The 4th CZU Prague hybrid seminar

„Biotechnology in small ruminant reproduction: an international experience 2025“

Advances in prediction of ram semen freezability

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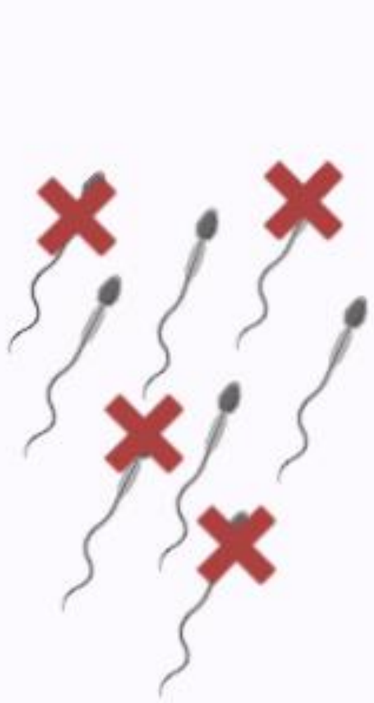
Cryopreservation



Survives about
30-50%

- Low cholesterol to phospholipid ratio in the plasma membrane
- Intracelular ice crystals
- Mitochondrial damage

Cryopreservation

Affected by other factors:

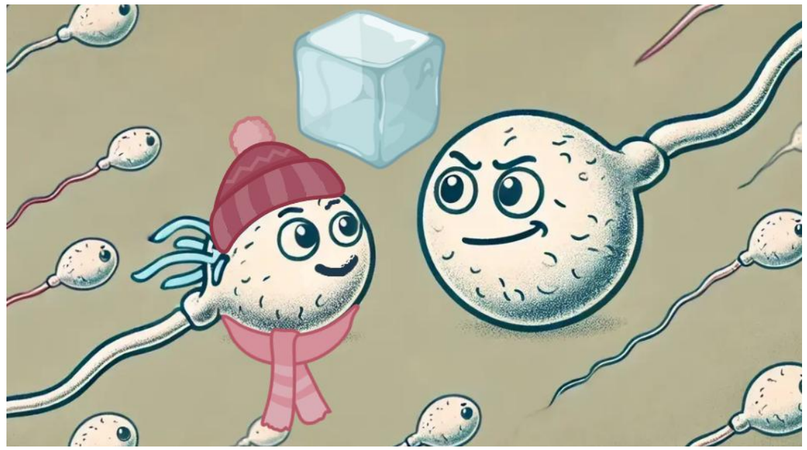
Breed

Environmental factors

Nutrition

„Good or bad“ freezers

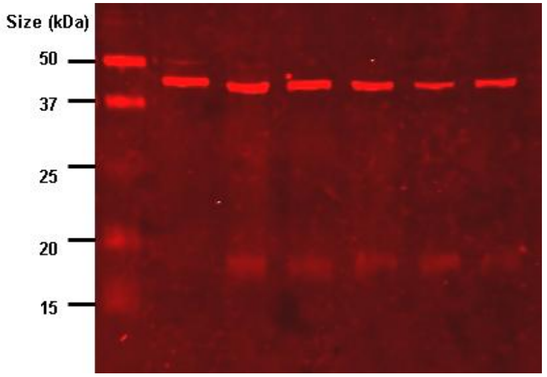
Ram semen shows inter-individual variability, that affects semen storage performance and freeze-thaw process



Prediction of sperm freezability

Cryotolerance in rams is associated with the expression of aquaporin 3 (Pequeno et al., 2023)

Sperm with high cryotolerance contained specific proteins associated with:
- the regulation of calcium (Ca^{2+}) transport: S100A8, S100A9, S100A12, S100A14
- **or providing antioxidant protection:** HYOU1, PRDX1 (Ren et al. 2023)



Western blott

microRNA analysis

Association between conventional semen variables and sperm freezability

- **Motility**
- **Concentration**
- **Volume**

- **Order of ejaculate sampling**
- **Breed**

ELSEVIER

Theriogenology

Volume 64, Issue 2, 15 July 2005, Pages 305-316

Does multivariate analysis of post-thaw sperm characteristics accurately estimate in vitro fertility of boar individual ejaculates?

M.A. Gil [a], J. Roca [a], T. Cremades [a], M. Hernández [a], J.M. Vázquez [a], H. Rodríguez-Martínez [a b], E.A. Martínez [a]

ELSEVIER

Animal Reproduction Science

Volume 112, Issues 1–2, May 2009, Pages 150-157

Short communication

Assessment of goat semen freezability according to the spermatozoa characteristics from fresh and frozen samples

J. Dorado [a], M. Hidalgo [a], A. Muñoz [b], I. Rodríguez [a]

Original Paper *Czech Journal of Animal Science, 70, 2025*

https://doi.org/10.17221/185/2024-CJAS

Association between conventional semen variables and sperm freezability in rams

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The effect of conventional variables on post-thawing total sperm motility

Simple regresion analysis:

	GLM procedure		Significance		
	R^2	P	VOL	CONC	FRESH
THAWED	0.007	n.s.	n.s.	–	–
	0.033	***	–	***	–
	0.057	****	–	–	****
	0.049	***	*	***	-
	0.071	****	*	–	****
	0.063	****	–	n.s.	**
	0.074	****	n.s.	n.s.	**

CONC = sperm concentration after collection (10^9/ml); FRESH = sperm motility after collection; GLM = generalised linear models; n.s. = not significant; *P*-model = *P*-value of the statistical model; *R*2 = coefficient of determination; THAWED = frozen-thawed total sperm motility; VOL = semen volume after collection (ml); $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

The effect of conventional variables on post-thawing total sperm motility

Regression analysis corrected for systematic effects:

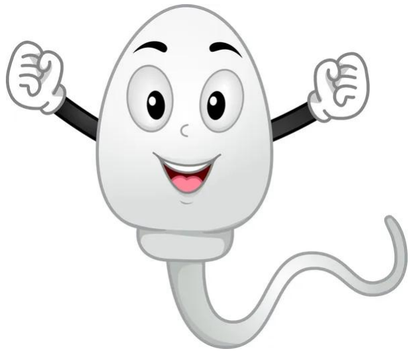
THAWED

GLM procedure		Significance (*F*-value)					
R^2	*P*	YEAR*SEASON	BREED	ORDER	VOL	CONC	FRESH
0.260	****	**** (3.20)	**** (26.82)	*** (11.91)	0.614 (2.26)	–	–
0.288	****	**** (3.07)	**** (33.17)	*** (13.20)	–	*** (12.44)	–
0.286	****	**** (2.80)	**** (29.68)	*** (12.06)	–	–	*** (11.67)
0.293	****	**** (3.10)	**** (30.28)	** (10.73)	0.133 (2.26)	*** (14.49)	–
0.289	****	**** (2.80)	**** (26.90)	** (10.06)	0.242 (1.38)	–	*** (12.79)
0.288	****	**** (2.84)	**** (30.41)	*** (12.63)	–	n.s. (0.90)	n.s. (0.16)
0.293	****	**** (2.89)	**** (28.58)	** (10.53)	0.146 (2.12)	n.s. (1.65)	n.s. (0.02)

BREED = fixed effect of breed; CONC = sperm concentration after collection (10^9/ml); FRESH = sperm motility after collection; GLM = generalised linear models; n.s. = not significant; ORDER = fixed effect of the order of ram semen collection; *P*-model = *P*-value of the statistical model; *R*2 = coefficient of determination; THAWED = frozen-thawed total sperm motility; VOL = semen volume after collection (ml); YEAR*SEASON = randomised combined year-seasonal effect; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.000\,1$

The effect of breed and order of ejaculate sampling on post-tawing total sperm motility

Summary

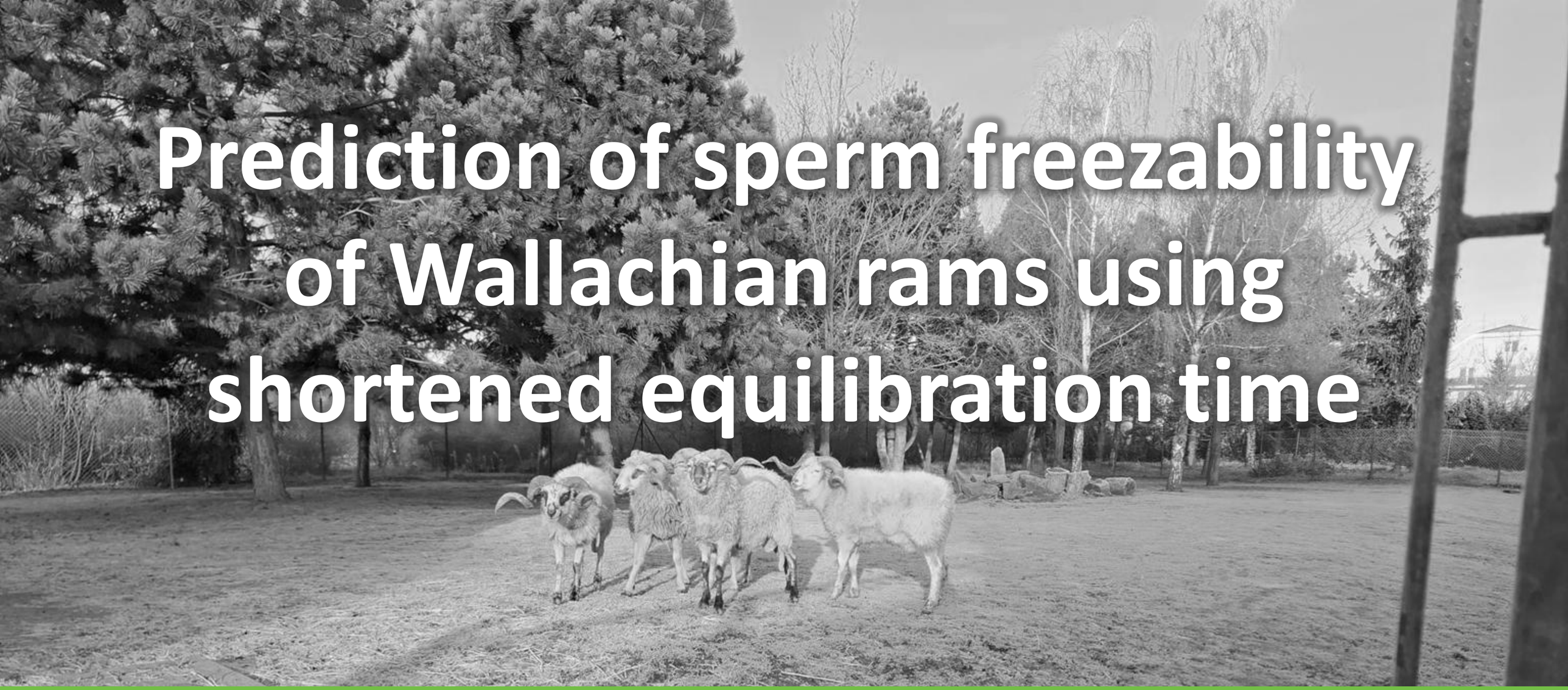


Semen does not need to be rejected based on motility, volume, and concentration, because these variables have very low predictive value for freezability

It would be possible to prepare 6% more doses for insemination (Nikitkina & Shapiev; 2010)

Other methods for predicting freezability should be explored

Prediction of sperm freezability of Wallachian rams using shortened equilibration time



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Introduction

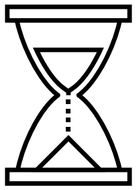
A 2 hour equilibration period improves the quality of ejaculates with low cryotolerance (Passareli et al., 2020)

Shortened equilibration time leads to decreased sperm viability
(Doležalová et al., 2016)

Aim of study

The aim of this study was to verify a defined procedure based on shortened equilibration during freezing, which could serve as a tool for predicting sperm freezability in rams of the Wallachian sheep breed.

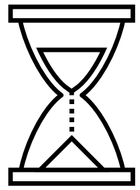
An experimental group



15 min. equilibration

VS.

A control group



120 min. equilibration

Rams and Semen collection

- A total of 7 collection days (with weekly intervals)
- **Method:** Artificial vagina

Ram housing

Analysis of collected semen quality and dilution

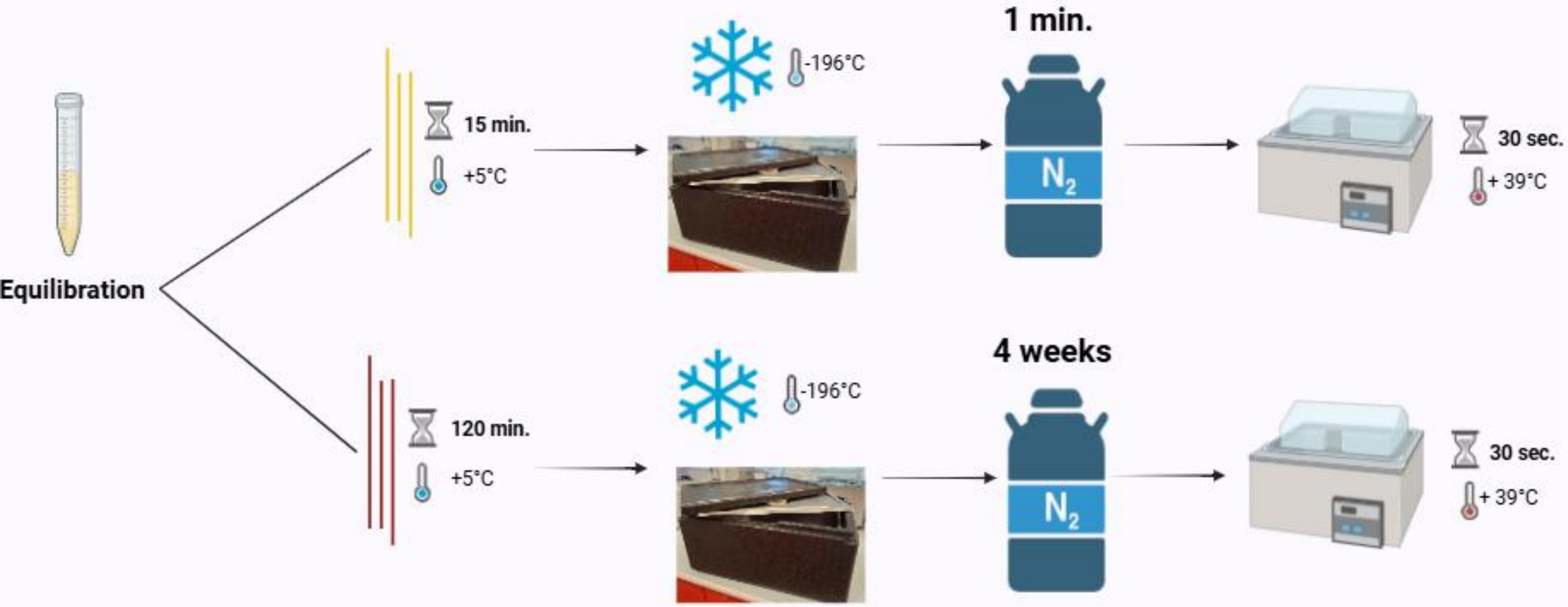
Organoleptically: colour, odour, density

Volume (ml)

Concentration (10^9/ml)

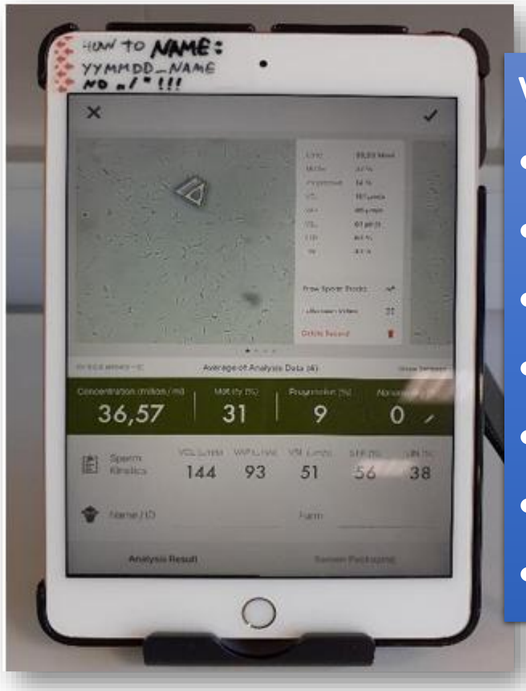
Mass motility
(0-5 points)

Concentration 350 mil/ml

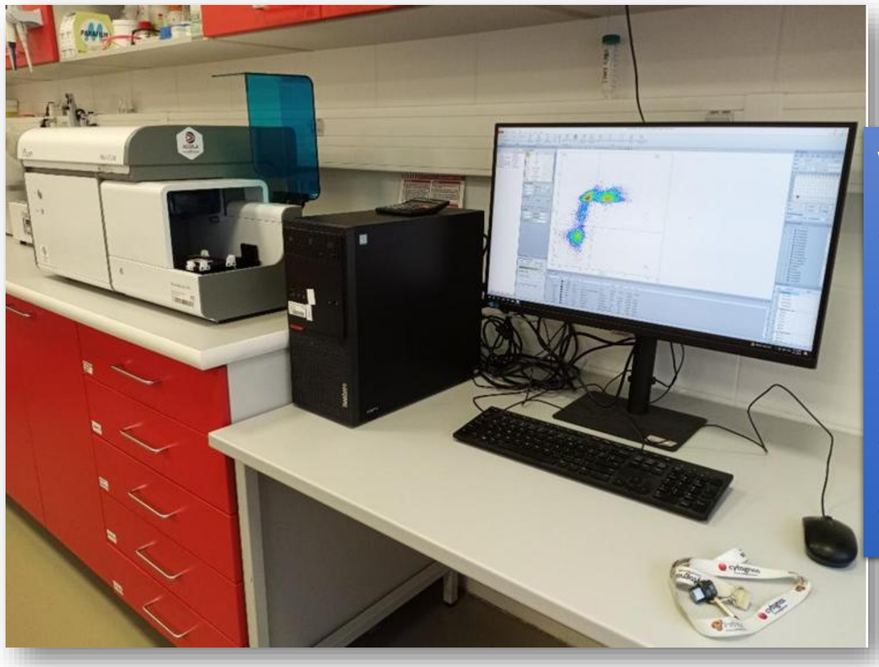

Equilibration
15 min.
+5°C
-196°C
1 min.
N_2
30 sec.
+ 39°C
120 min.
+5°C
-196°C
4 weeks
N_2
30 sec.
+ 39°C

Statistical analyses

Method: GLM (Generalized Linear Model)



Variables:
- MOT (%)
- PROGR (%)
- VAP (μm/s)
- VCL (μm/s)
- VSL (μm/s)
- LIN (%)
- STR (%)



Variables:
- VIA (%)
- PMD (%)
- PMAD (%)
- AD (%)
- MA (%)

$$Y_{ijk} = Ram_i + Day_i + b*x + e_{ijk}$$

Y_{ijk} = variables; Ram_i = fixed effect of the ram; Day_i = fixed effect of collection day; b*x = adjustment for equilibration time (15 min → 120 min); e_{ijk} = the residual error

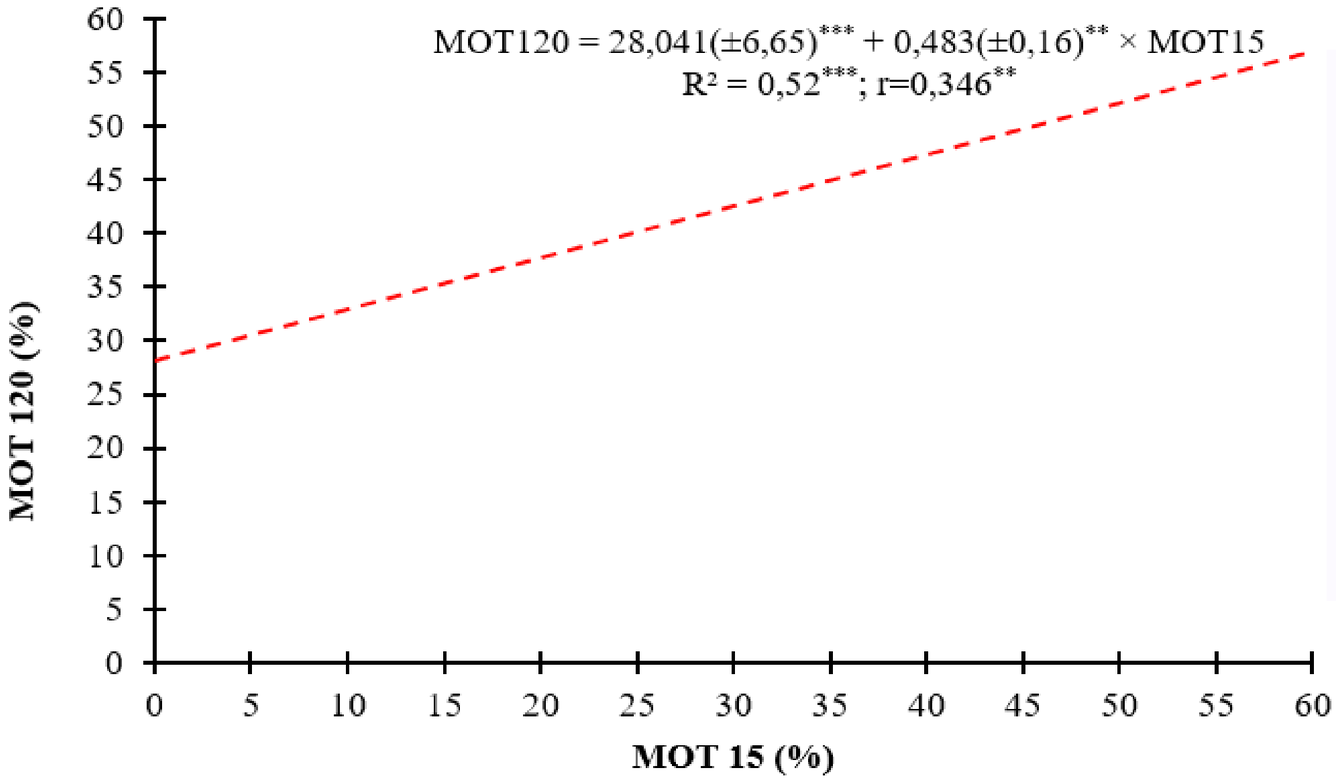
Assessment of sperm kinematic characteristics

VARIABLE	R^2	P	RAM	DAY	SHORTENED EQ
MOT	0,523	***	**	***	**
PROGR	0,356	**	*	n.s.	*
VAP	0,362	*	*	**	n.s.
VCL	0,338	**	**	n.s.	n.s.
VSL	0,371	**	n.s.	***	n.s.
LIN	0,261	*	n.s.	*	n.s.
STR	0,319	**	n.s.	*	n.s.

MOT = total motility (%); PROGR = progressive motility (%); VAP = average path velocity (µm/s); VCL = curvilinear velocity (µm/s); VSL = straight line velocity (µm/s); LIN = linearity (%); STR = straightness (%); R^2= coefficient of determination; P- value = P – value of the statistical model; SHORTENED EQ = Shortened equilibration - for the given parameter as a covariate; DAY = collection day; n.s. = not significant;
*P <0,05; **P <0,01; ***P <0,001

Predictive model for assessing total motility after thawing

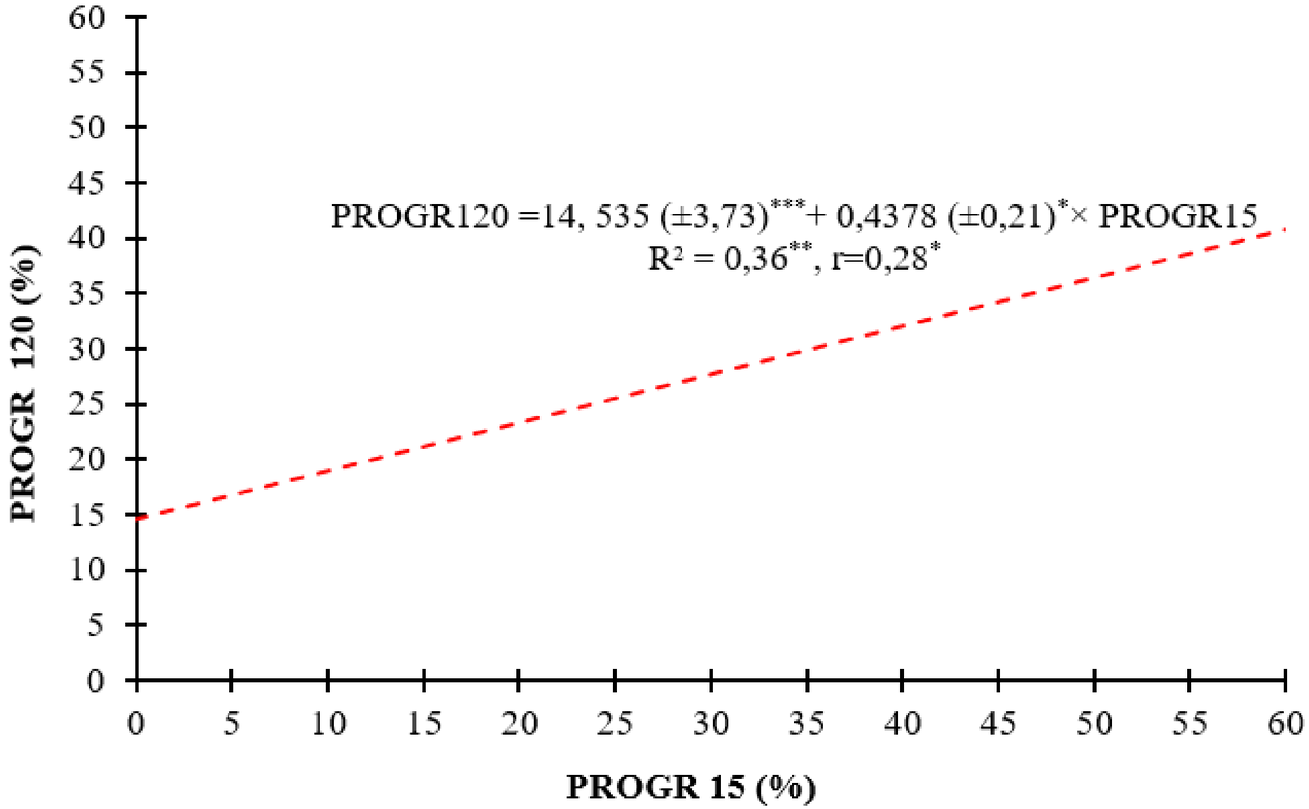
Linear regression correlation of MOT 15 and MOT 120



MOT15 (%) = % total motility after freezing with a 15 minute equilibration; MOT120 (%) = % total motility after freezing with a 120 minute equilibration

Predictive model for assessing progressive motilty after thawing

Linear regression correlation of PROGR 15 and PROGR 120



PROGR15 (%) = % progressive motility after freezing with a 15 minute equilibration; PROGR120 (%) = % progressive motility after freezing with a 120 minute equilibration

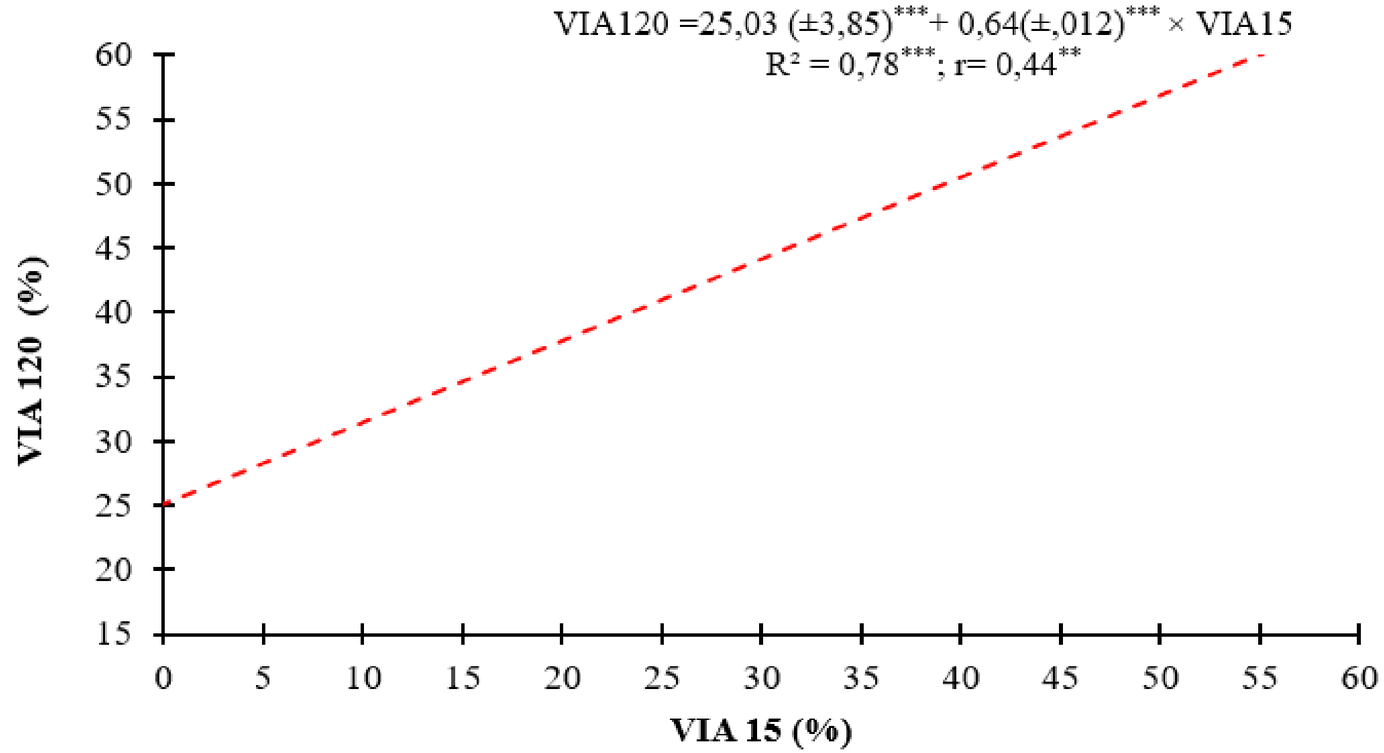
Assessment of sperm viability and membrane integrity indicators

VARIABLE	R^2	P	RAM	DAY	SHORTENED EQ
VIA	0,775	***	n.s.	***	***
PMD	0,617	***	*	**	***
PMAD	0,695	***	***	***	*
AD	0,769	***	n.s.	***	***
MA	0,724	***	n.s.	*	***

VIA = sperm viability (%); PMD = plasma membrane damage (%); PMAD = plasma membrane and acrosome damage (%); AD = acrosome damage (%); MA = mitochondrial activity (%); R = coefficient of deterioration; P-value = P – value of the statistical model; SHORTENED EQ = Shortened equilibration – for the given parameter as a covariate; DAY = collection day; n.s. = not significant; *P<0,05; **P<0,01; ***P<0,001

Predictive model for assessing sperm viability after thawing

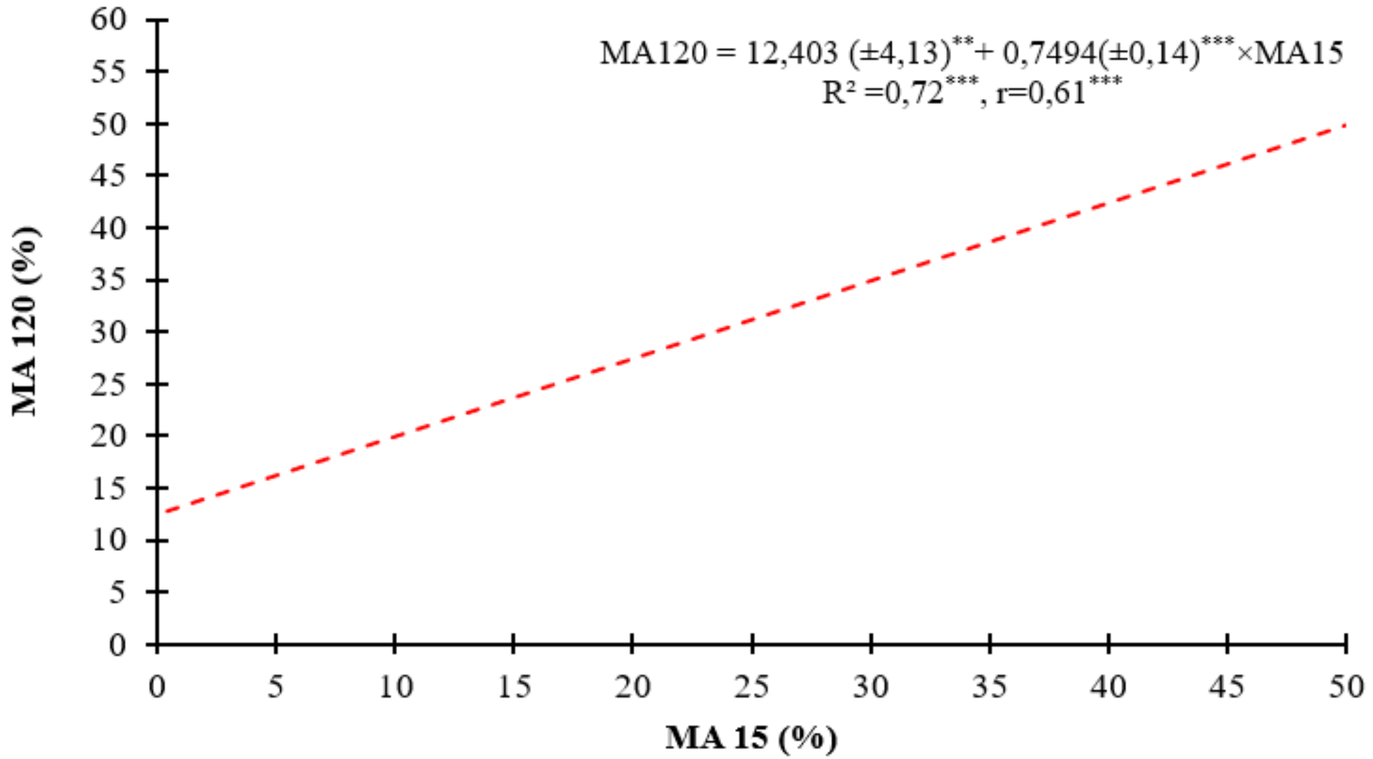
Linear regression correlation of VIA 15 and VIA 120



VIA15 (%) = % viable sperm after freezing with a 15 minute equilibration; VIA 120 (%) = % viable sperm after freezing with a 120 minute equilibration

Predictive model for assessing mitochondrial activity after thawing

Linear regression correlation of MA 15 and MA 120



MA15 (%) = % mitochondrial damage after freezing with a 15 minute equilibration; MA120 (%) = % mitochondrial damage after freezing with a 120 minute equilibration

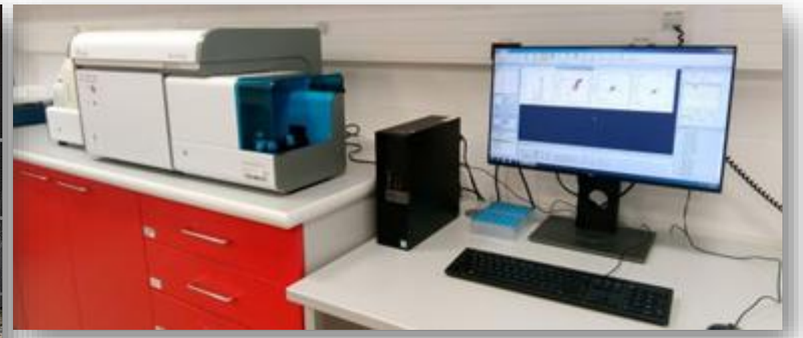
Conclusion

The prediction of sperm freezability in rams based on shortened equilibration time represents an effective tool

A significant predictive potential of sperm parameters after 120 minute equilibration based on the assessment at 15 minute equilibration was observed primarily in indicators evaluated by flow cytometry

<u>Further research should focus on</u>:

- **Increasing the sample size**
- **Different sheep breeds**
- **Other livestock species (on goats)**



Thank you for your attention !

Lucie Langerová,
Martin Ptáček,
Filipp Georgievič Savvulidi

I would like to thank:

Aizhan Makhanbetova,
Beybit Kulataev,
Nurlan Malmakov and the other members of the Kazakh team

AND

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for their cooperation !