	Phosphoglycerate mutase
PGK2	phosphoglycerate kinase 2
SOD1	Superoxide dismutase
SORD	Sorbitol dehydrogenase
VAT1	Synaptic vesicle membrane protein

In all these species, 83 proteins were more abundant in the epididymal sperm (using *Capra hircus database*), whereas 4 proteins were more abundant in the ejaculated sperm.

These proteins found more abundant in epididymal sperm should be explored as additives in extenders.

SEASONAL CHANGES IN PLASMA TESTOSTERONE AND PROLACTIN CONCENTRATIONS

Tropical and Subtropical Agroecosystems, 15 (2012) SUP 1: S47 – S70

REVIEW [REVISIÓN]

REPRODUCTIVE SEASONALITY AND ITS CONTROL IN SPANISH SHEEP AND GOATS

[ESTACIONALIDAD REPRODUCTIVA Y SU CONTROL EN RAZAS ESPAÑOLAS DE OVINOS Y CAPRINOS]

A. Gómez-Brunet, J. Santiago-Moreno, A. Toledano-Díaz and A. López-Sebastián

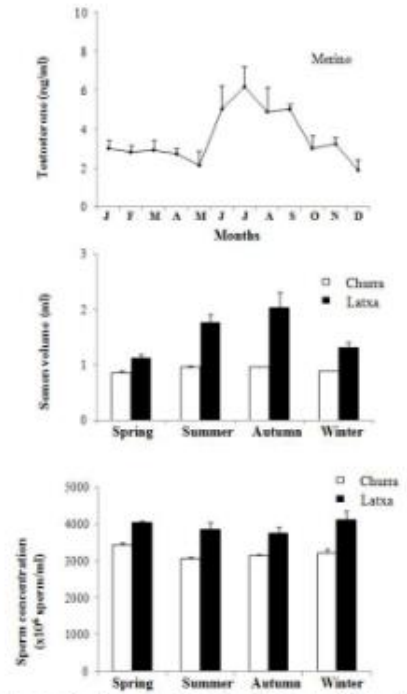


Figure 2. Seasonal changes (mean ± SEM) in testosterone secretion, semen volume and sperm concentration in different Spanish sheep breeds (Adapted from Beltran de Heredia and Gabiña, 2004; Santiago-Moreno et al., 2005; Mazariegos et al., 2010).

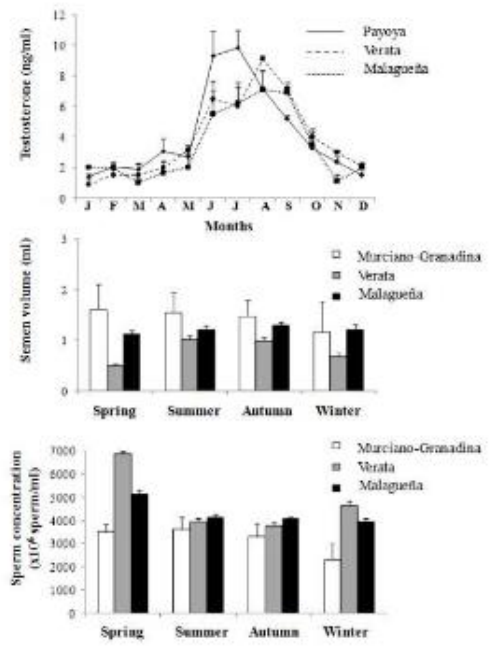
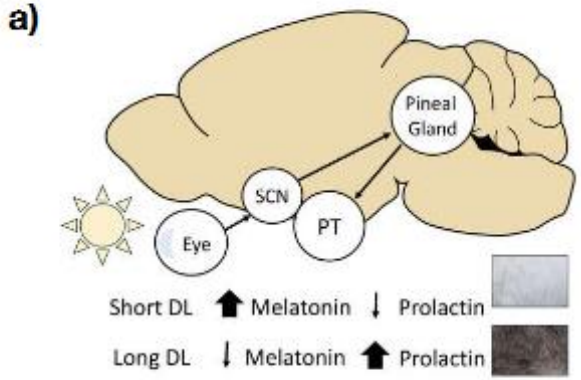
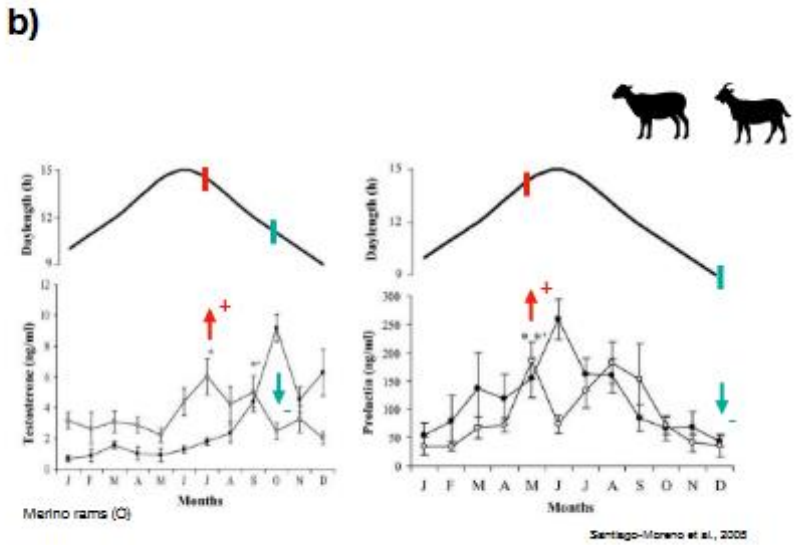


Figure 3. Seasonal changes (mean ± E.S.M) in testosterone secretion, semen volume and sperm concentration in different Spanish goats breeds (Adapted from Perez and Mateos, 1994; 1996; Arrebola et al., 2010; Gómez-Brunet et al., unpublished data).



Zimova et al., 2018



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Theriogenology 76 (2011) 1695–1705
Theriogenology
www.theriojournal.com

Seasonal variation in reproductive physiological status in the Iberian ibex (*Capra pyrenaica*) and its relationship with sperm freezability

M.A. Coloma, A. Toledano-Díaz, C. Castaño, R. Velázquez, A. Gómez-Brunet, A. López-Sebastián, J. Santiago-Moreno*

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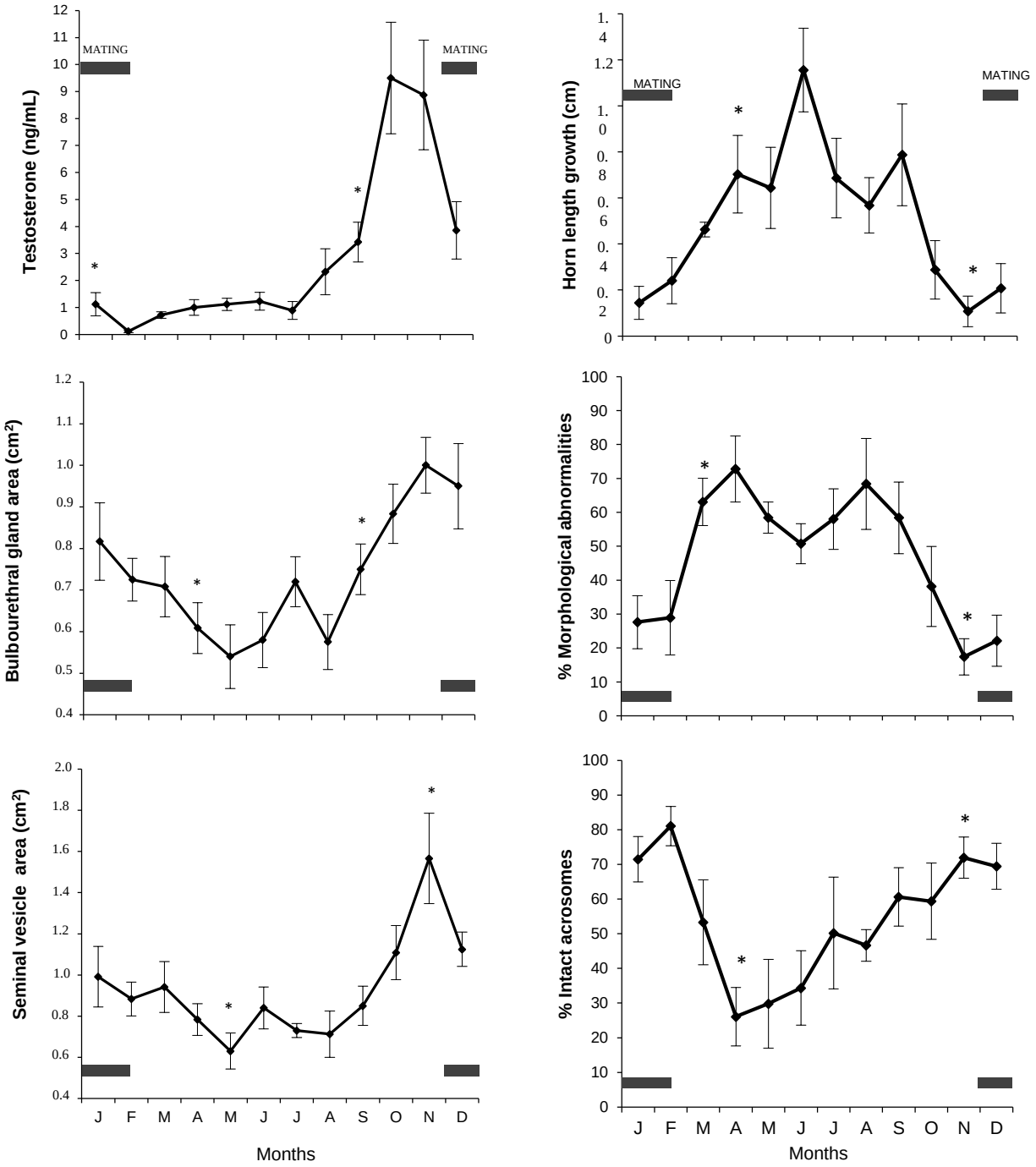

Cryobiology 61 (2010) 204–210

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Freezability of Iberian ibex (*Capra pyrenaica*) spermatozoa according to the glycerolization temperature and plasma testosterone concentration ☆

Miguel. A. Coloma, Amelia Gómez-Brunet, Rosario Velázquez, Adolfo Toledano-Díaz, Antonio López-Sebastián, Julián Santiago-Moreno *

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Theriogenology 76 (2011) 1695–1705

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Seasonal variation in reproductive physiological status in the Iberian ibex (*Capra pyrenaica*) and its relationship with sperm freezability

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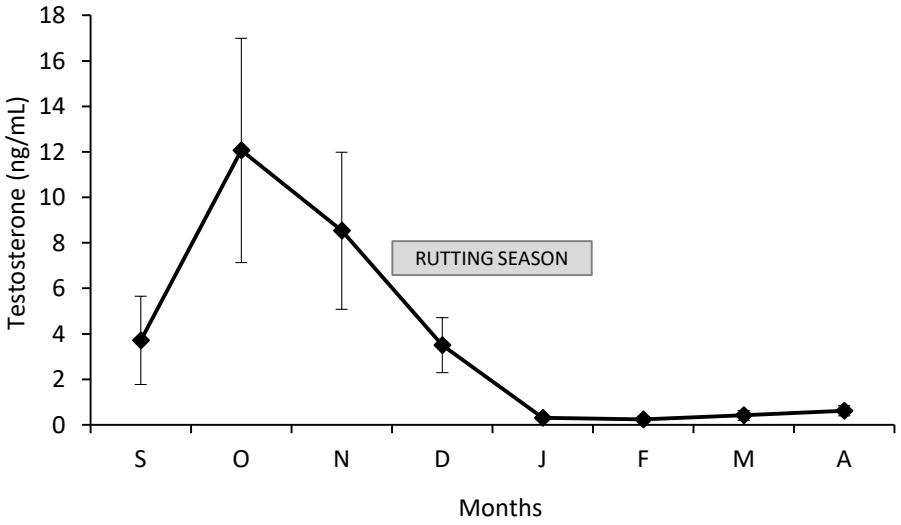
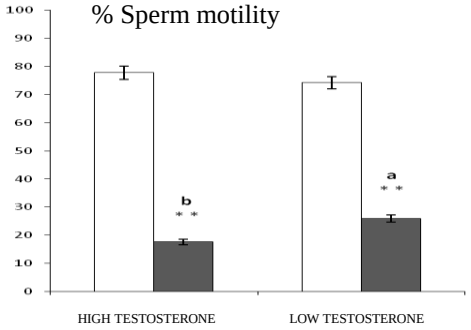
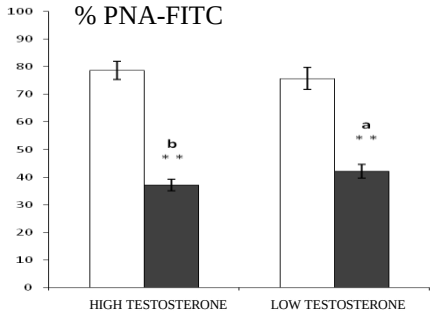
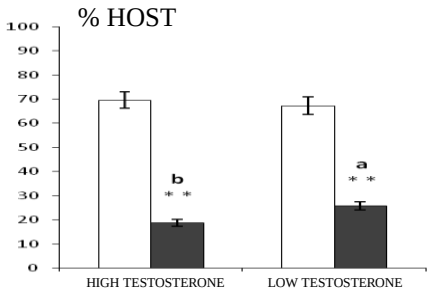








High plasma testosterone levels during the pre-rutting season may interfere with the freezing-thawing process, having a negative influence on sperm cryosurvival.

Livestock Science 249 (2021) 104513

Contents lists available at ScienceDirect

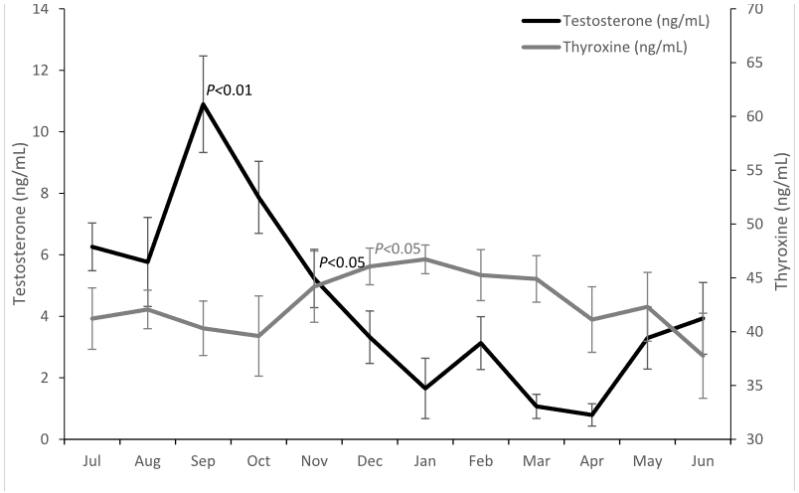
Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Seasonal changes in testosterone and thyroxine concentrations in Mediterranean rams and bucks and their relationship with sperm cryoresistance

Belén Martínez-Madrid [a,*], Cristina Castaño [b], Luis Pablo Ureña [c], Elena Flix [a], Rosario Velázquez [b], Antonio López-Sebastián [b], Rodolfo Ungerfeld [d], Francisco A. Arrebola [c,1], Julián Santiago-Moreno [b,1]

38°NL Florida breed bucks



Annual variation in sperm cryoresistance is related to seasonal endocrine changes in goat. Winter and spring are the most appropriate seasons to collect and freeze sperm from Florida goat

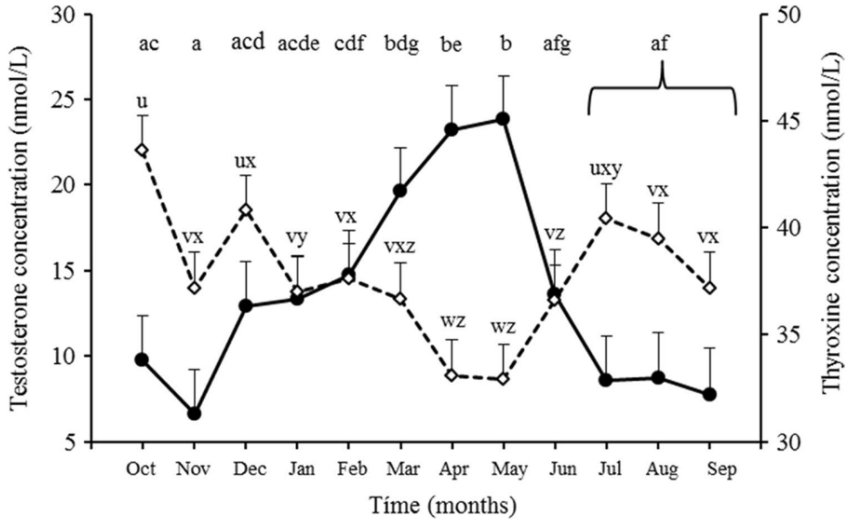
Tropical Animal Health and Production (2021) 53: 370
https://doi.org/10.1007/s11250-021-02830-z

REGULAR ARTICLES

Relationship between the seasonal changes in plasma testosterone and thyroxine concentrations with sperm cryoresistance in Gabon bucks

María Noel Viera[1] · Rodolfo Ungerfeld[1] · Rosario Velázquez[2] · Julián Santiago-Moreno[2]

35°SL Gabon breed bucks



It is recommended to freeze sperm of Gabon bucks in the period coinciding with the rise of testosterone concentration, before reaching maximum concentrations.

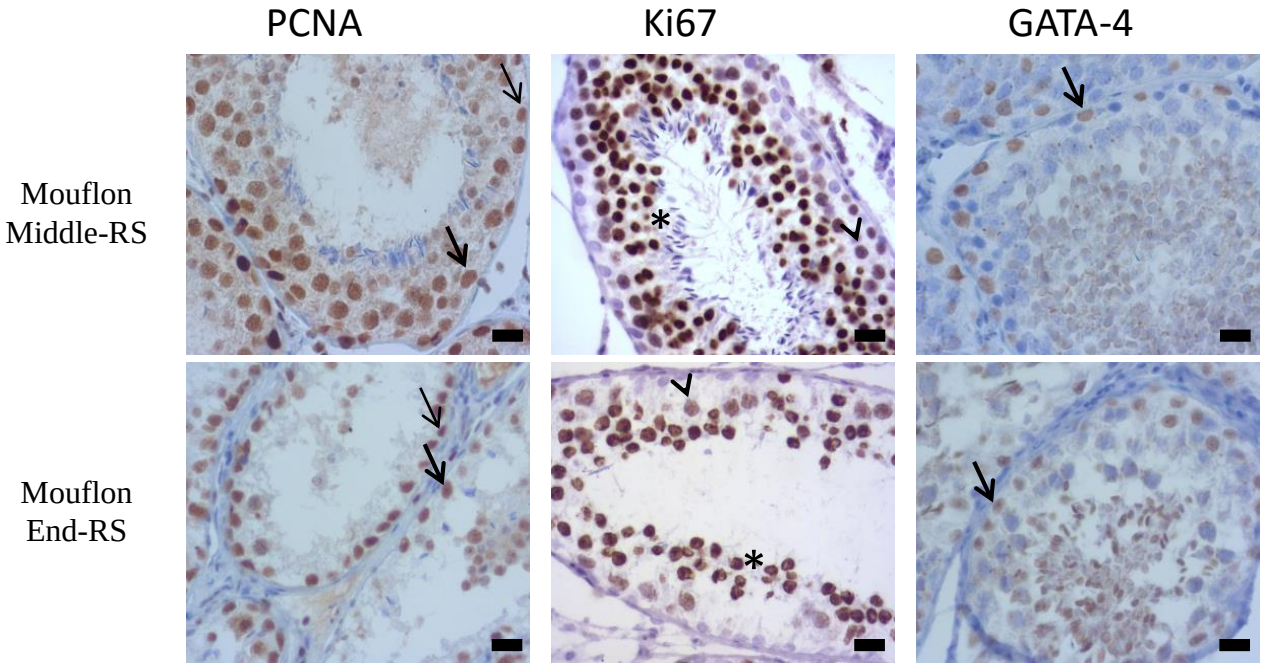
CSIRO PUBLISHING

Reproduction, Fertility and Development
https://doi.org/10.1071/RD18511

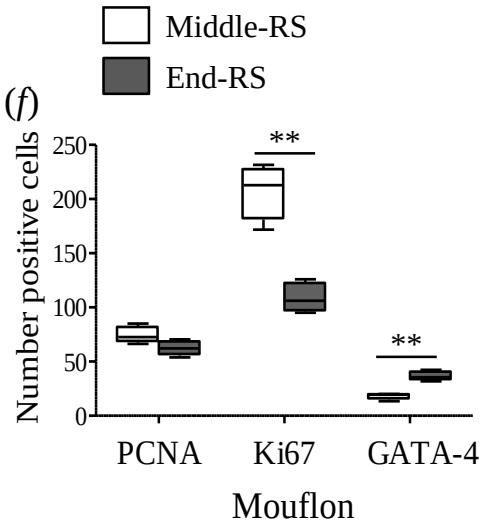
Seasonal variation in sperm freezability associated with changes in testicular germinal epithelium in domestic (*Ovis aries*) and wild (*Ovis musimon*) sheep

Lucía Martínez-Fresneda[A,B,C], *Emma O'Brien*[A], *Rosario Velázquez*[A], *Adolfo Toledano-Díaz*[A], *Carlos M. Martínez-Cáceres*[D], *Dawit Tesfaye*[B], *Karl Schellander*[B], *Francisco A. García-Vázquez*[C,E] and *Julian Santiago-Moreno*[A,F]

Testosterone influences Sertoli and germ cells' proliferative activity during spermatogenesis, which might affect sperm cryotolerance



400x seminiferous tubules Scale bar = 20 µm.



PCNA (proliferating cell nuclear antigen) immunolabeling was quantified in Sertoli cells (thick arrows) and spermatogonias (thin arrows), proliferation marker protein Ki-67 (Ki67) in spermatocytes (arrowheads) and round spermatids (asterisks) and GATA-4 in Sertoli cells (thick arrows). Asterisks indicate statistically significant differences between groups (*P=0.004; **P<0.001; ***P<0.0001).

Received: 10 September 2020 | Revised: 4 February 2021 | Accepted: 4 March 2021
DOI: 10.1111/andr.12998

ORIGINAL ARTICLE

ANDROLOGY WILEY

Influence of circulating testosterone concentration on sperm cryoresistance: The ibex as an experimental model

Paula Bóveda[1] | Milagros Cristina Esteso[1] | Rosario Velázquez[1] | Cristina Castaño[1] | Adolfo Toledano-Díaz[1] | Antonio López-Sebastián[1] | Octavio Mejía[2] | María Gemma Millán de la Blanca[1] | Rodolfo Ungerfeld[3] | Julián Santiago-Moreno[1]

Group (1) 200 mg of cyproterone acetate (CA) intramuscularly diluted in 2 mL of olive oil (vehicle) twice weekly throughout December (coinciding with the period in which testosterone plasma levels were high). Group (2) control.

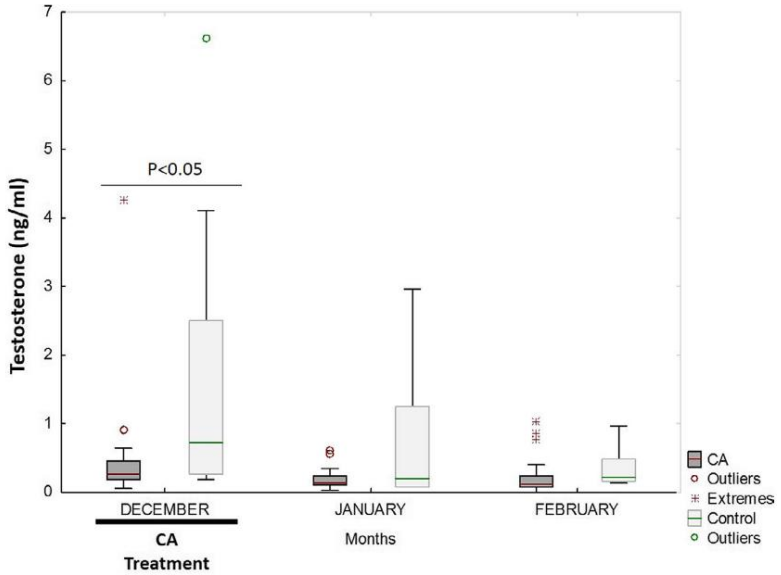


TABLE 2 Cryoresistance ratios (means ±SE) for sperm variables in control and CA-treated animals following the different protocols

	CR slow freezing				CR ultra-rapid freezing			
	Frozen-thawed during treatment		Frozen-thawed after treatment		Frozen-thawed during treatment		Frozen-thawed after treatment	
Sperm variables	Control	CA	Control	CA	Control	CA	Control	CA
Motility	28.5 ± 6.1	54.1 ± 10.5	61.4 ± 13.7	60 ± 10.4	7.2 ± 0.8	6.7 ± 1.6	18.6 ± 4.4	13.2 ± 3
VCL	87.8 ± 17.2	69.3 ± 7.6	95.9 ± 6.2^{a}	65.5 ± 3.5^{b}	45.2 ± 4.9	59.8 ± 11.4	67.1 ± 11.4	53.7 ± 9.3
VSL	117.1 ± 26.6	109.6 ± 24.4	145.3 ± 26	83.8 ± 13	43.9 ± 12.8	50.4 ± 7.6	97.5 ± 25.5	55.2 ± 14.1
VAP	101.4 ± 22.4	87.1 ± 16.1	125.3 ± 22.4	72.1 ± 9.2	41.9 ± 9.5	56.2 ± 10.7	77.4 ± 17.7	48.9 ± 10.8
ALH	87.7 ± 14.4	73.5 ± 5.8	62.2 ± 7.3	77.4 ± 4.4	50.3 ± 18.6	42.6 ± 13	61.3 ± 13.1	46.6 ± 14.9
Viability	48.2 ± 10.8^{b}	91.4 ± 15^{a}	46.4 ± 7.2	46.4 ± 12.3	22.4 ± 8.7^{B}	44.7 ± 5.4^{A}	23.8 ± 3.5	33.4 ± 7.6
Acrosome integrity	60.7 ± 13	75.3 ± 5.9	66.3 ± 6.8	82.8 ± 3.1	52.5 ± 11.2	65 ± 6.9	69 ± 7.2	75.1 ± 5.5
Normal spermatozoa	112.4 ± 39.5	105.6 ± 8	63.8 ± 11.8	111.2 ± 35	97.1 ± 30.4	105.4 ± 7.1	66 ± 13	128.9 ± 35.5

Different letters indicate significant differences ($p < 0.05$) between the control group and the treatment group for slow freezing (lower case letters) and for ultra-rapid freezing (upper case letters).

Abbreviations: ALH, amplitude of lateral head displacement; CA, cyproterone acetate; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

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Group (1) received 25 mg of testosterone propionate (TP) subcutaneously diluted in 2 mL of olive oil (vehicle) twice weekly in January (coinciding with the seasonal fall in plasma testosterone), and Group (2) control.

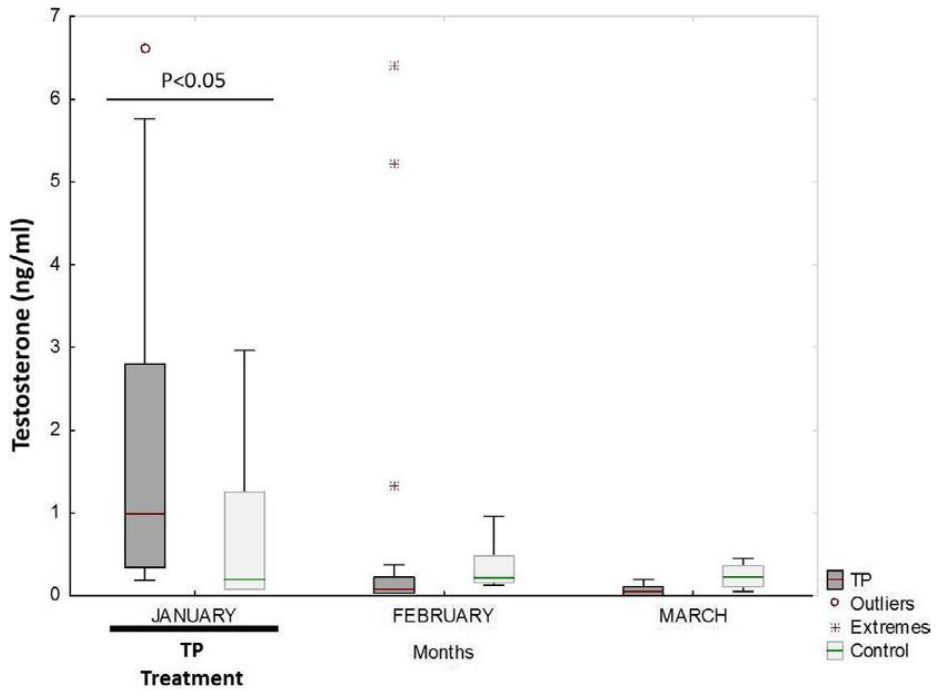

TABLE 5 Cryoresistance ratios (means ±SE) for sperm variables in control and TP-treated animals

	CR slow freezing				CR ultra-rapid freezing			
	Frozen-thawed during treatment		Frozen-thawed after treatment		Frozen-thawed during treatment		Frozen-thawed after treatment	
Sperm variables	Control	TP	Control	TP	Control	TP	Control	TP
Motility	62.9 ± 21.6	40.1 ± 7.3	41.8 ± 11	42.9 ± 6.1	18.3 ± 4.4^{A}	1.9 ± 0.4^{B}	17.9 ± 6.7	11.6 ± 1.5
VCL	96.9 ± 9.1	85.1 ± 22.8	65.7 ± 16.8	90 ± 12.2	67.6 ± 16.3	36.4 ± 12	47.4 ± 14	63.5 ± 7.5
VSL	159.8 ± 38.5	112.5 ± 38.8	76.7 ± 27.3	163.7 ± 40.8	101.9 ± 31.3	34.7 ± 17.1	59.9 ± 31.5	75.2 ± 13.8
VAP	135.2 ± 33.1	92.6 ± 28.6	68.7 ± 22.6	116.6 ± 25.4	85.4 ± 23	27.7 ± 11.4	43.2 ± 18.7	64.2 ± 10.9
ALH	59.2 ± 11.1	62.27 ± 31.2	43.2 ± 14.6	53 ± 10.9	55.1 ± 19.6	19.1 ± 14.2	42.7 ± 18.1	21.2 ± 6
Viability	49.9 ± 10.8	64.3 ± 8.4	47.9 ± 9	67.6 ± 13.1	27.2 ± 4.3	45.8 ± 22.1	18.9 ± 3.3	29.9 ± 3.5
Acrosome integrity	70.1 ± 9.8	60 ± 16.7	66.1 ± 5.8	80.8 ± 3.5	73.8 ± 9.4	49.9 ± 18.3	65.1 ± 8.5	72.3 ± 4
Normal spermatozoa	54.7 ± 14.8	98.8 ± 40.4	126.7 ± 44.6	81.5 ± 6.9	56.1 ± 15.8	94.5 ± 45.1	125.6 ± 48.6	82.5 ± 9

Different letters indicate significant differences ($p < 0.05$) between control and treatment groups for ultra-rapid freezing (upper case letters).
Abbreviations: TP, testosterone propionate; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement.

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Paula Bóveda[1] | Milagros Cristina Esteso[1] | Rosario Velázquez[1] | Cristina Castaño[1] | Adolfo Toledano-Díaz[1] | Antonio López-Sebastián[1] | Octavio Mejía[2] | María Gemma Millán de la Blanca[1] | Rodolfo Ungerfeld[3] | Julián Santiago-Moreno[1]

Our findings support the hypothesis that testosterone negatively influences sperm cryoresistance.

Variations in the sperm cryoresistance related to testosterone concentrations occur during the sperm transit through the epididymis, and during the short exposition of sperm to seminal plasma at the ejaculation.

TABLE 2 Cryoresistance ratios (means ±SE) for sperm variables in control and CA-treated animals following the different protocols

	CR slow freezing				CR ultra-rapid freezing			
	Frozen-thawed during treatment		Frozen-thawed after treatment		Frozen-thawed during treatment		Frozen-thawed after treatment	
Sperm variables	Control	CA	Control	CA	Control	CA	Control	CA
Motility	28.5 ± 6.1	54.1 ± 10.5	61.4 ± 13.7	60 ± 10.4	7.2 ± 0.8	6.7 ± 1.6	18.6 ± 4.4	13.2 ± 3
VCL	87.8 ± 17.2	69.3 ± 7.6	95.9 ± 6.2[a]	65.5 ± 3.5[b]	45.2 ± 4.9	59.8 ± 11.4	67.1 ± 11.4	53.7 ± 9.3
VSL	117.1 ± 26.6	109.6 ± 24.4	145.3 ± 26	83.8 ± 13	43.9 ± 12.8	50.4 ± 7.6	97.5 ± 25.5	55.2 ± 14.1
VAP	101.4 ± 22.4	87.1 ± 16.1	125.3 ± 22.4	72.1 ± 9.2	41.9 ± 9.5	56.2 ± 10.7	77.4 ± 17.7	48.9 ± 10.8
ALH	87.7 ± 14.4	73.5 ± 5.8	62.2 ± 7.3	77.4 ± 4.4	50.3 ± 18.6	42.6 ± 13	61.3 ± 13.1	46.6 ± 14.9
Viability	48.2 ± 10.8[b]	91.4 ± 15[a]	46.4 ± 7.2	46.4 ± 12.3	22.4 ± 8.7[B]	44.7 ± 5.4[A]	23.8 ± 3.5	33.4 ± 7.6
Acrosome integrity	60.7 ± 13	75.3 ± 5.9	66.3 ± 6.8	82.8 ± 3.1	52.5 ± 11.2	65 ± 6.9	69 ± 7.2	75.1 ± 5.5
Normal spermatozoa	112.4 ± 39.5	105.6 ± 8	63.8 ± 11.8	111.2 ± 35	97.1 ± 30.4	105.4 ± 7.1	66 ± 13	128.9 ± 35.5

Different letters indicate significant differences ($p < 0.05$) between the control group and the treatment group for slow freezing (lower case letters) and for ultra-rapid freezing (upper case letters).

Abbreviations: ALH, amplitude of lateral head displacement; CA, cyproterone acetate; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

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Domestic Animal Endocrinology

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Capítulo 1

Influence of testosterone administration at the end of the breeding season on sperm cryoresistance in rams (*Ovis aries*) and bucks (*Capra hircus*)

V.N. Flores-Gil, M.G. Millan de la Blanca, R. Velázquez, A. Toledano-Díaz*, J. Santiago-Moreno, A. López-Sebastián

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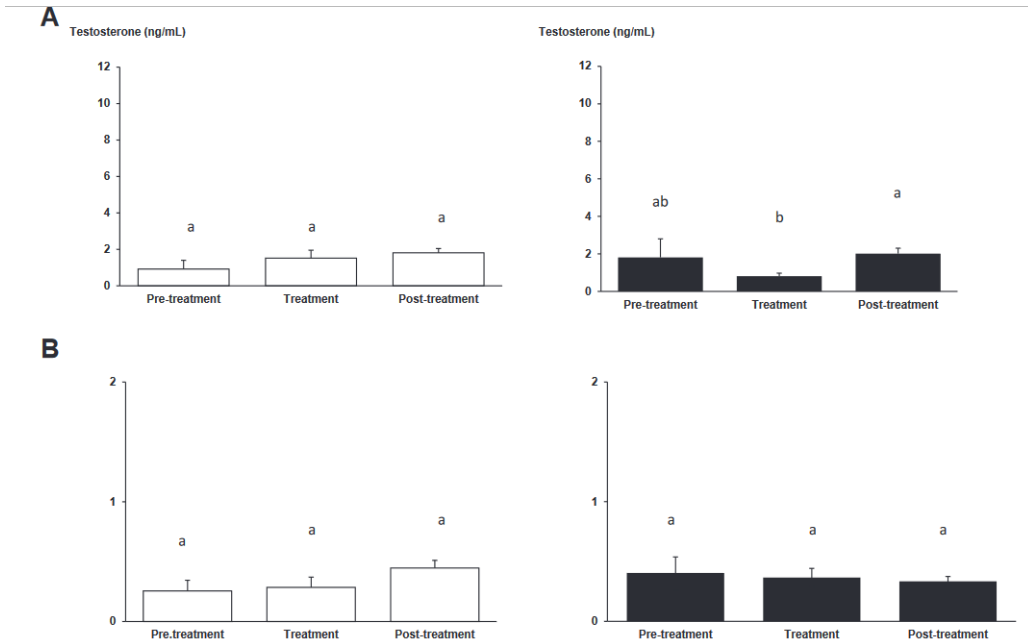

Fig. 1. Changes in blood plasma (A) and seminal plasma (B) testosterone concentrations (ng/mL) (mean ± SEM) in ram controls (open bars; n = 5) and rams treated with 25 mg TP in olive oil (solid bars; n = 5) over pre-treatment, treatment, and post-treatment. Different letters (a and b) indicate differences within a group between periods. TP, testosterone propionate

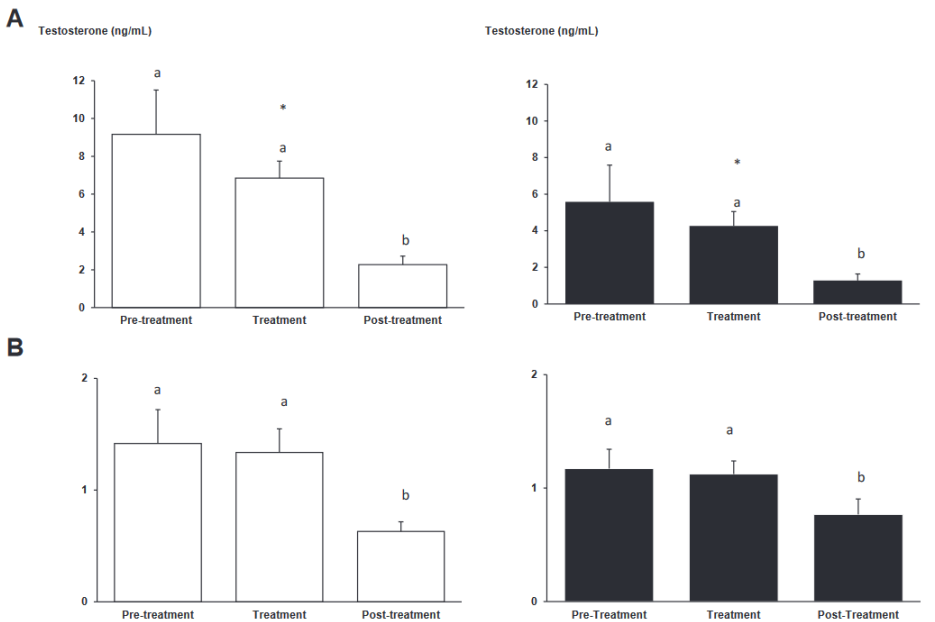

Fig. 2. Changes in blood plasma (A) and seminal plasma (B) testosterone concentrations (ng/mL) (mean ± SEM) in buck controls (open bars; n = 5) and bucks treated with 25 mg TP in olive oil (solid bars; n = 5) over pre-treatment, treatment, and post-treatment. Different letters (a and b) indicate differences within a group between periods. Asterisks indicate differences ($P < 0.05$) between groups. TP, testosterone propionate

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Influence of testosterone administration at the end of the breeding season on sperm cryoresistance in rams (*Ovis aries*) and bucks (*Capra hircus*)

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Table 1
Characteristics of fresh and frozen-thawed ram sperm in control and treated (TP, testosterone propionate) rams (mean ± SE).

Sperm Parameters	Fresh		Frozen-thawed	
	Control	TP	Control	TP
Motile sperm (%)	81.5 ± 2.0^a	72.7 ± 4.0^a	50.4 ± 7.0^A	46.2 ± 6.3^A
Progressive motility (%)	34.6 ± 3.7^a	42.7 ± 2.4^a	20.0 ± 3.2^A	20.8 ± 2.2^A
VCL (μm/s)	140.8 ± 8.0^a	150.7 ± 5.3^a	98.6 ± 5.9^A	113.4 ± 5.5^A
VSL (μm/s)	76.4 ± 7.4^b	101.1 ± 4.4^a	54.4 ± 5.9^B	73.0 ± 4.0^A
VAP (μm/s)	109.5 ± 9.7^a	126.7 ± 5.2^a	75.6 ± 6.0^B	97.0 ± 5.0^A
LIN (%)	53.7 ± 4.4^b	67.3 ± 2.0^a	54.1 ± 2.9^B	64.5 ± 1.8^A
STR (%)	69.5 ± 3.6^b	80.0 ± 1.4^a	70.8 ± 2.2^A	75.3 ± 1.4^A
WOB (%)	76.2 ± 3.8^b	83.9 ± 1.3^a	75.9 ± 1.8^B	85.5 ± 1.0^A
ALH (μm)	4.0 ± 0.2^a	3.7 ± 0.2^a	3.2 ± 0.1^A	2.7 ± 0.1^B
BCF (Hz)	8.0 ± 0.3^a	8.7 ± 0.2^a	7.0 ± 0.4^A	7.4 ± 0.2^A
Viability (%)	92.0 ± 1.0^a	92.0 ± 2.0^a	68.0 ± 6.0^A	68.0 ± 6.0^A
Intact acrosome (%)	98.0 ± 1.0^a	98.0 ± 1.0^a	90.0 ± 3.0^A	83.0 ± 4.0^A
Morphological abnormalities (%)	4.0 ± 1.0^a	10.0 ± 3.0^a	5.0 ± 1.0^A	12.0 ± 3.0^A

Abbreviations: ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; LIN, linearity; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity; WOB, Wobble.
Different lowercase letters indicate significant differences ($P < 0.05$) between control and TP-treated samples for fresh (a and b) and frozen-thawed (A and B) samples.
n = 4 samples/ram collected after the last day of treatment, at intervals of 15 d.

Table 2
Characteristics of fresh and frozen-thawed buck sperm in control and treated (TP, testosterone propionate) bucks (mean ± SE).

Sperm Parameters	Fresh		Frozen-thawed	
	Control	TP	Control	TP
Motile sperm (%)	78.9 ± 2.2^a	76.1 ± 3.1^a	34.4 ± 2.1^A	42.5 ± 2.4^A
Progressive motility (%)	51.7 ± 2.2^a	49.0 ± 3.65^a	22.1 ± 2.8^A	29.28 ± 3.2^A
VCL (μm/s)	152.3 ± 4.4^a	140.2 ± 5.4^a	114.2 ± 3.8^A	118.8 ± 3.9^A
VSL (μm/s)	113.1 ± 4.5^a	104.1 ± 4.9^a	79.8 ± 3.7^B	91.1 ± 3.2^A
VAP (μm/s)	135.6 ± 4.9^a	124.3 ± 5.9^a	95.5 ± 4.0^A	104.9 ± 3.9^A
LIN (%)	73.9 ± 1.6^a	73.9 ± 1.8^a	69.6 ± 2.0^B	76.7 ± 1.1^A
STR (%)	83.3 ± 1.1^a	83.8 ± 1.3^a	83.4 ± 1.3^B	86.9 ± 0.8^A
WOB (%)	88.6 + 1.2^a	88.1 + 1.4^a	83.1 + 1.5^B	88.1 + 0.7^A
ALH (μm)	2.7 ± 0.1^a	2.7 ± 0.1^a	2.8 ± 0.1^A	2.6 ± 0.1^A
BCF (Hz)	9.7 ± 0.2^a	9.4 + 0.1^a	9.2 ± 0.2^A	9.1 ± 0.2^A
Viability (%)	94.0 ± 1.0^a	95.0 ± 0.0^a	59.0 ± 6.0^A	67.0 ± 4.0^A
Intact acrosome (%)	98.0 ± 0.0^a	98.0 ± 0.0^a	71.0 ± 4.0^A	74.0 ± 4.0^A
Morphological abnormalities (%)	3.0 + 1.0^a	3.0 + 1.0^a	3.0 + 1.0^A	3.0 + 0.0^A

Abbreviations: ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; LIN, linearity; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity; WOB, Wobble.
Different lowercase letters indicate significant differences ($P < 0.05$) between control and TP-treated samples for fresh (a and b) and frozen-thawed (A and B) samples.
n = 4 samples/buck collected after the last day of treatment, at intervals of 15 d.

Administration of testosterone at the end of the rutting season improves the sperm quality in rams and the sperm cryoresistance in rams and bucks

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In vitro supplementation of testosterone or prolactin affects spermatozoa freezability in small ruminants

L. Martínez-Fresneda[a,b,c], E. O'Brien[a], A. López Sebastián[a], R. Velázquez[a], A. Toledano-Díaz[a], D. Tesfaye[b], K. Schellander[b], F.A. García-Vázquez[c,d], J. Santiago-Moreno[a,*]

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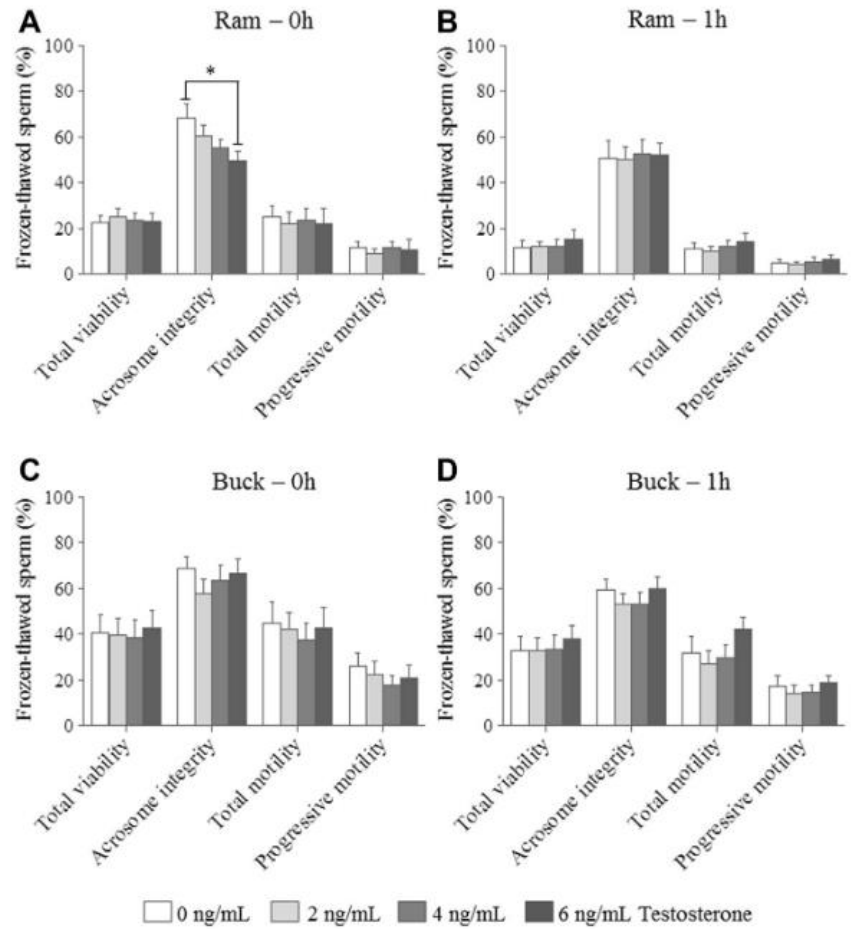

Fig. 1. Frozen-thawed quality parameters of ram (A and B) and buck (C and D) sperm cryopreserved at time 0 h and after 1 h incubation with 0, 2, 4, or 6 ng/mL of testosterone (Exp. 1). Data are expressed as mean + SEM (*$P < 0.05$).

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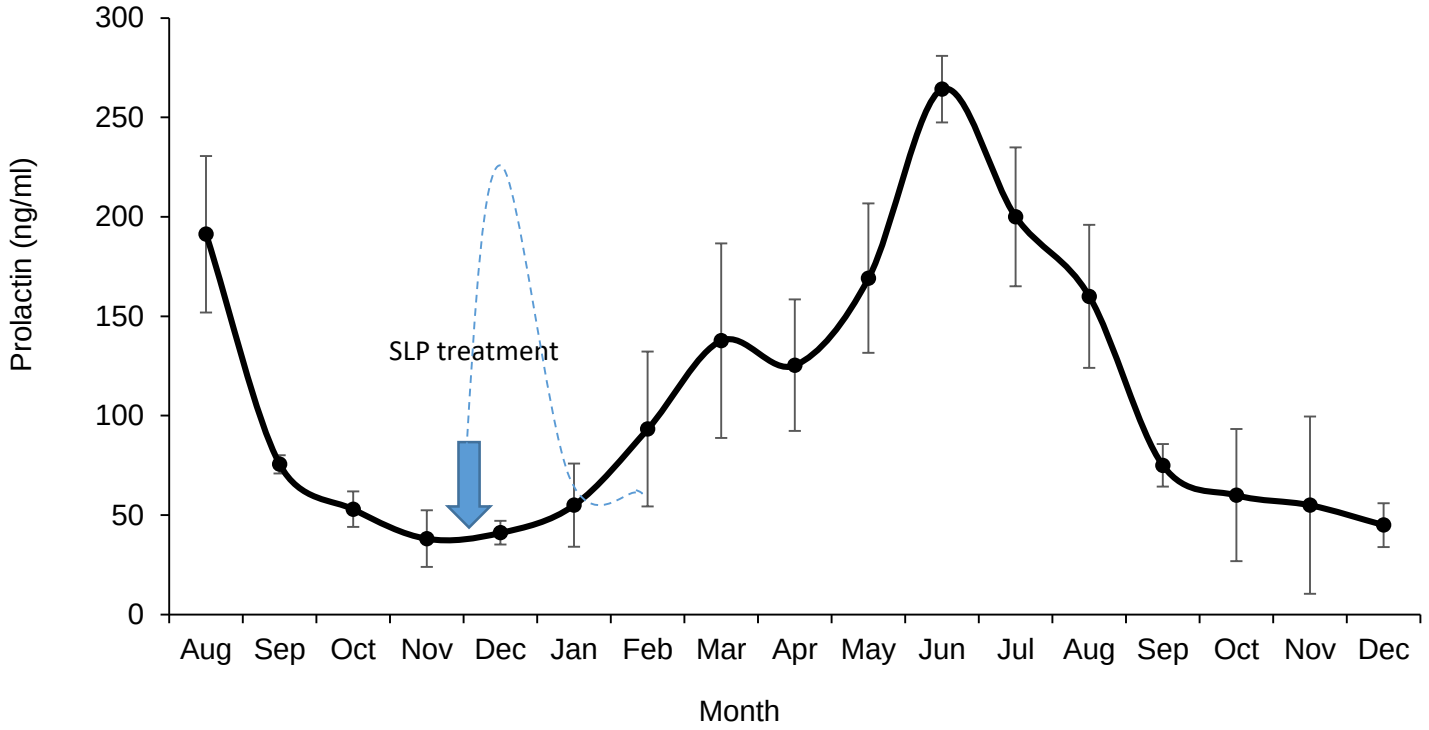
Sulpiride (SLP) treatment (100 mg) s.c. daily administered from December 15th to January 15th.

Influence of Prolactin Secretion Changes on Sperm Head Size and Freezability in Ibex and Mouflon

Paula Bóveda Gómez,[1] Rosario Velázquez,[1] Lucía Martínez-Fresneda,[1] Octavio Mejía,[2] Marta Oteo,[3] Adolfo Toledano-Díaz,[1] Cristina Castaño,[1] Milagros Cristina Esteso,[1] Rodolfo Ungerfeld,[4] Antonio López-Sebastián,[1] and Julian Santiago-Moreno[1]



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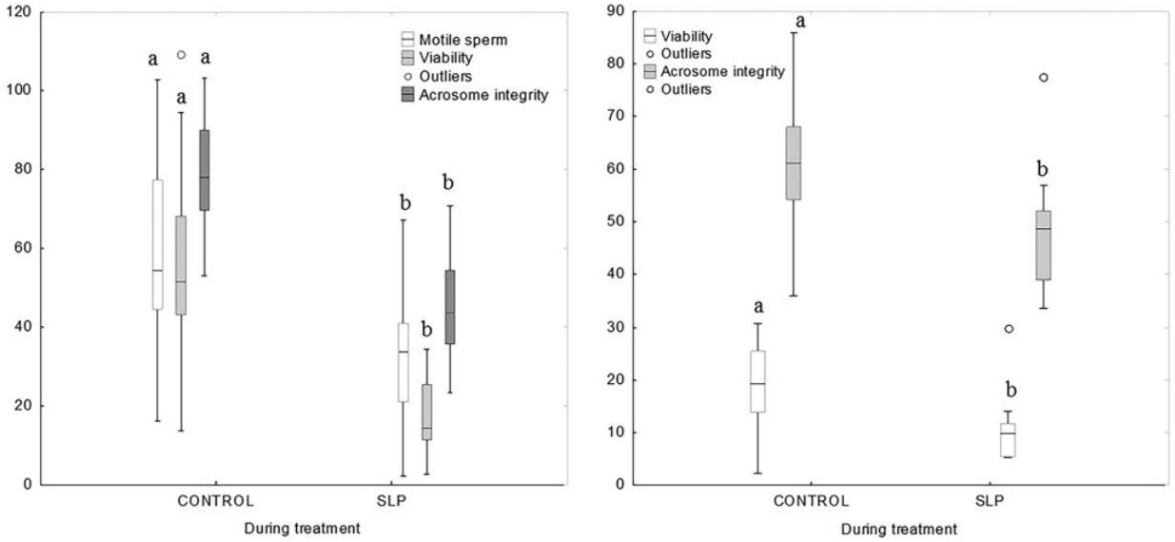

FIG. 3. Cryoresistance ratios in control and SLP-treated mouflons, after slow-freezing (*left*) and ultra-rapid freezing (*right*). *Different letters* indicate differences ($p < 0.05$) between the control and treatment group.

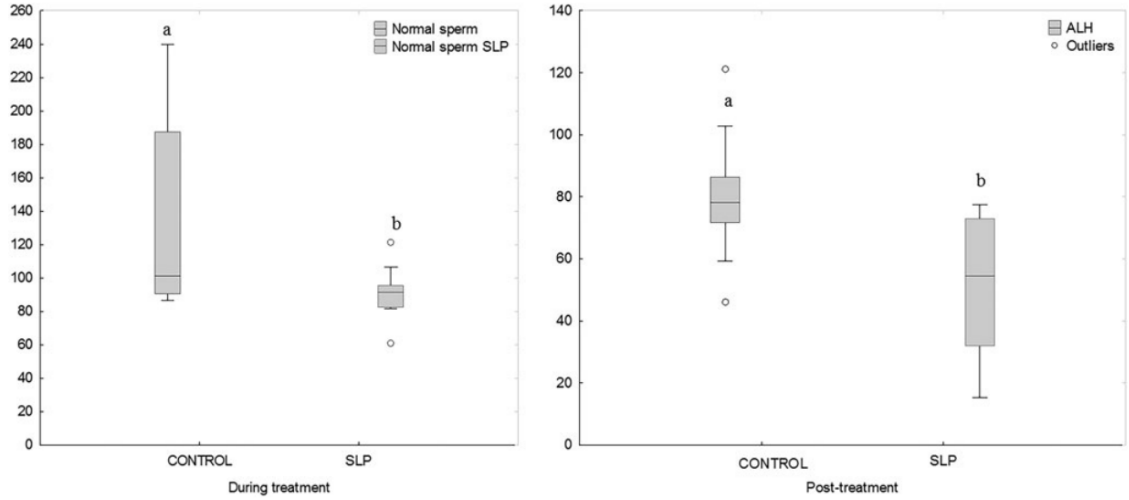

FIG. 2. Cryoresistance ratios in control and SLP-treated ibexes, after slow-freezing (*left*) and ultra-rapid freezing (*right*). *Different letters* indicate differences ($p < 0.05$) between the control and treatment group. ALH, amplitude of lateral head displacement.

Sulpiride (SLP) treatment (daily administered to s.c from December 15th to January 15th) which increased blood plasma PRL concentrations–induced a negative influence on sperm freezability.

WILD RUMINANTS

It is not recommended to freeze sperm at the beginning of the rutting season (autumn), coinciding with maximum testosterone concentrations.

We recommend to freeze sperm around the end of the rutting season (i.e. towards the end of autumn and beginning of winter) → spermatozoa are still preserving good quality; **testosterone** levels have already decreased; **prolactin** concentrations remain low.

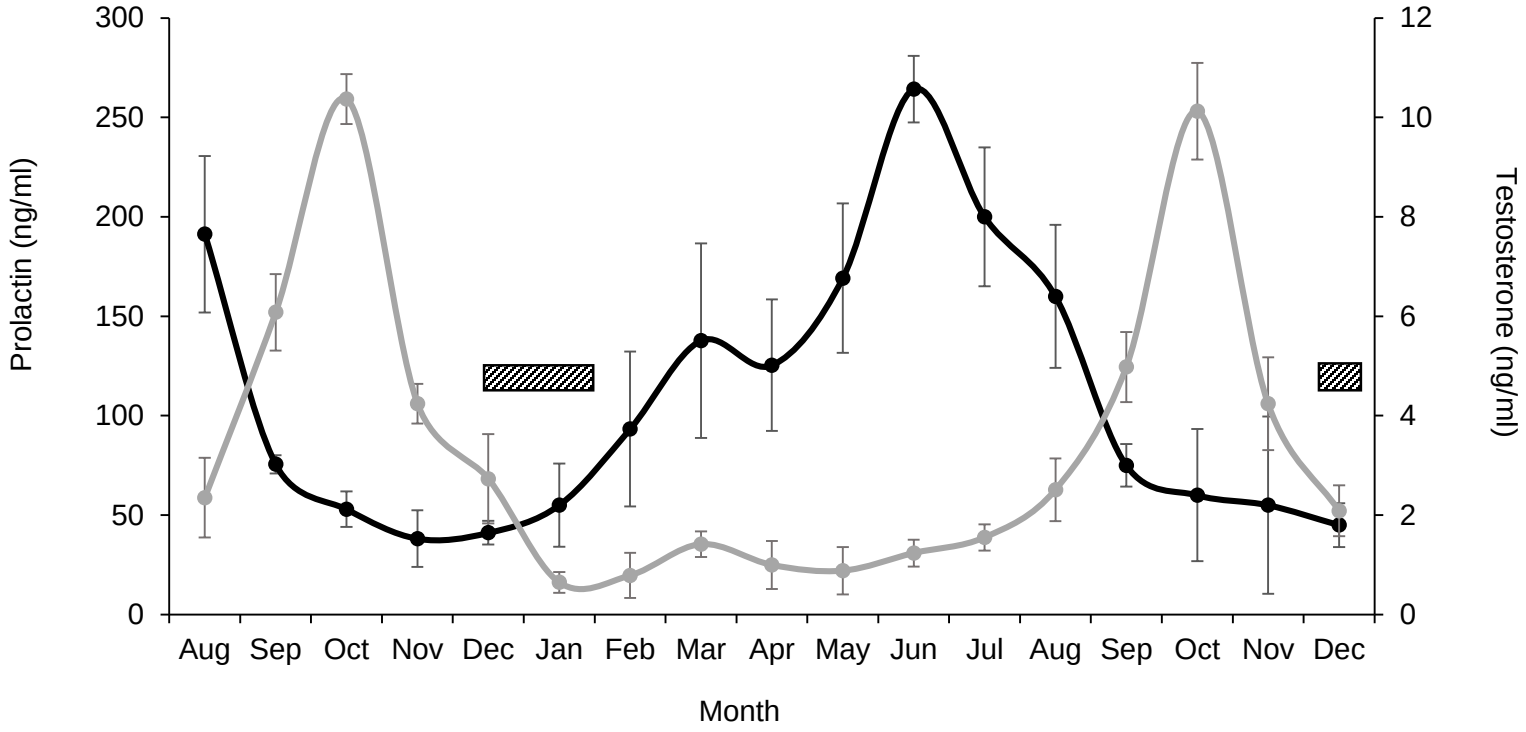

Figure : Monthly variation (mean ± SE) in testosterone (grey line) and prolactin (black line) concentration in mouflon as a model of wild ruminant species. The bar indicates the best period of the year to cryopreserve spermatozoa

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Role of changes in plasma prolactin concentrations on ram and buck sperm cryoresistance

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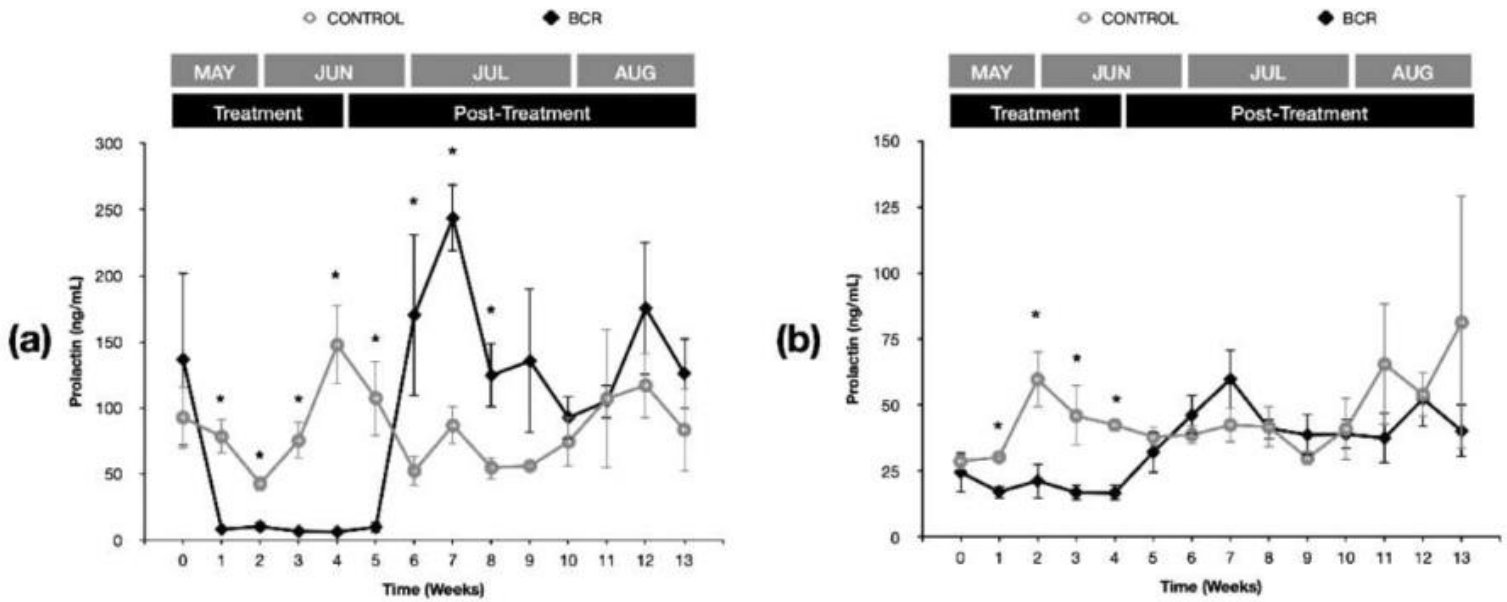

Fig. 1. Weekly change in plasma prolactin concentration (ng/mL; means ± SEM) in rams (a) and bucks (b) during the experimental period; control group (○), bromocriptine (BCR) group (●). Asterisks indicate differences ($P < 0.05$, ANOVA) between groups.

Table 2
Ram and buck sperm cryoresistance ratios - control and bromocriptine (BCR) groups (mean ± S.E.).

CR BCR	RAMS		BUCKS	
	CONTROL	BCR	CONTROL	BCR
Motile sperm (%)	64.08 ± 7.18	75.59 ± 6.44	53.49 ± 8.14	51.08 ± 5.64
Progressive motility (%)	78.09 ± 7.84	88.70 ± 9.70	64.49 ± 14.88	61.25 ± 11.39
VCL (μm/s)	63.60 ± 2.41	72.95 ± 2.42*	68.29 ± 3.46	68.93 ± 5.00
VSL (μm/s)	81.81 ± 6.73	90.26 ± 9.10	76.04 ± 4.76	77.20 ± 8.69
VAP (μm/s)	71.97 ± 3.85	79.70 ± 5.51	71.15 ± 4.33	73.31 ± 7.48
LIN (%)	128.44 ± 8.82	121.90 ± 10.07	112.63 ± 7.29	109.02 ± 7.23
STR (%)	111.98 ± 3.93	110.12 ± 4.79	108.04 ± 6.32	104.02 ± 3.43
WOB (%)	113.01 ± 3.72	108.28 ± 5.12	104.16 ± 3.12	103.94 ± 4.30
ALH (μm)	57.24 ± 3.14	73.76 ± 4.76**	71.57 ± 6.20	74.93 ± 5.70
BCF (Hz)	96.97 ± 3.67	88.48 ± 2.17	90.86 ± 5.81	87.77 ± 3.87
Viability (%)	56.05 ± 5.78	58.18 ± 4.47	47.25 ± 7.88	46.00 ± 6.24
Intact acrosome (%)	96.09 ± 2.36	95.93 ± 1.90	85.17 ± 3.83	85.71 ± 3.84
Morphological abnormalities (%)	105.77 ± 25.54	92.90 ± 10.94	85.00 ± 23.63	84.72 ± 41.36

Cryoresistance ratio (CR) = (Post-thaw value / Fresh value) x 100. Asterisks indicate significant differences (*$P<0.05$, **$P<0.01$, ANOVA) between the control and BCR groups within each species.

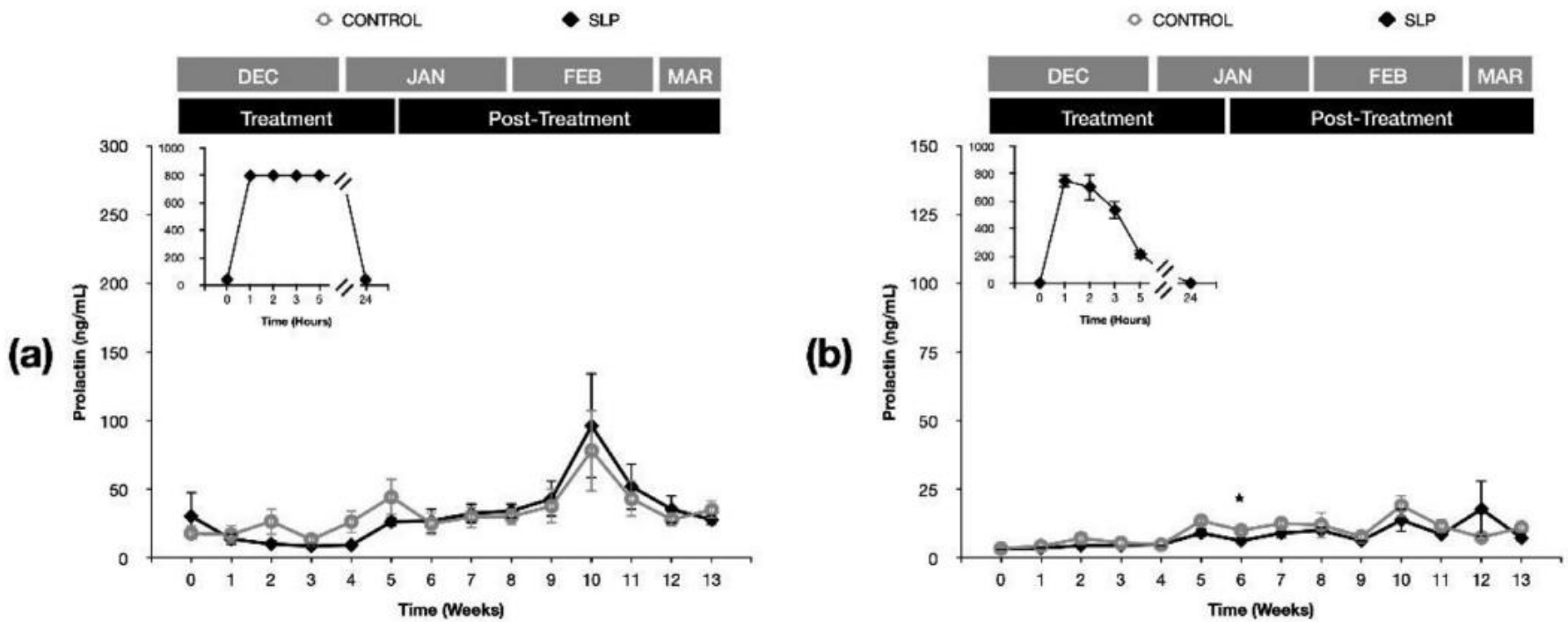

Fig. 2. Weekly change in plasma prolactin concentration (ng/mL; means ± SEM) in rams (a) and bucks (b) during the experimental period; control group (○), sulpiride (SLP) group (●). Asterisks indicate differences ($P < 0.05$, ANOVA) between groups. *Inserted graph*: Change in plasma PRL over the first 5 h and at 24 h following SLP administration.

Table 6
Ram and buck sperm cryoresistance ratios - control and sulpiride (SLP) groups (mean ± S.E.).

CR SLP	RAMS		BUCKS	
	CONTROL	SLP	CONTROL	SLP
Motile sperm (%)	67.19 ± 8.22	79.26 ± 7.16	54.67 ± 8.93	57.42 ± 9.46
Progressive motility (%)	96.86 ± 15.27	57.18 ± 6.27*	67.05 ± 12.62	60.54 ± 9.08
VCL (μm/s)	72.07 ± 3.69	72.47 ± 3.08	64.65 ± 5.66	69.68 ± 4.06
VSL (μm/s)	93.46 ± 6.60	65.10 ± 5.93**	71.04 ± 5.66	71.38 ± 4.03
VAP (μm/s)	79.60 ± 4.79	66.59 ± 4.06*	66.33 ± 5.66	69.33 ± 4.21
LIN (%)	129.16 ± 6.65	88.31 ± 5.37**	111.97 ± 8.00	102.72 ± 3.43
STR (%)	116.70 ± 3.49	96.07 ± 3.74**	107.76 ± 4.17	103.20 ± 2.09
WOB (%)	109.98 ± 2.92	91.13 ± 2.53**	103.18 ± 3.99	99.38 ± 1.72
ALH (μm)	73.08 ± 2.49	95.48 ± 3.73**	82.86 ± 11.56	85.12 ± 3.76
BCF (Hz)	93.11 ± 2.02	84.12 ± 2.69*	93.57 ± 3.88	89.54 ± 2.80
Viability (%)	54.86 ± 7.79	65.17 ± 5.33	37.99 ± 8.46	37.30 ± 8.12
Intact acrosome (%)	92.58 ± 3.11	$94.67 \pm 2.38_A$	80.03 ± 5.35	$75.60 \pm 9.59_B$
Morphological abnormalities (%)	122.06 ± 20.55	116.57 ± 12.69	152.38 ± 43.49	116.67 ± 17.57

Cryoresistance ratio (CR) = (Post-thaw value / Fresh value) x 100.
Asterisks indicate significant differences (*$P<0.05$, **$P<0.01$, one way ANOVA) between the control and SLP groups within each species. Different capital letter indicate significant differences ($P<0.05$) between species.

In conclusion, high levels of PRL in rams exert a negative effect on sperm cryoresistance. Reducing PRL levels by BCR near the summer solstice when the seasonal PRL secretion is maximum, leads to an improvement in ram sperm quality and freezability. Buck sperm characteristics and cryoresistance, however, seem to be unaffected.

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[d] Biomedical Research Institute of Murcia (IMIB-Arrixaca), University of Murcia, 30120 Murcia, Spain

*Fresh sperm samples diluted in **Tyrode medium** PRL. Incubation: 38.5ºC, 5%CO2 and humidified atmosphere prior its cryopreservation. Tyrode medium: NaCl 120 mM, KCl 1 mM, $CaCl_2$ 2 mM, $MgSO_4$ 0.4 mM, Hepes 16.6 mM, sodium lactate 21.7 mM, sodium pyruvate 0.11 g/L, glucose 5.5 mM and BSA 5 g/L (pH 7.5;290 mOsm/kg).*

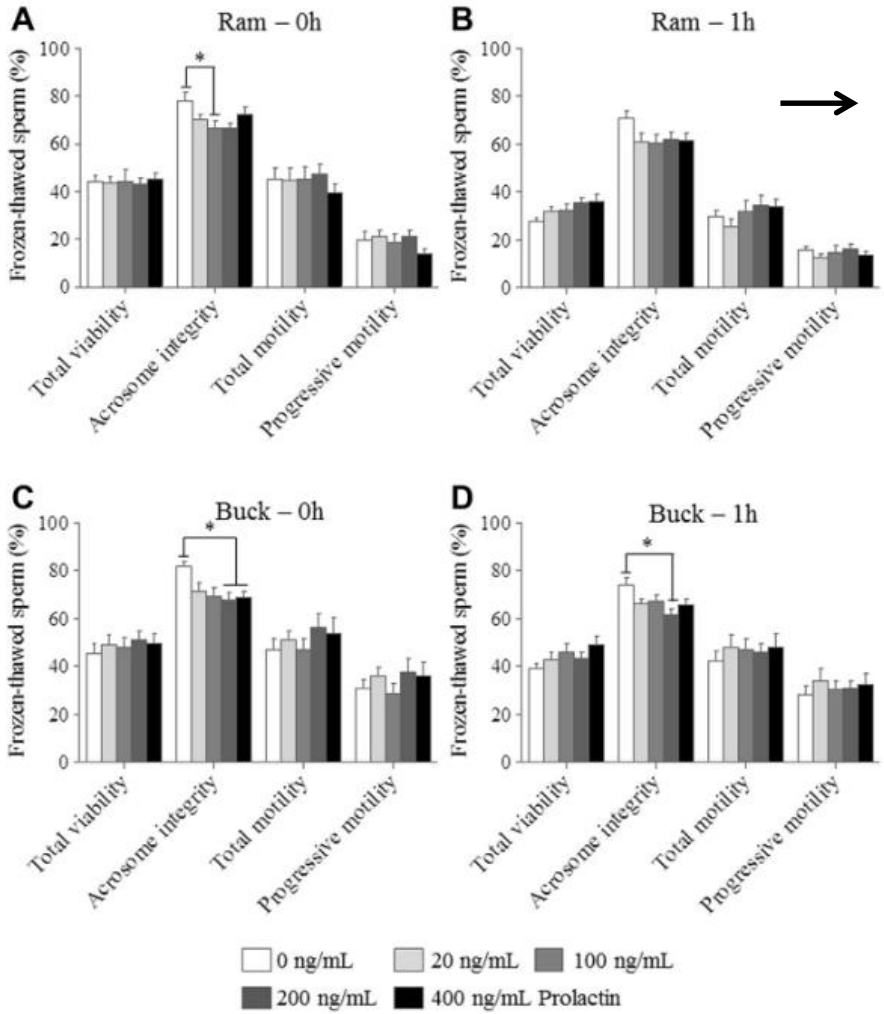
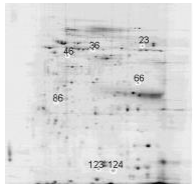


Fig. 2. Frozen-thawed quality parameters of ram (A and B) and buck (C and D) sperm cryopreserved at time 0 h and after 1 h incubation with 0, 20, 100, 200, or 400 ng/mL of prolactin (Exp. 2). Data are expressed as mean ± SEM (*$P < 0.05$).

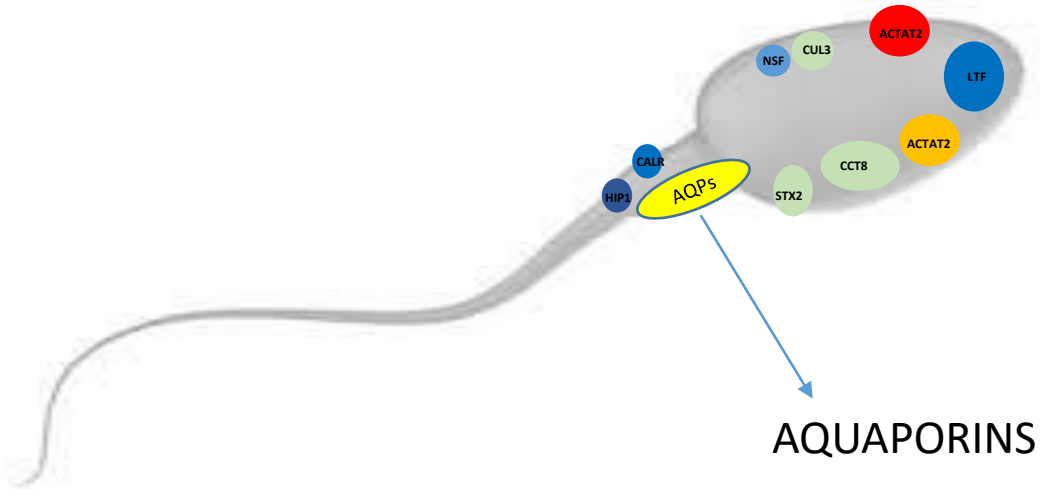
Prolactin also increased the post-thaw amplitude of lateral head displacement (ALH) in ram sperm (from 3.3 ± 0.1 µm to 3.8 ± 0.2 µm; $P < 0.05$).

Prolactin decreased the post-thaw acrosome integrity of ram and buck sperm.

Sperm cryoresistance is higher at the end of rutting season



Looking for proteins differently expressed in sperm at the end and at the middle of rutting season



AQUAPORINS

AQUAPORINS

- Membrane channels for the selective passage of water.
- Identification (AQP1) in erythrocytes and renal proximal tubule in mammals.
- Functional characterization in African clawed frog oocytes.

Peter Agre 2003 Nobel Prize in Chemistry for discoveries concerning channels in cell membranes.

Since then, identification of several types of aquaporins (AQP0, AQP2, AQP3... AQP11) in different cell types with involvement in physiological processes (e.g. kidney, intestinal function, cellular metabolism, immune system) and pathological processes (e.g. glaucoma, tumor metastasis)

Aquaglyceroporins (AQP3, AQP7, AQP9, AQP10) are membrane proteins responsible for the transport of water and solutes such as glycerol. These water channels are crucial to regulate sperm volume during freezing-thawing processes and, therefore, they are involved in the functional sperm response to cryopreservation.

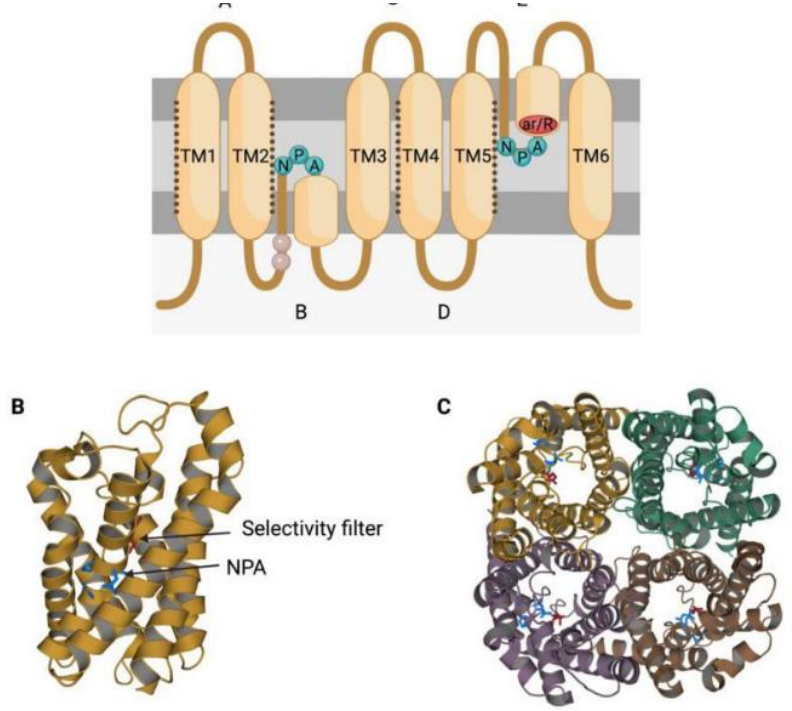


Figure 1. *Structural characteristics of the family of aquaporins (AQPs) (Delgado-Bermudez et al. Animals 2022, 12(5), 573).*

International Journal of
Molecular Sciences

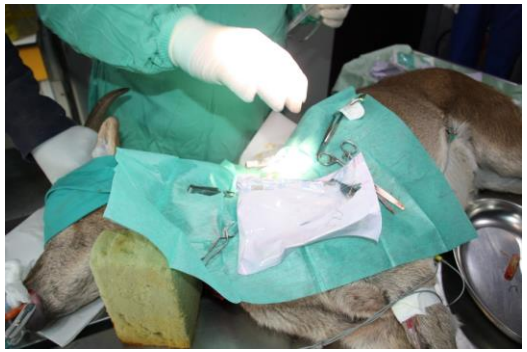
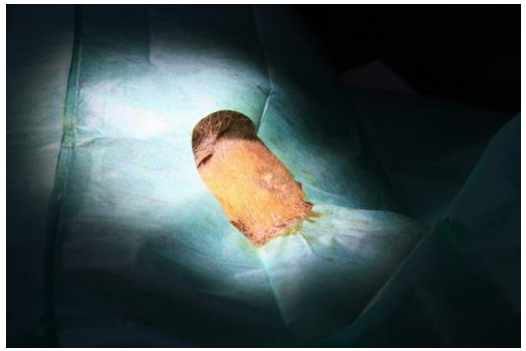
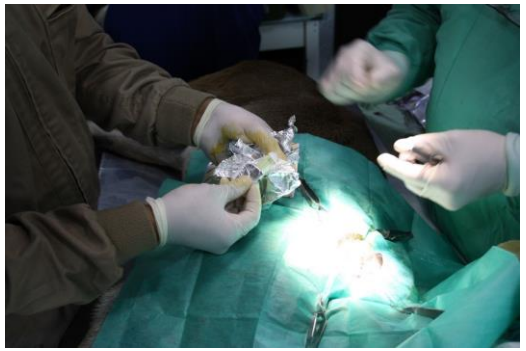
MDPI

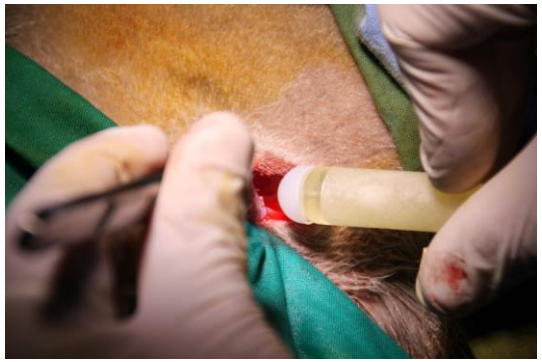
Article

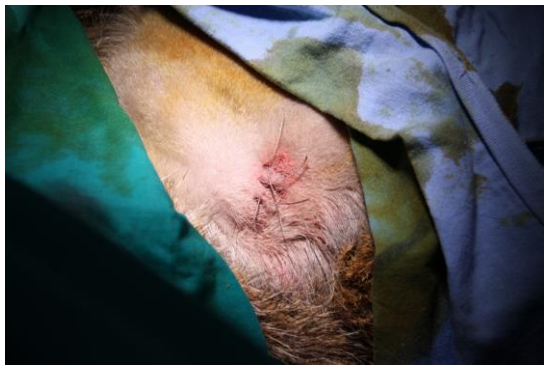
Expression of Aquaglyceroporins in Spermatozoa from Wild Ruminants Is Influenced by Photoperiod and Thyroxine Concentrations

Julián Santiago-Moreno [1,*], Belén Pequeño [1], Belen Martinez-Madrid [2], Cristina Castaño [1], Paula Bóveda [1], Rosario Velázquez [1], Adolfo Toledano-Díaz [1], Manuel Álvarez-Rodríguez [3,†] and Heriberto Rodríguez-Martínez [3,†]

Thyroxine and photoperiod signals were modified during the periods in which testosterone secretion is high and basal.





International Journal of
Molecular Sciences

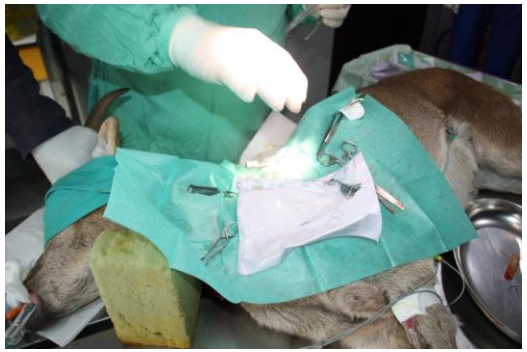
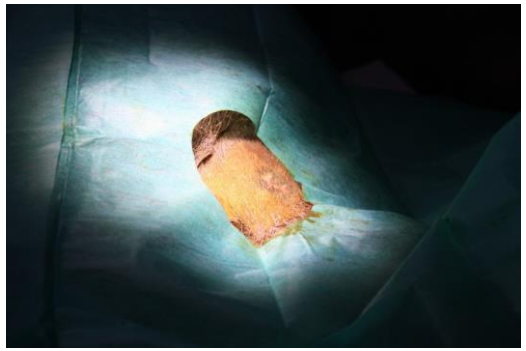
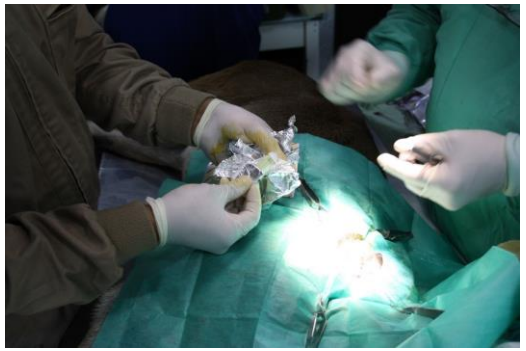
MDPI

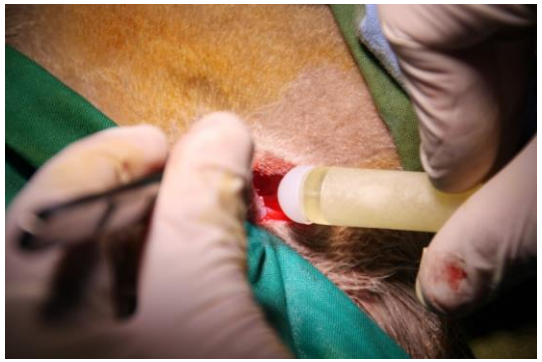
Article

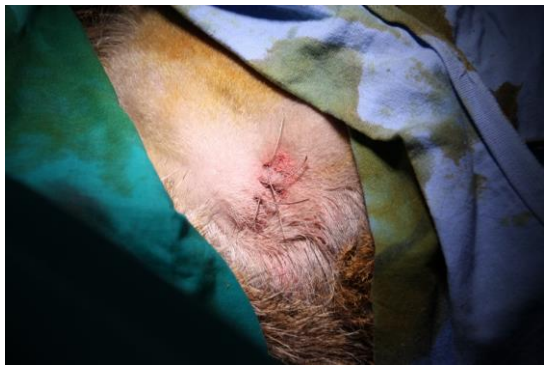
Expression of Aquaglyceroporins in Spermatozoa from Wild Ruminants Is Influenced by Photoperiod and Thyroxine Concentrations

Julián Santiago-Moreno [1,*], Belén Pequeño [1], Belen Martinez-Madrid [2], Cristina Castaño [1], Paula Bóveda [1], Rosario Velázquez [1], Adolfo Toledano-Díaz [1], Manuel Álvarez-Rodríguez [3,†] and Heriberto Rodríguez-Martínez [3,†]

Melatonin group received subcutaneous melatonin implants, on the winter solstice. This provided a continuous short day-signal from the winter solstice onward that stimulated reproductive activity.





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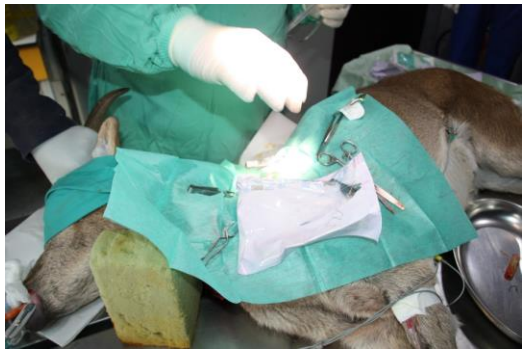
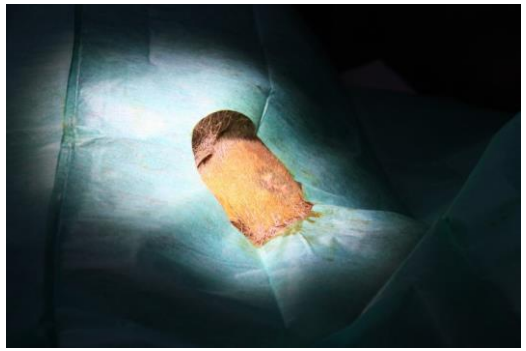
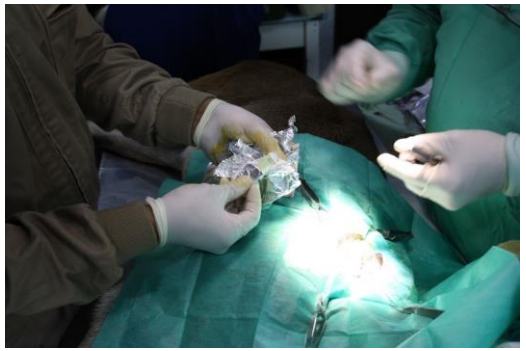
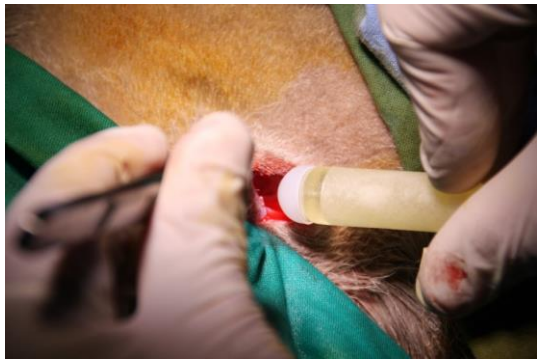
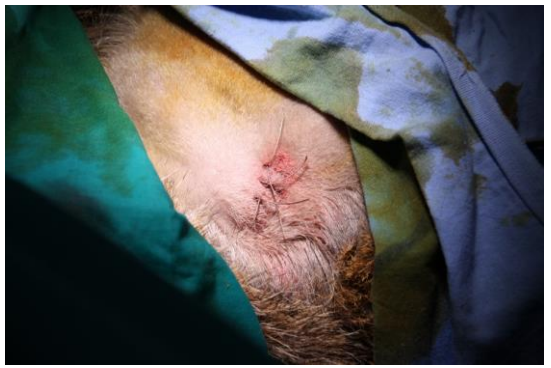
Implantation of osmotic pumps (2 mL Alcet®), under anesthesia, in the lateral shoulder to increase plasma concentrations of thyroxine; no pathological hyperthyroidism was induced.





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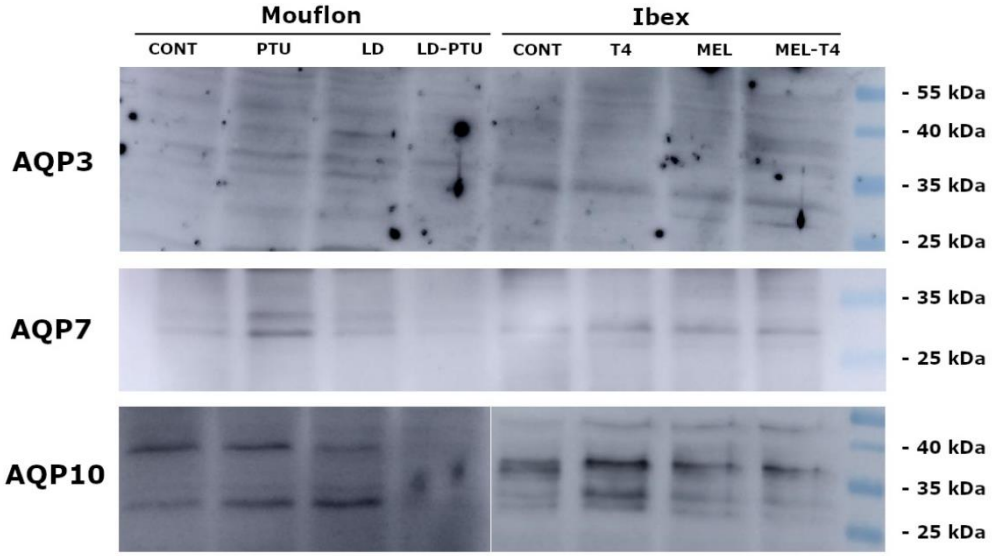
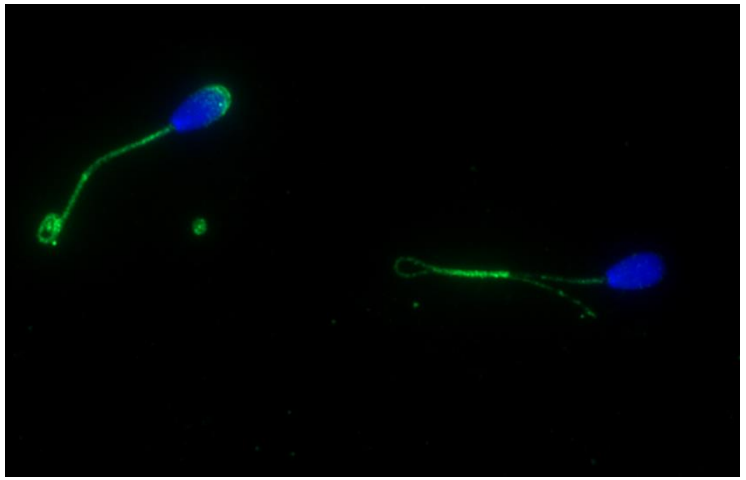
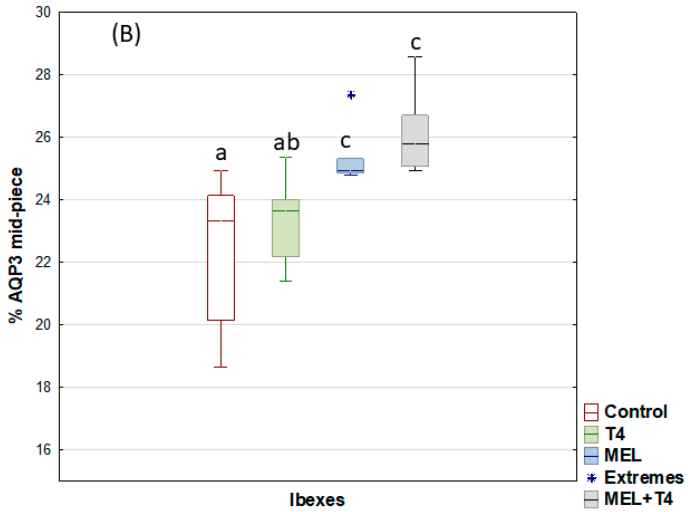
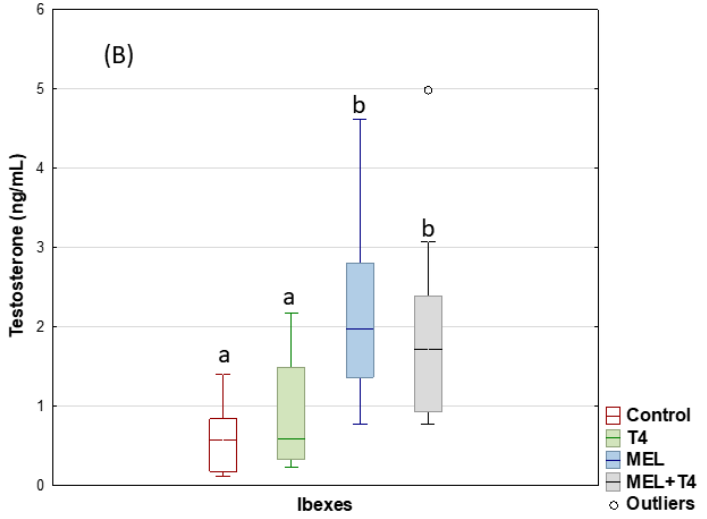




Immunolabelling of AQP3 (located in different regions: acrosome, post acrosomal region, midpiece, principal piece and the end piece of the tail) in ibex sperm.



AQP 3 expression by ICC in mid-piece (B) of ibex sperm of the following groups: Control (red), treated with thyroxin (T4, green), treated with melatonin implants (MEL, blue), and treated with melatonin implants plus thyroxin (MEL+T4, grey)



Plasma testosterone (B) concentrations in ibexes of the following groups: Control (red), treated with thyroxin (T4, green), treated with melatonin implants (MEL, blue), and treated with melatonin implants plus thyroxin (MEL+T4, grey).

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Animal Reproduction - Theriogenology,

Variation of existence and location of aquaporin 3 in relation to cryoresistance of ram spermatozoa

Belén Pequeño[1], Cristina Castaño[1], Manuel Alvarez-Rodriguez[1], Paula Bóveda[1], María Gemma Millán de la Blanca[2], Adolfo Toledano-Díaz[1], Diego Andres Galarza[2], Heriberto Rodriguez-Martinez[3], Belén Martínez-Madrid[4] and Julián Santiago-Moreno[1]*

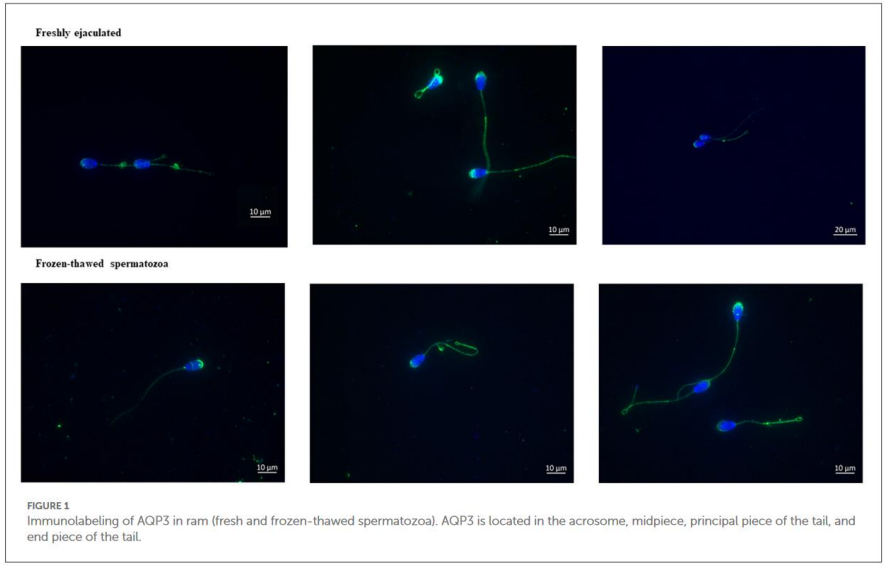

FIGURE 1
Immunolabeling of AQP3 in ram (fresh and frozen-thawed spermatozoa). AQP3 is located in the acrosome, midpiece, principal piece of the tail, and end piece of the tail.

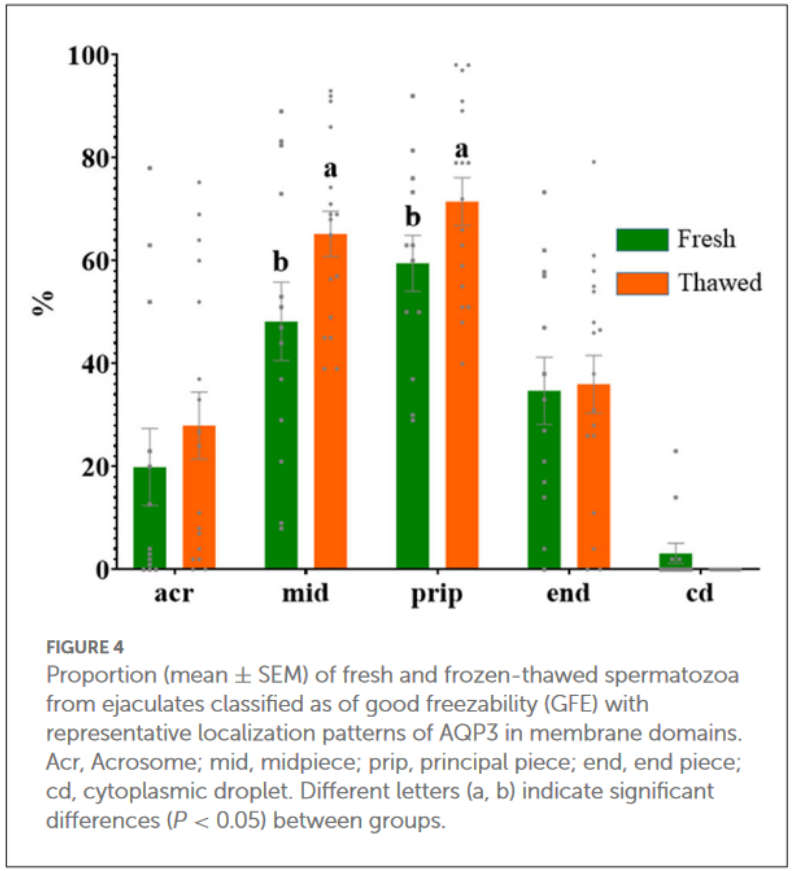

FIGURE 4
Proportion (mean ± SEM) of fresh and frozen-thawed spermatozoa from ejaculates classified as of good freezability (GFE) with representative localization patterns of AQP3 in membrane domains. Acr, Acrosome; mid, midpiece; prip, principal piece; end, end piece; cd, cytoplasmic droplet. Different letters (a, b) indicate significant differences ($P < 0.05$) between groups.

AQP3 relocalisation could be linked to an increase the osmo-adaptative capacity of ejaculates with better capacity to withstand freeze-thawing processes.

Theriogenology 223 (2024) 29–35

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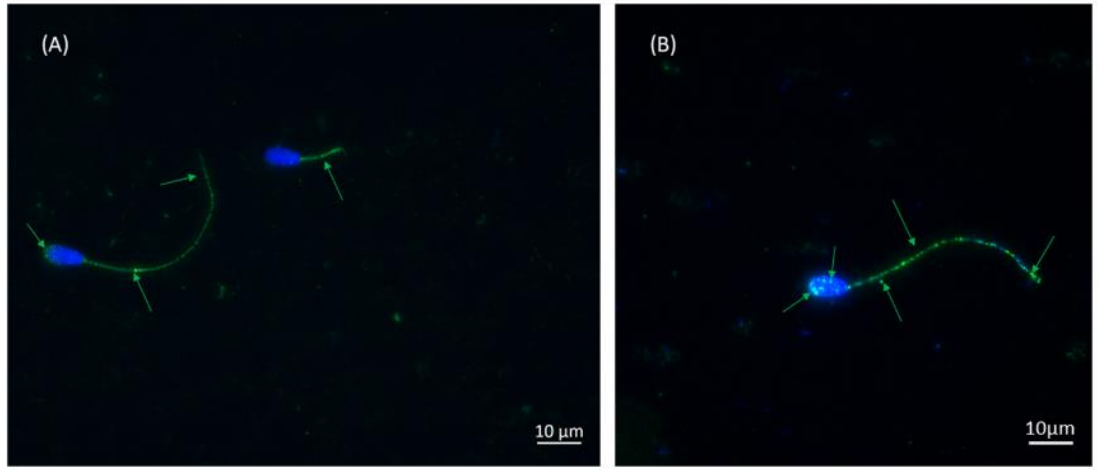
Original Research Article

Cooling rate modifies the location of aquaporin 3 in spermatozoa of sheep and goat

Belén Pequeño [a], María Gemma Millán de la Blanca [a], Cristina Castaño [a], Adolfo Toledano-Díaz [a], Milagros Cristina Esteso [a], Esther Alba [a], Francisco A. Arrebola [b], Rodolfo Ungerfeld [c], Belén Martínez-Madrid [d], Manuel Alvarez-Rodriguez [a], Heriberto Rodriguez-Martinez [e], Julián Santiago-Moreno [a,*]



Immunolabelling of AQP3 located in acrosome, post-acrosomal region, mid-piece, principal piece, and end-piece in buck spermatozoa (similarly to ram).

Fig. 3. Immunolabeling of AQP3 in conventional and ultra-rapid freezing-thawing. Immunolabeling of AQP3 located in the acrosome, post-acrosomal region, mid-piece, principal piece, and end piece in buck spermatozoa (shown with arrows).

Cooling rate modifies the location of aquaporin 3 in spermatozoa of sheep and goat

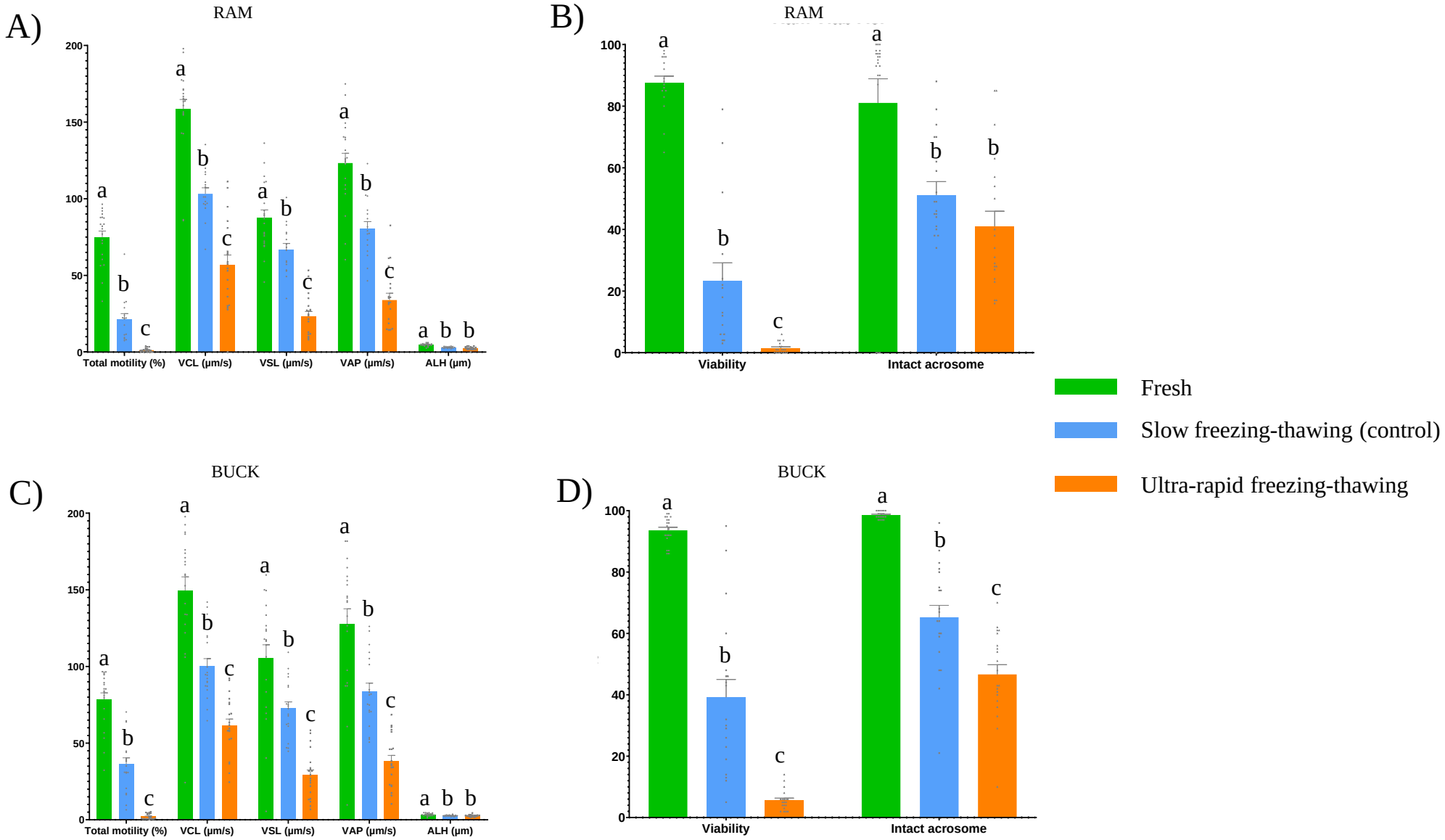


Fig 1. Sperm quality variables (mean±SEM) of Murciano-Granadina buck and Merino ram semen samples. Motility and kinematics variables in rams (A) and bucks (C). Percentage of viable spermatozoa and spermatozoa with acrosome integrity in rams (B) and bucks (D). Different letters (a, b, b) indicate significant differences ($p < 0.01$) between cryopreservation methods for each sperm variable. Curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), amplitude of lateral head (ALH). Results are expressed as mean ± SEM.ca

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The proportion of spermatozoa showing immunolabeling of AQP3 in post-acrosome, mid-piece, and principal piece were greater after slow freezing-thawing than after ultra-rapid freezing-thawing in both ram and buck sample

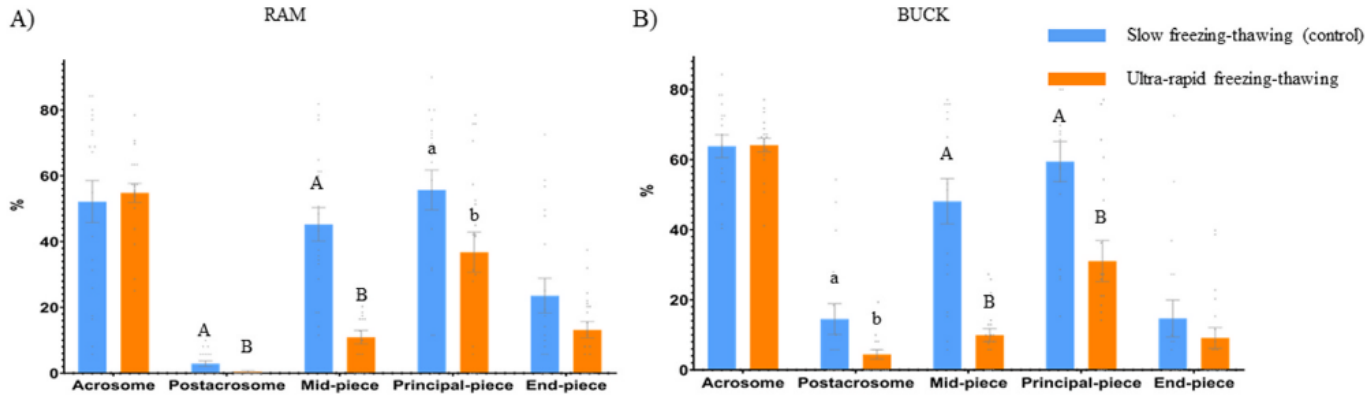

Fig. 4. Proportion (mean ± SEM) of fresh and frozen-thawed spermatozoa showing AQP3 in different membrane domains of spermatozoa. Different letters indicate significant differences (A-B $p < 0.01$, a-b $p < 0.05$) between cryopreservation methods.

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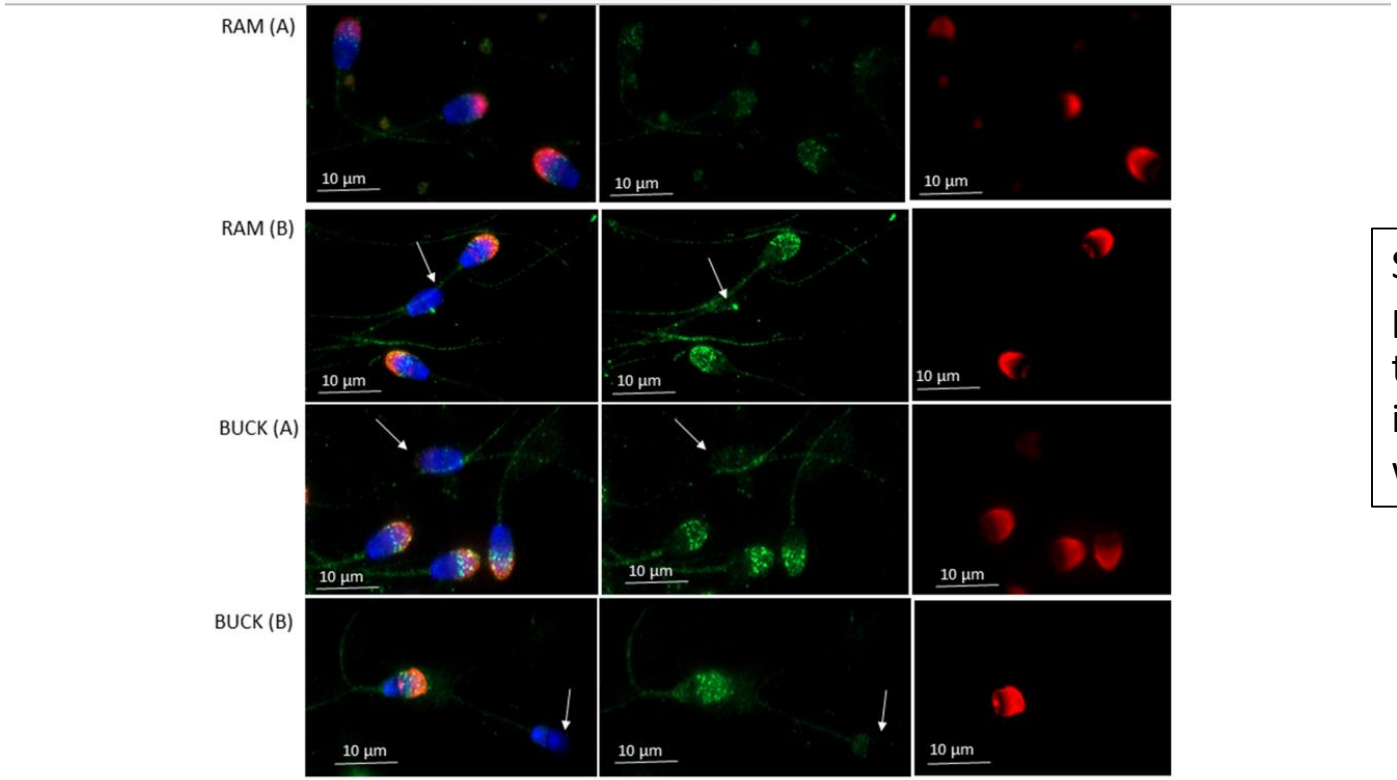
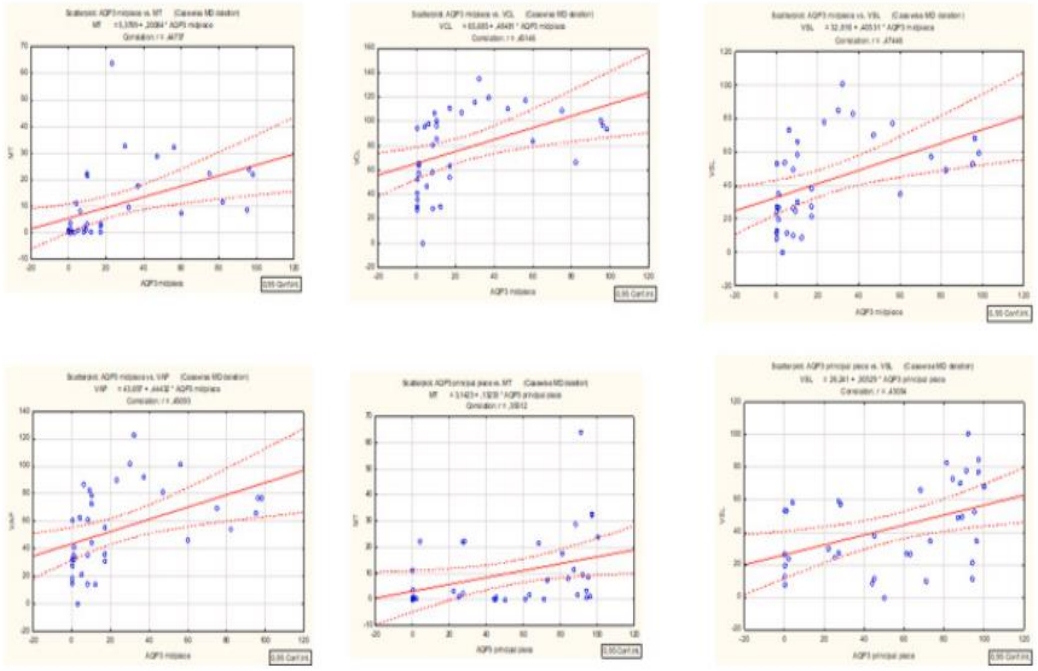


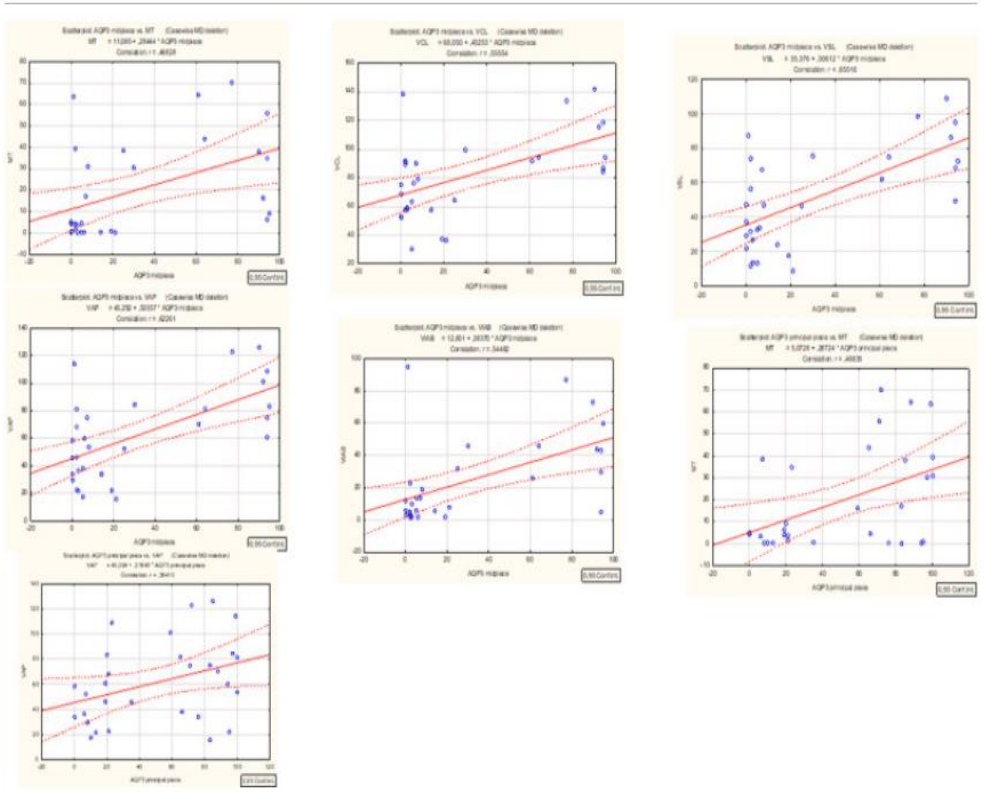
Fig. 5. Immunolabeling of AQP3 in slow (A) and ultra-rapid (B) frozen-thawed spermatozoa. Sperm nuclei were stained with Hoechst (blue) and acrosomes by PNA staining (red). Immunolabeling of AQP3 (green) located in intact acrosome, but was not seen when acrosome was damaged (arrows) in either ram or buck. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Simultaneous evaluation of acrosome integrity and presence of AQP3 in this membrane domain revealed that immunolabeling of AQP3 was always present in intact acrosomes, but was not seen when acrosome was damaged.

In frozen-thawed samples, the proportion of sperm with AQP3 located in the mid-piece was correlated with sperm motility and kinematic sperm variables (VCL, VSL, VAP)in both bucks and rams



Supplementary Fig. 2. Correlation between the proportions of sperm with AQP3 located in different membrane domains and sperm motility variables in ram frozen-thawed samples (samples cryopreserved using slow and ultra-rapid methods).



Supplementary Fig. 3. Correlation between the proportions of sperm with AQP3 located in different membrane domains and sperm motility variables in buck frozen-thawed samples (samples cryopreserved using slow and ultra-rapid methods).

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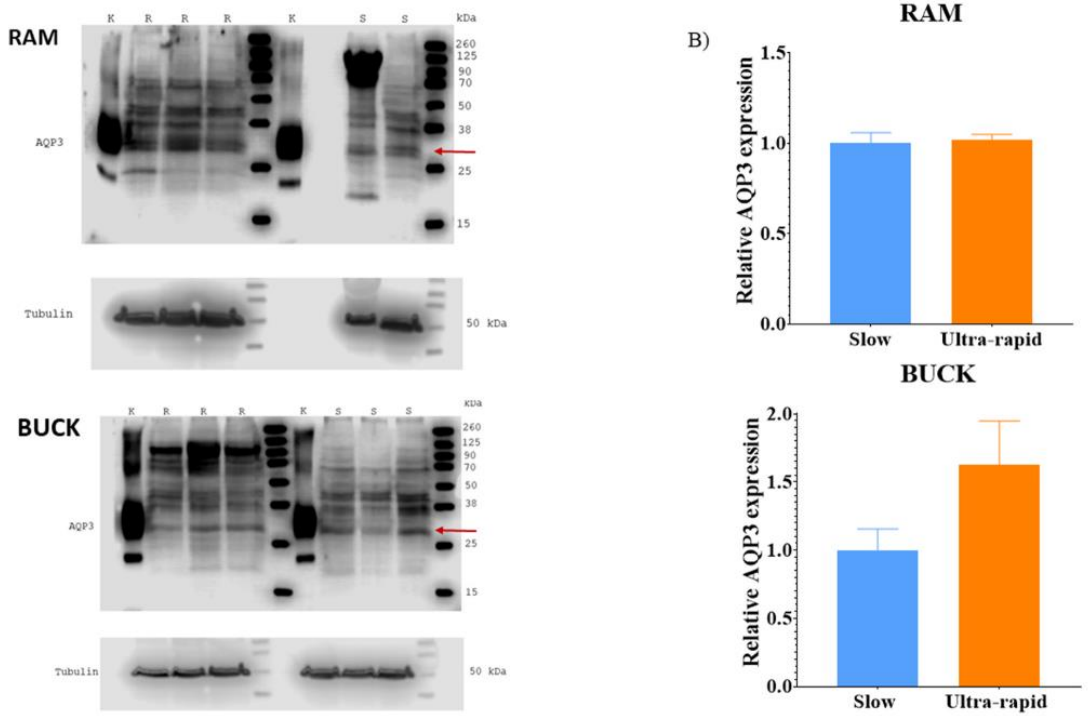

Fig. 6. Identification of AQP3 by Western blotting. A) Immunoblots for AQP3 in Merino ram and Murciano-Granadina buck spermatozoa of ejaculates frozen-thawed by slow (S) and ultra-rapid (R) methods; K: mouse kidney tissue lysate. Arrow indicates AQP3 band of about 32 kDa in either slow or ultrarapid freezing samples, and K. B) Relative abundances of AQP3 bands (as mean ± SEM) from the samples cryopreserved by slow (control) and ultra-rapid cryopreservation method. No significant differences were found between cryopreservation methods after quantification of 32 kDa bands and normalization using tubulin protein as an internal standard.

- WB identified AQP3 as a band of about 32 kDa in either slow or ultrarapid freezing samples.

- Relative abundances of AQP3 32 kDa band did not show significant differences between slow freezing-thawing and ultra-rapid freezing-thawing samples

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Location of aquaporins 3, 7 and 10 in frozen-thawed ejaculated and cauda epididymal spermatozoa from the Iberian ibex, mouflon, and chamois

Belén Pequeño[a], Belén Martínez-Madrid[b], Cristina Castaño[a], Adolfo Toledano-Díaz[a], Paula Bóveda[a], Milagros C. Esteso[a], Félix Gómez-Guillamón[c], Paloma Prieto[d], Jaime L. Marcos-Beltrán[e], Manuel Alvarez-Rodriguez[a], Heriberto Rodriguez-Martinez[f], Julián Santiago-Moreno[a,*]

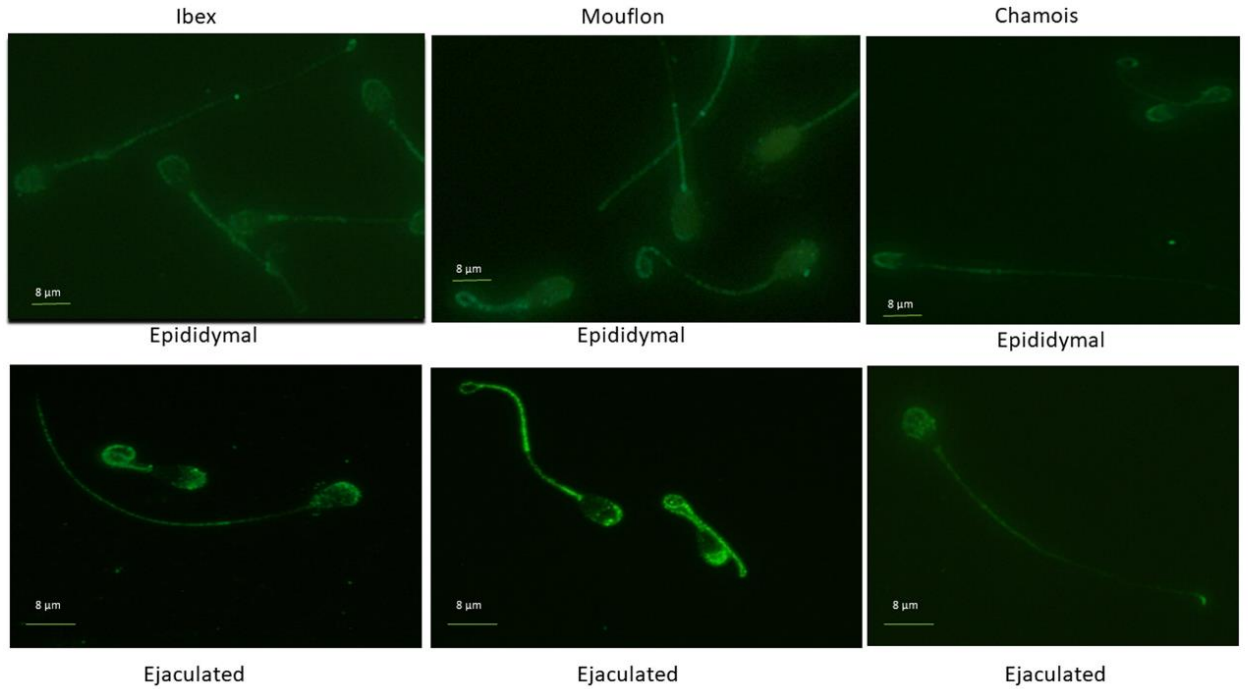

Fig. 1. Immunolabelling of AQP3 in ibex, mouflon and chamois sperm. AQP3 was located in the acrosome, post-acrosomal region, mid piece, principal piece, and end piece.

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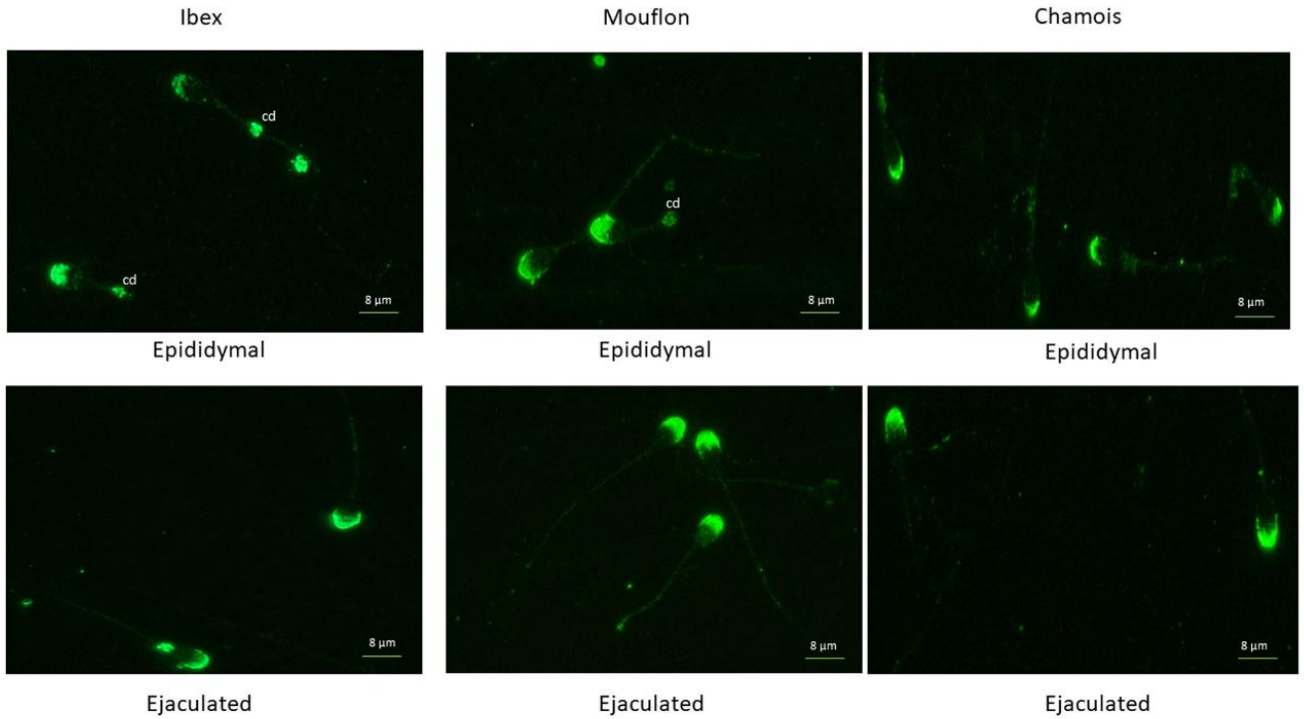

Fig. 3. Immunolabelling of AQP7 in ibex, mouflon and chamois sperm. AQP7 was located in the acrosome and cytoplasmic droplet (cd).

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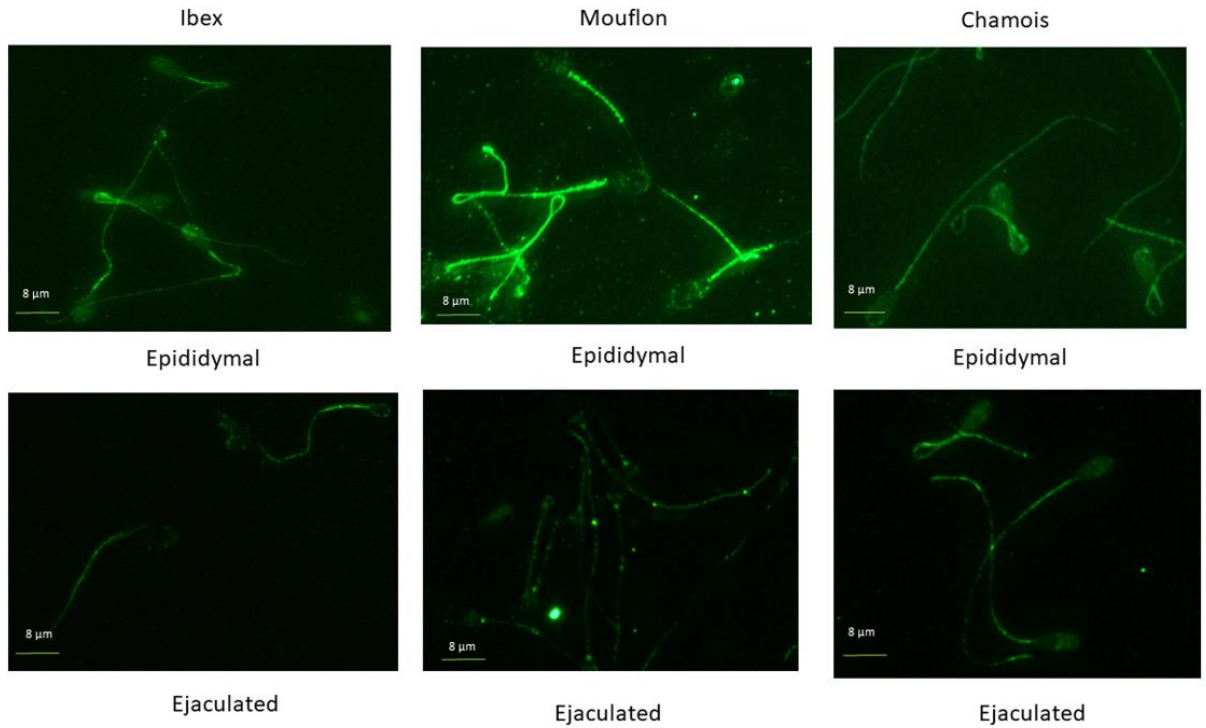

Fig. 5. Immunolabelling of AQP10 in ibex, mouflon and chamois sperm. AQP10 was located in the mid piece, principal piece and end piece. Immunolabelling in equatorial zone of the acrosome is a nonspecific union, as revealed by the AQP10-blocking peptide (S1 Fig).

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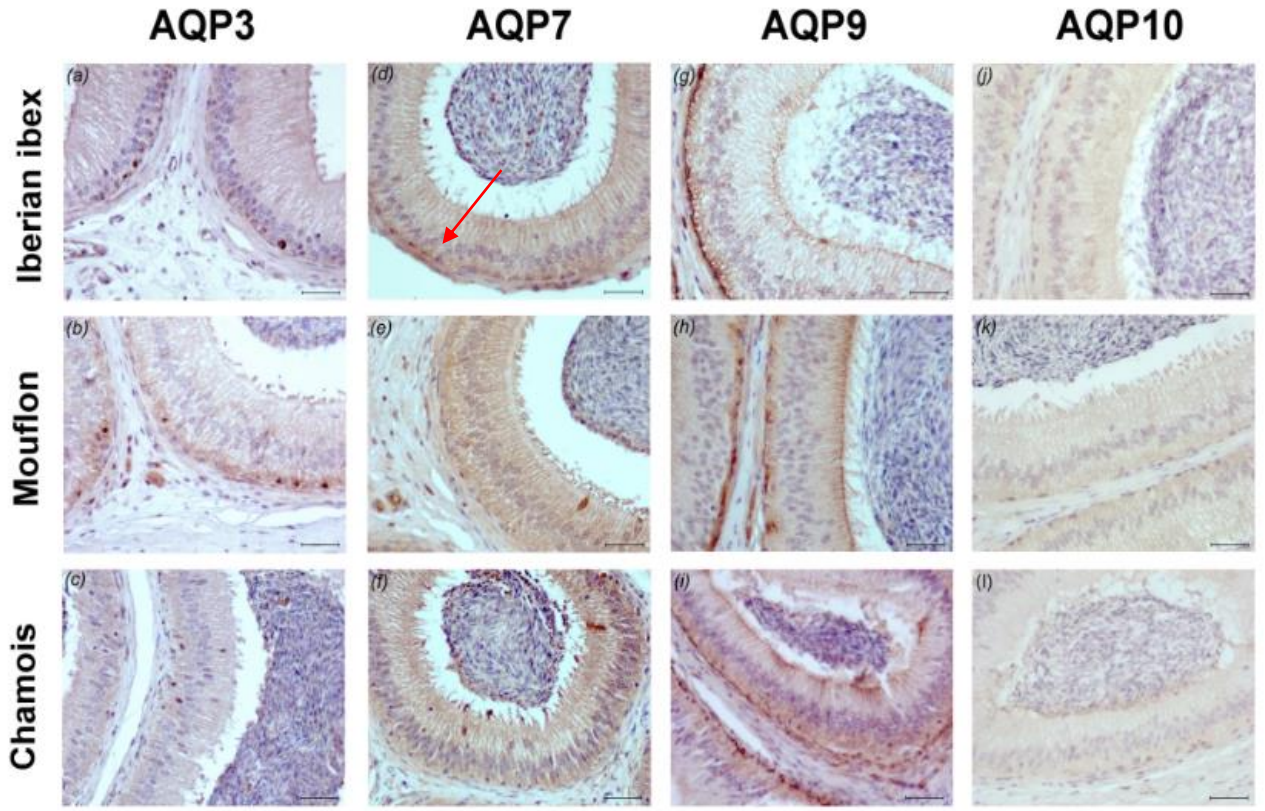
Reproduction, Fertility and Development

Immunolocalisation of aquaporins 3, 7, 9 and 10 in the epididymis of three wild ruminant species (Iberian ibex, mouflon and chamois) and sperm cryoresistance

Belen Martinez-Madrid[A,*], Carlos Martínez-Cáceres[B], Belén Pequeño[C], Cristina Castaño[C], Adolfo Toledano-Díaz[C], Paula Bóveda[C], Paloma Prieto[D], Manuel Alvarez-Rodriguez[C], Heriberto Rodriguez-Martinez[E] and Julián Santiago-Moreno[C]

AQP7 was observed in principal cells in all species, whereas only in basal cells of Iberian ibex and apical cells of mouflon and chamois ducts.



Aquaporin 3, 7, 9, and 10 immunohistochemistry in **caput region** of Iberian ibex, mouflon and chamois epididymis. 400X. Bar = 50 µm.

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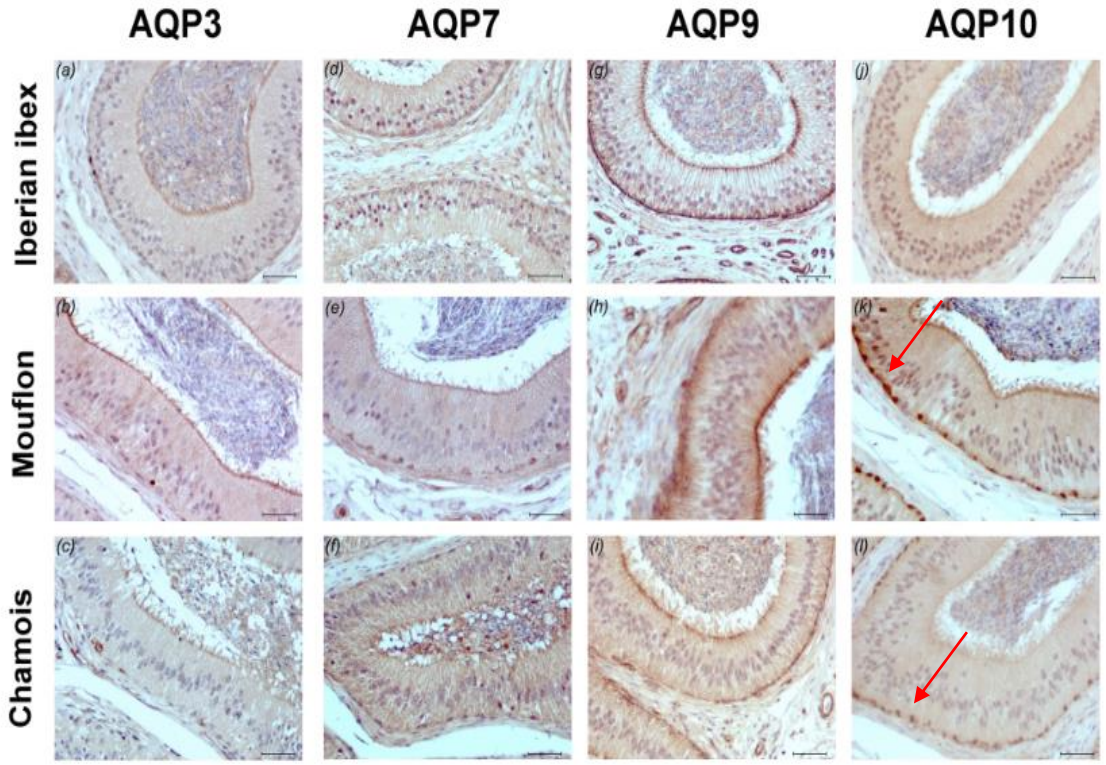
Reproduction, Fertility and Development

Immunolocalisation of aquaporins 3, 7, 9 and 10 in the epididymis of three wild ruminant species (Iberian ibex, mouflon and chamois) and sperm cryoresistance

Belen Martinez-Madrid[A,*], Carlos Martínez-Cáceres[B], Belén Pequeño[C], Cristina Castaño[C], Adolfo Toledano-Díaz[C], Paula Bóveda[C], Paloma Prieto[D], Manuel Alvarez-Rodriguez[C], Heriberto Rodriguez-Martinez[E] and Julián Santiago-Moreno[C]

No immunostaining for AQP10 was observed in ibex



Aquaporin 3, 7, 9, and 10 immunohistochemistry in **corpus region** of Iberian ibex, mouflon and chamois epididymis. 400X. Bar = 50 µm.

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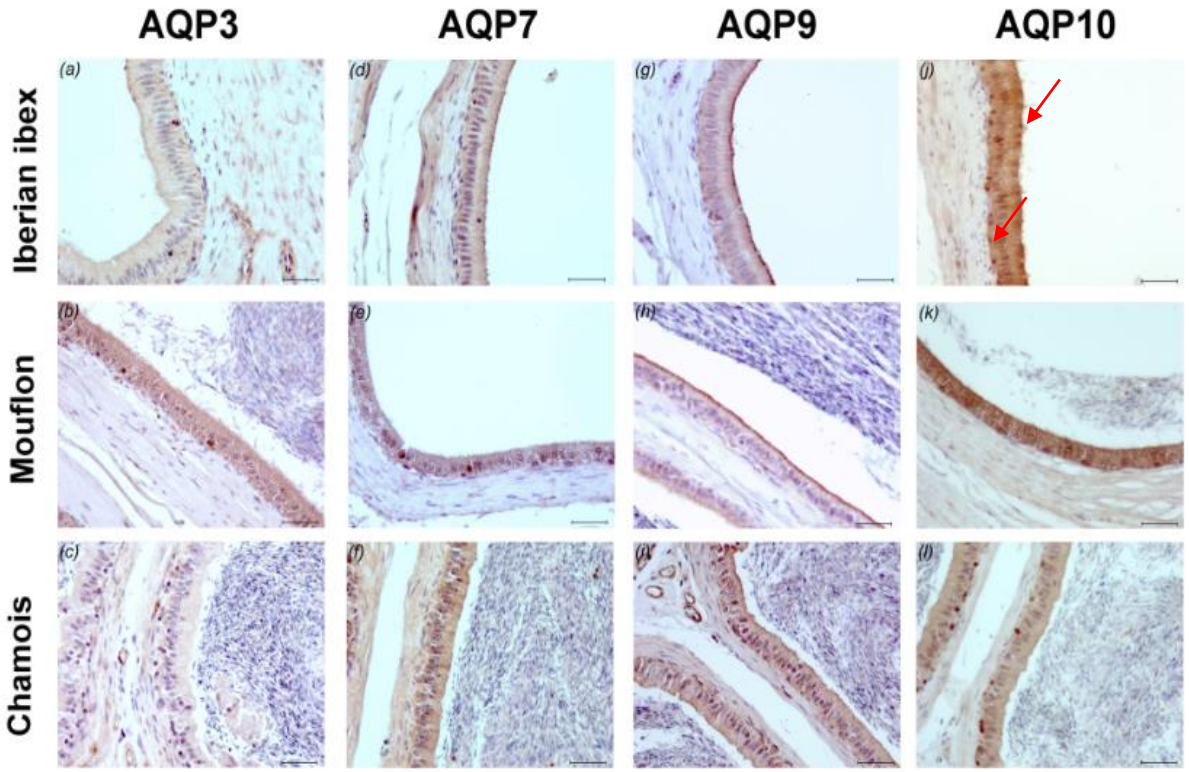
Positive immunostaining for AQP10 was observed in principal (apical membrane) and basal cells in ibex, whereas in mouflon and chamois was restricted to basal cells.



Aquaporin 3, 7, 9, and 10 immunohistochemistry in **cauda region** of 708 Iberian ibex, mouflon and chamois epididymis. 400X. Bar = 50 µm.

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Reproduction, Fertility and Development

Immunolocalisation of aquaporins 3, 7, 9 and 10 in the epididymis of three wild ruminant species (Iberian ibex, mouflon and chamois) and sperm cryoresistance

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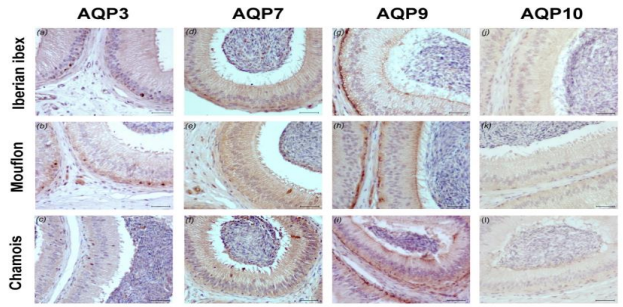
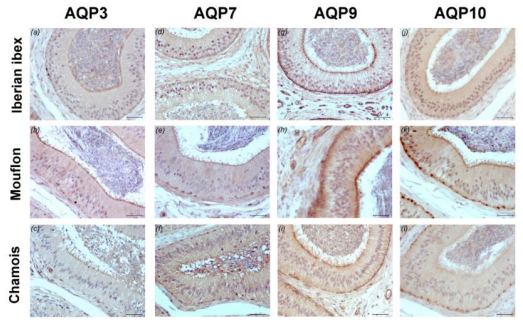
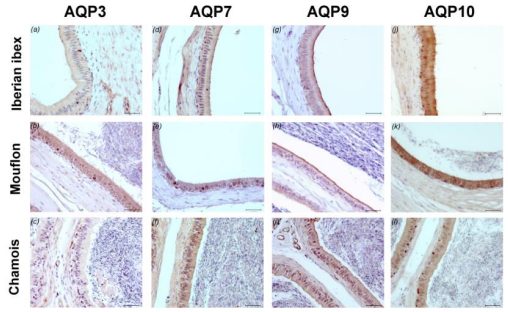
Caput



Corpus



Cauda

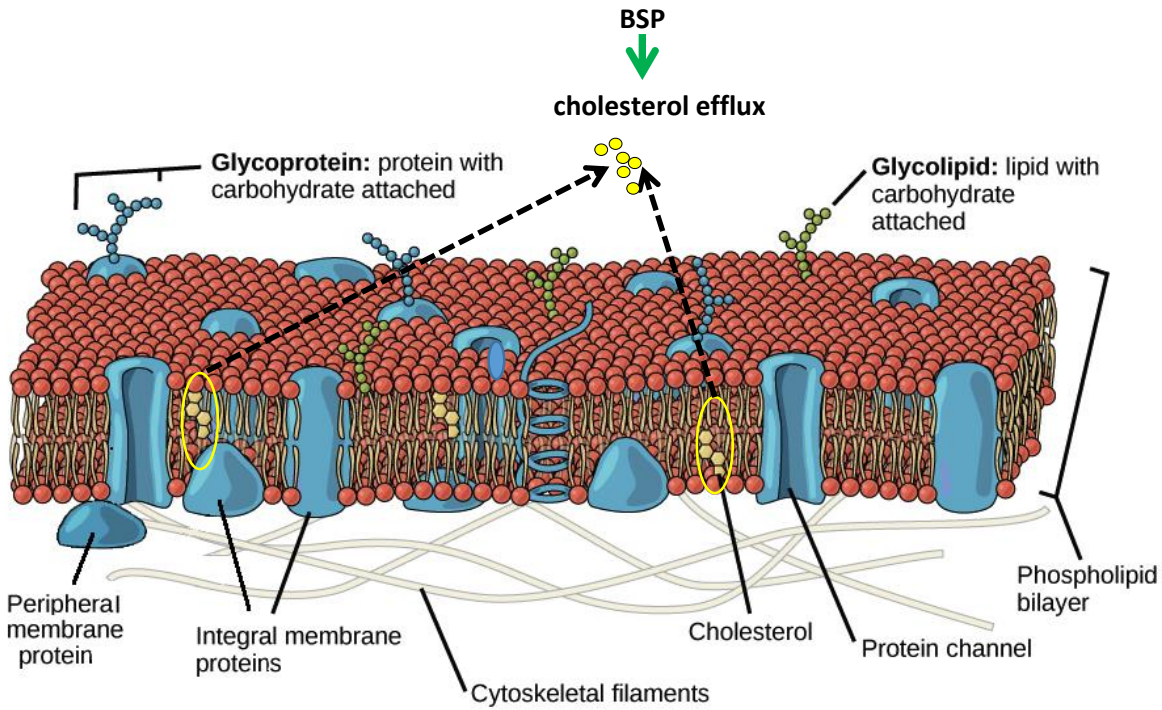


The expression patterns of AQP3 and AQP9 in the epididymal epithelial cells were identical for the three wild ungulates.

Ibex varied in the immunolocalisation of AQP7 in caput and cauda regions and AQP10 in corpus and cauda epididymis, compared to mouflon and chamois.

It could partially explain changes in the environment where sperm maturation occurred that could influence the higher sperm resistance to osmotic stress in Ibex.

BSP (*Major Proteins of Bovine Seminal Plasma*): BSP-A1, BSP-A2, BSP-A3, BSP-30-kDa



- BSP stimulate the efflux of cholesterol and phospholipids from the bull sperm membrane, making it more sensitive to cryopreservation processes.

- Cholesterol has a stabilizing effect on the plasma membrane by imposing conformational order on lipids (cholesterol increases fluidity at low temperatures, but decreases fluidity at high temperatures) → variations in the cholesterol/phospholipid ratio across mammalian species has been linked to the ability to survive cryopreservation

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The influence of washing Spanish ibex (*Capra pyrenaica*) sperm on the effects of cryopreservation in dependency of the photoperiod

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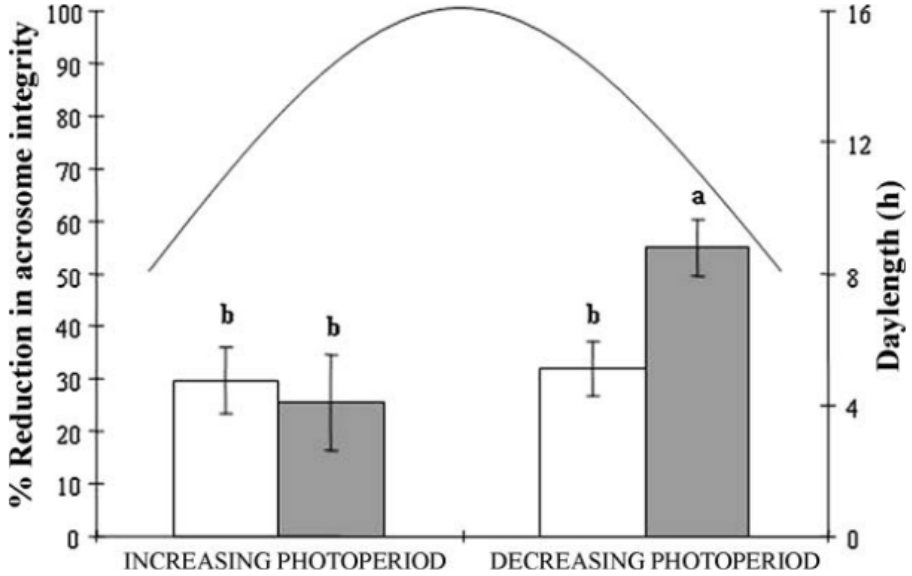

Fig. 2. Reduction in acrosome integrity (mean ± SE) after freezing-thawing in washed (open bars) and nonwashed (solid bars) sperm samples during the time of increasing (January to June) and decreasing (July to December) photoperiod. [a,b]Different letters between bars indicate differences ($P < 0.05$).

- Phospholipase is secreted from the bulbourethral glands in caprines.

- Phospholipase activity produces sperm-deteriorating effect through the hydrolysis of membrane phospholipids of sperm or the production of toxic derivative from egg yolk phospholipids.

- Increased phospholipase activity during the rutting season.

AMINO ACIDS OF SEMINAL PLASMA ARE ASSOCIATED WITH SPERM CRYORESISTANCE

PLOS ONE

RESEARCH ARTICLE

Seminal plasma amino acid profile in different breeds of chicken: Role of seminal plasma on sperm cryoresistance

Julián Santiago-Moreno[1]*, Berenice Bernal[1], Serafín Pérez-Cerezales[1], Cristina Castaño[1], Adolfo Toledano-Díaz[1], Milagros C. Esteso[1], Alfonso Gutiérrez-Adán[1], Antonio López-Sebastián[1], María G. Gil[2], Henri Woelders[3], Elisabeth Blesbois[4]

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frontiers in Cell and Developmental Biology

ORIGINAL RESEARCH
published: 14 January 2020
doi: 10.3389/fcell.2019.00347

Amino Acids of Seminal Plasma Associated With Freezability of Bull Sperm

*Muhammet Rasit Ugur[1], Thu Dinh[1], Mustafa Hitit[1,2], Abdullah Kaya[3], Einko Topper[4], Bradley Didion[4] and Erdogan Memili[1]**

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PLOS ONE

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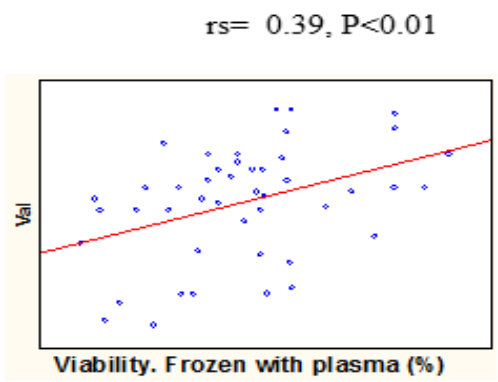

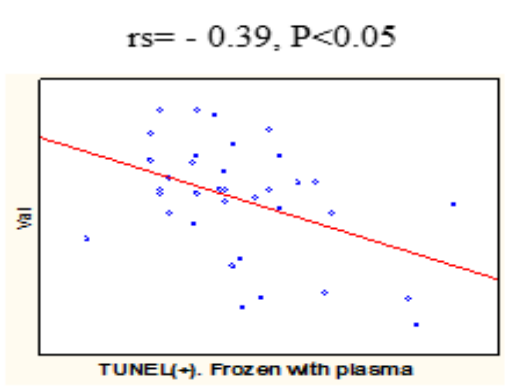

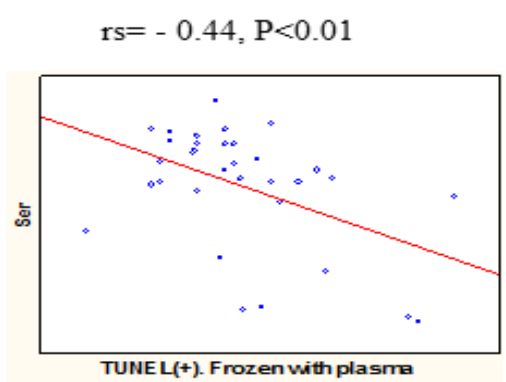

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Amino Acid Concentration in Bull Seminal Plasma

Twenty-one amino acids were detected in bull seminal plasma (**Figure 1**). Free amino acid concentrations of bull seminal

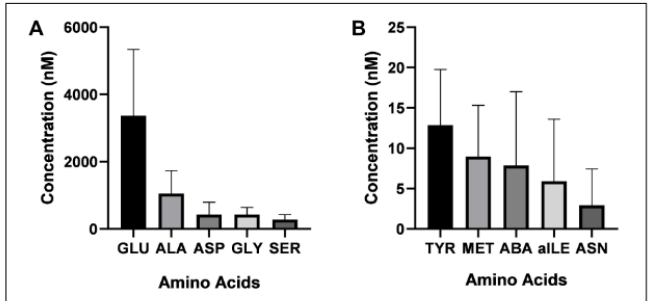

FIGURE 2 | Concentrations of the most and the least abundant amino acids in bull seminal plasma. **(A)** The most abundant amino acids in bull seminal plasma was glutamic acid. Alanine, glycine, aspartic acid, and serine were the other predominant amino acids in bull seminal plasma. **(B)** The least predominant amino acids were tyrosine, methionine, alpha aminobutyric acid, allo-isoleucine, and asparagine.

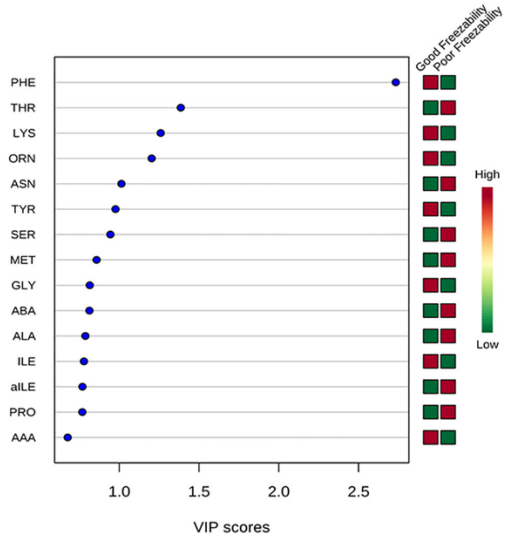

FIGURE 5 | Variable importance in projection (VIP) plot displays the top 15 most important amino acid features identified by PLS-DA. Colored boxes on right indicate concentration of corresponding amino acid from GF and PF samples. VIP score is a weighted based on PLS-DA model.

Phenylalanine is more abundant in seminal plasma of the GF (good freezability) bulls than in that of PF (poor freezability) and could be considered as a freezability biomarker.

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Group of Physiology and Technologies of Reproduction in Wild Species, INIA-CSIC

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