Ex situ Conservation Group - Ad hoc workshop on small ruminant semen cryopreservation
Swedish University of Agricultural Sciences, Uppsala, Sweden
10-11 June 2025

Field-based semen collection and cryopreservation in small ruminants: protocols and practices of the Cryobank of Animal Germplasm - National Research Council of Italy

FEDERICA TURRI
Institute of Agricultural Biology and Biotechnology
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AnGR Ex Situ Conservation in ITALY

Lack of National Coordination for the Cryobank of Germplasm for Animal Genetic Resources

Over the years, genetic material has been collected by Ministry, Breeders Associations, Research Institutions, Universities, Regional authorities, independently on multiple species and breeds in the framework of various projects

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An example…
The Cryobank of Animal Germplasm IBBA-CNR

**Institute of Agricultural Biology and Biotechnology (CNR),
within the Department of Veterinary Medicine and Animal Sciences (UNIMI),
Via dell'Università 6, 26900 Lodi – Italy.**

Formally recognized in 2023 by Ministry of Agriculture, Food Sovereignty and Forests

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divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

- ✓ **IBBA-CNR provides: qualified scientific and technical staff, running costs**
- ✓ **DIVAS-UNIMI provides: facilities**

- ✓ **The cryobank benefits from funding through regional and national research projects.**

1. PRESERVE

Genetic diversity of various Italian local breeds

2. GENETIC MATERIAL

To support in vivo populations through the establishment of a genetic reserve

3. FACILITATE RESEARCH

Fertility evaluation, cryobiology, molecular analysis, genetic characterization

Objectives of the Cryobank of Animal Germplasm IBBA-CNR

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The development of the Cryobank of Animal Germplasm IBBA-CNR

1996 ITALIAN LOCAL PIG BREEDS

- Eu project "European gene banking project for pig genetic resources"
- 4 breeds: Nero Siciliano, Cinta Senese, Mora Romagnola, Casertana
- 42 donors, 15625 semen doses

2008 LOMBARDIA FARM Angr CRYOBANK

- Accordo quadro CNR – Regione Lombardia
- 5 breeds: Varzese, Orobica, Frisa Valtellinese, Verzaschese, Brianzola
- 46 donors, 2429 semen doses

2018 ITALIAN LOCAL SHEEP AND GOAT BREEDS

- National Project, PSRN 2014 – 2020, CHEERS
- 6 breeds: Comisana, Massese, Ovino delle Langhe, Massese, Gentile di Puglia, Garganica, Nicastrese
- 59 donors, 1025 semen doses

2021 LOMBARDIA FARM Angr CRYOBANK 2.0

- Regional Project, PSR 2014 – 2020, BIODIVAL
- 7 breeds: Varzese, Modenese, Orobica, Frisa V., Verzaschese, Brianzola, Pecora di Corteno
- 58 donors, 2508 semen doses

2021 ITALIAN LOCAL SHEEP AND GOAT BREEDS

- National Project, PSRN 2021 – 2023, SHEEP&GOAT
- 6 breeds: Leccese, Rossa Mediterranea, Ionica, Massese, Orobica, Frisa Valtellinese, Verzaschese
- 31 donors, 705 semen doses

2021 ITALIAN LOCAL CATTLE BREEDS

- National Project, PSRN 2021 – 2023, I-BEEF2
- 4 breeds: Sarda, Sardo-Bruna, Sardo-Modicana, Calvana
- 5 donors, 970 semen doses

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The Cryobank of Animal Germplasm IBBA-CNR

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divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

IMAGE
Innovative Management of Animal Genetic Resources

4 SPECIES
31 BREEDS
287 DONORS
27539 DOSES

SPECIES	BREEDS	DONORS (n°)	SPERM DOSES (n° paillettes)	GENOTYPED (%)
Cattle	Varzese	9	2206	56
Cattle	Modenese	5	1150	0
Cattle	Burlina	9	1079	44
Cattle	Sarda	1	342	0
Cattle	Sardo-Bruna	3	333	0
Cattle	Sardo-Modicana	1	70	0
Cattle	Calvana	2	465	0
Cattle	Garfagnina	1	259	0
Cattle	Pontremolese	1	364	0
CATTLE TOT (9 breeds)		**32**	**6268**	**28**
Goat	Frisa	32	601	31
Goat	Orobica	32	391	28
Goat	Verzaschese	26	933	35
Goat	Garganica	6	84	0
Goat	Nicastrese	18	294	11
Goat	Rossa Mediterranea	2	34	0
Goat	Ionica	1	47	0
Goat	Cilentana	4	92	0
Goat	Bionda dell'Adamello	2	70	0
GOAT TOT (9 breeds)		**123**	**2546**	**24**
Sheep	Brianzola	17	437	35
Sheep	Pecora Ciuta	12	207	0
Sheep	Comisana	4	179	100
Sheep	Massese	19	670	53
Sheep	Gentile di Puglia	16	171	19
Sheep	Ovino delle Langhe	4	92	0
Sheep	Leccese	1	54	0
Sheep	Pecora di Corteno	1	146	0
SHEEP TOT (8 breeds)		**74**	**1956**	**31**
Swine	Casertana	11	3804	100
Swine	Cinta Senese	14	5244	79
Swine	Mora Romagnola	1	478	100
Swine	Nero Siciliano	30	7210	100
Swine	Ecotipo Nero delle Alpi	2	119	0
SWINE TOT (5 breeds)		**58**	**16855**	**91**
TOT CRYOBANK		**287**	**27625**	**40**

SHEEP-GOAT-CATTLE SPECIES
SEMEN COLLECTION ON FARM

Estrus females (natural or induction)

Semen collection on farm with AV

Epididymal sperm recovery at slaughter

Dilution 1:1 with commercial extender

Refrigeration at +5 ºC, transport to the lab

Semen evaluation and cryoconservation

Semen storage in the IBBA-CNR CRYOBANK

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✓ Livestock exhibition



✓ On farm




ON FIELD - Standardized protocols

Semen collection and semen quality evaluation

BUCKS AND RAMS SEMEN COLLECTION

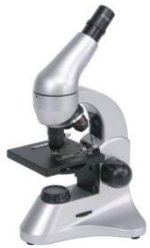
Prior to Collection

- Collect the donor's information (animal ID, date of birth, farm ID).
- Clean the prepuce using sterile normal saline solution.
- Thaw the frozen medium and warm it to 37 °C.
- Maintain this temperature throughout all dilution steps.

Collection Process

Buck and ram semen collection is performed directly on the farm. Semen is collected from each animal by the CNR team through repeated collections, using an artificial vagina and estrous females as mounts (goats or sheep used as teasers).

- Label a sample tube with the animal's name and/or identification number.
- Collect semen from sexually mature bucks/rams using an artificial vagina.
- Check the sample to ensure it is free of urine and other contaminants. Maintain the sample at 35–37 °C.
- Measure the semen volume and determine sperm concentration. Sperm motility and kinetics are assessed using a computer-assisted semen analyzer.
- Dilute the sample 1:1 with 37 °C cryopreservation medium or adjust to the ideal concentration to obtain **300 × 10^6 sperm** per semen dose (0.5 ml).
- Keep the sealed tube at 20 °C for 30 minutes.
- Cool the sperm suspension to 5 °C within 30 minutes of collection using a portable refrigerator, and transport it to our laboratory in Lodi.



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IN LAB - Standardized protocols

Facilities for cryopreservation

Cryopreservation Process

- Label the semen straws with an identification code (breed code, animal ID, and production date) using a labeling printer.
- Fill 0.5 ml straws with the sperm sample using a semi-automatic filling system, ensuring a final concentration of **300 × 10^6 sperm/ml**.
- Freeze the semen straws 5 cm above liquid nitrogen vapors for 20 minutes, then transfer them into liquid nitrogen storage tanks.

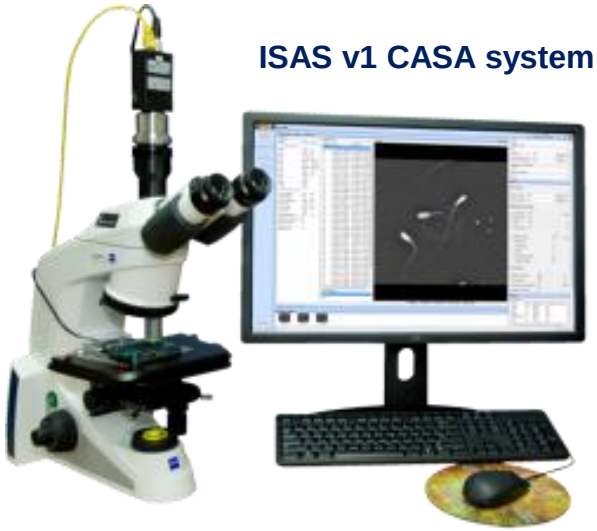
Multicoder printer for straws and gobelets (Minitube)

Semiautomatic filling and sealing machine (Minitube)

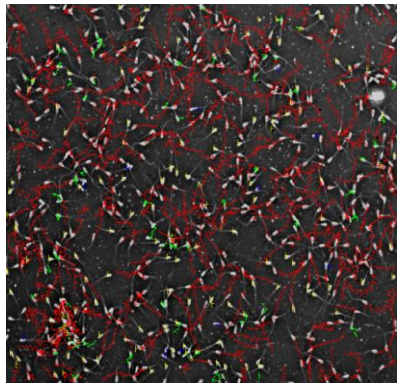
Nitrogen vapour

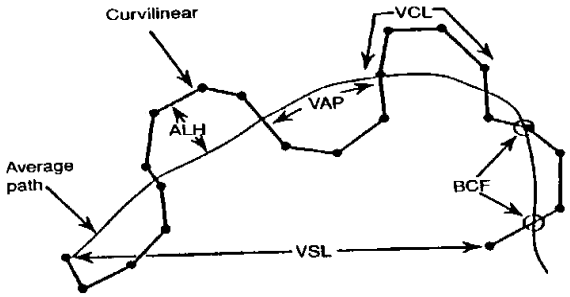
IN LAB Semen quality evaluation - advanced technologies
Fresh and frozen samples

CASA SYSTEM: MOTILITY AND KINETICS
SPERM PARAMETERS



ISAS v1 CASA system (Proiser)





Average path velocity (VAP) µm/sec,
Curvilinear velocity (VCL) µm/sec,
Straight line velocity (VSL) µm/sec,
Linearity (LIN=VSL/VCL) %
Amplitude of lateral head displacement (ALH) µm
Straightness VAP/VCL (STR) %
Beat cross frequency (BCF) beats/s
Wobble movement (WOB) %

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SEMINAL QUALITY CHARACTERIZATION BY FLOW CYTOMETRY

EASYCYTE PLUS/5HT GEN4 with IMV SOFT (IMV Technologies)

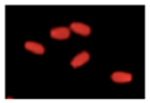
Viability

Membrane permeability is an indicator of cellular viability. Through membrane integrity, this test indicates percentage of viable spermatozoa.

Live | Dead

Sperm count

Assess accurate cell numbers and population percentages without reference beads.



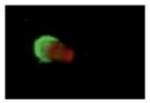
Oxidation level

This assay measures the intracellular level of Reactive Oxygen Species needed for sperm function but that are harmful at a high level.

Stongly oxydized

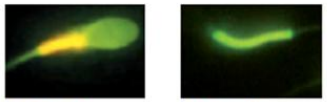
Viability and acrosome integrity

The acrosome is essential for fertilization.
A new combination of three stains was developed by IMV. Two of the stains monitor the integrity of the acrosome and of the membrane, simultaneously. The third fluorochrome is sperm-specific and thus allows to remove debris from the analysis.



Mitochondria activity

To reach the ovocyte, the sperm needs energy which is produced by the mitochondria. This assay analyzes membrane potential (polarized, depolarized) to show the integrity of the mitochondria.



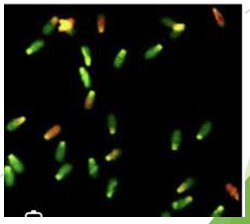
High potential | Low potential

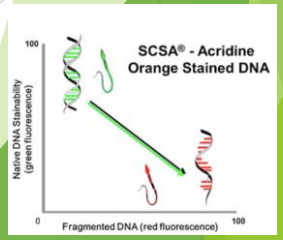
Membrane fluidity

Membrane fluidity is critical for proper cell function and exchange with the outside environment. This assay assesses phospholipid disorder in the membrane.

Normal organization | Membrane phospholipid disorder

- DNA compaction
- DNA fragmentation





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Difficulties associated with cryopreservation in local breeds

- ✓ Limited use of AI in small ruminants
- ✓ Lack of donors trained for semen collection
- ✓ Training more difficult in rams than bucks
- ✓ Lack of facilities and expertise for cryopreservation in the farming area

EMERGING TECHNIQUE: EPIDIDYMAL SPERM RECOVERY

1. Recovery of genetic material

- ✓ traditional collection is not possible
- ✓ accidental death of breeding animals
- ✓ end of reproductive career
- ✓ after castration

2. Cost reduction through the creation of cryobanks

- ✓ simple technology
- ✓ bypasses the need for animal training

Italian Journal of Animal Science

Taylor & Francis

Implementation and cost analysis of a regional farm animal cryobank: an Italian case study

Flavia Pizzi, Federica Turri, Teresa M. Gliozzi & Gustavo Gandini

Setting-up of operational protocols - CATTLE SPECIES

Reproduction in Domestic Animals

Reprod Dom Anim doi: 10.1111/j.1439-0531.2011.01948.x
ISSN 0936-6768

Influence of Recovery Methods and Extenders on Bull Epididymal Spermatozoa Quality

F Turri[1,2], M Madeddu[1], TM Gliozzi[1], G Gandini[2] and F Pizzi[1]

[1]*Istituto di Biologia e Biotecnologia Agraria, Unità Organizzativa di Supporto di Lodi, Consiglio Nazionale delle Ricerche, Lodi, Italy;*
[2]*Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università degli Studi di Milano, Milano, Italy*

Table 1. Sperm kinetics and viability parameters (LMS ± SEM) in fresh epididymal sperm samples, by extraction technique

Parameter	Experimental group[1] FL (n = 25)	RF (n = 21)
Total motility (%)	71.6 ± 2.0[a]	80.3 ± 2.3[b]
Active cells (%)	9.0 ± 1.6	6.8 ± 1.8
Hyperactive cells (%)	55.0 ± 4.2	52.6 ± 4.8
Average path velocity (μm/s)	53.4 ± 1.8	49.4 ± 2.1
Curvilinear velocity (μm/s)	112.8 ± 3.5	109.6 ± 4.0
Straight-line velocity (μm/s)	13.7 ± 0.9	12.4 ± 1.1
Amplitude of lateral head displacement (μm)	6.3 ± 0.4	6.6 ± 0.4
Beat cross-frequency (Hz)	9.5 ± 0.7	8.3 ± 0.8
Linearity index (%)	12.1 ± 0.9	11.2 ± 1.1
Straightness index (%)	30.4 ± 1.9	28.8 ± 2.2
Viability (%)	77.2 ± 1.3[a]	84.5 ± 1.5[b]

[1]Extraction technique: FL, floated samples; RF, flushed samples.
Values within each row with different letters are significantly different ($p < 0.05$).

FLOAT-UP

RETROGRADE FLUSHING

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Setting-up of operational protocols - GOAT SPECIES

Animal, page 1 of 8 © The Animal Consortium 2013
doi:10.1017/S1751731113002279

animal

Effect of testicle *postmortem* storage on goat frozen-thawed epididymal sperm quality as a tool to improve genebanking in local breeds

F. Turri[1,2], M. Madeddu[1a], T. M. Gliozzi[1], G. Gandini[2] and F. Pizzi[1†]

[1]*Institute of Agricultural Biology and Biotechnology, Lodi Unit, National Research Council, c/o Parco Tecnologico Padano, via Einstein, 26900 Lodi, Italy;*
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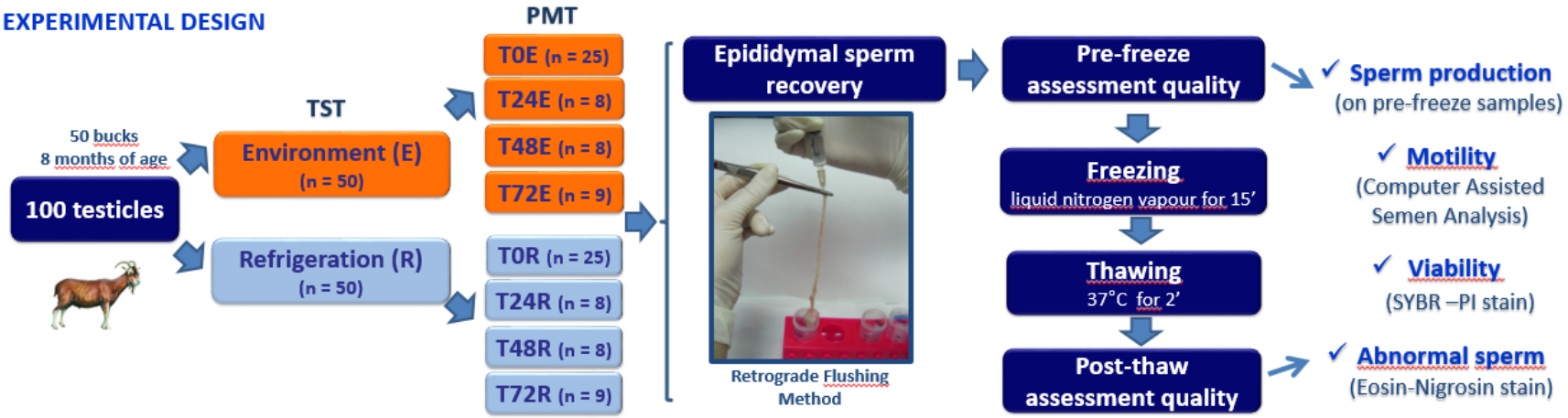
INTRODUCTION

Epididymal spermatozoa obtained from slaughtered or castrated animals associated to the possibility of managing rather long periods between animal death, sperm recovery and freezing would increase the opportunities to create semen cryobanks.

AIM

The aim was the assessment of pre-freeze/post-thaw quality of goat epididymal spermatozoa as function of testicles storage temperature **(TST)** and time elapsed between animal death or castration and sperm recovery **(PMT)** in order to establish optimal protocols for the recovery and cryopreservation of epididymal sperm.

EXPERIMENTAL DESIGN



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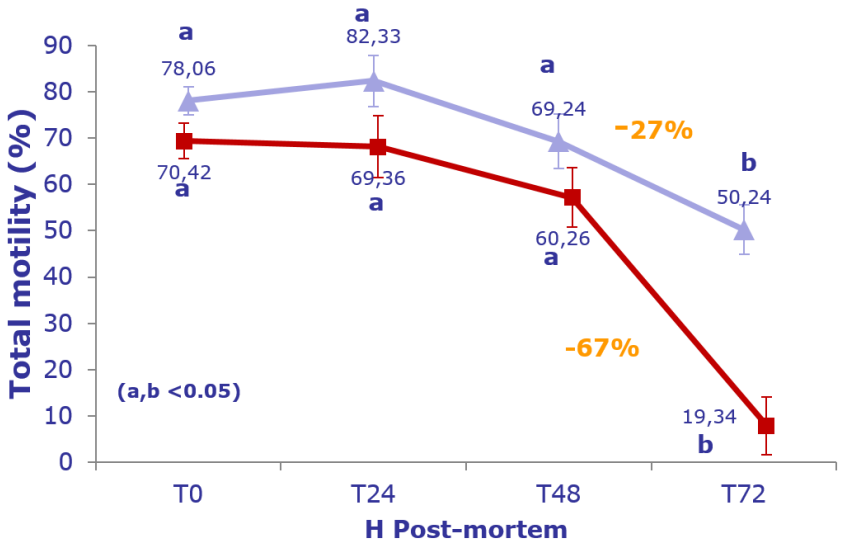
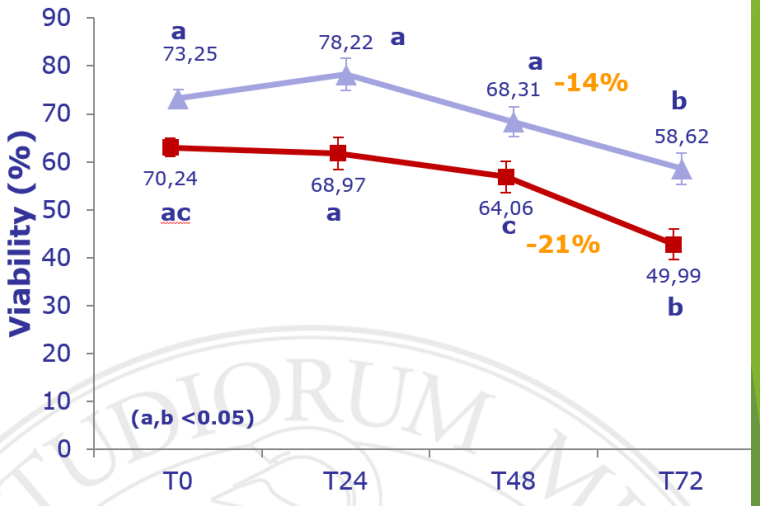
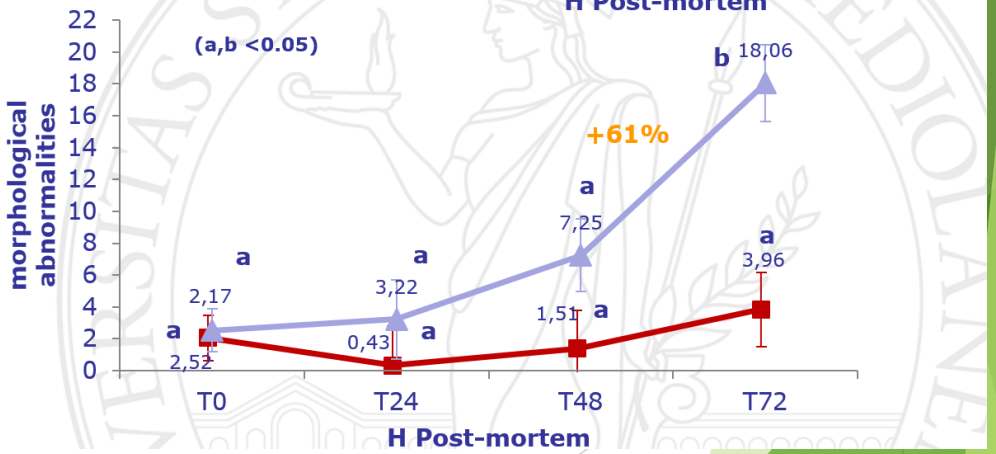




TIME POST MORTEM
(0, 24, 48, 72 h)

■ **Room temp**

▲ **+5° C**



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✓**RETROGRADE FLUSHING**

Suitable also for caprine species

✓ **SPERM QUALITY**

High semen quality maintained up to 48 hours post mortem (Pre-freezing e post-thawing)

✓**After 48 h post mortem**

Refrigeration of the samples is necessary

When sperm cryopreservation is not immediately practicable goat testicles should be transported and stored at refrigeration temperature (5°C) up to a maximum of 48 h post mortem to ensure an acceptable sperm quality.

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FIELD APPLICATION: EPIDIDYMAL SPERM RECOVERY IN SHEEP AND GOAT SPECIES

- Difficulties in training breeding males for semen collection in different sheep breeds (longer training period respect to goat and catlle)
- Donors of high genetic value important for the in vivo population

Check of the sanitary status of the male donors before and after death

⇩

Recovery of testicles during slaughter

⇩

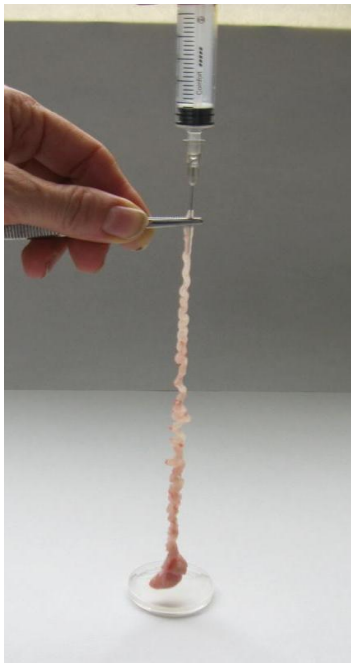
Processing of seminal material at IBBA-CNR LAB

⇩

Semen evaluation and cryopreservation

⇨

Storage of seminal material in the cryobank



assonapa

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The breeds involved – SHEEP

BRIANZOLA

CIUTA

DELLE LANGHE

MASSESE

PECORA DI CORTENO

COMISANA

GENTILE DI PUGLIA

LECCESE

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The breeds involved – GOAT

CILENTANA

FRISA VALTELLINESE

GARGANICA

OROBICA

CILENTANA

IONICA

BIONDA DELL'ADAMELLO

VERZASCHESE

NICASTRESE

FIELD APPLICATION:
CREATION OF THE GENETIC RESERVE OF ITALIAN SHEEP AND GOAT BREEDS

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	GOAT BREED	Genetic Strategy	RISK STATUS FAO	TYPE OF MATERIAL	N. DONORS	N. DOSES	VOLUME (ML)	CONC (sperm/ml)
1	Bionda Adamello	Conservation	Endangered	Ejaculate	2	70	3,65	$1,95x10^9$
2	Cilentana	Conservation	Endangered	Ejaculate	4	92	2,4	$2,64x10^9$
3	Frisa Valtellinese	Conservation	Endangered	Ejaculate	32	601	4,35	$1,32x10^9$
4	Garganica	Conservation	Endangered	Ejaculate	6	84	1,25	$2,70x10^9$
5	Jonica	Conservation	Critical	Ejaculate	1	47	5	$2,10x10^9$
6	Orobica	Conservation	Endangered	Ejaculate	32	391	2,6	$1,40x10^9$
7	Rossa Mediterranea	Conservation	Endangered	Ejaculate	2	34	2,5	$1,32x10^9$
8	Nicastrese	Conservation	Vulnerable	Ejaculate/ Epididymis	18	294	1,33	$2,40x10^9$
9	Verzaschese	Conservation	Endangered Manteined	Ejaculate	26	933	1,7	$2,10x10^9$
					123	2546	2,75	$1,99x10^9$
	SHEEP BREED	Genetic Strategy	RISK STATUS FAO	TYPE OF MATERIAL	N. DONORS	N. DOSES	VOLUME (ML)	CONC (sperm/ml)
1	Comisana	Genetic improvement	Endangered Manteined	Epididymis	4	179	3,54	$1,80x10^9$
2	Gentile di Puglia	Conservation	Vulnerable	Ejaculate/ Epididymis	16	171	2,26	$1,09x10^9$
3	Delle Langhe	Genetic improvement	Endangered	Epididymis	4	92	3,6	$2,20x10^9$
4	Leccese	Conservation	Endangered	Ejaculate	1	54	1,8	$3,80x10^9$
5	Massese	Genetic improvement	Vulnerable	Ejaculate/ Epididymis	19	670	3,76	$1,70x10^9$
6	Ciuta	Conservation	Endangered	Epididymis	12	207	3,57	$1,32x10^9$
7	Pecora di Corteno	Conservation	Endangered	Epididymis	1	146	11	$3,99x10^9$
8	Pecora Brianzola	Conservation	Endangered Manteined	Ejaculate/ Epididymis	17	437	3,3	2,54x109
					74	1956	4,10	$2,30x10^9$
					197	4502		

- ✓ **AVAILABLE as GENETIC RESERVE FOR THE BREEDS**
- ✓ **PROCEDURE INITIATED TO AUTHORIZE SEMEN DOSES FOR AI WITH THE MINISTRY**
- ✓ **SEMEN FROM THE DIFFERENT BREEDS IS BEING CHARACTERIZED FOR FREEZABILITY AND QUALITY USING FLOW CYTOMETRY**

FIELD APPLICATION:
EPIDIDYMAL SPERM RECOVERY IN CATTLE SPECIES

- **Difficulties in training breeding males for semen collection in different cattle breeds (cost and time of the project)**
- **Health requirements not adequate for entrance in bull AI CENTER**
- **Donors of high genetic value important for the in vivo population**

Check of the sanitary status of the male donors before and after death

⇩

Recovery of testicles during slaughter

⇩

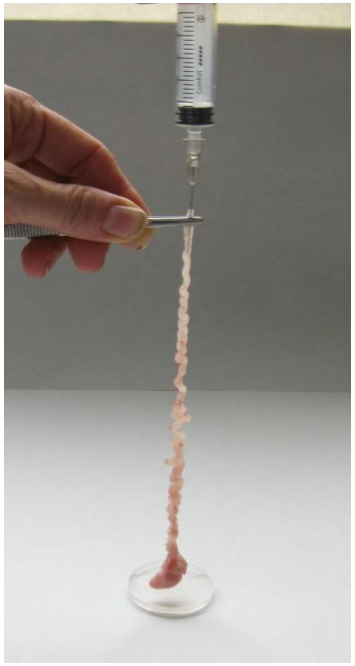
Processing of seminal material at the AI center

⇩

Semen evaluation and cryopreservation

⇨ Storage of seminal material in the cryobank

⇨ Semen dose available for AI



The breeds involved

Sardo Modicana

Sardo Bruna

Sarda

Garfagnina

Varzese

Calvana

Modenese

Pontremolese

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The semen doses produced

N°	BREED	Sample Cryobank IBBA-CNR	Date of collection	N. SEMEN DOSES
1	Sardo-Bruna	SB01	12.04.23	179
2	Sardo-Bruna	SB02	12.04.23	154
3	Sarda	SA01	12.04.23	342
4	Calvana	CA01	31.01.24	218
5	Sardo- Modicana	SM01	08.02.24	67
6	Garfagnina	GAR01	20.05.24	259
7	Calvana	CA02	05.12.24	240
8	Pontremolese	PO01	04.02.25	364
9	Garfagnina	GAR02		550
10	Varzese	VAR06	29.03.2022	119
11	Varzese	VAR07	02.11.2022	285
12	Varzese	VAR08	19.06.2023	142
13	Varzese	VAR09	14.02.2024	502
14	Modenese	MO01	23.11.2022	46
15	Modenese	MO02	01.12.2022	190
16	Modenese	MO03	13.02.2023	500
17	Modenese	MO04	13.02.2023	142
18	Modenese	MO05	06.11.2023	272
				4571

✓ **AVAILABLE for AI to SUPPORT IN VIVO-POPULATION**

Semen samples were collected under National sanitary criteria

- ✓ **Donors Health Examinations**
- ✓ **Sanitary condition of the herds**

CATTLE	SHEEP / GOAT
Trichomonas foetus – Prepuce	Brucella abortus/melitensis
Campylobacter – Prepuce	Caprine Arthritis Encephalitis Virus (CAEV) / Visna-Maedi
BHV-1 – Infectious Bovine Rhinotracheitis, gB, gE	Chlamydia abortus
Mycobacterium avium paratubercolosis	Q Fever - Coxiella burnetii
Blue Tongue	Mycobacterium avium paratub.
BVD – Bovine Viral Diarrhea	Leptospira spp
Leptospira spp	Toxoplasma gondii
Brucella abortus/melitensis	Blue Tongue
Enzootic Bovine Leukosis	Mycoplasma agalactiae

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SOME RESEARCH ACTIVITIES

Identify the most suitable parameters that would provide reliable prediction of fertility

Animal, page 1 of 8 © The Animal Consortium 2017
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animal

The combination of kinetic and flow cytometric semen parameters as a tool to predict fertility in cryopreserved bull semen

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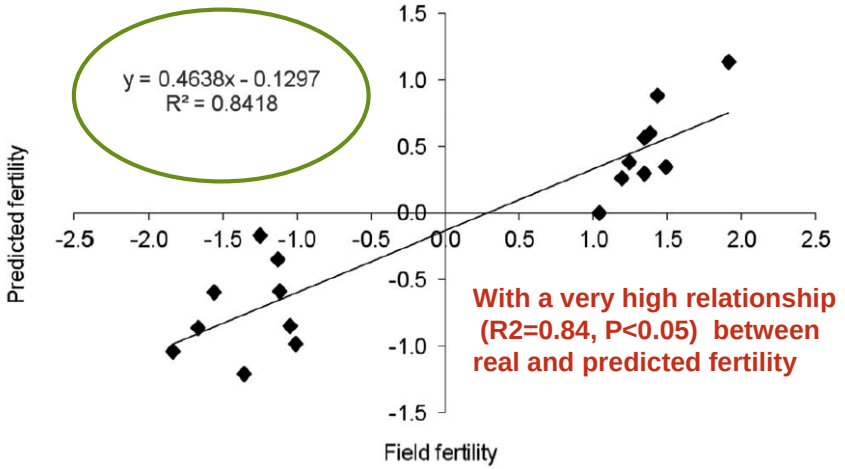
Table 1 *Computer-assisted semen analysis and flow cytometry semen quality parameters of 18 Holstein bulls based on fertility ranking (LSM ± SEM)*

	Fertility			
	High		Low	
Parameters	LSM	SEM	LSM	SEM
MTOT (%)	75.72[a]	2.09	71.56[b]	1.59
ACT (%)	52.45[A]	1.53	43.92[B]	1.40
HYP (%)	24.15	1.88	27.01	1.58
BCF (Hz)	10.87	0.29	10.73	0.27
VCL (µm/s)	112.28	2.78	113.04	2.36
VAP (µm/s)	56.30	1.25	57.99	1.14
STR (%)	64.86[A]	1.03	59.26[B]	0.94
ALH (µm)	6.59	0.18	6.49	0.15
VSL (µm/s)	36.23	0.88	34.21	0.81
LIN (%)	30.15[A]	0.80	27.71[B]	0.69
HMMP (%)	45.07	1.76	44.46	1.51
AIV (%)	38.84	2.19	38.06	1.84
ARV (%)	2.67	0.18	2.29	0.16
VIAB (%)	48.87[a]	2.15	44.70[b]	1.80
LDV (%)	24.80	1.85	23.83	1.52
HDV (%)	5.03	0.24	4.52	0.21
NPV (%)	46.26	2.49	44.32	2.00
PV (%)	0.73	0.15	0.69	0.12
NPVFe (%)	45.93	2.48	43.64	1.99
PVFe (%)	1.18	0.20	1.44	0.17
Alpha-T	0.5237	0.0005	0.5230	0.0005
ATSD	0.0144	0.0003	0.0136	0.0003
%DFI (%)	3.10[A]	0.16	4.04[B]	0.15
%HG (%)	3.09	0.13	3.18	0.11

Prediction model that explained almost half of the variation in conception rate, included 9 variables



With a very high relationship (R2=0.84, P<0.05) between real and predicted fertility

Figure 1 Relationship between fertility rates predicted by an equation based on the combination of nine sperm quality parameters and field fertility (expressed as the estimated relative conception rate) observed in 18 Holstein bulls ($R^2 = 0.84$, $P < 0.05$). The line indicates the trend in the data.

Correlations were observed between ERCR and some kinetic parameters, and viability and %DFI.

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APPLICATION OF FREEZE-DRIED PROCEDURE IN RAM SPERMATOZOA WITH TWO COOLING PROTOCOLS

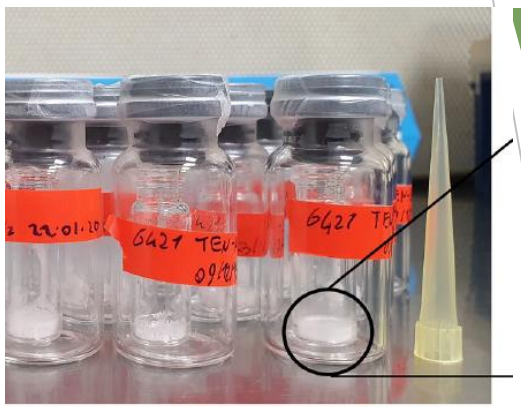
UNIVERSITÁ DEGLI STUDI DI TERAMO

scientific reports

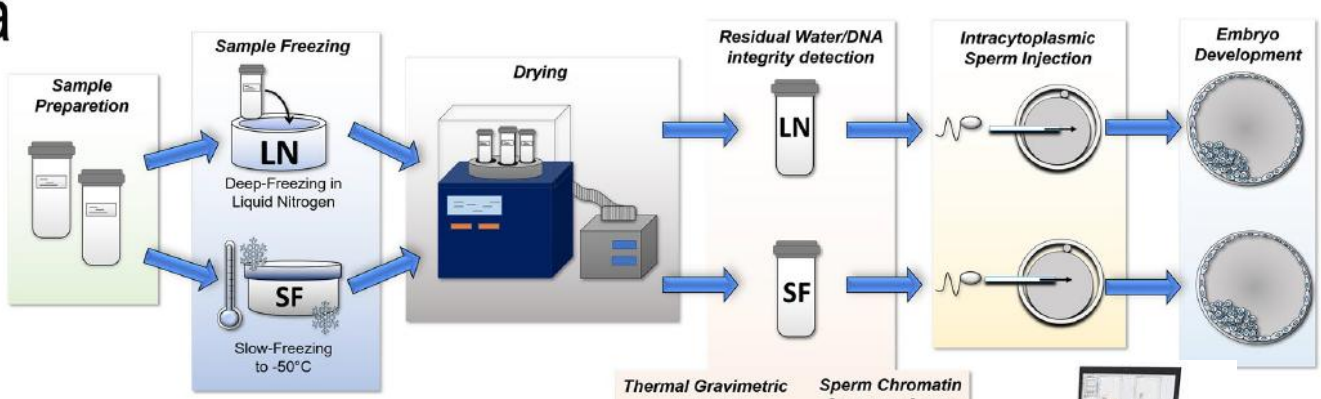
OPEN

Whole genome integrity and enhanced developmental potential in ram freeze-dried spermatozoa at mild sub-zero temperature

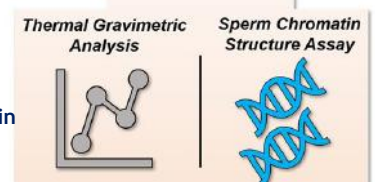
Luca Palazzese[1,6], Debora Agata Anzalone[1,6], Federica Turri[2], Marco Faieta[3], Anna Donnadio[4], Flavia Pizzi[2], Paola Pittia[3], Kazutsugu Matsukawa[5] & Pasqualino Loi[1]✉



a



minor DNA damage, Double-Strand Break (DSB) characterized by a lower degree of abnormal chromatin structure (Alpha-T)



the best embryonic development (42% vs 25,3%)

Significant technological advancement for the development of lyophilization as a valuable and cheaper alternative to deep-freezing in LN for ram semen.

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APPLICATION OF VACUUM-DRYING ENCAPSULATION TECHNIQUE IN RAM SPERMATOZOA

frontiers | Frontiers in Veterinary Science

TYPE Original Research
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Reviving vacuum-dried encapsulated ram spermatozoa via ICSI after 2 years of storage

Luca Palazzese[1†], Federica Turri[2†], Debora Agata Anzalone[3], Joseph Saragusty[3‡], Jacques Bonnet[4,5], Marthe Colotte[6], Sophie Tuffet[6], Flavia Pizzi[2], Alessia Luciani[3], Kazutsugu Matsukawa[7], Marta Czernik[1,3] and Pasqualino Loi[3*]

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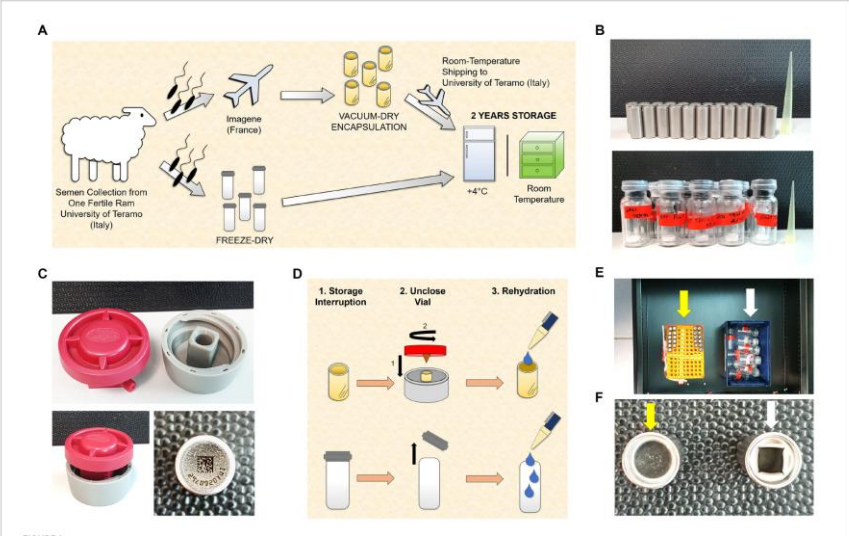

Adaptation of vacuum-drying encapsulation (VDE) technique, originally developed for nucleic acid conservation, to ram spermatozoa, and compared it to canonical lyophilization (FD), testing long-term storage at room temperature (RT) and 4°C.

VDE
better structural stability and lipid composition, higher DNA integrity and embryonic development (12.8% Vs 8.7%)

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