Ing. **Filipp Georgijevič Savvulidi**, Ph.D.
Ing. Martin Ptáček, Ph.D.

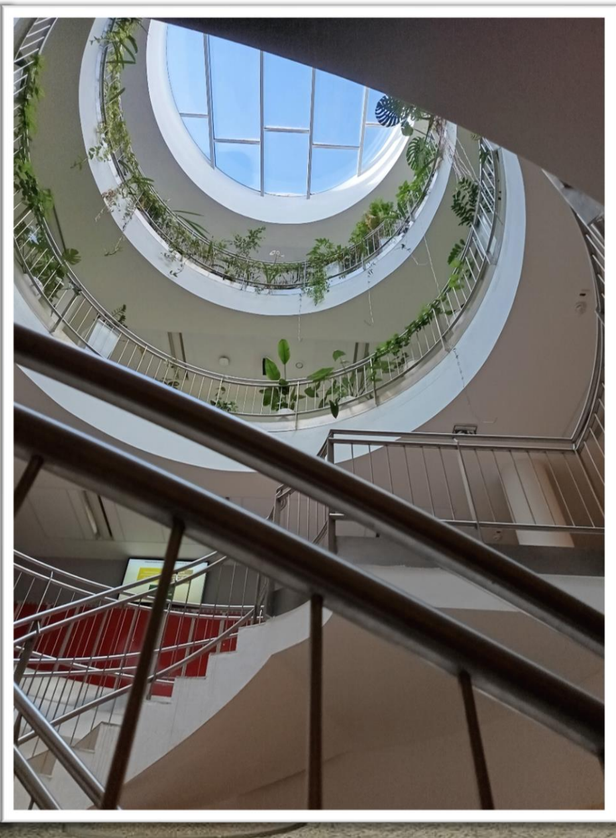
Reproductive biotechnologies in native sheep: the Czech experience

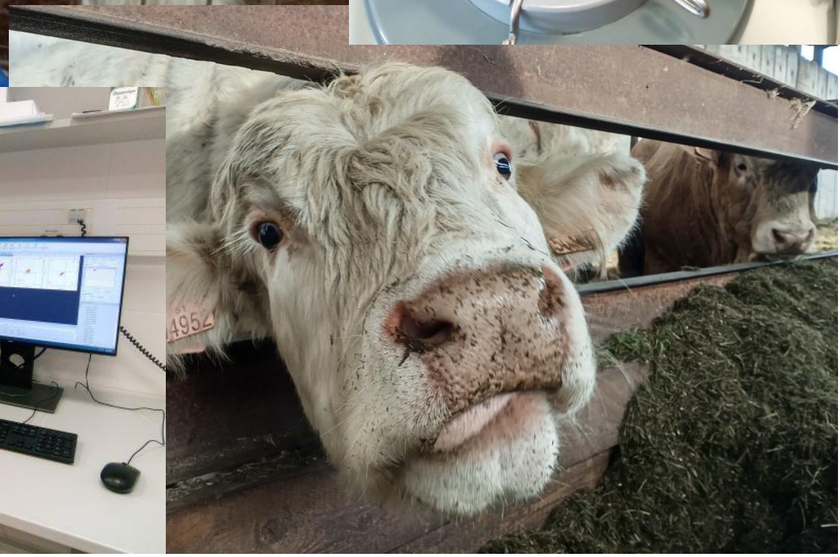
Czech University of Life Sciences Prague

University full of life

Name: Filipp Georgijevič
Surname: Savvulidi

Department of Animal Science



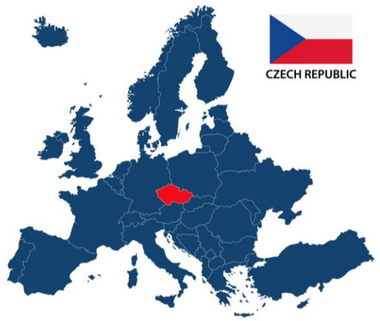


Filipp Georgijevič Savvulidi

Ing., Ph.D. · Assistant professor at Czech University of Life Sciences Prague

Studying the factors affecting the cryosurvivality of livestock spermatozoa. Optimizing/development of AI methods.

Czech University of Life Sciences Prague



Native small ruminants in the Czech Republic

NÁRODNÍ PROGRAM PRO ZEMĚDĚLSKOU BIODIVERZITU

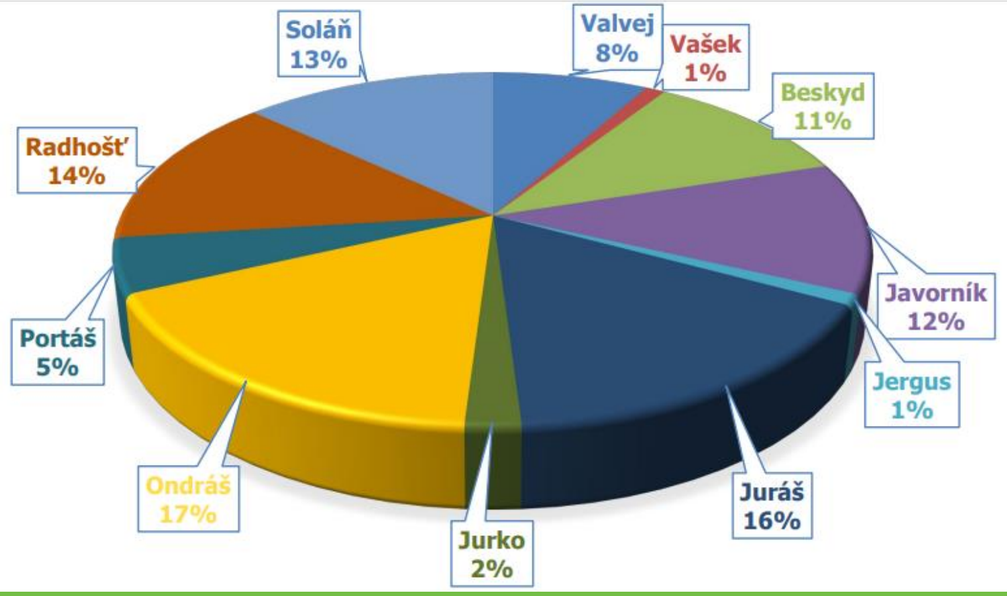
The National Programme on Conservation and Utilization of Plant, Animal and Microbial Genetic Resources Important for Food and Agriculture

Czech Sheep genetic recources

2024: 1095 animals (76 rams), 56 flocks

Wallachian Sheep

Graf 30: zastoupení jednotlivých genealogických linií beranů v ročníku jehňat 2024



Czech Goat genetic recources

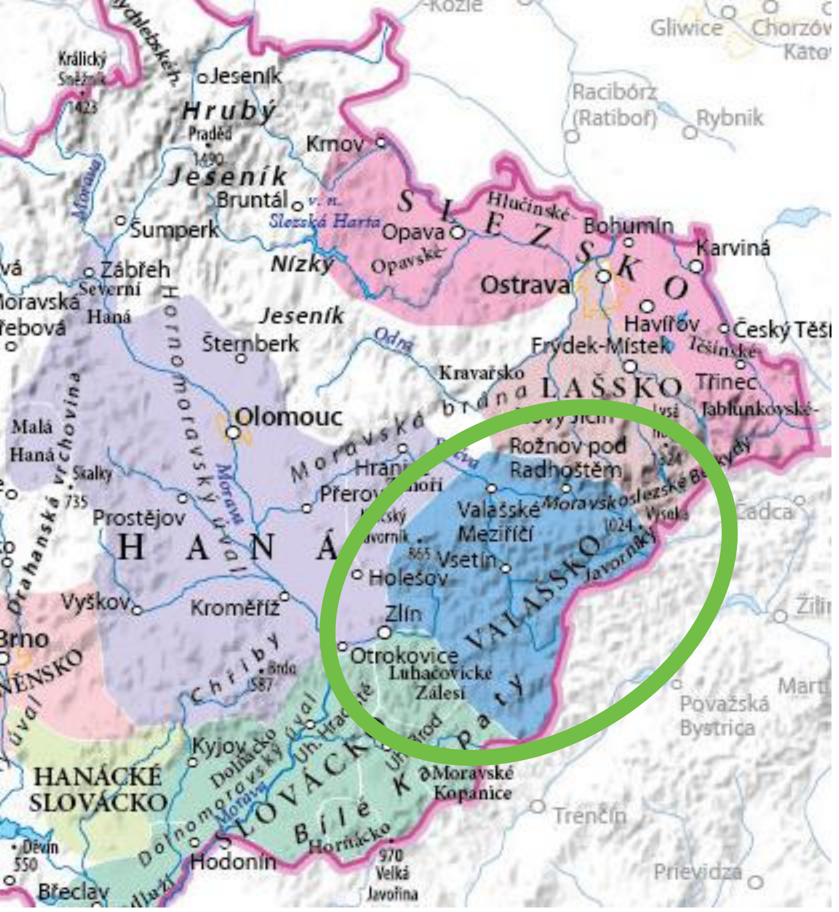
flocks 2022: 627 animals in 52 flocks

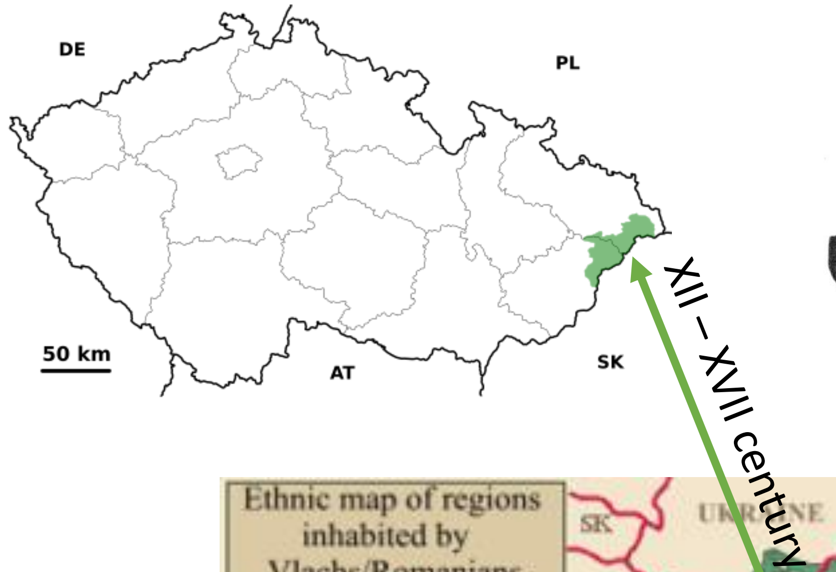
BSH goat

Czech University of Life Sciences Prague

University full of life

czu.cz






Valach z roku 1787 (zdroj: P.M)

Czech national heritage

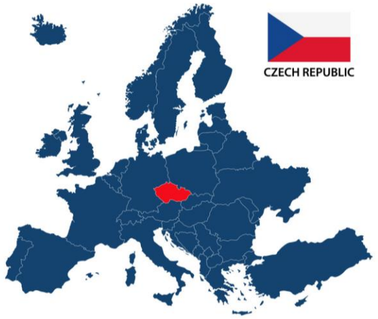
1. Traditional cheeses made from the milk of Wallachian ewes grazed on mountain meadows full of medicinal herbs,
2. Lean lamb meat with fine muscle bundles,
3. Leather with attractive long hair or handicrafts made from Wallachian wool,
4. Wallachian sheep can also find application in hybridization programs as a mother breed capable of using poor mountain pastures
5. Can be used to maintain forest-free areas in mountain areas through extensive grazing,
6. Wallachian sheep increase landscape attractiveness for tourists by their effect on the landscape and their direct presence in it...

Original Paper *Czech Journal of Animal Science, 68, 2023 (11): 460–468*

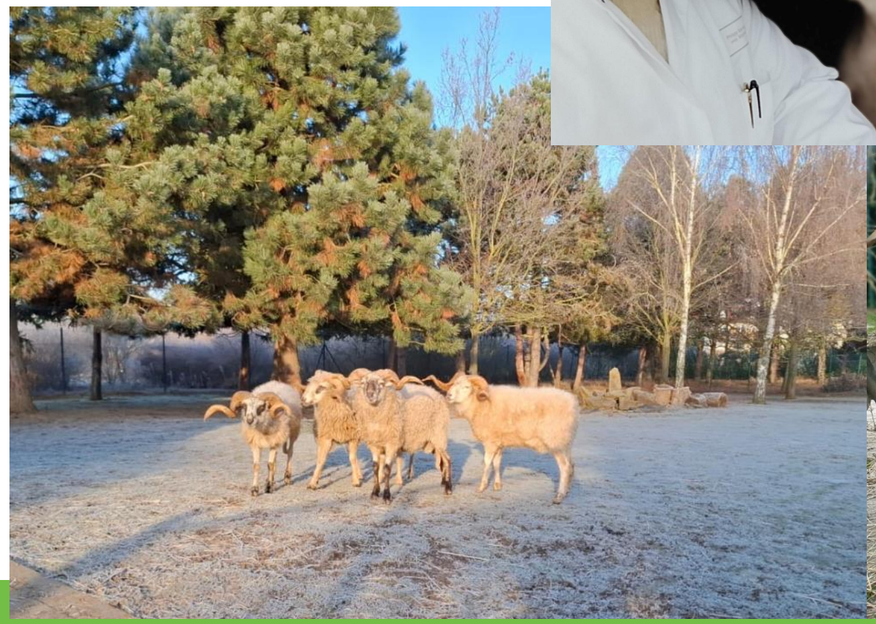
https://doi.org/10.17221/85/2023-CJAS

Monitoring of milk performance of Wallachian sheep grazed under traditional Carpathian management in Western Beskids location

Martin Ptáček[1], Michal Milerski[2], Tereza Michlová[3], Jaromír Ducháček[1], Vladimír Tančin[4,5], Michal Uhrinčať[4], Jitka Schmidová[2], Filipp Georgijevič Savvulidi[1], Luděk Stádník[1]*



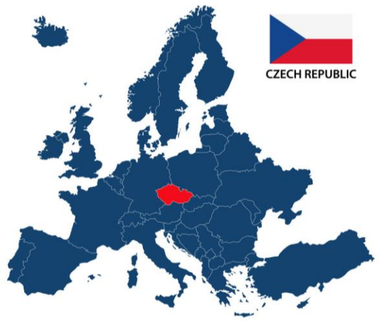
Wallachian rams in CULS campus



Czech University
of Life Sciences Prague

University full of life

czu.cz



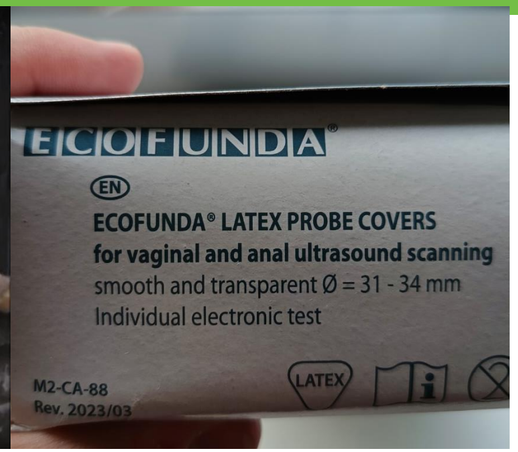
Czech cryobank for native small ruminants

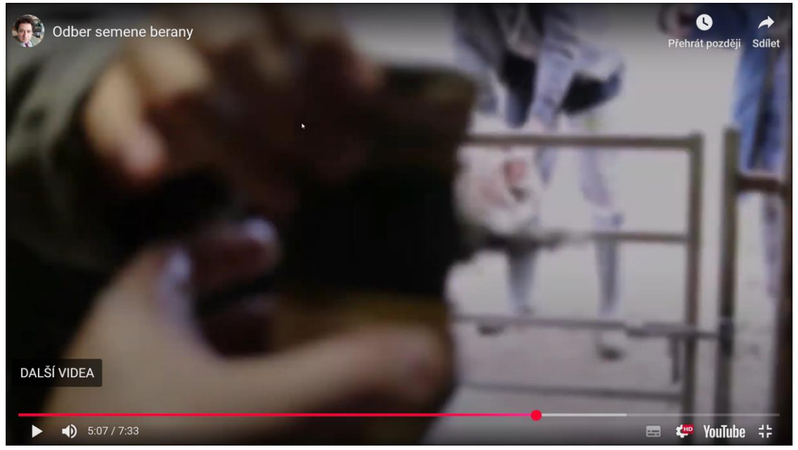
NÁRODNÍ CENTRUM
pro genetické zdroje zvířat

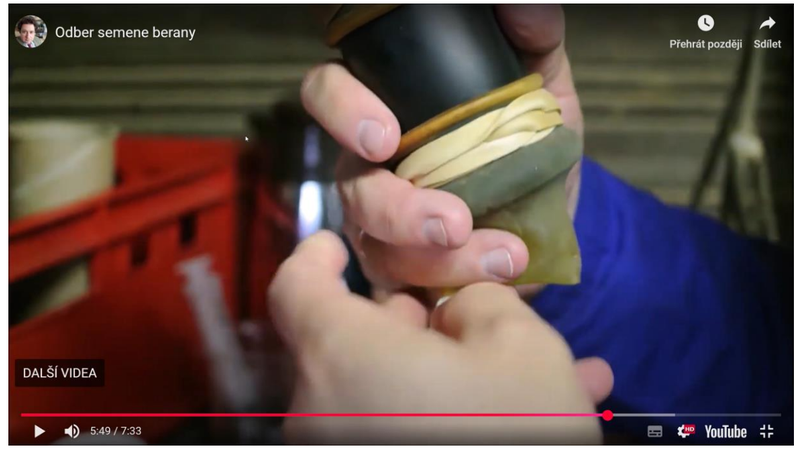
	No. of Sires	No. of IDs
Wallachian sheep	15	283*
Sumava sheep	58	1116
White short-haired goat	17	1597
Brown short-haired goat	10	304

*additional 360 IDs planned for the year 2025

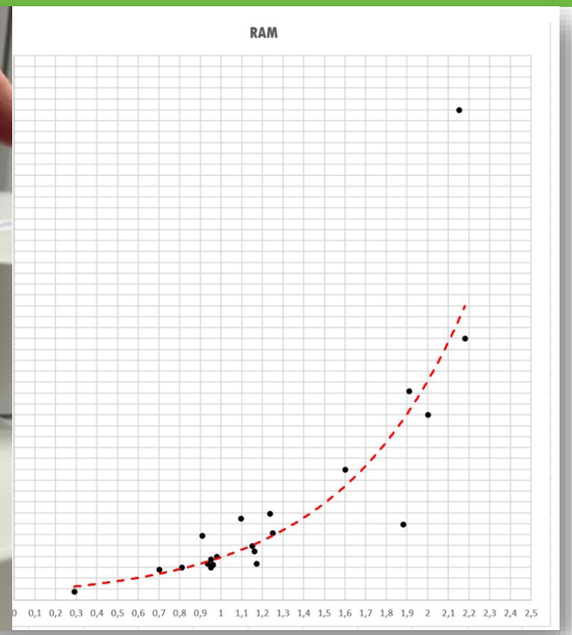
Ejaculate collection

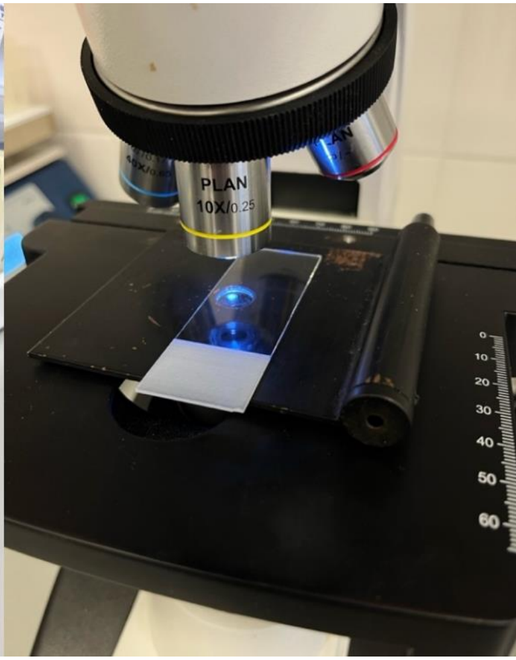






Ejaculate quality evaluation





Sperm motility and freezability

- The association between spermatozoa motility before sperm freezing and sperm freezability in rams is quite low

Czech J. Anim. Sci., 2025, 70(3):93-101 | DOI: 10.17221/185/2024-CJAS

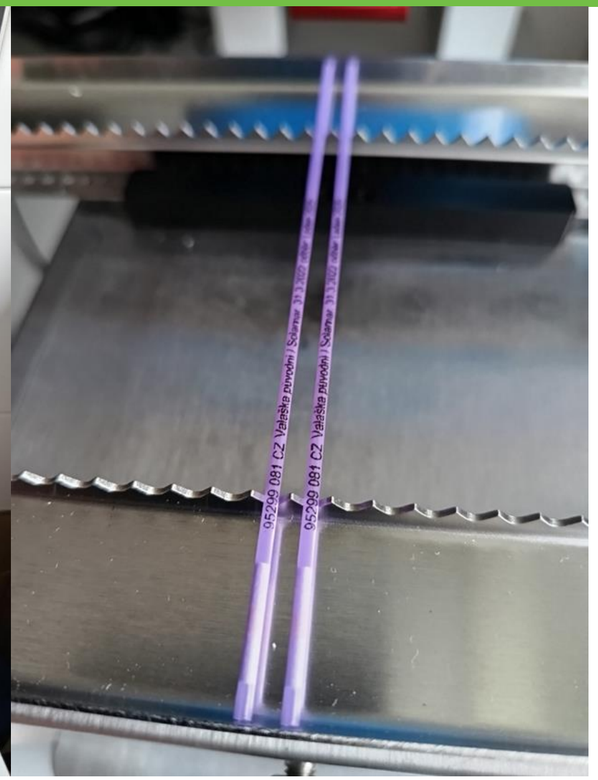
Association between conventional semen variables and sperm freezability in rams

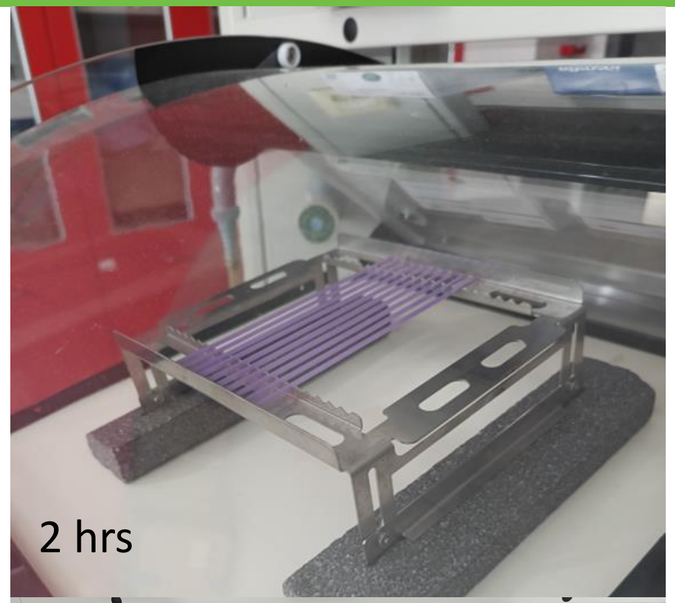
Aizhan Makhanbetova [1], Filipp Georgijevič Savvulidi [2], Martin Ptáček [2], Lucie Langerová [2], Beybit Kulataev [1], Nurlan Malmakov [1]

[1] Meat Sheep Breeding Department, Kazakh Research Institute of Livestock and Fodder Production, Almaty, Republic of Kazakhstan

[2] Department of Animal Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sceinces (CULS) Prague, Prague, Czech Republic

Straws processing





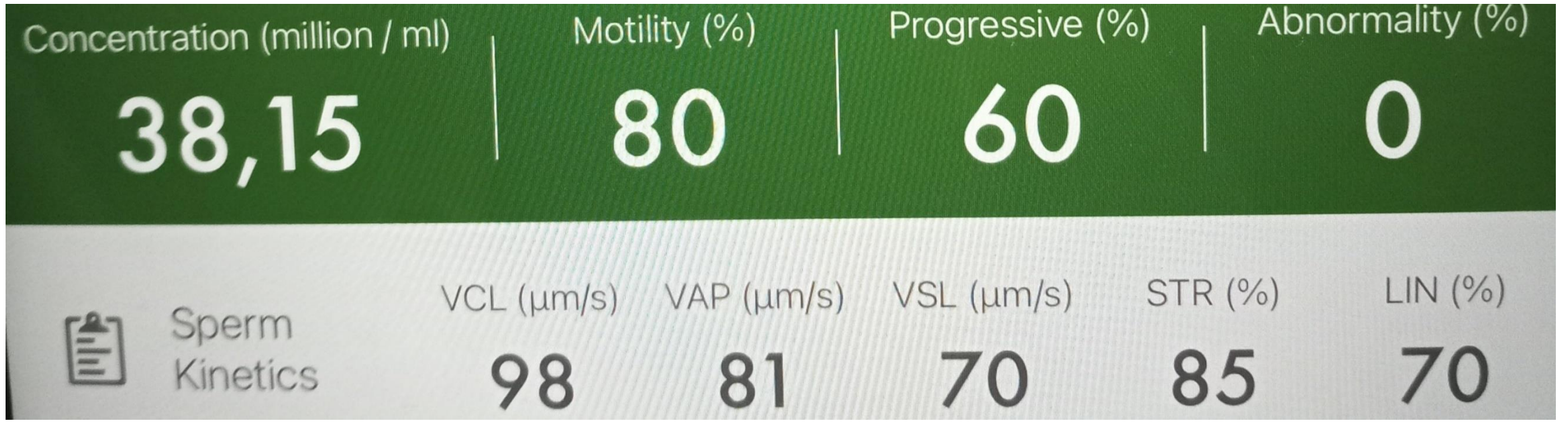
Straws freezing and thawing

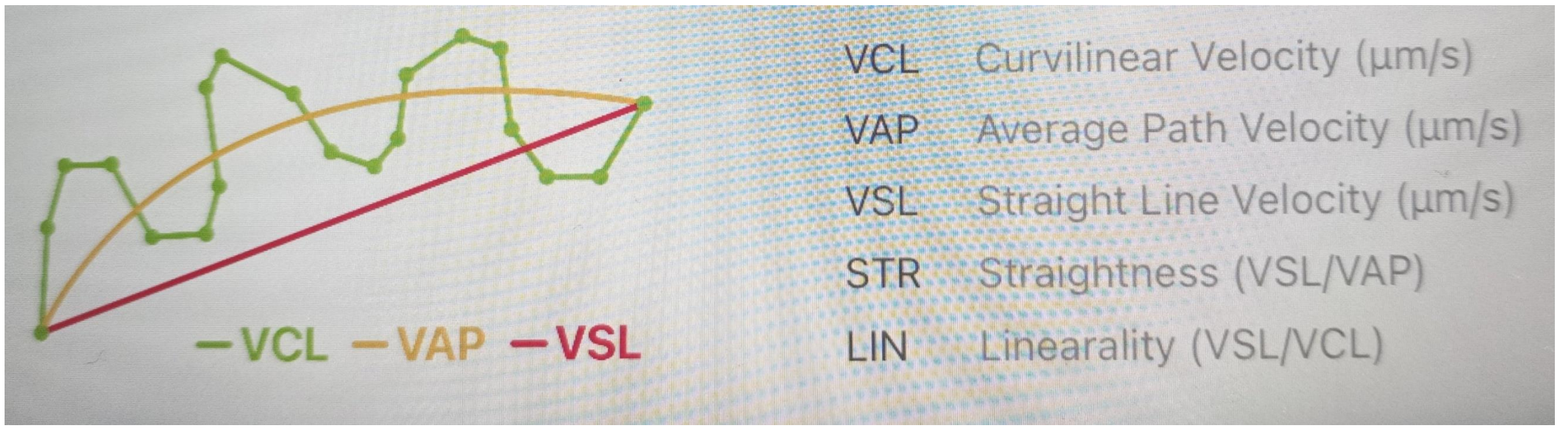
Analytical equipment for thawed sperm analysis

mCASA

Flow cytometer

mCASA


Concentration (million / ml)
38,15
Motility (%)
80
Progressive (%)
60
Abnormality (%)
0
Sperm Kinetics
VCL (µm/s)
98
VAP (µm/s)
81
VSL (µm/s)
70
STR (%)
85
LIN (%)
70


—VCL —VAP —VSL
VCL Curvilinear Velocity (µm/s)
VAP Average Path Velocity (µm/s)
VSL Straight Line Velocity (µm/s)
STR Straightness (VSL/VAP)
LIN Linearality (VSL/VCL)

Clustering analysis

Sperm Motility Tracker v2.0

- Novel software is a versatile tool with a very intuitive graphic interface allowing **clustering analysis without programming knowledge**

COMPUTER METHODS IN BIOMECHANICS AND BIOMEDICAL ENGINEERING: IMAGING & VISUALIZATION
2022, VOL. 10, NO. 6, 585–598
https://doi.org/10.1080/21681163.2021.2012831

Clustering and classification software for sperm subpopulation analysis

Francisco Buchelly Imbachí[a], Lucía Zalazar[b,c*], Juan Ignacio Pastore[a,c], Anabella Nicolli[b,c], Alba Ledesma[c,d], Federico A. Hozbor[d], Andreina Cesari[b,c*] and Virginia Ballarin[a*]

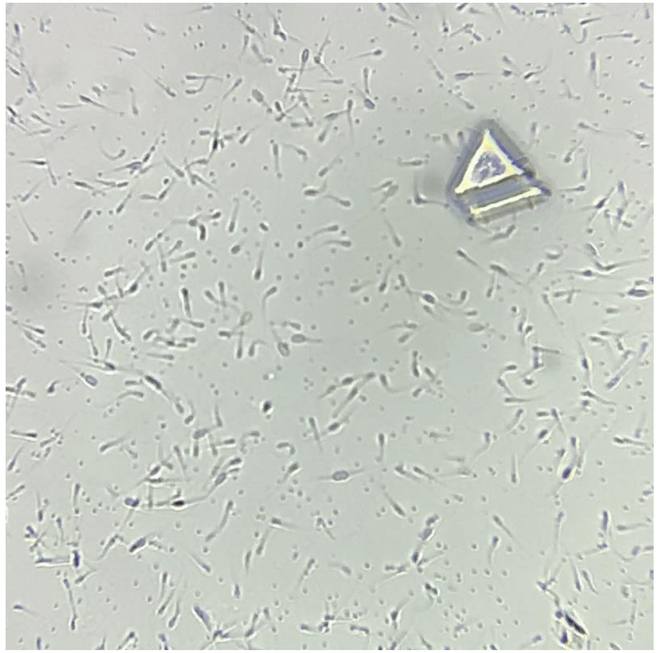
[a]Image Processing Lab. ICYTE-CONICET. Electronics Engineering Department Universidad Nacional de Mar del Plata, Laboratorio de Procesamiento de Imágenes Icyte Unmdp – Conicet, Buenos Aires, Argentina; [b]Instituto de Investigaciones Biológicas (IIB) Unmdp – Conicet, Mar del Plata, Buenos Aires, Argentina; [c]Consejo Nacional de Investigaciones Científicas Y Técnicas Conicet, Buenos Aires, Argentina; [d]Biotecnología de La Reproducción, Departamento de Producción Animal, Instituto Nacional de Tecnología Agropecuaria (Inta), Balcarce, Argentina

Final remarks

Our software module is a versatile tool with a very intuitive graphic interface allowing an automatic analysis (clustering, classification, and validation) without programming deep knowledge or expertise in clustering. As benefits, *SMT v2.0* performs hierarchical clustering by default classifier parameters or by choosing the number and which ones. The user can perform the motility parameters analysis with this software or with any other CASA system only importing the parameters' data set regarding the proposed structure. Additionally, other multiparametric studies can be adapted as *SMT v2.0* input datasets. The output gives information about the characteristics and relative abundance of each cluster as well as information related to which individual cell is belonging to each cluster.

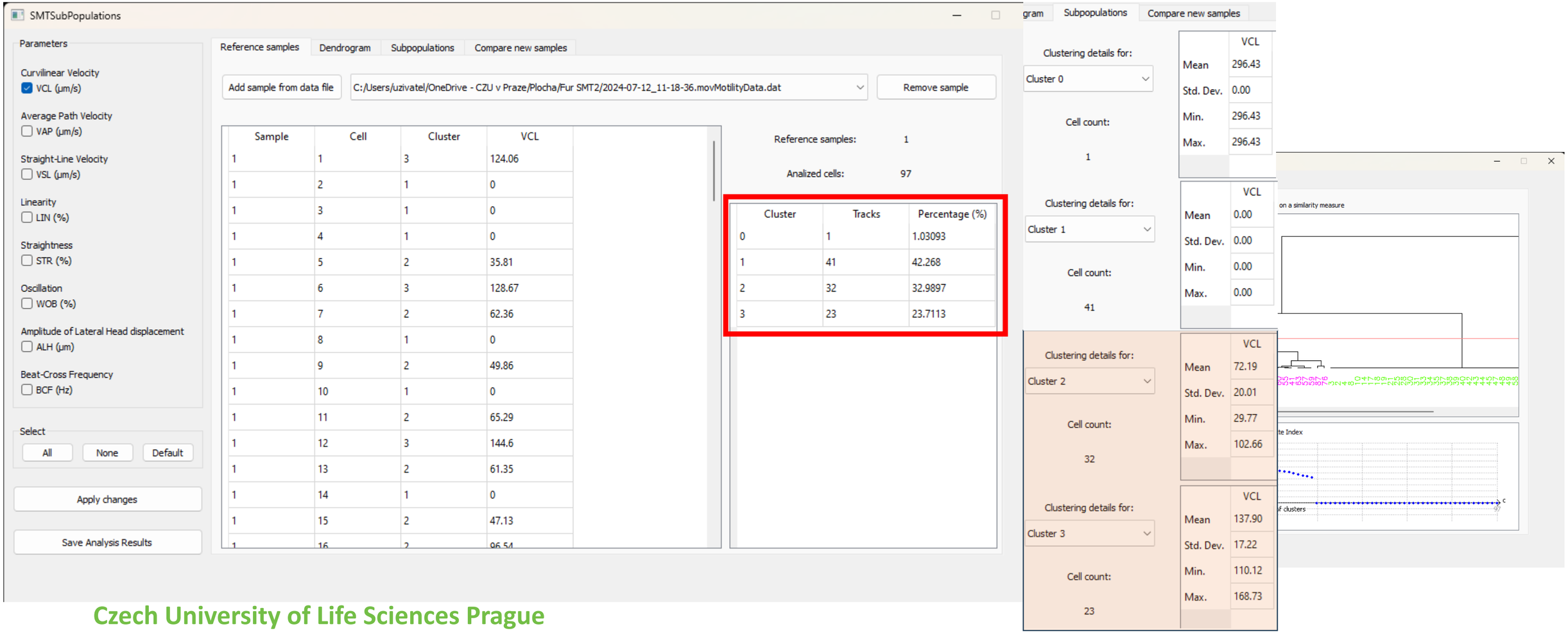
The software is free for rese… …ver-sion could be downloaded from http://smt.fi.mdp.edu.ar/ …ro-viding an agreement the licence terms and conditions. The instrumental considerations and settings for users are the same that for *SMT v1.0* (Buchelly Imbachí et al. 2018).

Ovine sperm sample: results of conventional CASA analysis

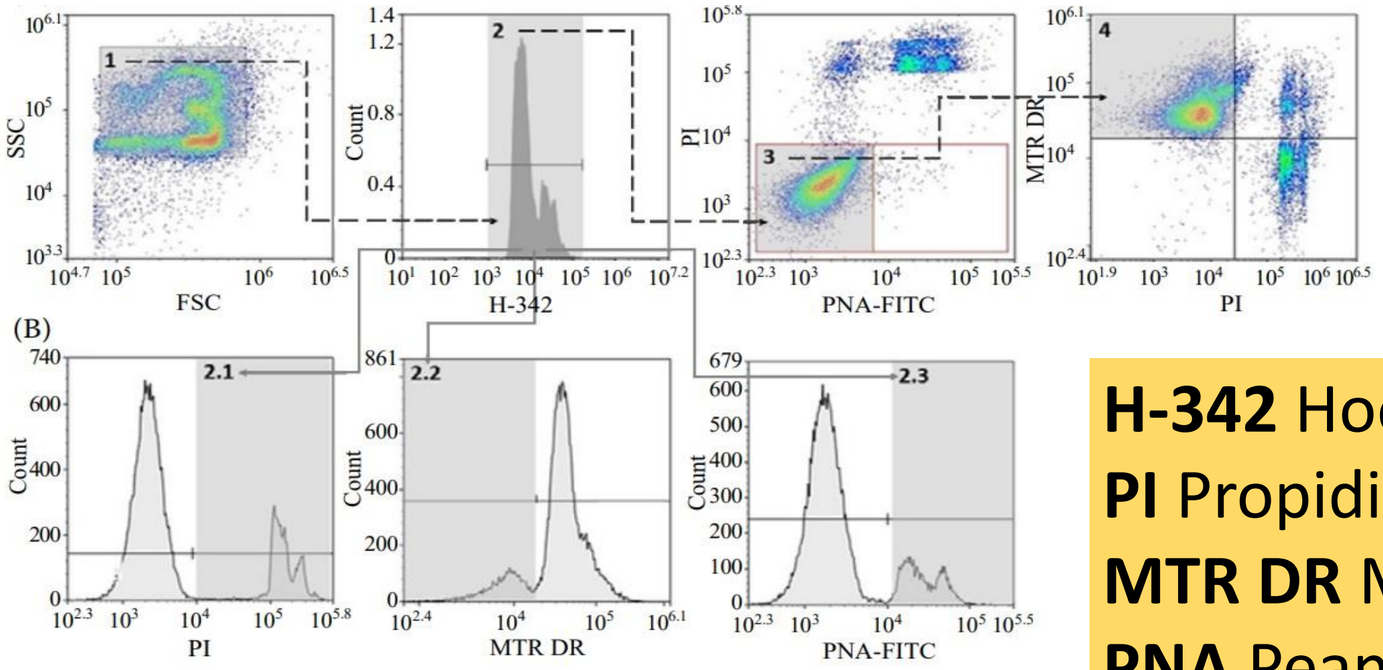


	A	B	C	D	E	F	G	H	I
1	Column1	Column2	Column3	Column4	Column5	Column6	Column7	Column8	Column9
2	Experiment								
3	Animal Name	Farm Name	Progressive Cutoff	Motile Cutoff	Analysis Frames	Semen Type	Genus	Time	
4	240712 33 T0		STR >= 70 & VAP >= 50	VAP >= 20 & VSL >= 0	22	N/A	N/A	2024-07-12 11:18:51	
5									
6	Analysis								
7	ID	Concentration (million/ml)	Motile (%)	Progressive (%)	VAP (um/s)	VCL (um/s)	VSL (um/s)	LIN (%)	STR (%)
8	Average	56,61	30	20,9	110,86	122,85	99,8	84,86	78,7

Ovine sperm sample: results of Sperm Motility Tracker v2.0



Flow cytometry

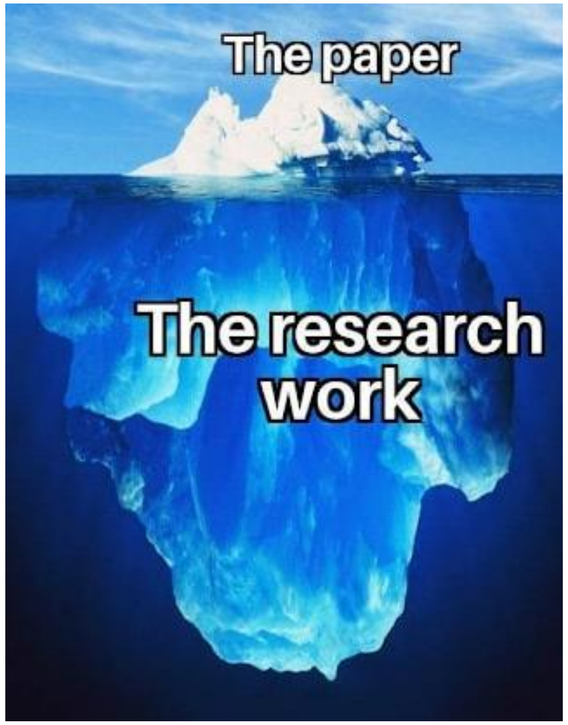


H-342 Hoechst 33342 (DNA content)
PI Propidium Iodide (Plasma membrane dam.)
MTR DR Mitotracker Deep Red (Mito- status)
PNA Peanut agglutinin (Acrosome dam.)

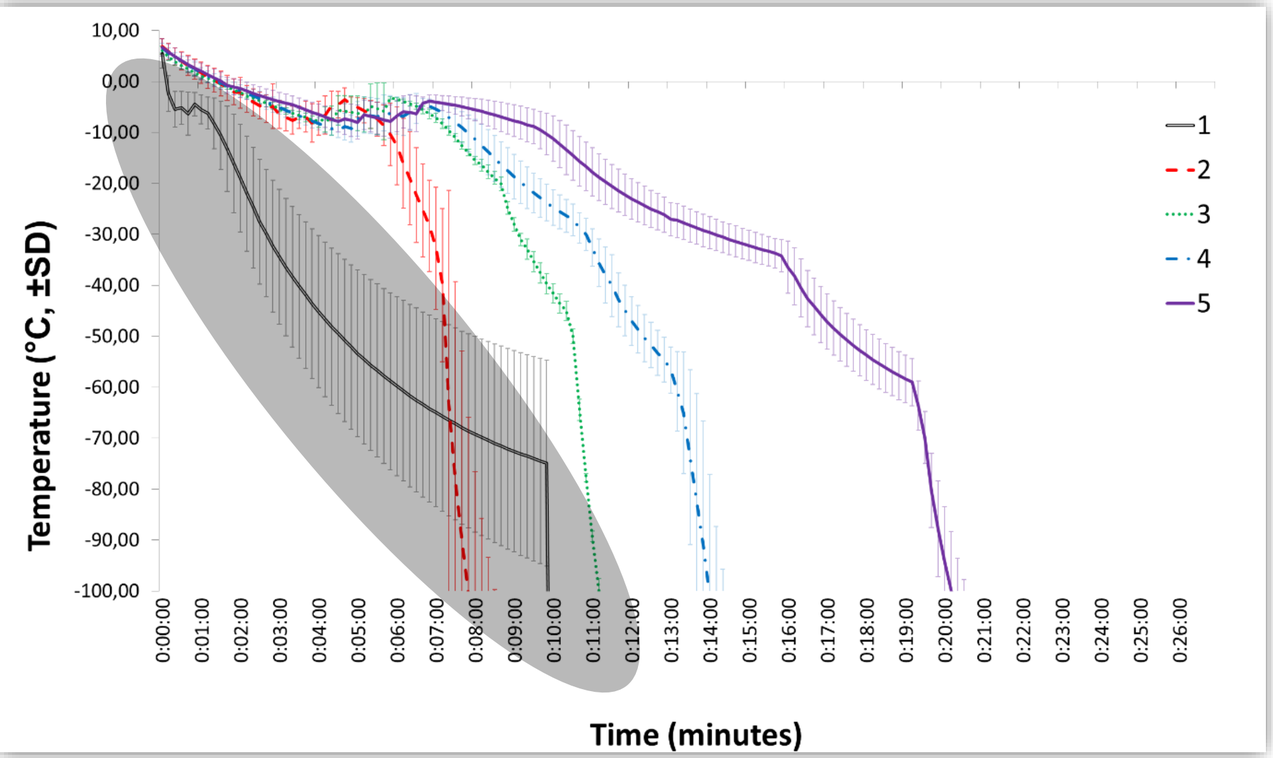
Our (un)published experience to share

- I) Optimization of cryopreservation process for Czech native sheep
  1. Optimization of freezing curve
  2. Optimization of Glycerolation
  3. Optimization of thawing curve
  4. Quality evaluation of thawing sperm

- II) Artificial insemination of Czech native sheep
  5. with cryopreserved sperm



1. Freezing curve



Czech University of Life Sciences Prague

czu.cz

Original Paper *Czech Journal of Animal Science, 66, 2021 (02): 55–64*

https://doi.org/10.17221/226/2020-CJAS

Optimizing the conventional method of sperm freezing in liquid nitrogen vapour for Wallachian sheep conservation program

Filipp Georgijevic Savvulidi, Martin Ptacek, Anezka Malkova, Jakub Beranek, Ludek Stadnik*

Table 2. The impact of freezing curve, intersire, and daily variation on the viability of thawed Wallachian ram sperm (least square means ± standard error)

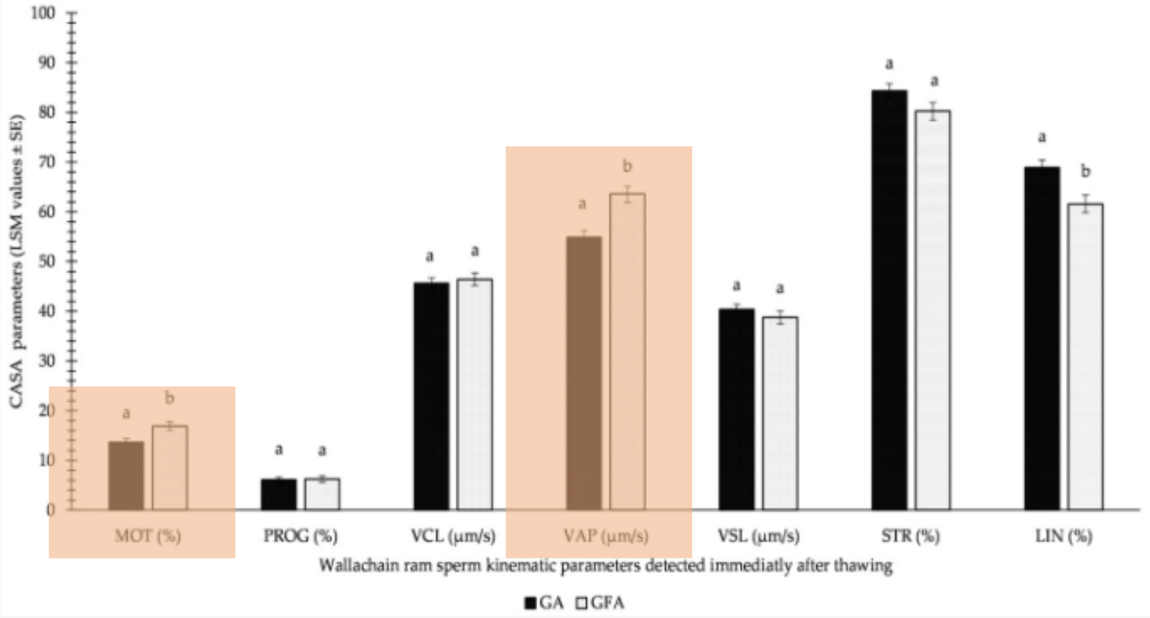
	THW	THW(PM)	THW(ACR)	THW(MTCH)
Curve	***	***	***	***
1 (n = 56)	8.91 ± 0.36^{a}	87.4 ± 0.39^{a}	70.0 ± 0.56^{c}	78.2 ± 0.48^{a}
2 (n = 56)	12.7 ± 0.57^{b}	82.9 ± 0.62^{b}	52.8 ± 0.87^{a}	73.5 ± 0.76^{b}
3 (n = 136)	12.6 ± 0.80^{b}	83.2 ± 0.88^{b}	58.5 ± 1.24^{b}	73.3 ± 1.07^{b}
4 (n = 28)	13.2 ± 0.57^{b}	82.5 ± 0.62^{b}	57.8 ± 0.87^{b}	74.0 ± 0.76^{b}
5 (n = 43)	12.9 ± 0.65^{b}	81.7 ± 0.71^{b}	61.4 ± 1.00^{b}	75.9 ± 0.87^{ab}
Sire	***	***	***	***
1 (n = 232)	14.0 ± 0.33^{a}	81.1 ± 0.36^{a}	47.0 ± 0.59^{a}	73.3 ± 0.44^{a}
2 (n = 87)	10.1 ± 0.53^{b}	86.0 ± 0.58^{b}	61.5 ± 0.96^{b}	76.6 ± 0.71^{b}
Day	***	***	***	***
b*EQ	n.s.	–	–	–
b*EQ(PM)	–	n.s.	–	–
b*EQ(ACR)	–	–	n.s.	–
b*EQ(MTCH)	–	–	–	***

b*EQ = initial percentage of viable sperm after equilibration as covariate; b*EQ(ACR) = initial percentage of sperm with acrosome damage after equilibration as covariate; b*EQ(MTCH) = initial percentage of sperm with deteriorated mitochondrial activity after equilibration as covariate; b*EQ(PM) = initial percentage of sperm with plasma membrane damage after equilibration as covariate; Curve = fixed effect of the freezing curve (1 = Curve 1; 2 = Curve 2; 3 = Curve 3; 4 = Curve 4; 5 = Curve 5); Day = fixed effect of the control day of semen collection; Sire = fixed effect of ram; THW = percentage of viable sperm after thawing; THW(ACR) = percentage of sperm with acrosome damage after thawing; THW(MTCH) = percentage of sperm with deteriorated mitochondrial activity after thawing; THW(PM) = percentage of sperm with plasma membrane damage after thawing

***Significance of particular effects in the model equation at $P < 0.001$; $^{a-c}$different superscripts within columns indicate that the means differ at $P < 0.05$ level of significance

2. Glycerolation

Figure 3. Influence of GA (glycerolation during the whole equilibration process) vs. GFA (glycerolation 10 min before the freezing procedure) on CASA parameters in Wallachian ram sperm detected immediately after thawing; MOT: total motility; PROG: progressive motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity; STR: straightness; LIN: linearity; a–b: different letters within particular parameters indicate significant differences at a $p < 0.05$ level of significance.



animals

MDPI

Communication

Glycerol-Free Equilibration with the Addition of Glycerol Shortly before the Freezing Procedure: A Perspective Strategy for Cryopreservation of Wallachian Ram Sperm

Anežka Málková [1], Filipp Georgijevič Savvulidi [1,*], Martin Ptáček [1], Karolína Machová [2], Martina Janošíková [1], Szabolcs Nagy [3] and Luděk Stádník [1]

Table 2. Significance of factors used in statistical models for flow cytometric parameters.

	GA (%)	GFA (%)	Significance of Factors in Statistical Model			
			b_{1-2} × EQ	DAY	VAR	DAY × VAR
PAI_{EQ}	60.48 ± 1.61 [a]	36.99 ± 1.61 [b]	---	***	***	***
PAI_{T0}	22.38 ± 0.87 [a]	27.12 ± 0.82 [b]	***	***	***	***
PAI_{T1}	10.92 ± 0.74 [a]	22.55 ± 0.69 [b]	***	***	***	***
PAI_{T2}	9.61 ± 0.68 [a]	20.53 ± 0.63 [b]	***	***	***	***
MMP_{EQ}	64.20 ± 1.75 [a]	54.92 ± 1.75 [b]	---	***	***	***
MMP_{T0}	36.67 ± 1.40 [a]	41.03 ± 1.30 [b]	***	***	*	***
MMP_{T1}	7.16 ± 0.82 [a]	18.97 ± 0.77 [b]	*	***	***	***
MMP_{T2}	7.49 ± 0.75 [a]	11.72 ± 0.71 [b]	***	***	***	***

3. Thawing curve

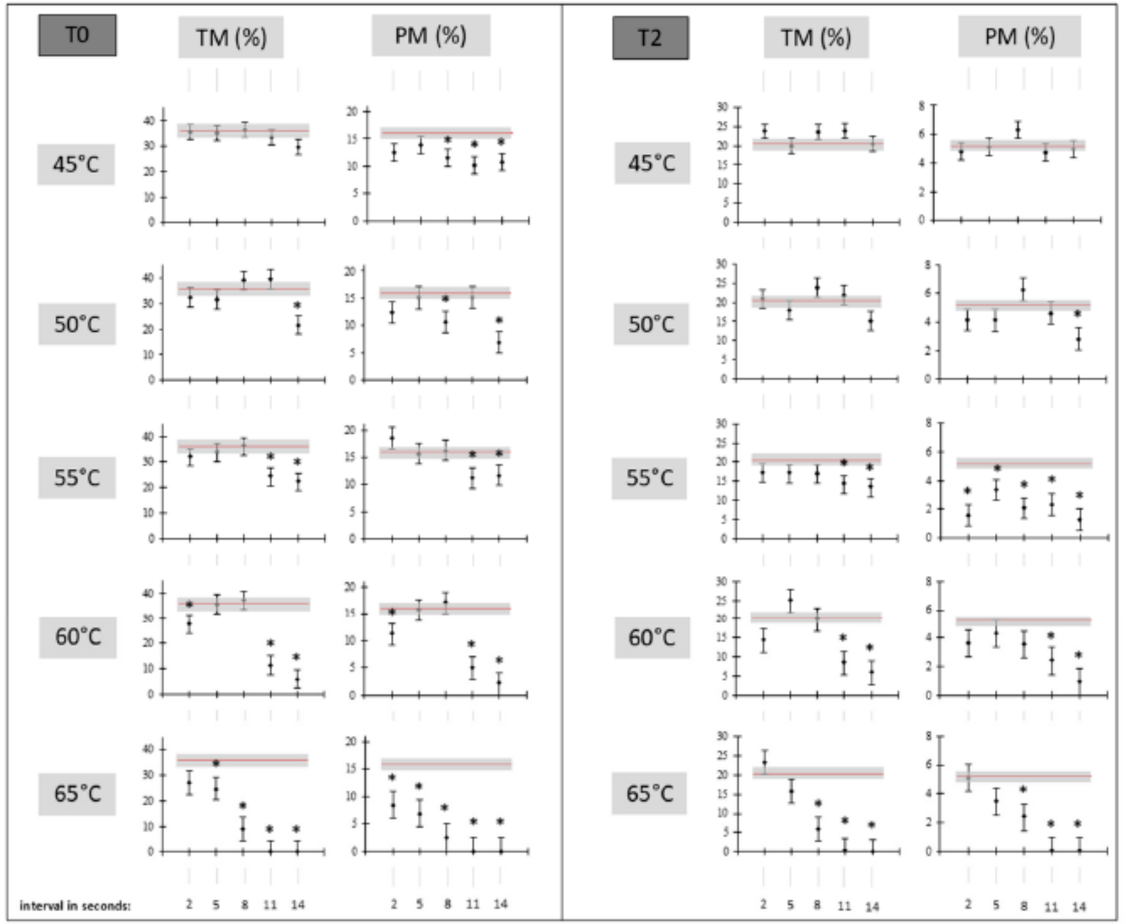


Figure 2. Total (TM) and progressive (PM) motility modified thawing protocols compared to the reference **39 °C/30 s** . T0 = results detected immediately after thawing; T2 = results detected after 2 h of incubation in a water bath heated at 39 °C; black points with bounded lines indicate LSM values ± SE for modified thawing protocols; the red line with a grey marked area indicates the reference thawing protocol (LSM ± SE); * = indicates a significant difference in the modified thawing protocol to the reference thawing protocol at $p < 0.05$ level of significance.

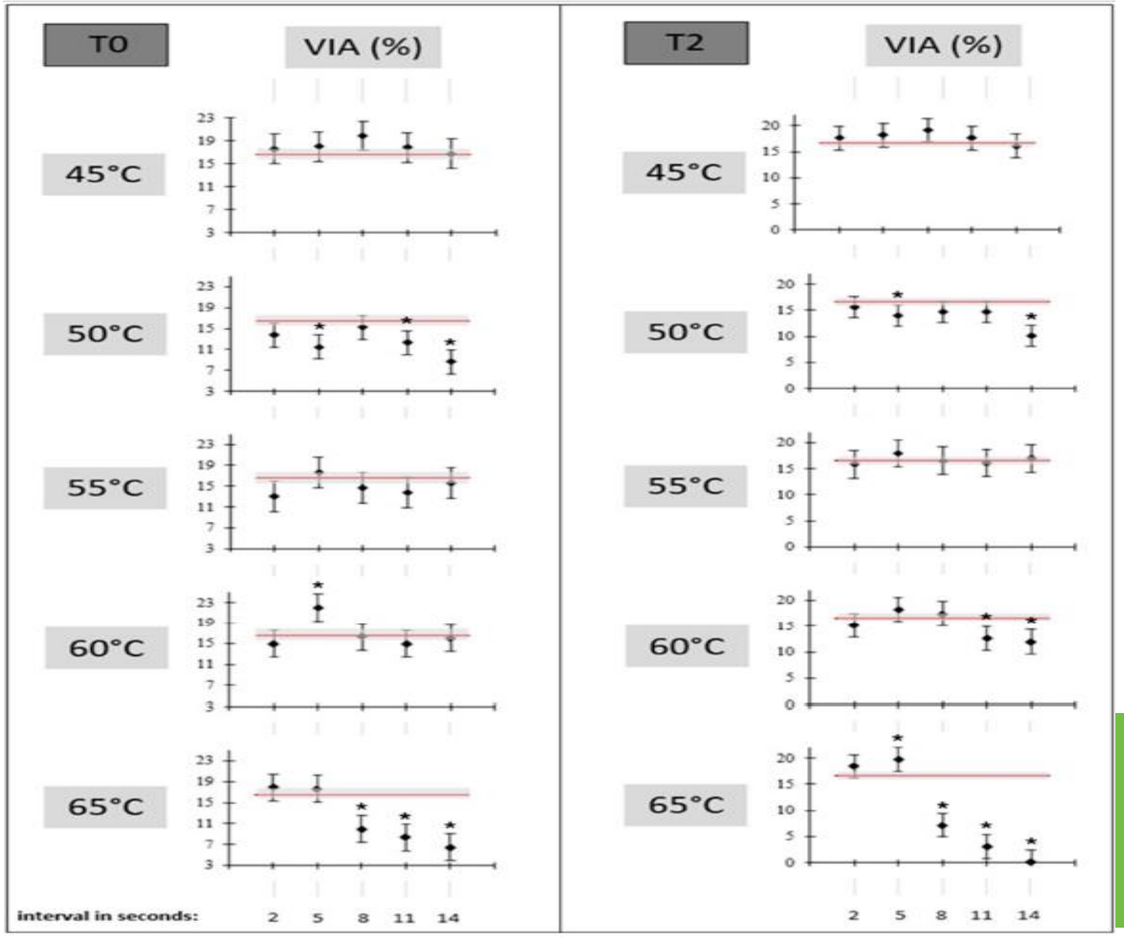
veterinary sciences

MDPI

Article

Effect of Different Thawing Regimes on Cell Kinematics and Organelle Integrity of Nitrogen-Stored Wallachian Ram Spermatozoa

Martin Ptáček [1,*], Filipp Georgijevič Savvulidi [1], Christopher LeBrun [1], Martina Janošíková [1], Temirkhan Kenzhebaev [2], Kairly Omashev [2], Beybit Kulataev [2] and Nurlan Malmakov [2]



Quality of cryoconserved sperm

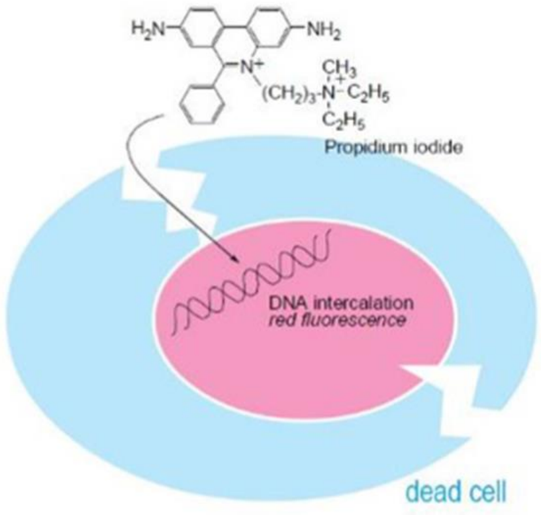
	Total motility (%)	Progressive motility (%)	Viability (%)	Mitochondrial potential (%)
Wallachian sheep	10.3	6.0	17.2	8.3
Sumava sheep	14.0	7.8	16.8	9.1
Czech white-short haired goat	24.1	19.1	32.3	33.8
Czech brown-short haired goat	22.6	17.6	30.1	31.3

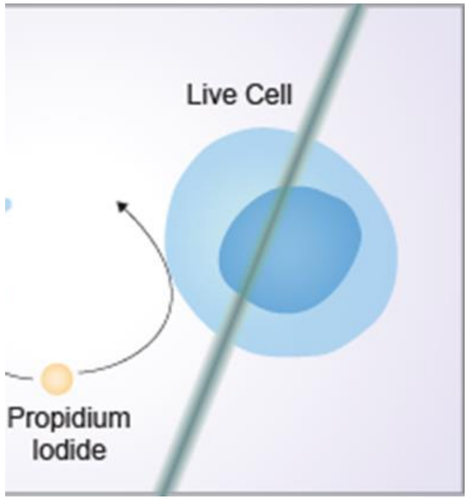
(Interim results from screening sperm analyses)

Dye-exclusion assays

A number of fluorescent dyes are used for flow cytometry determination of sperm viability in livestock species

- Dye-exclusion assays with propidium iodide (PI)
- Classic interpretation: PI penetrates cells only through damaged plasma membranes
- But…





Pannexin channels!

…But previous studies show that pannexin channels increase PI uptake in frozen-thawed dog spermatozoa (Torres et al. 2017)

- Suggesting that the estimated percentage of PI-positive spermatozoa used as an indicator of non-viable cells may lead to their overestimation.
- Pannexins are proteins that form functional membrane channels, but they have not yet been described in ovine.

CSIRO PUBLISHING

Reproduction, Fertility and Development, 2017, **29**, 2269–2276
https://doi.org/10.1071/RD16267

Pannexin channels increase propidium iodide permeability in frozen–thawed dog spermatozoa

J. L. Torres[B], *J. Palomino*[A], *R. D. Moreno*[B] *and M. De los Reyes*[A,C]

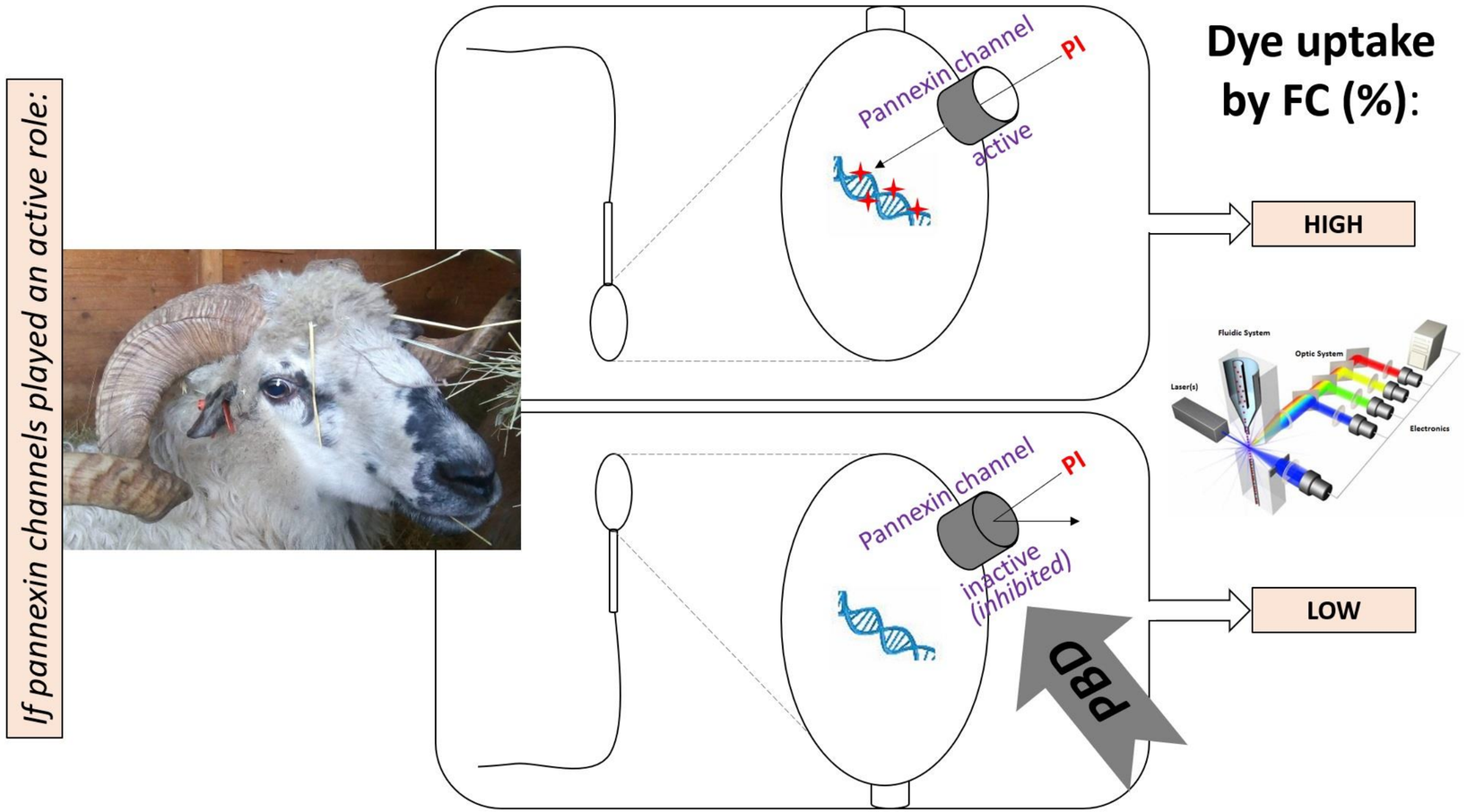
[A]Laboratory of Animal Reproduction, Faculty of Veterinary Sciences, University of Chile, Casilla 2, Correo 14, La Granja, Santiago, Chile.
[B]Physiology Department, Biological Sciences Faculty, Pontifical Catholic University of Chile, Alameda 340, Santiago, Chile.
[C]Corresponding author. Email: mdlreyes@uchile.cl

Original research on the pannexin channels and ovine sperm

In our recent research, we aimed at the:

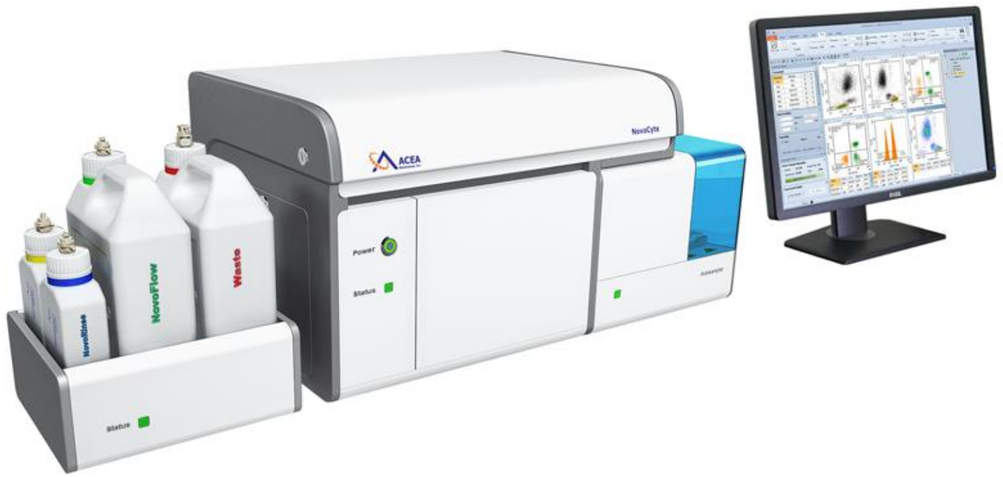
- Evaluation of the role of pannexins in PI uptake by frozen-thawed Wallachian ram spermatozoa

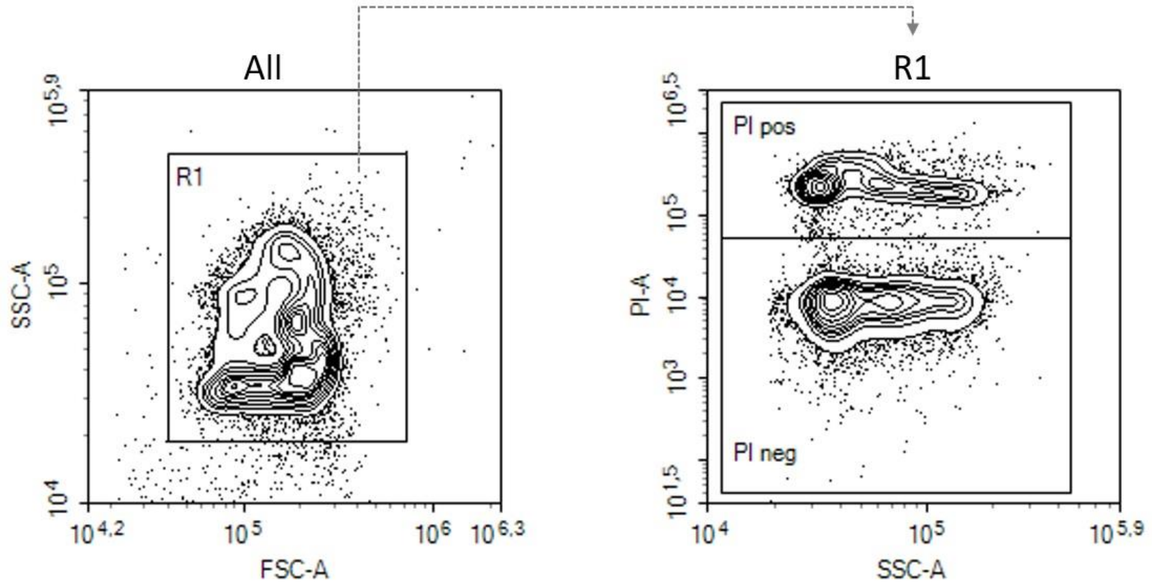
MM: graphical representation of the hypothesis



PBD - Probenecid

MM: PI-exclusion assay by flow cytometry





Results: Pannexin channels played an active role in the process of PI uptake in frozen-thawed ram spermatozoa

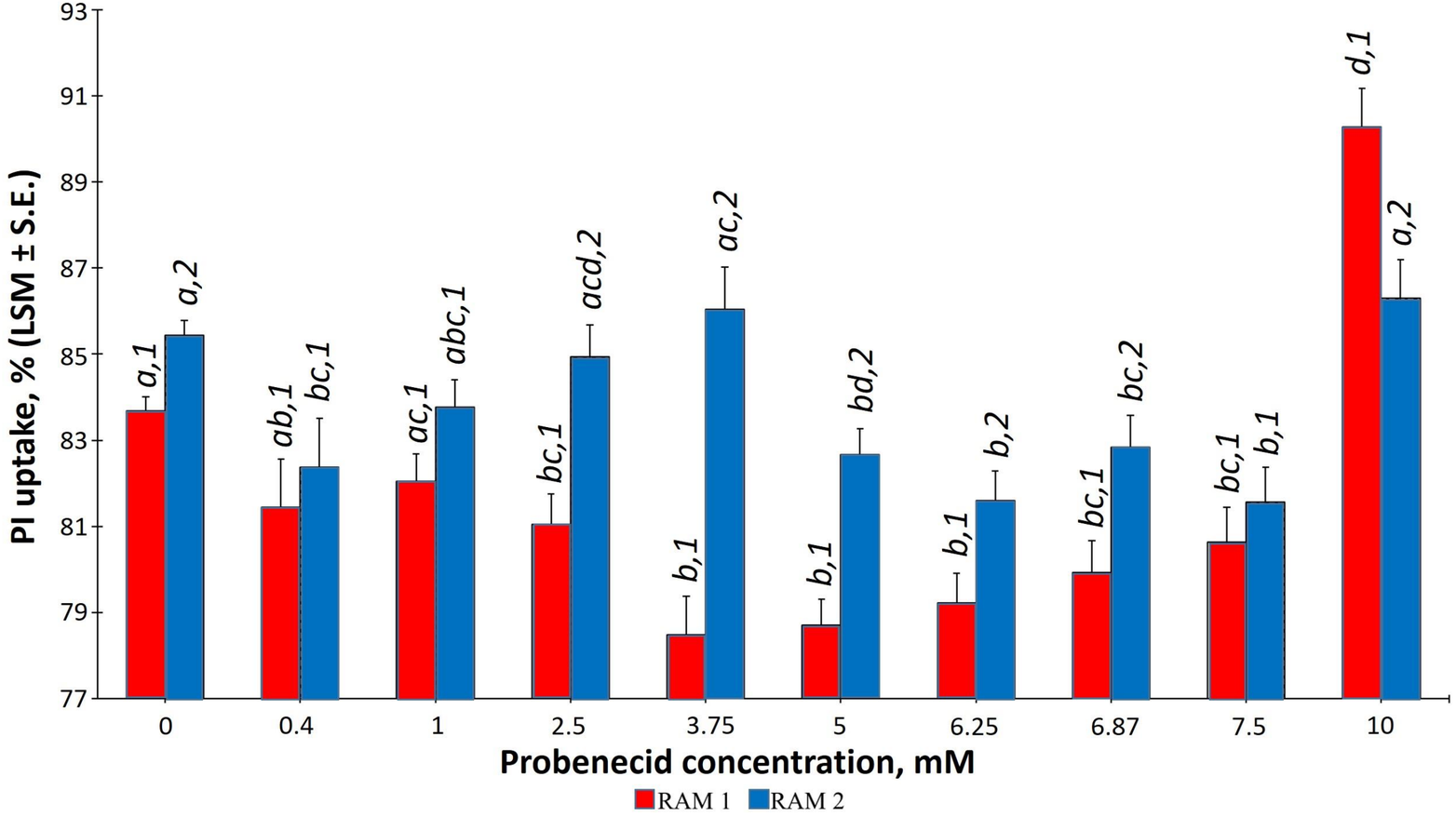
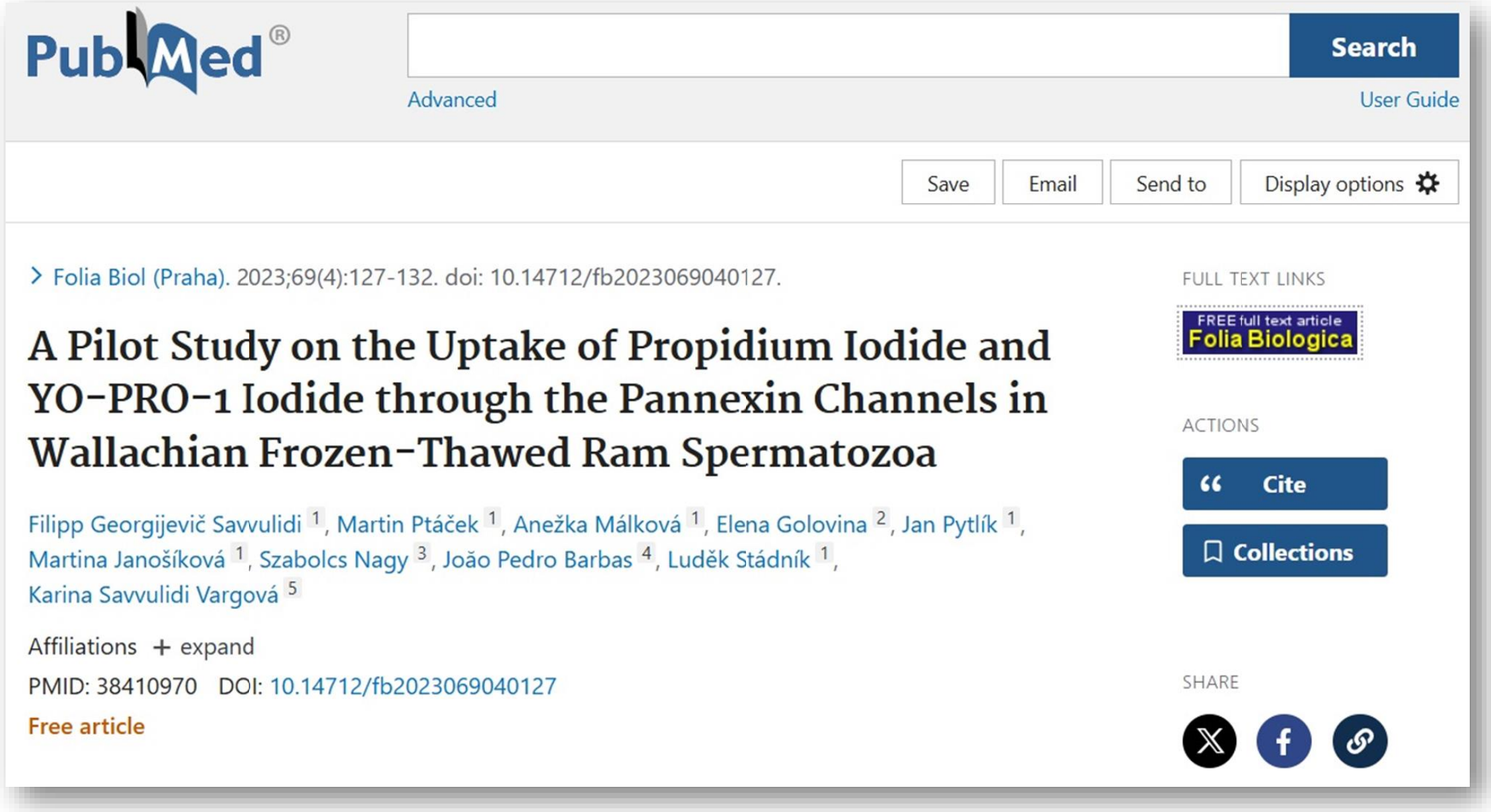


Figure 1. PI uptake as measured by flow cytometry. Values with the different notifications (a-d intra-ram differences; 1-2 ram-to-ram differences) indicate significant differences at $p < 0.05$ level of significance

Conclusion: PI-exclusion assay: a non-0 % chance of detecting false positives

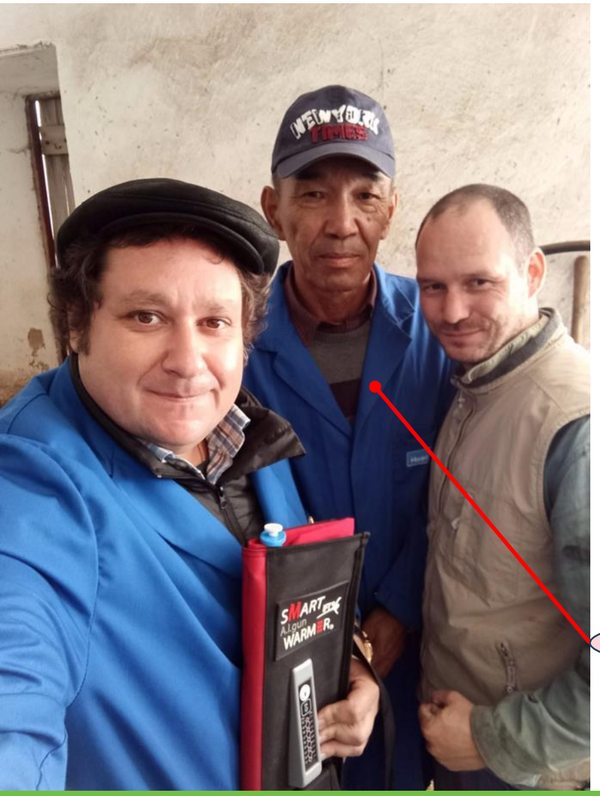
- According to our results, the PI assay has a 6 % false positivity using flow cytometry ($p < 0.05$)




AI
(Artificial Insemination)

4. Laparoscopic AI

The Welfare of Sheep



HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

الجمعية السعودية لعلوم الحياة
SAUDI BIOLOGICAL SOCIETY

Original article

Optimal time for laparoscopic intrauterine insemination performed on ewes detected in natural heat

Nurlan Malmakov [a], Martin Ptacek [b], Filipp Georgijevic Savvulidi [b,*], Ludek Stadnik [b]

[a] Department of Sheep and Goat Artificial Insemination and Sperm Cryoconservation, Scientific Research Institute of Sheep Breeding Branch, Mynbaev, Almaty Region 040622, Kazakhstan

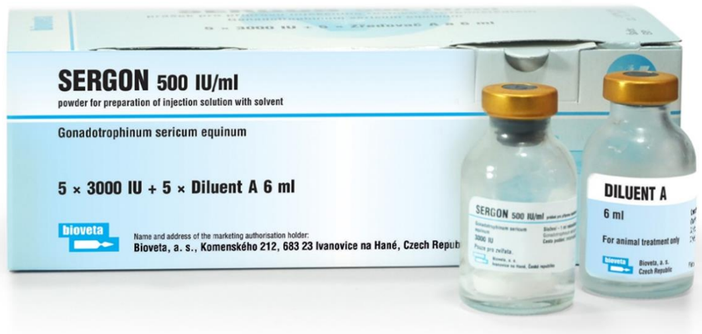
[b] Department of Animal Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamycka 129, 165 00 Prague, Suchdol, Czech Republic

4. Intra-cervical AI

4. (Trans-cervical AI)

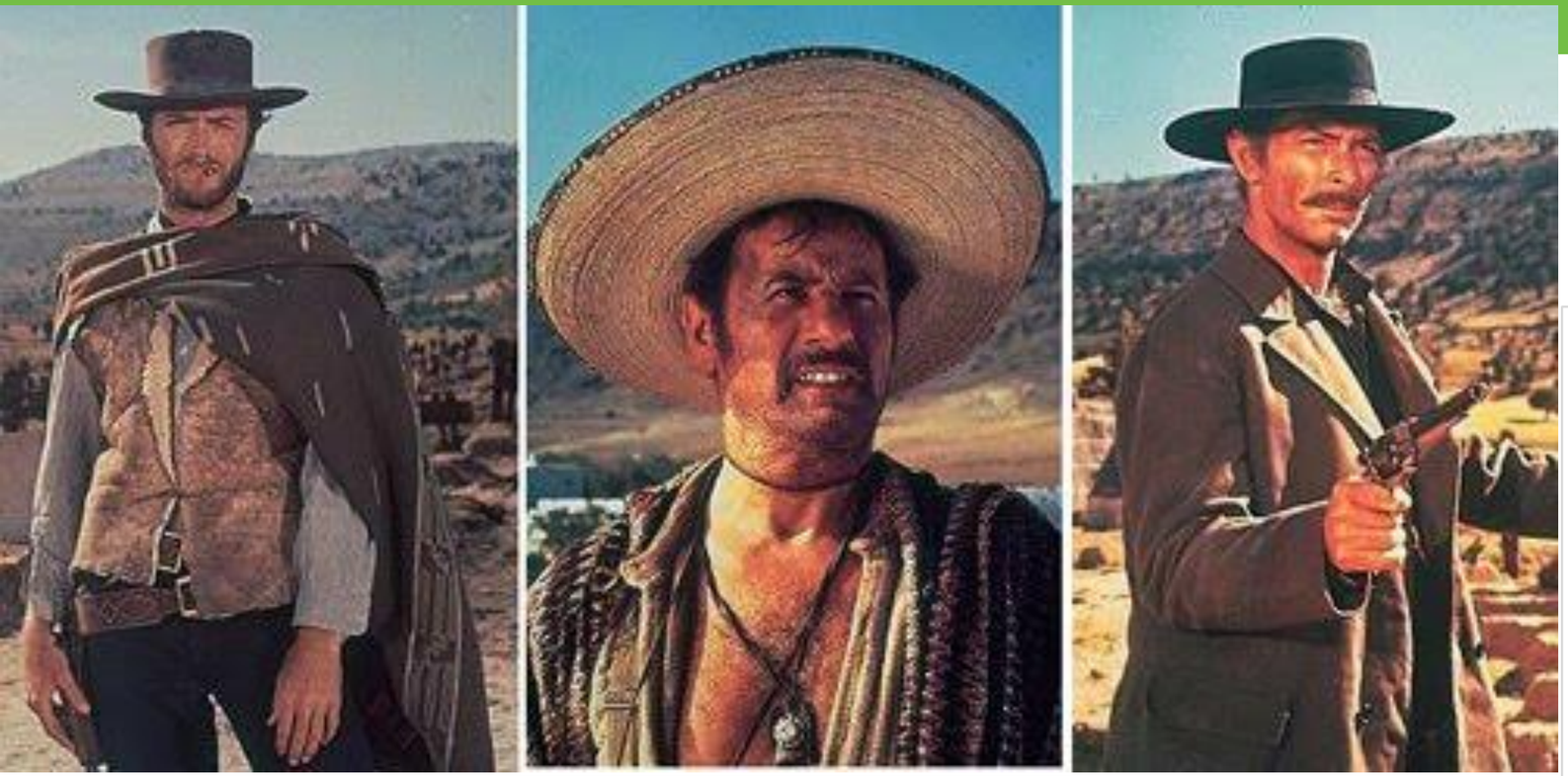
Sheep cervix penetration: 40 – 60 %

4. Synchro-program

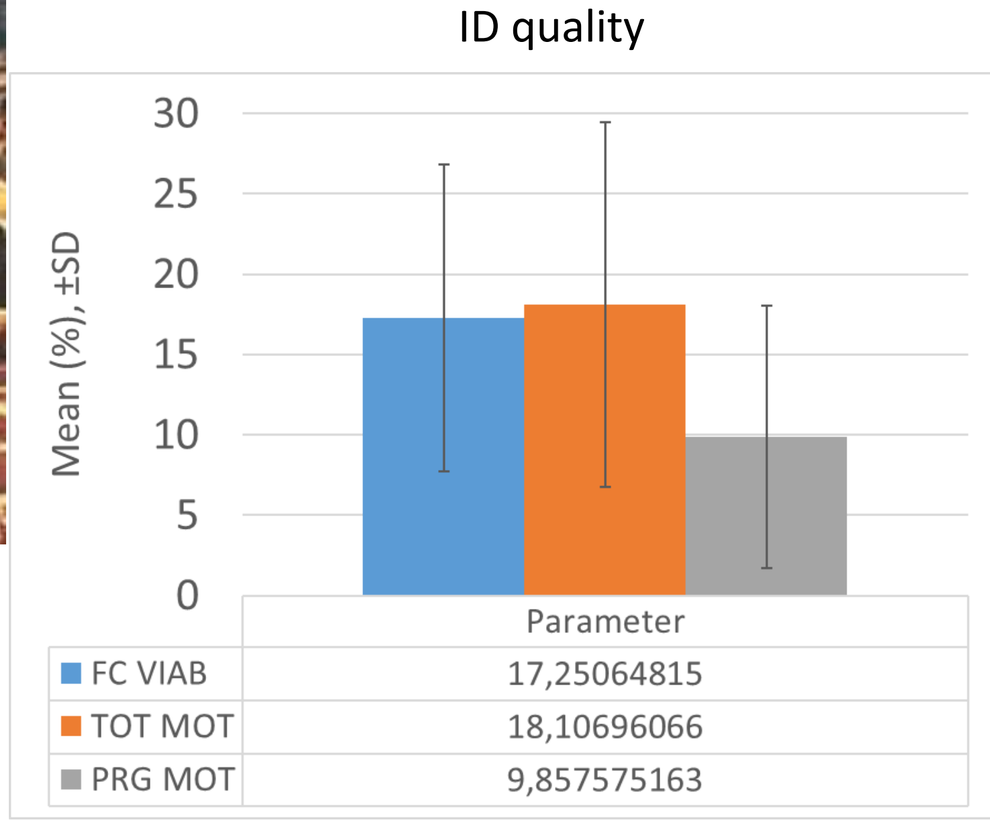


DAY	Heat synchroniztion for ICI
0	Insert sponges (T+)
14	Remove sponges (T-) + 300 – 500 IU eCG (PMSG) application
16	Expected time of sheep/goat ovultion: 48 – 72 hrs. from T-
	Double ICI at 54/58 hrs. from T-

First field trial: intra-cervical double-AI



Lambing rate: 3 out of 30
(based on the lambing date)



European Regional Focal Point for Animal Genetic Resources

The Research School, Swedish University of Agricultural Sciences

Thank you for your attention

savvulidi@af.czu.cz

Martin Ptáček
Lucie Langerová

Czech University of Life Sciences Prague

For your interest

MDPI

Special Issue

Sperm Preservation and Sexing Technologies in Large- and Small-Ruminant Reproduction: Current Status and Future Perspectives

Guest Editors

Dr. Filipp Savvulidi
Dr. Martin Ptáček

Deadline

15 February 2026

animals

IMPACT FACTOR 2.7

Indexed in: PubMed

CITESCORE 5.2

ByTech

University spin-off

Est. 2025

Czech University
of Life Sciences Prague

University full of life