Mouflon *(Ovis musimon)* sperm cryosurvival is better at the end of the rutting season coinciding with low plasma testosterone concentrations

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Introduction:

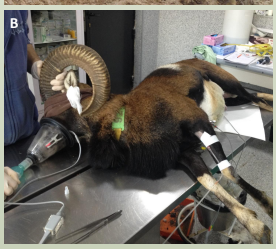
Cryosurvival of spermatozoa in Iberian ibex (*Capra pyrenaica*) is poorer at the onset and in the middle of the rutting season, when plasma testosterone levels are the highest, than at the end of the rutting season coinciding with fall of testosterones levels. We hypothesized that high plasma testosterone concentration might have a negative effect on sperm cryosurvival, and thus a similar situation may be found in other wild ruminants, such as the mouflon (*Ovis musimon*).

Objectives:

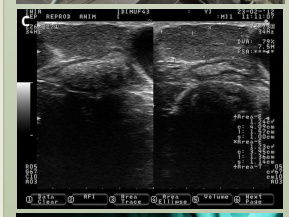
- To study the effect of mouflon endocrine status on sperm cryosurvival.
- To compare two protocols for mouflon sperm cryopreservation: the traditional freezing protocol in straws and an ultrarapid cryopreservation protocol in sperm pellets.

Semen collection:

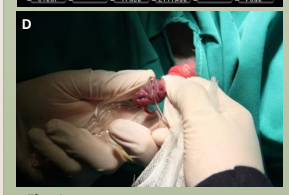
Samples were obtained from 22 mouflons maintained at different locations in Spain: INIA experimental farm (Department of Animal Reproduction INIA, Madrid, Spain), Guadalajara Zoological Garden (Guadalajara, Spain) and Córdoba Zoological Garden (Fig. 1A, Córdoba, Spain).



Sperm samples were collected from anesthetized animals (Fig. 1B) during autumn (October) when plasma testosterone concentrations are high, and at the end of the rutting season (January), when levels of testosterone tend to decrease to basal levels.



Semen was collected from all animals by transrectal ultrasound-guided massage of the accessory sex glands (TUMASG). TUMASG was performed with the ultrasonographic probe placed on the ampulla of the vas deferens and seminal vesicles (Fig. 1C).



The penis was manually protruded during the TUMASG and the semen sample was collected in a tube (Fig. 1D).

Fig. 1

Sperm evaluation:

- Sperm motility was assessed with a computer-aided sperm analysis system (Fig. 2A).
- Membrane integrity and acrosomal status were evaluated by the eosin-nigrosin technique (EN) and by fluorescence with a fluorochrome combination of propidium iodide (PI), and fluorescein isothiocyanate-conjugated peanut (*Arachis hypogaea*) agglutinin (PNA-FITC) (Fig.2B).
- Morphological abnormalities and acrosome integrity were evaluated in samples fixed in buffered 2% glutaraldehyde (Fig. 2C).

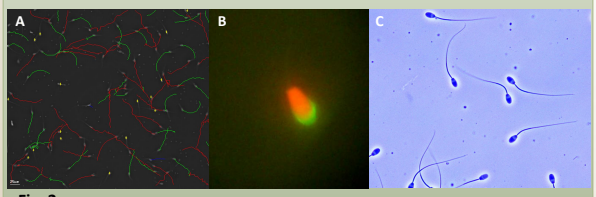


Fig. 2

Sperm cryopreservation

Each sample was cryopreserved following two different protocols.

Fig. 3A. Traditional freezing protocol in straws. TES-glucose-based medium + 6% egg yolk + glycerol 5%

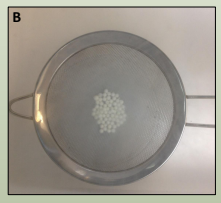


Fig. 3B. Ultrarapid cryopreservation protocol in pellets. TES-glucose-based medium + 6% egg yolk + sucrose 100 mM

Results:

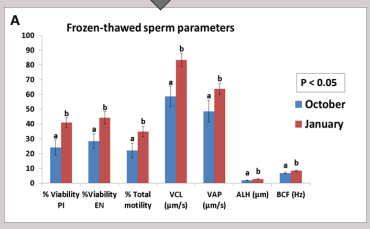


Fig. 4A. Frozen-thawed sperm cryopreserved in January had a total sperm motility, curvilinear velocity (VCL), average path velocity (VAP), amplitude of lateral head displacement (ALH) and beat-cross frequency (BCF) greater than samples collected in October ($p < 0.05$). Sperm viability assessed by fluorescence and by the eosin-nigrosin (EN) technique was also higher in frozen-thawed samples collected in January than in October ($p < 0.05$). Different letters (a, b) indicate significant differences ($p < 0.05$) between October and January frozen-thawed sperm parameters. Vertical bars represent S.E.M.

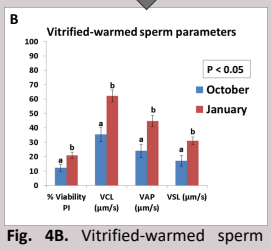


Fig. 4B. Vitrified-warmed sperm had a VCL ($p < 0.01$), VAP ($p < 0.01$), straight-line velocity (VSL) ($p < 0.05$) and viability assessed by fluorescence (PI) higher in samples collected in January than in October ($p < 0.05$). Different letters (a, b) indicate significant differences ($p < 0.05$) between October and January vitrified-warmed sperm parameters. Vertical bars represent S.E.M.

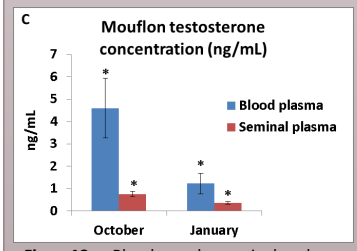


Fig. 4C. Blood and seminal plasma testosterone concentration measured by radioimmunoassay in October and January. Asterisks indicate significant differences between October and January testosterone concentration values ($p < 0.05$). Vertical bars represent S.E.M.

- There were no differences in fresh sperm variables between samples collected in October and in January ($p > 0.05$).
- Frozen-thawed sperm quality parameters were higher than vitrified-warmed sperm values ($p < 0.05$).

➢ Statistical analysis was performed by one-way ANOVA using Statistica for Windows v.12.0 software (StatSoft, Tulsa, OK, USA). Data are expressed as means. Vertical bars represent S.E.M.

Conclusions:

- Sperm quality of frozen-thawed and vitrified-warmed samples was higher in January, when levels of testosterone are decreasing, than in October.
- These results confirmed the hypothesis that the pick of plasma testosterone concentration that occurs in October, could affect negatively to mouflon sperm cryosurvival.

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