CREATION OF A REGIONAL BANK OF NORTHERN SPAIN BREEDS *BERMEYA GOAT* AND *XALDA SHEEP*: PRACTICAL ASPECTS

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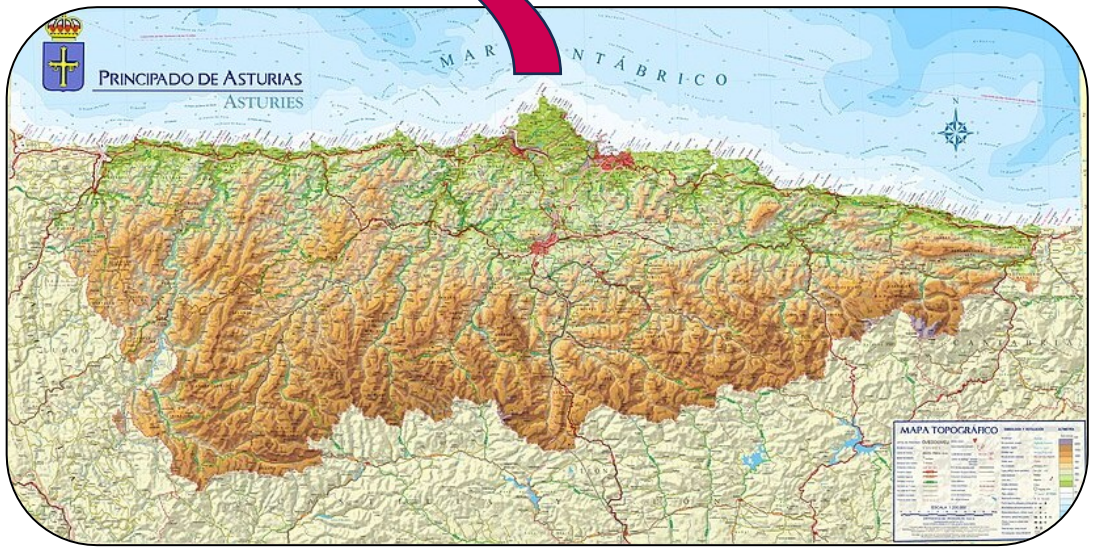
AHA "ADVANCES IN SEMEN CRYOPRESERVATION IN SMALL RUMINANTS". UPPSALA, 2025

OVINE AND CAPRINE BREEDS IN SPAIN

BREEDS AND VARIETIES INCLUDED IN THE OFFICIAL CATALOGUE OF LIVESTOCK BREEDS OF SPAIN		
BREEDS	**SHEEP**	**GOAT**
Natives	47	22
Natives at risk	37	17
Integrated in Spain	8	0
Other breeds	1	0
TOTAL	56	22

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

MATERIAL AND METHODS

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

ACOXA for Xalda sheep

ACRIBER for Bermeya goat

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

RAMS	BUCKS
Brucella mellitensis, B.ovis, Paratuberculosis, Maedi/Cae, Border Disease (Ag/Ac), Agalactia, Scrapie.	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. Assessment of semen/spermatozoa quality: **parameters fully studied.**

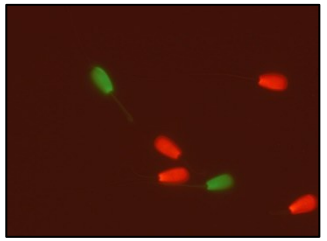
- Semen appeareance
- Volume
- Sperm concentration
- Mass motility (0-5)
- Total/progressive motility
- Sperm morphology
- Sperm viability

ACUCELL (IMV Technologies®, France)

ISAS® v. 1.19 (Proiser, Spain)

Morphology

Acrosome integrity



Viability

MATERIAL AND METHODS

6. Ram and buck **semen processing.**

Dilution (1/10)

Rams: TTFC Medium (Tes, Tris, Fructose, Citric Acid)

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

Centrifugation 900 X *g*/15 min.

Room temperature

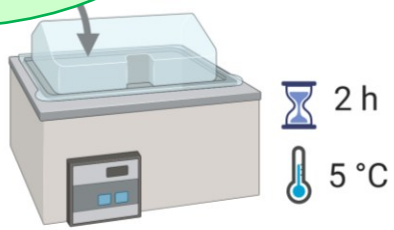
Pellet resuspension with freezing medium

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

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

Final sperm concentration 400 X 10^6 spz/ mL

Equilibration prior to freezing

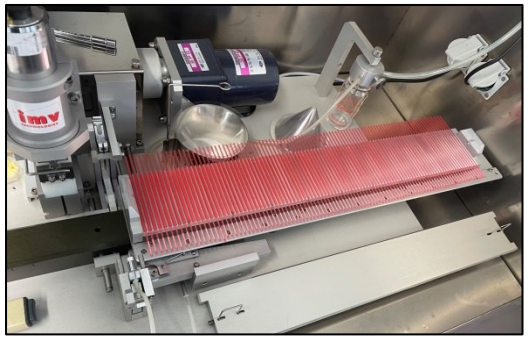


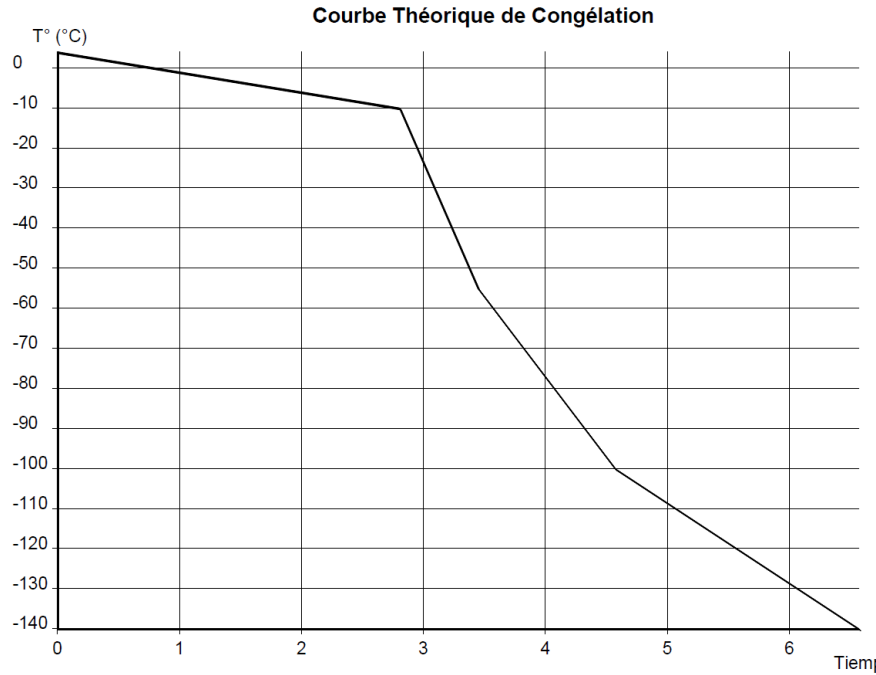
MATERIAL AND METHODS

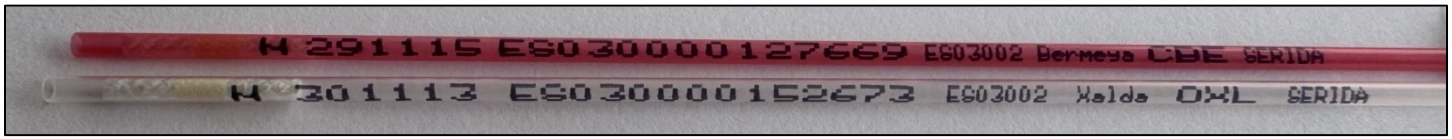
7. Ram and buck **semen cryopreservation.**

Machine filled and sealed of 0.25 mL straws

Straw frozen in programmable freezer



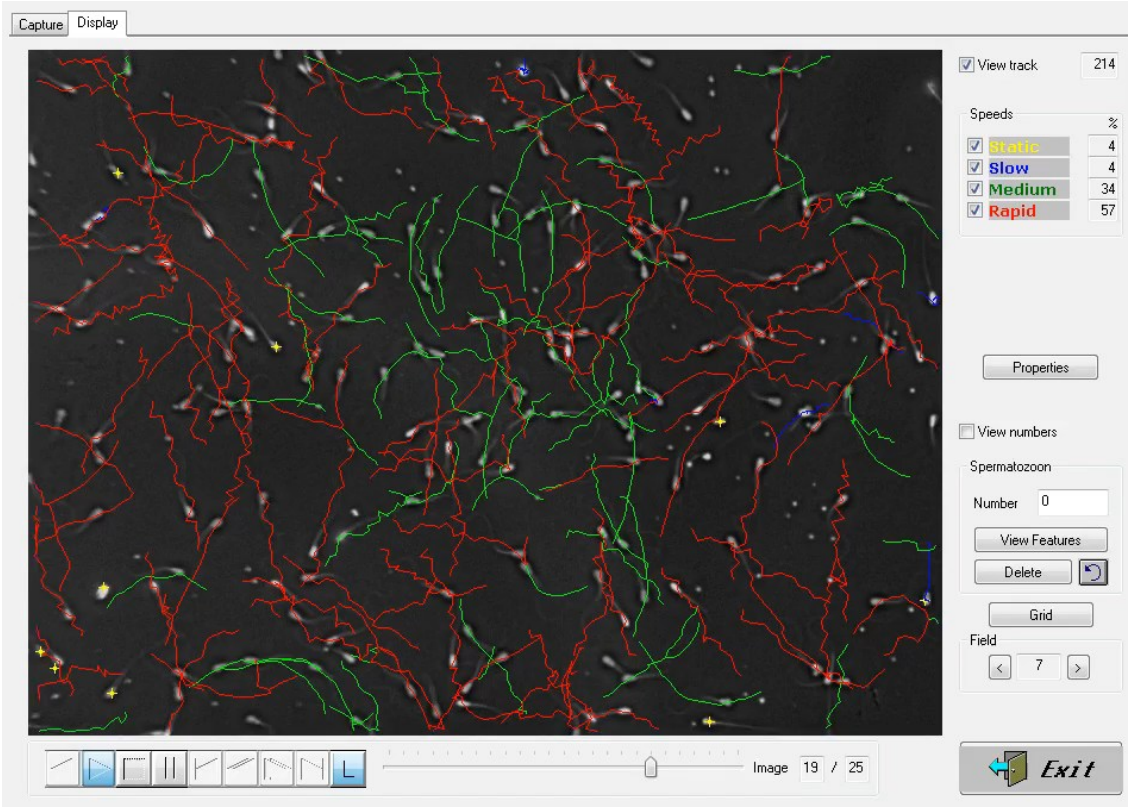




MATERIAL AND METHODS

8.1. Post-thawed sperm evaluation: CASA (fully studied).

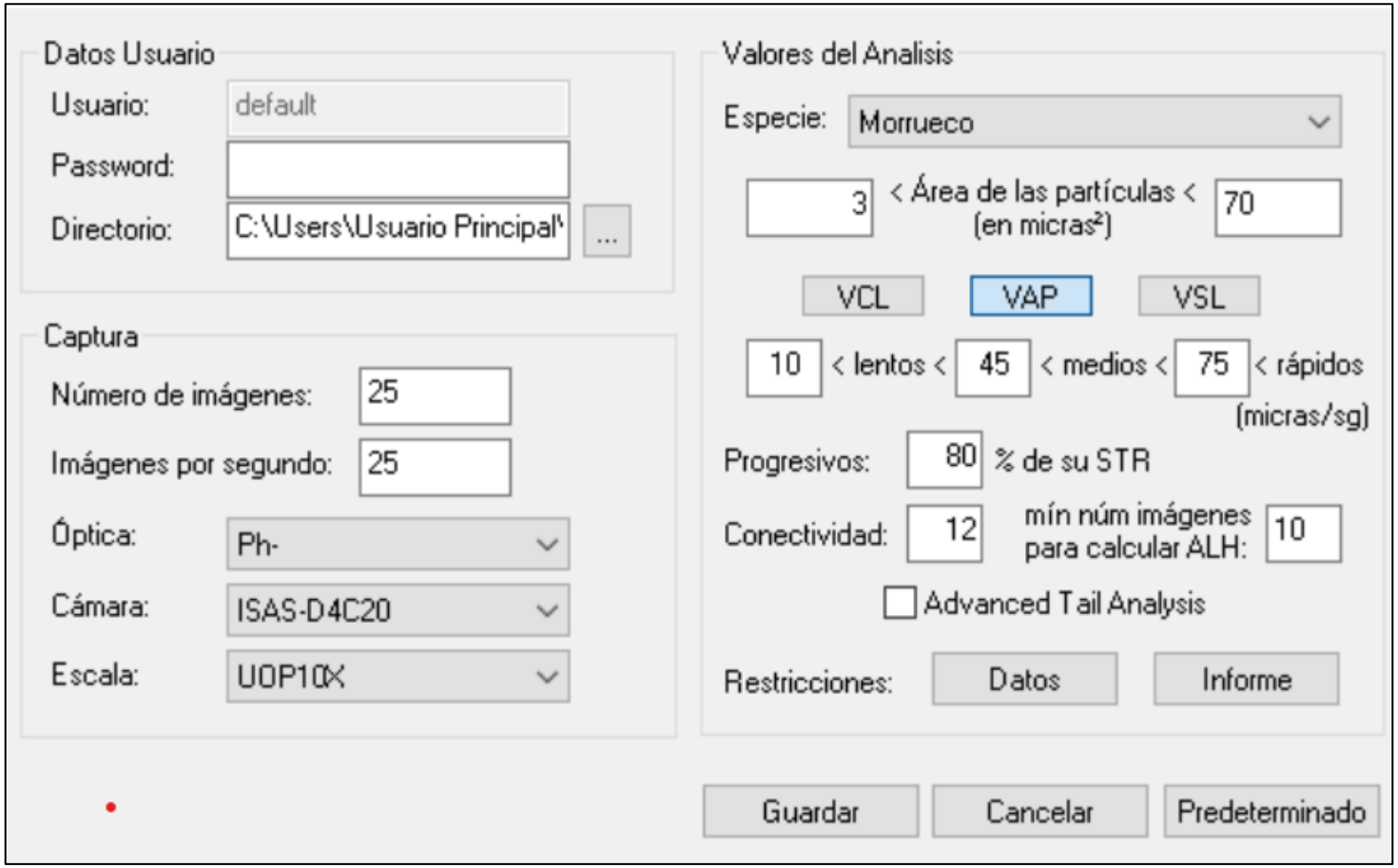
- Total motility
- Progressive motility
- VCL
- VSL
- VAP
- LIN
- STR
- WOB
- ALH
- BCF



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

MATERIAL AND METHODS

Motility: CASA ram and buck settings.



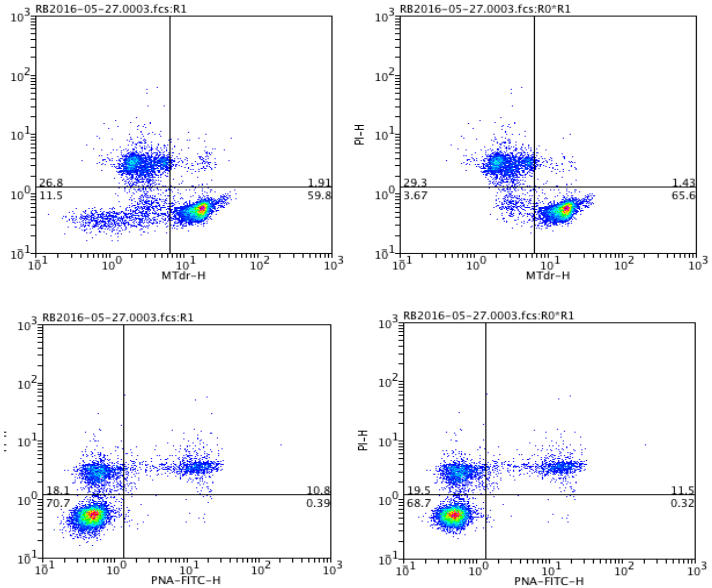
At least 1000 spermatozoa in 4-6 fields analyzed in a pre-warmed ISAS or Makler chamber

MATERIAL AND METHODS

8.2. Post-thawed sperm evaluation: FLOW CYTOMETER (not fully studied).

- 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

Table 1. Number of animals and ejaculates collected, frozen and stored samples in the Germplasm Bank of each breed.

Specie/Breed	N	Ejaculates collected	Ejaculates frozen	Seminal doses frozen	Doses stored in the bank
Xalda rams	**31**	**352**	**324**	**14,218**	**13,420**
Bermeya bucks	**38**	**321**	**294**	**15,549**	**15,189**

Rams
90.4 % Collection success rate
96.4 % Freezing success rate

Bucks
97.4 % Collection success rate
97.4 % Freezing success rate

Fresh semen samples thereshold values for freezing: 2000 x 10^6 spz/mL; 75% total motility; 90% normal morphology.
Post-thaw values for storage: 40% total motility.

RESULTS

Table 2. Characteristics (Mean±SD) of native Xalda ram and Bermeya buck semen.

Specie	N (ejaculates)	Volume (mL.)	Concentration (10^9/mL.)	Mass motility (0-5)	Fresh motility (%)	Post-thaw motility (%)
Rams	352	1.03 ± 0.15	4.25 ± 0.51	4.36 ± 0.18	78.13 ± 1.45*	57.76 ± 2.01*
Bucks	321	0.89 ± 0.21	4.64 ± 0.76	4.68 ± 0.23	80.03 ± 0.89*	51.38 ± 0.95*

* Indicates differences among values (P < 0.05)

Data were analyzed using SPSS® software

Recovery rate
Rams: 0.74
Bucks: 0.64

Volume and spermatozoa concentration differed significantly among individuals.
Significant differences between fresh and post-thaw sperm motility.

TWO DIFFERENT PRACTICAL ASPECTS

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

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CONCLUSIONS

1. The **establishment of a Genetic Resources Bank in Asturias**, as an *ex situ* conservation tool, to protect local breeds. It works as a complement to breeding on farms, storing genetic material to ensure long-term biodiversity.

2. It provides essential information on **sperm quality, functionality**, and, in some cases, **fertility** of the Xalda and Bermeya as autochthonous breeds.

3. Despite **individual differences**, most of the frozen samples showed good quality, but further studies are needed to **optimize the freezing protocol**.

4. **Regular evaluation of cryobank procedures** may be useful to characterizing stored batches and defining strategies to guaranteeing success in their future use.

IN THE FUTURE

1. Improve the **genetic and morphological selection criteria** of donors to ensure the greatest genetic representation of the *in vivo* population.

2. Implement an extended conservation program involving cryopreservation of **oocytes and embryos**.

3. Complete *in vitro* **semen quality evaluations** and **field fertility** probes.

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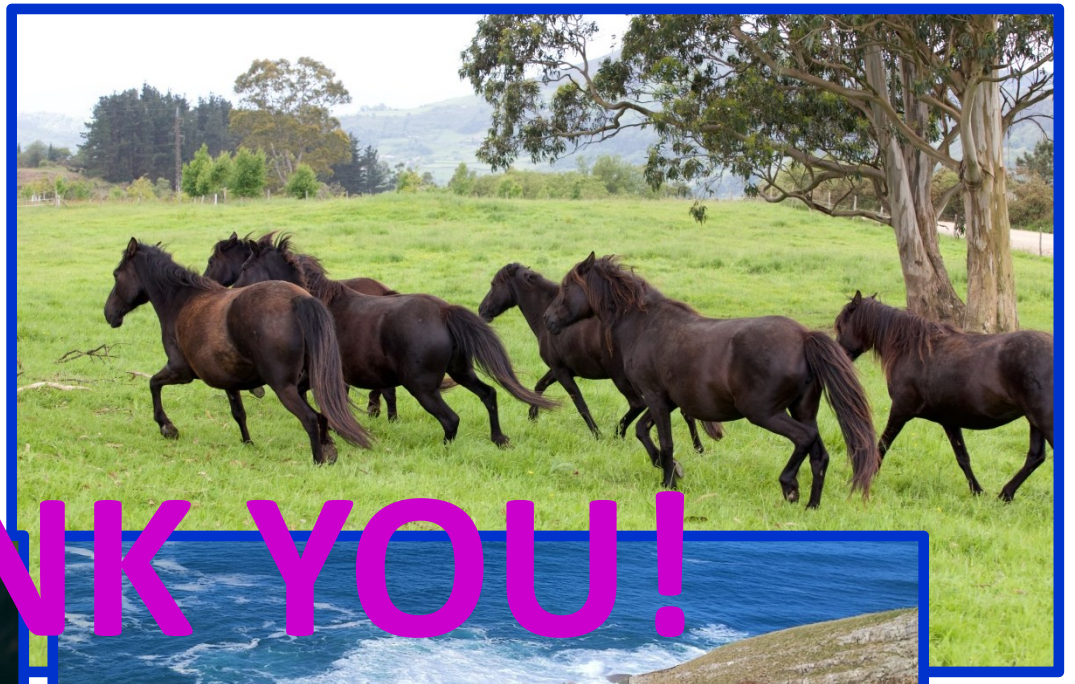

THANK YOU!