ADDING TAXIFOLINE, AN ANTIOXIDANT, TO THE SEMEN EXTENDER OF BERMEYA GOAT, A LOCAL BREED

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Use of the flavonoid taxifolin for sperm cryopreservation from the threatened Bermeya goat breed

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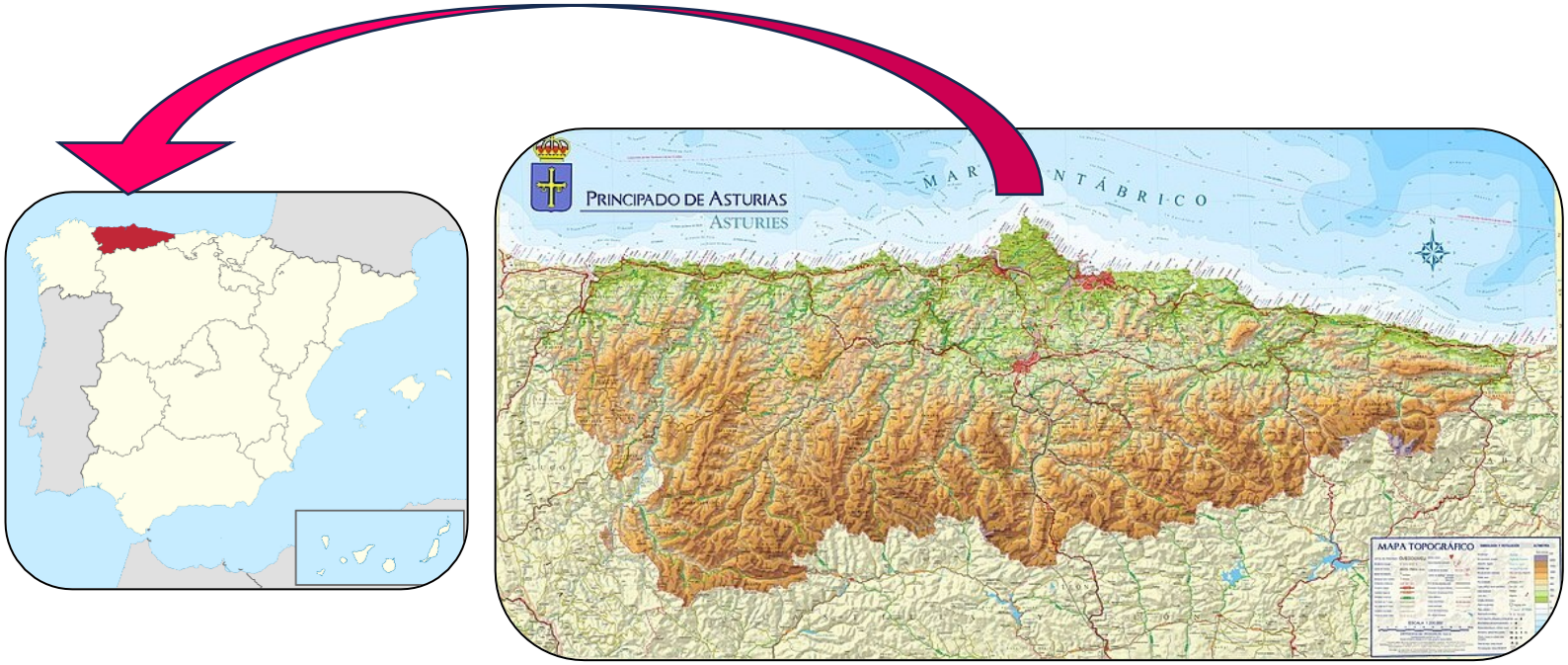
AD HOC WORKSHOP ON SMALL RUMINANT SEMEN PRESERVATION. 26 MAYO, 2026

OVINE AND CAPRINE BREEDS IN ASTURIAS



INTRODUCTION

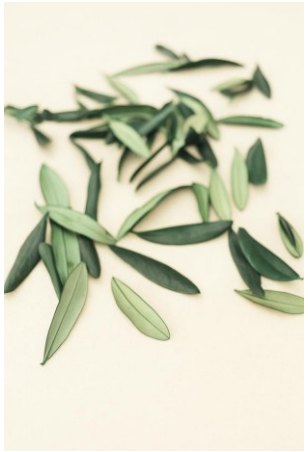
- **Xalda sheep and Bermeya goat** are endangered and officially protected breeds.
- Cryobank with semen doses in **Centro de Biotecnología Animal** (Gijón, Asturias), SERIDA.


Xalda ram


Bermeya buck

INTRODUCTION

- Assess the effect of **adding Taxifoline** to the semen extender during the **cooling period** before freezing on the overall post-thawing sperm variables of Bermeya goats.



Bermeya breed

MATERIAL AND METHODS

1. Genetic and morphology **male donor selection** by breeders´associations:

ACRIBER for Bermeya goat

2. Bucks transport to Animal Biotechnology Centre, after negative **sanitary test** results (pre-quarantine and quarantine).

BUCKS
Brucella mellitensis, B.ovis, Paratuberculosis, Tuberculosis, Maedi/Cae, Border Disease (Ag/Ac), Agalactia, Scrapie.

MATERIAL AND METHODS

3. Training to mount, and **male sexual activity** study (Flehmen response, incomplete mounts...).

4. **Semen collection** by artificial vagina (40-42°C), 2 days per week in breeding season, Autumn (from October to December) between 2007 and 2020.

MATERIAL AND METHODS

5. EXPERIMENTAL DESIGN

EXPERIMENT 1: 8 bucks, 4 replicates (dose-response)

- Control (CTL)
- T10 (10 µg/mL)
- T50 (50 µg/mL)
- T100 (100 µg/mL)

EXPERIMENT 2: 7 bucks, 3 replicates.

- Control (CTL)
- 5 µM taxifolina (TFL)
- 1 mM Glutation (GSH)
- Taxifoline+GSH (TXH)

Artificial insemination trial using 29 Bermeya does

MATERIAL AND METHODS

6. Buck semen processing.

Dilution (1/10)

Centrifugation 900 X *g*/15 min.

Pellet resuspension with freezing medium

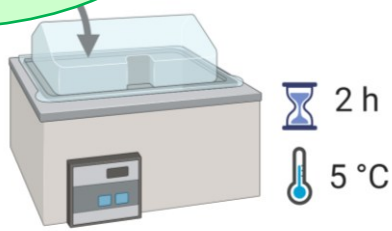
Bucks: TGC Medium (Tris, Glucose, Citric Acid)

Room temperature

TGC/TTFC Medium + 12% egg yolk (v/v) and 4% glycerol

Refrigeration to 5 °C in 90 min (0.5 °C/min)

Equilibration prior to freezing



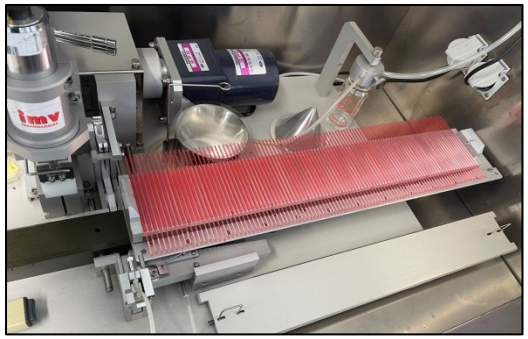
Final sperm concentration 400 X 10^6 spz/ mL

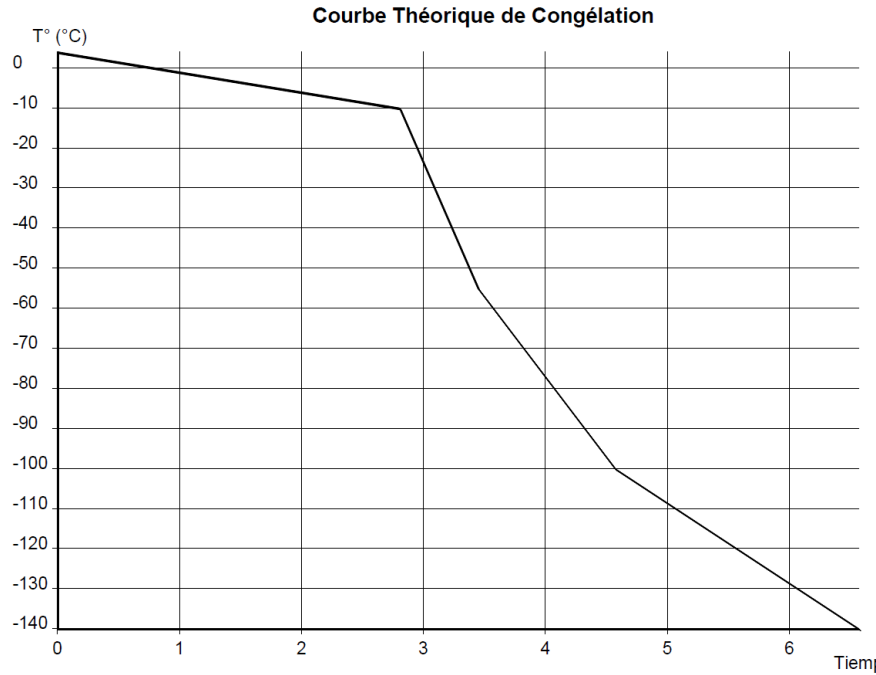
MATERIAL AND METHODS

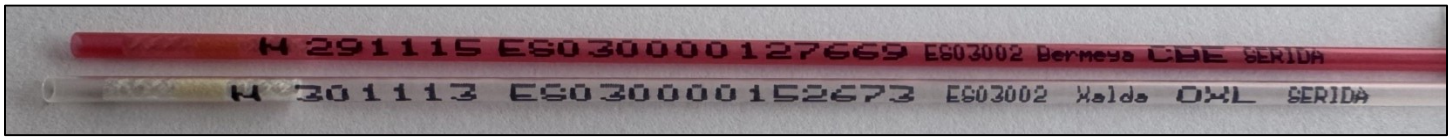
7. Buck semen cryopreservation.

Machine filled and sealed of 0.25 mL straws

Straw frozen in programmable freezer



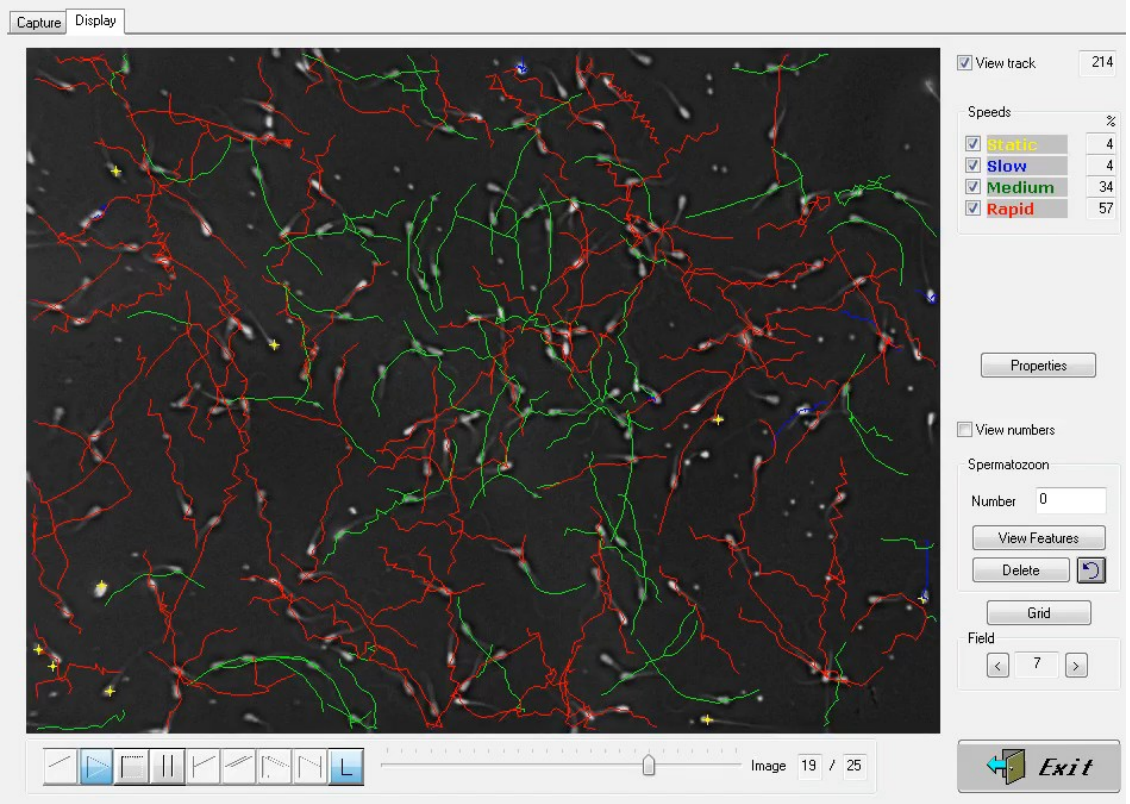




MATERIAL AND METHODS

8.1. Post-thawed sperm evaluation: CASA.

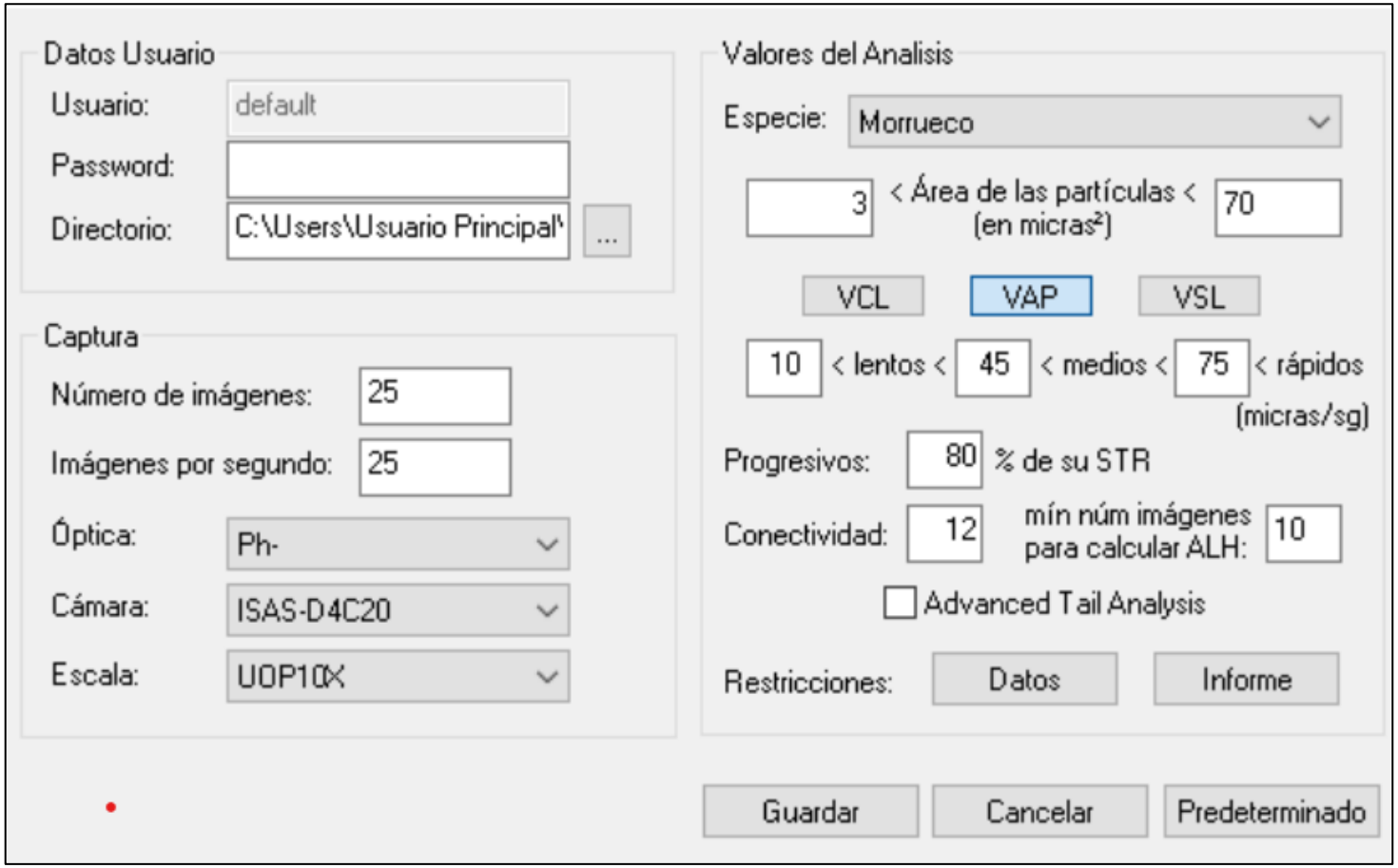
- Total motility
- Progressive motility
- VCL
- VSL
- VAP
- LIN
- STR
- WOB
- ALH
- BCF



*2 straws per batch thawed in a water bath (**37°C/30s**) and assessed after **15 m, 2h** and **5 h***

MATERIAL AND METHODS

Motility: CASA buck settings.



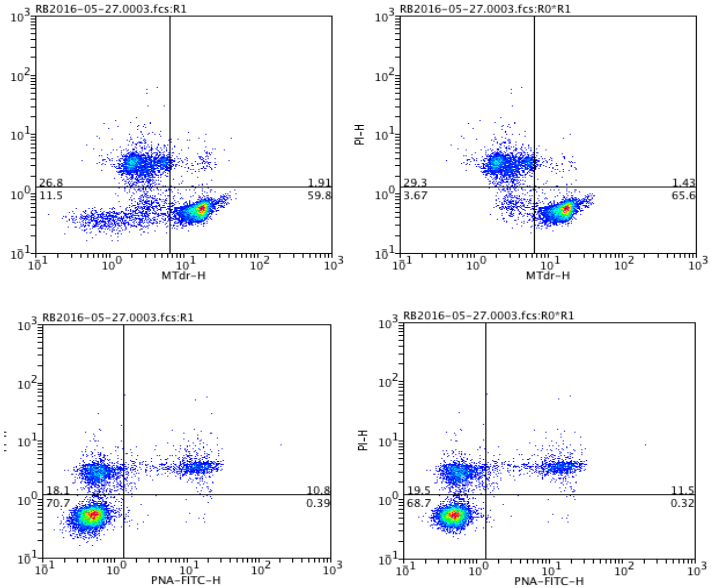
At least 1000 spermatozoa in 4-6 fields analyzed in a pre-warmed ISAS or Makler chamber

MATERIAL AND METHODS

8.2. Post-thawed sperm evaluation: FLOW CYTOMETER.

- Apoptosic changes
- Acrosoma integrity
- Mitochondrial status
- Capacitation
- Cytoplasmic ROS
- Mitochondrial superoxide production
- Sperm chromatin assessment

Cell Lab Quanta ™ (Beckman Coulter, USA)



MATERIAL AND METHODS

9. Liquid nitrogen storage in the *Genetic Resource Bank for Endangered Domestic Native Animal Species of Principado de Asturias.*

CERTIFICATE OF MEMBERSHIP

THIS CERTIFICATE IS AWARDED TO

Servicio Regional de Investigación y Desarrollo Agroalimentario

AS MEMBER OF THE EUROPEAN GENEBANK NETWORK FOR ANIMAL GENETIC RESOURCES (EUGENA)

MONTSERRAT CASTELLANOS

Chair of the European Regional Focal Point for Animal Genetic Resources

Date 14th August, 2024

Member of the European Genebank Network for Animal Genetic Resources (EUGENA)

RESULTS: EXP. 1/CASA

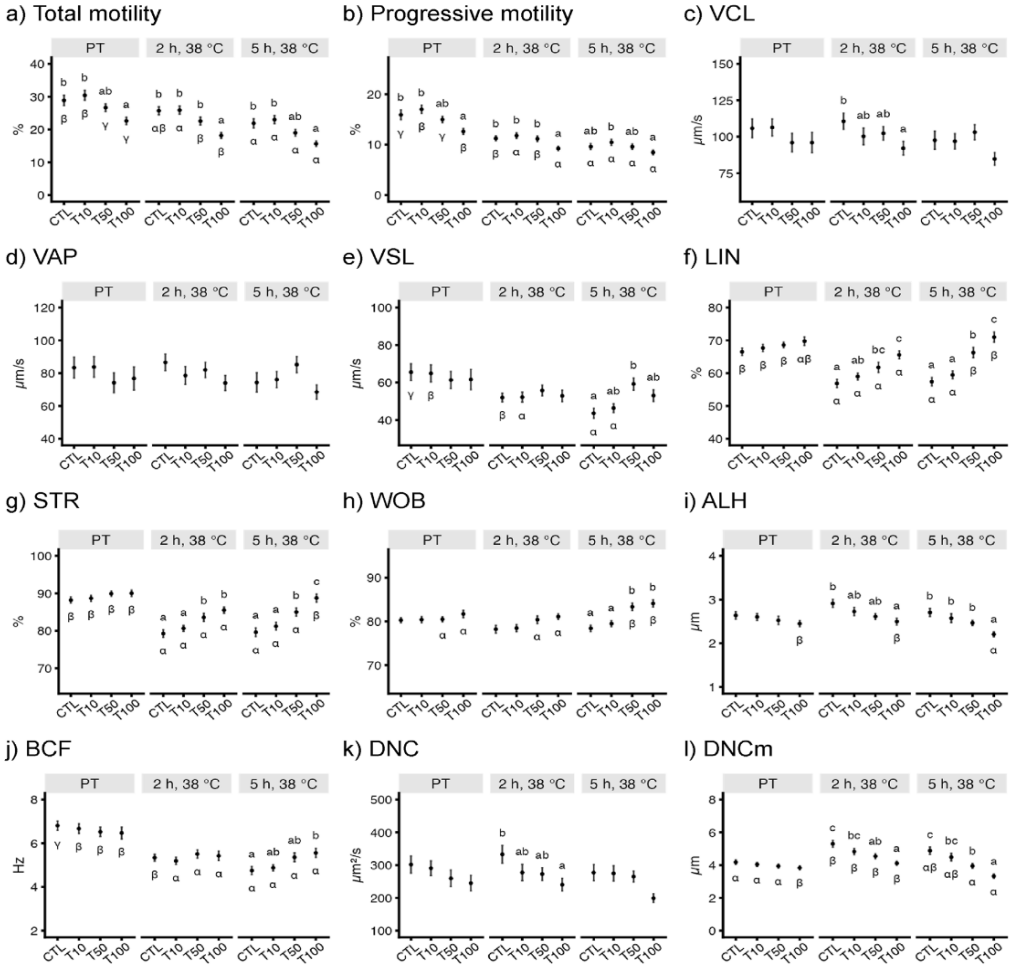


Fig. 1. CASA results (mean ± SEM) for Experiment 1, showing the effect of taxifolin at 10, 50, and 100 µg/ml (T10, T50, and T100; CTL: Control) on motility and kinematics variables directly post-thawing (PT), and after 2 and 5 h of incubation at 37 °C. VCL: Curvilinear velocity; VAP: Average-path velocity; VSL: Straight-path velocity; LIN: Linearity (VSL/VCL × 100); STR Straightness (VSL/VAP); WOB: Wobble (VAP/VCL × 100); ALH: Amplitude of the lateral displacement of the sperm head; BCF: Frequency of the flagellar beat; DNC: Dance (ALH × VCL); DNCm: Mean Dance (ALH × VSL/VCL). Treatments with different Latin letters differ by $P < 0.05$ within each incubation group, and those with different Greek letters differ by $P < 0.05$ among incubation groups and within the same treatment.

In experiment 1 and compared to the control, T10 increased progressive motility ($P < 0.001$) but taxifolin decreased total and progressive motility at higher concentrations ($P < 0.001$), both post-thawing and after the incubation.

RESULTS: EXP. 1/CYTOMETRY

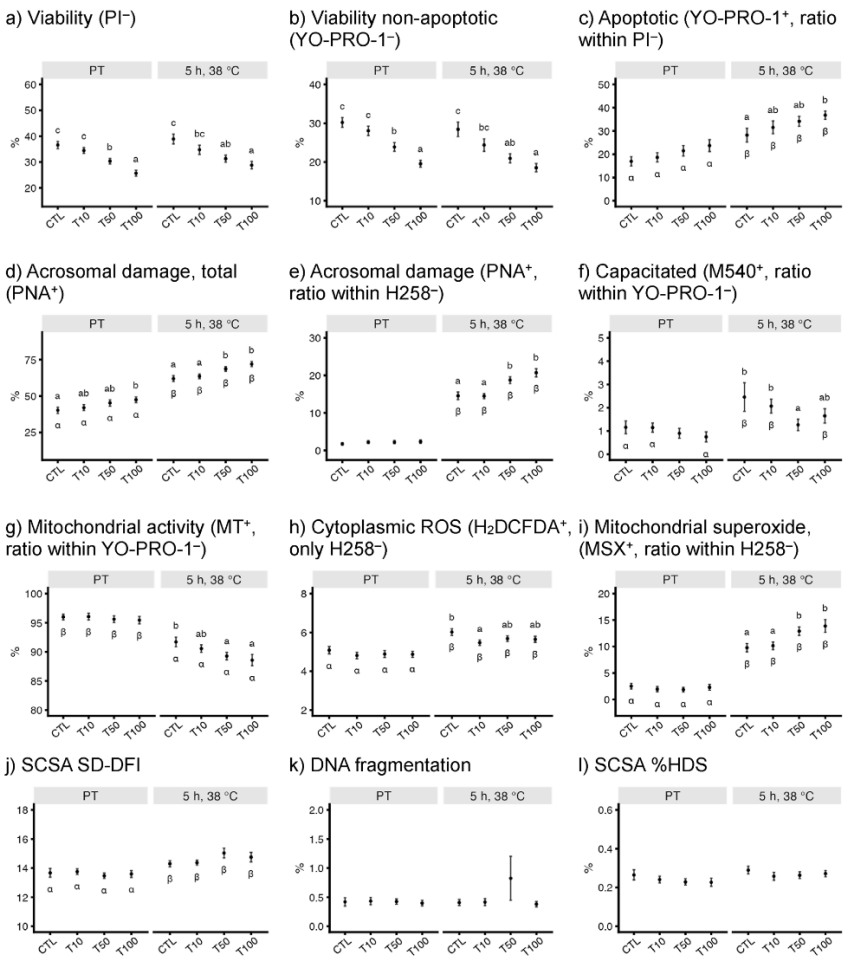


Fig. 2. Flow cytometry results (mean ± SEM) for Experiment 1, showing the effect of taxifolin at 10, 50, and 100 μg/ml (T10, T50, and T100; CTL: Control) on physiology and chromatin structure variables directly post-thawing (PT), and after 2 and 5 h of incubation at 37 °C. PI: Propidium iodide; H258: Hoechst 33258; PNA: Peanut agglutinin (Alexa 647 conjugated); M540: Merocyanine 540; MT: MitoTracker deep red; MSX; MitoSOX; SCSA: Sperm chromatin structure assay; SD-DFI: Standard deviation of the DNA fragmentation index; %DFI: % spermatozoa with high DFI (fragmented DNA); %HDS: % spermatozoa with high DNA stainability (low compaction or chromatin immaturity). Treatments with different Latin letters differ by $P < 0.05$ within each incubation group, and those with different Greek letters differ by $P < 0.05$ between incubation groups and within the same treatment.

Viability decreased post-thawing in the three concentrations ($P < 0.001$). Cytoplasmic ROS decreased at 0 and 5 h at T10 ($P < 0.049$), and all doses decreased mitochondrial superoxide post-thawing ($P < 0.024$).

RESULTS: EXP. 2/CASA

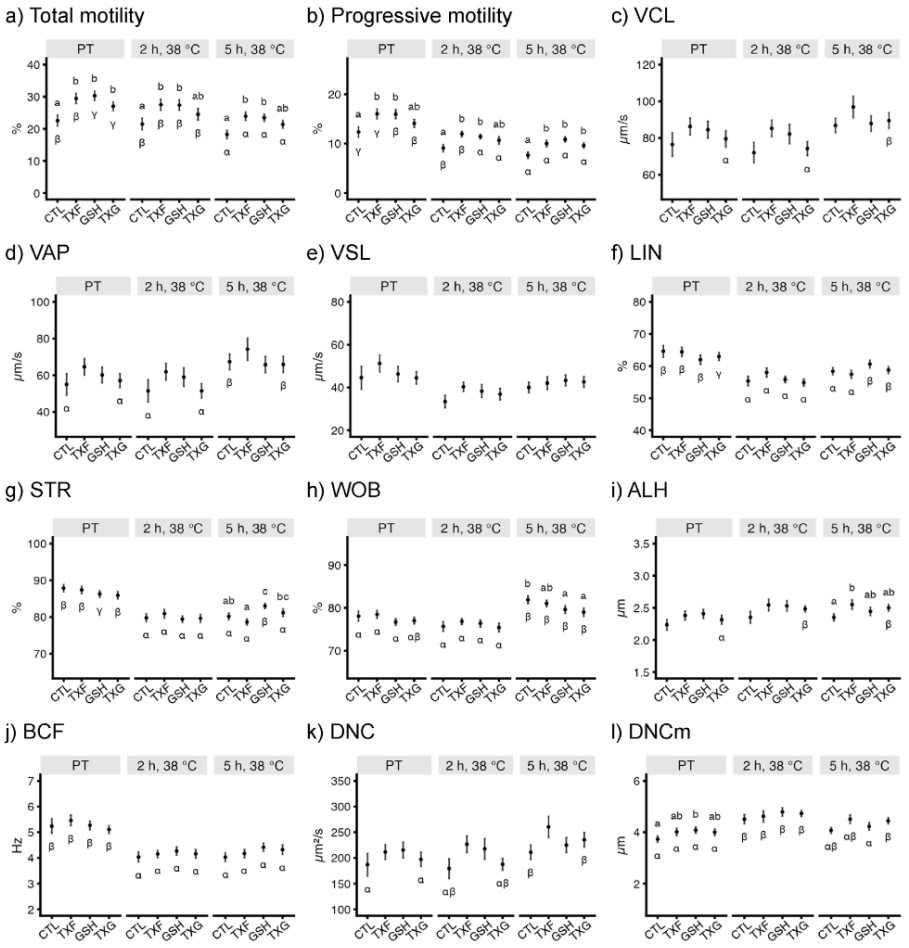


Fig. 3. CASA results (mean ± SEM) for Experiment 2, showing the effect of taxifolin at 5 μM (1.5 μg/ml) (TXF), GSH at 1 mM (GSH), their combination (TXG), or no supplement (CTL: Control) on motility and kinematics variables directly post-thawing (PT), and after 2 and 5 h of incubation at 37 °C. VCL: Curvilinear velocity; VAP: Average-path velocity; VSL: Straight-path velocity; LIN: Linearity (VSL/VCL × 100); STR Straightness (VSL/VAP); WOB: Wobble (VAP/VCL × 100); ALH: Amplitude of the lateral displacement of the sperm head; BCF: Frequency of the flagellar beat; DNC: Dance (ALH × VCL); DNCm: Mean Dance (ALH × VSL/VCL). Treatments with different Latin letters differ by $P < 0.05$ within each incubation group, and those with different Greek letters differ by $P < 0.05$ among incubation groups and within the same treatment. Whereas the treatments showed few significant effects within each incubation time (except for total and progressive motility), the main effects analysis indicated significant effects on CTL for TXF ($P < 0.01$ for VCL, VSL, VAP, ALH, and DNC, and $P < 0.05$ for DNCm), for GSH ($P < 0.05$ for VCL, ALH, DNC, and DNCm), and TXG ($P < 0.05$ for WOB and $P < 0.01$ for DNCm).

5 μM taxifolin or 1 mM GSH (alone or combined) increased total and progressive motility vs. the control ($P < 0.01$), and taxifolin increased kinematic parameters such as VCL, ALH, and DNC ($P < 0.05$).

RESULTS: EXP. 2/CYTOMETRY

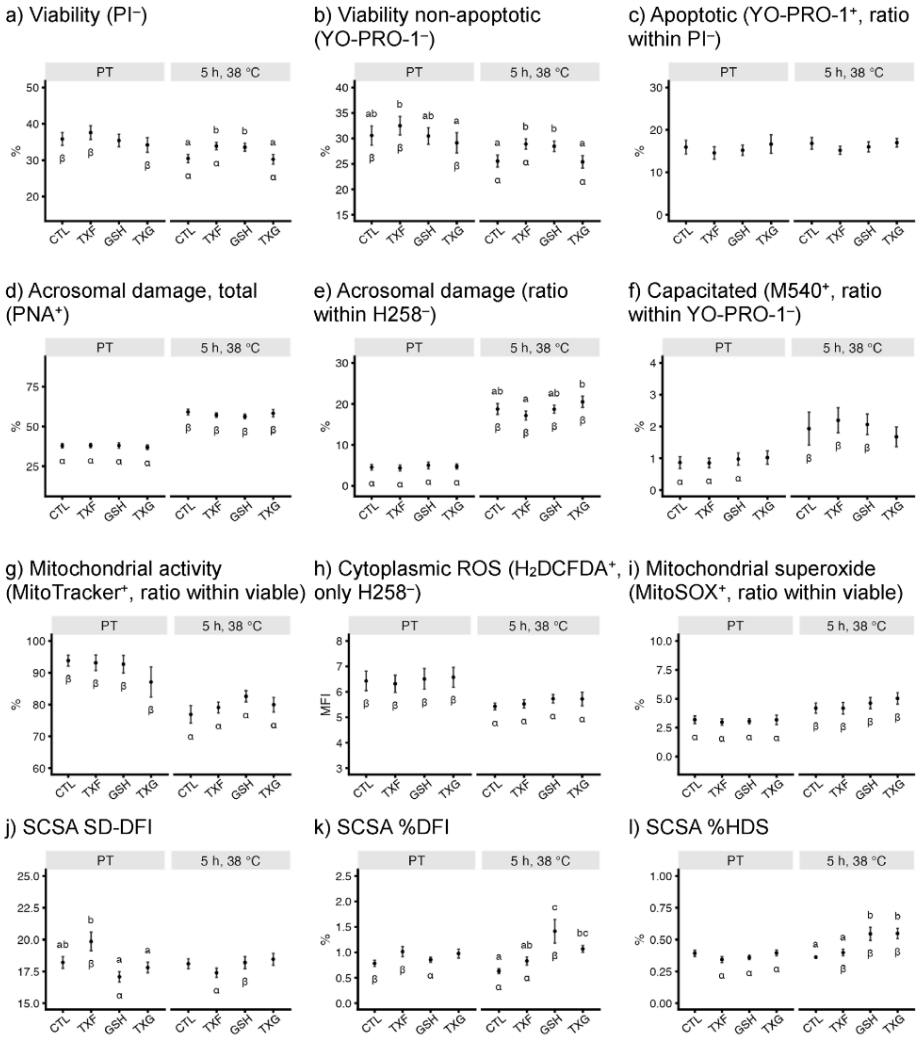
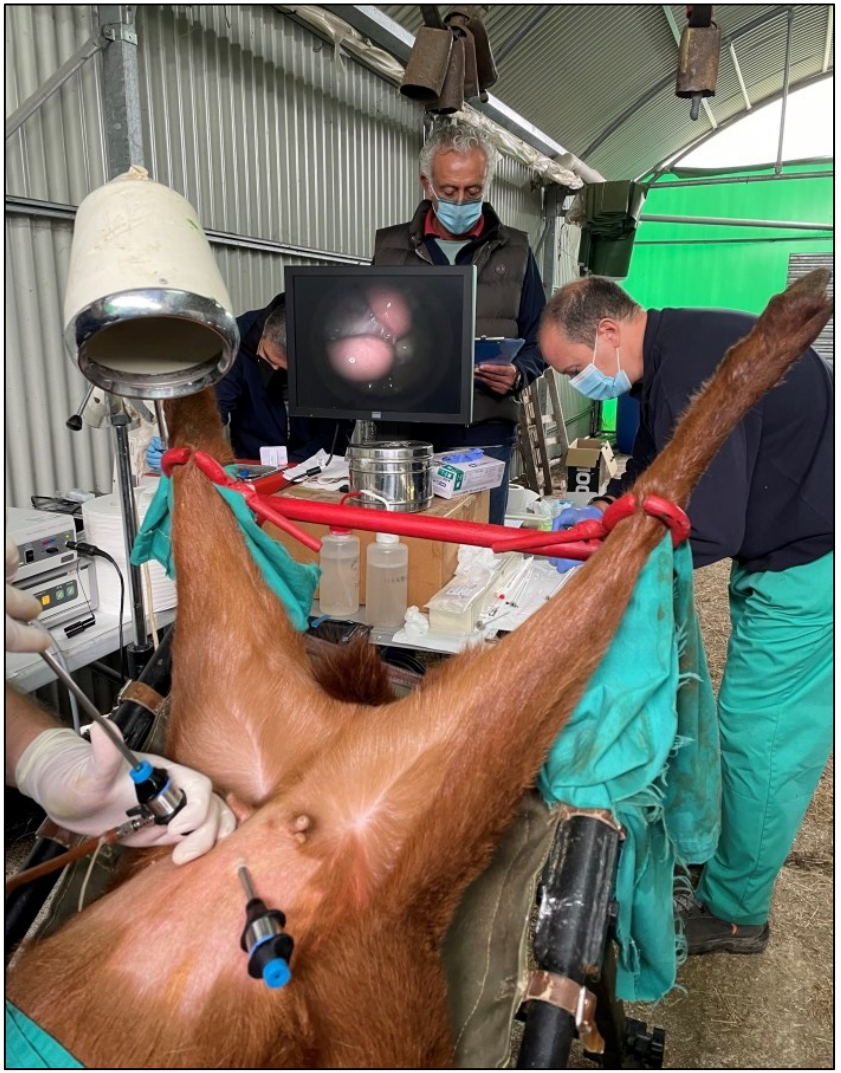


Fig. 4. Flow cytometry results (mean ± SEM) for Experiment 2, showing the effect of taxifolin at 5 µM (1.5 µg/ml) (TXF), GSH at 1 mM (GSH), their combination (TXG), or no supplement (CTL: Control) on physiology and chromatin structure variables directly post-thawing (PT), and after 2 and 5 h of incubation at 37 °C. PI: Propidium iodide; H258: Hoechst 33258; PNA: Peanut agglutinin (Alexa 647 conjugated); M540: Merocyanine 540; MT: MitoTracker deep red; MSX; MitoSOX; SCSA: Sperm chromatin structure assay; SD-DFI: Standard deviation of the DNA fragmentation index; %DFI: % spermatozoa with high DFI (fragmented DNA); %HDS: % spermatozoa with high DNA stainability (low compaction or chromatin immaturity). Treatments with different Latin letters differ by $P < 0.05$ within each incubation group, and those with different Greek letters differ by $P < 0.05$ between incubation groups and within the same treatment.

Viability was not affected by taxifolin. Both antioxidants did not significantly affect other sperm physiology parameters. The incubation significantly affected all the parameters ($P < 0.004$), overall decreasing sperm quality.

RESULTS: ARTIFICIAL INSEMINATION

During the fertility trial, three does die for reasons unrelated to the study, with 26 does diagnosed by echography. Pregnancy diagnostic was 9/13 (69.2%) in the control group and 10/13 (76.9%) in the TXF group

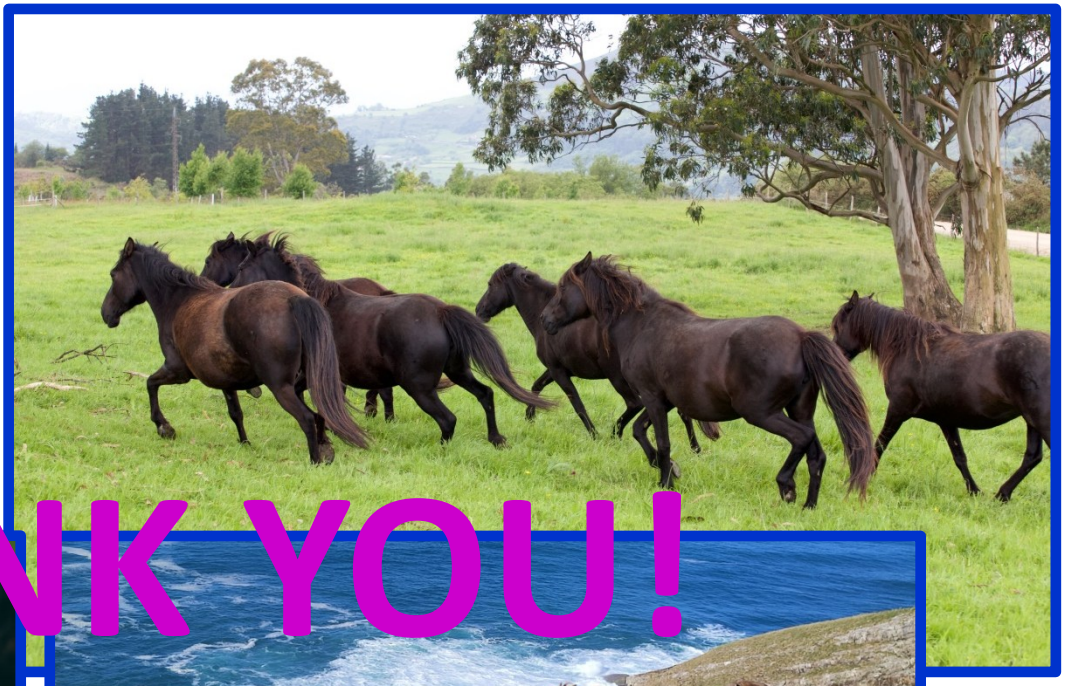


CONCLUSIONS

1. This is a practical case to improve sperm cryopreservation to preserve a local endangered breed, the Bermeya goat. The fertility trial using frozen-thawed semen yielded excellent results in pregnancy rates, showing the potential of reproductive biotechnologies to recover and preserve this native breed.
2. Taxifolin showed a lack of toxicity in the low micromolar range and some encouraging effects for cryopreserving goat semen. Although taxifolin and GSH did not show evidence of synergic effects, each one improved the post-thawing results. In
3. This information could help the conservation of other autochthonous, non-commercial, or endangered breeds.
4. Future experiments could test these treatments on commercial breeds, which are more apt for extensive fertility trials.

ACKNOWLEDGEMENTS

Grant RZP2013-006-00-00 y RZP2017-00008-00-00 (MINECO)



THANK YOU!