



HAL
open science

Laparoscopic insemination method in sheep allows the use of an animal protein-free and inexpensive freezing medium

Lucie Gavin-Plagne, Lionel Boyer, Anne Baudot, Magda Guedes Teixeira, Gérard Louis, Loris Commin, S. Buff, Thierry Joly

► To cite this version:

Lucie Gavin-Plagne, Lionel Boyer, Anne Baudot, Magda Guedes Teixeira, Gérard Louis, et al.. Laparoscopic insemination method in sheep allows the use of an animal protein-free and inexpensive freezing medium. Annual Conference of the International Embryo Technology Society, Jan 2020, New York, United States. 32 (2), pp.206, 2020, 10.1071/RDv32n2Ab159 . hal-02450391

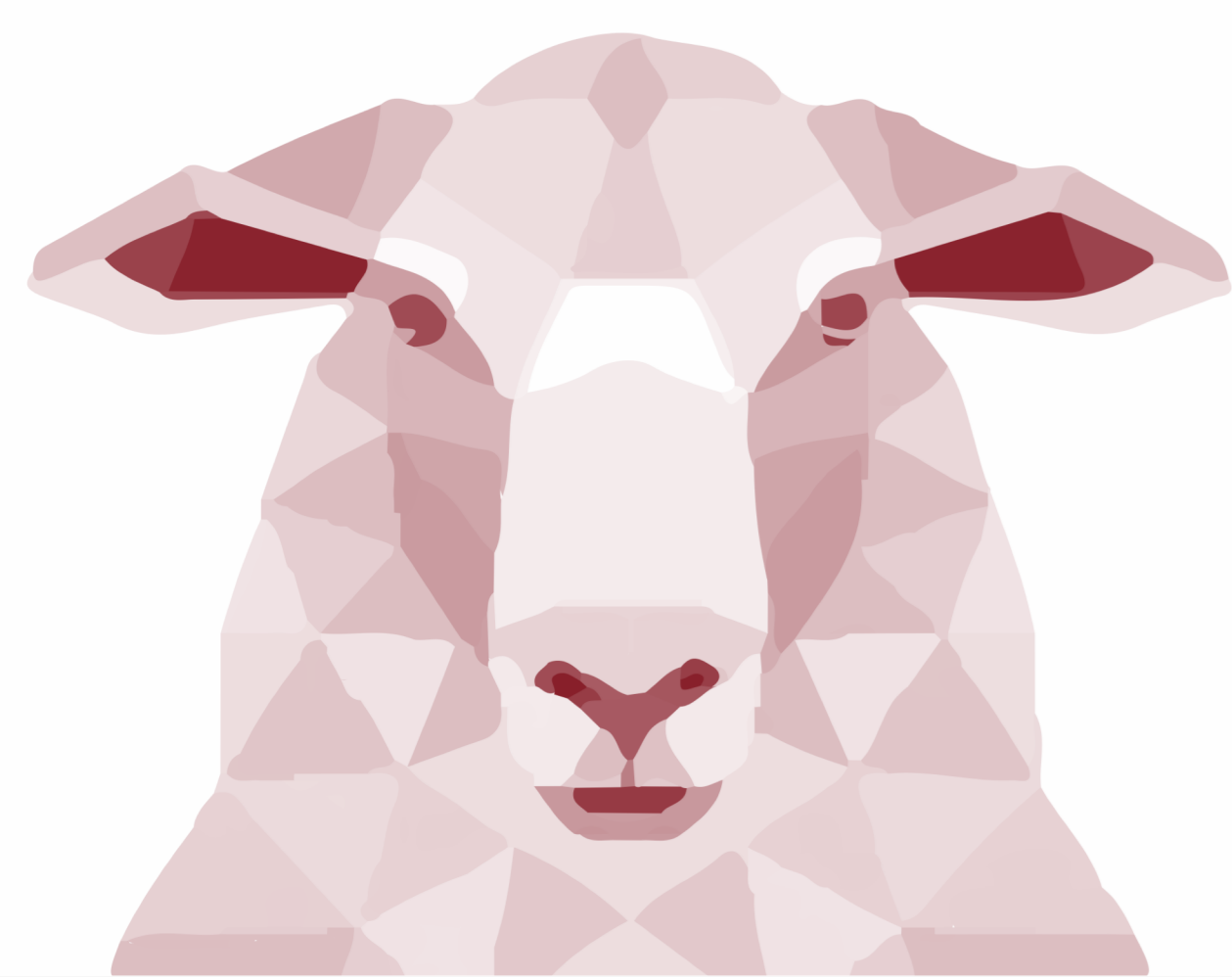
HAL Id: hal-02450391

<https://vetagro-sup.hal.science/hal-02450391v1>

Submitted on 22 Jan 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Laparoscopic insemination method in sheep allows the use of an animal protein-free and inexpensive freezing medium

Lucie GAVIN-PLAGNE^{1,2}, Lionel BOYER^{3,4}, Anne BAUDOT⁵, Magda GUEDES TEIXEIRA¹, Gérard LOUIS⁵, Loris COMMIN¹, Samuel BUFF¹, Thierry JOLY¹



¹UPSP ICE 2016.A104, VetAgro Sup, Marcy l'Etoile, France,

²IMV Technologies, L'Aigle, France,

³Fedatest, Mazeyrat d'Allier, France,

⁴GIE Us Rom, Mazeyrat d'Allier, France,

⁵INSERM U1148, Université Paris Descartes, Paris, France

+33 (0)676675790 | lucie.gavinplagne@imv-technologies.com

CONTEXT

In breed societies managing ovine species, sperm is cryopreserved according to a method adapted from Colas [1], using egg yolk and milk in extenders. Using such media is acceptable within a short-term commercial strategy. However, it sounds necessary to develop the use of stable, synthetic and chemically defined medium in biobank to overcome any update of the regulations by the future. STEMALPHA.CRYO3 (Ref 5617, Stem Alpha, Saint-Genis-l'Argentiere, France) called 'CRYO3' is a chemically defined preservation medium currently used for freezing human tissue and adult stem cells.

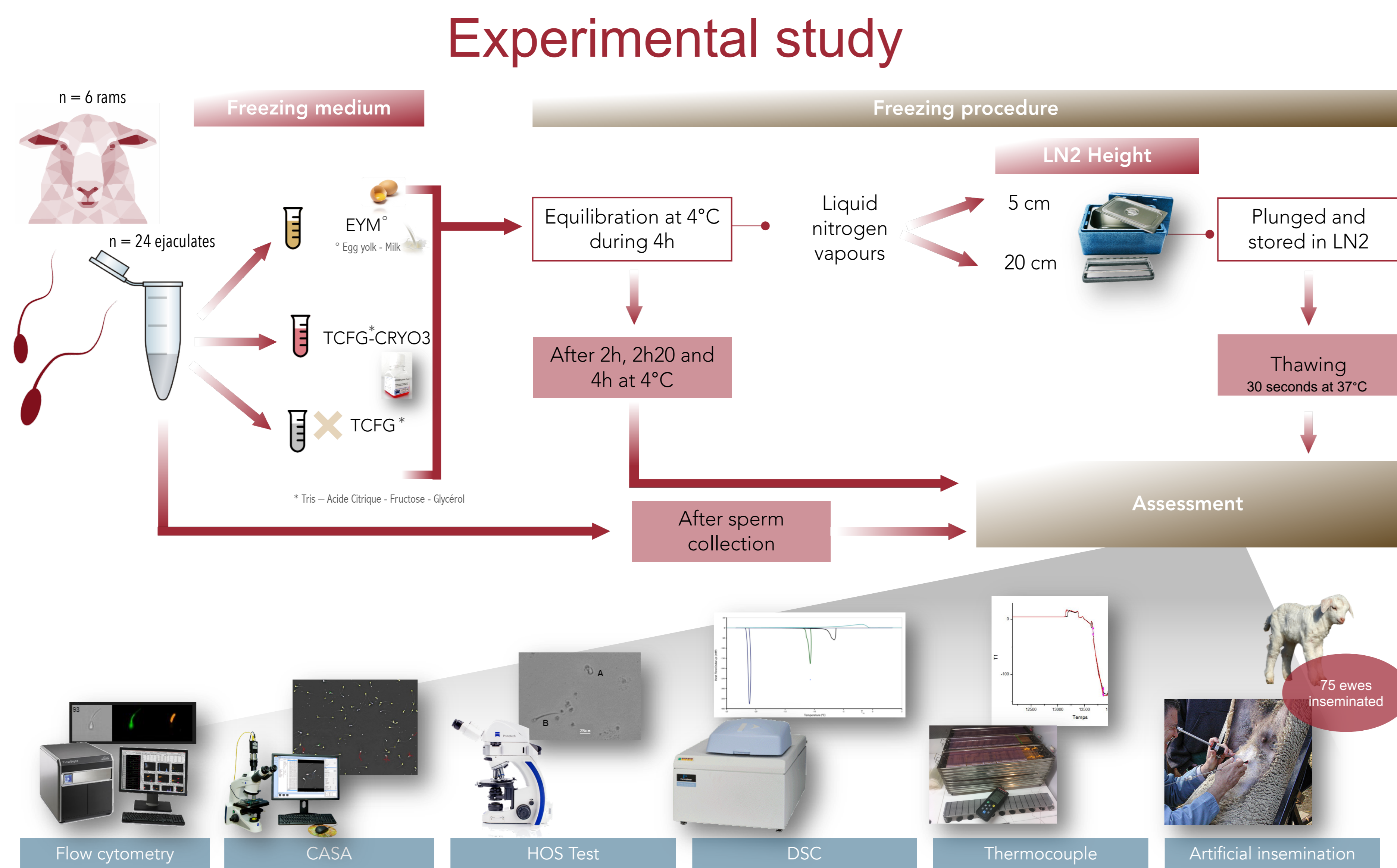
[1] Colas G. Effect of initial freezing temperature addition of glycerol and dilution on the survival and fertilizing ability of deep-frozen ram semen. Journal of the Society for Reproduction and Fertility. 1975;277-85.

OBJECTIVE

The objectives of this study were firstly to avoid all forms of derived (plant or animal origin), or unstable and variable products, and secondly to test a faster cooling rate. The aim of the present study was to evaluate the effects of a CRYO3-based medium and of two cooling rates on *in vitro* parameters and *in vivo* fertility of ram sperm.

1

MATERIALS & METHODS



Medium composition

Table 1. Composition of extender 1 and extender 2 for each freezing medium.

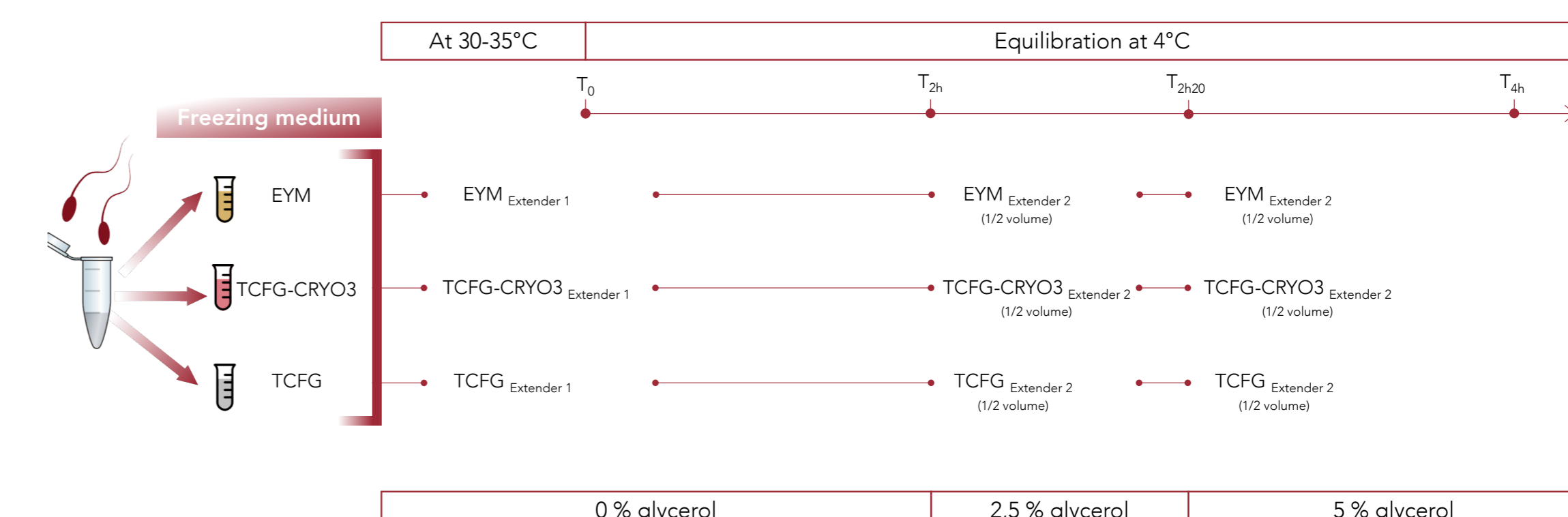
	EYM1	TCFG-CRYO3	TCFG
Extender 1 (at 30°C)	Sugar: Lactose (102.96 g/L) Additive: 20% (v/v) hen egg yolk Buffer: Gentamicin (Gibco, 10 mg/mL) in sterile water	20% CRYO3 TCF	- TCF
Extender 2 (at 4°C)	Sugar: - Glycerol: 10% (v/v) glycerol Buffer: 90% (w/v) of milk powder diluted in sterile water (Regilaat, 40 g/L of semi-skimmed milk) and gentamicin (Gibco, 10 mg/mL)	0.2 M trehalose 10% (v/v) glycerol TCF	0.2 M trehalose 10% (v/v) glycerol TCF
pH	Adjusted at 6.8	7.0	7.0

TCF: Tris buffer supplemented with citric acid and fructose (Tris-hydroxymethyl-aminomethane, 27 g/L; citric acid, 14 g/L; fructose, 10 g/L; pH = 7.0).

Semen diluted up to 800 × 10⁶ spz/mL

Final concentration of 400 × 10⁶ spz/mL

Dilution procedure of ram sperm



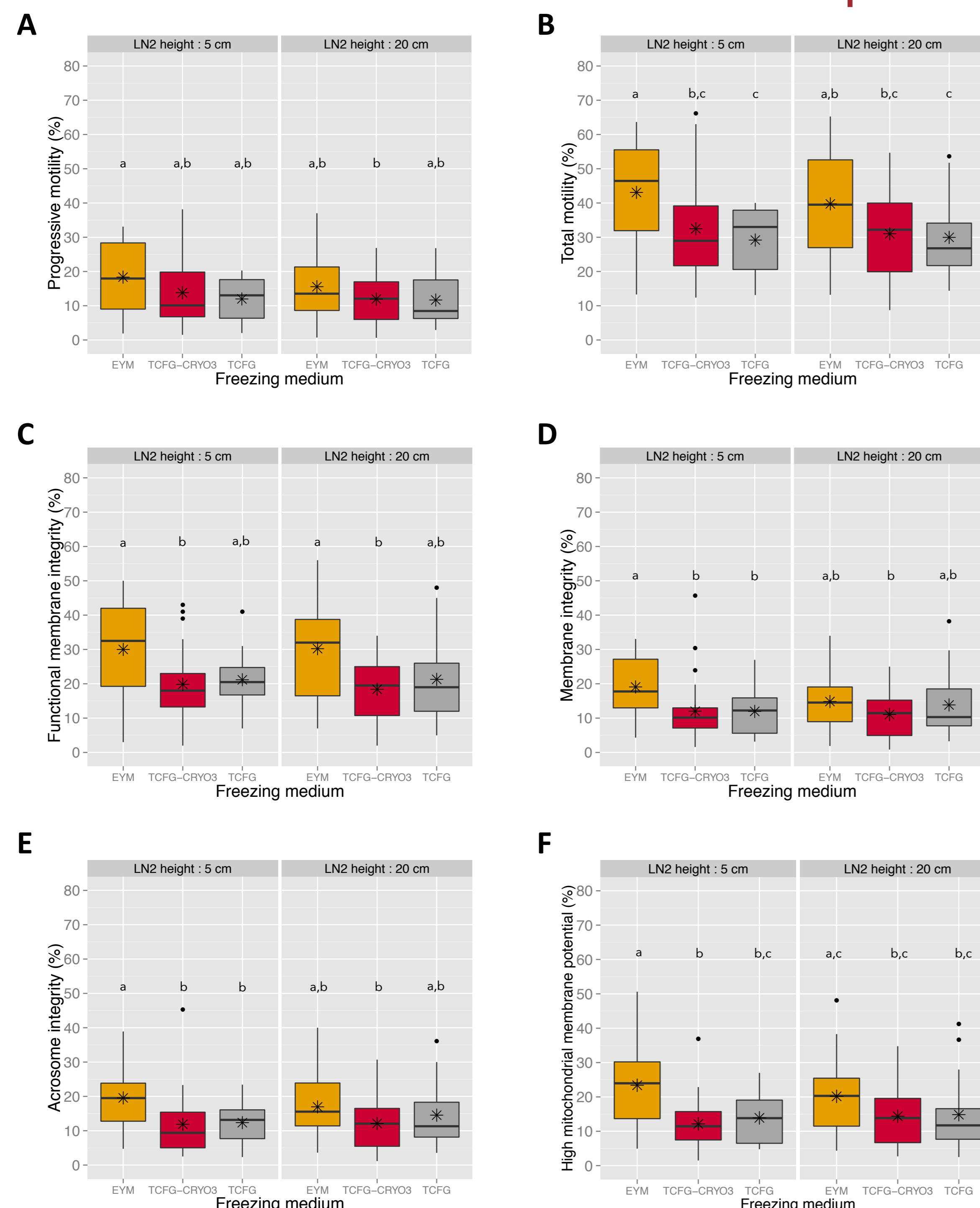
Statistics

• R software
• *in vitro* parameters: a mixed model including the ram as a random effect and the medium and the distance above the surface of liquid nitrogen as fixed effects with three and two levels respectively.
• Pregnancy and parturition rates (binomial distribution) & prolificacy (Poisson distribution): generalized linear models, including the medium as a fixed effect and the ram as a random effect.
• Differences with p < 0.05 were considered statistically significant. Thermodynamic values were analysed using descriptive statistics.

2

RESULTS

in vitro assessment of frozen sperm



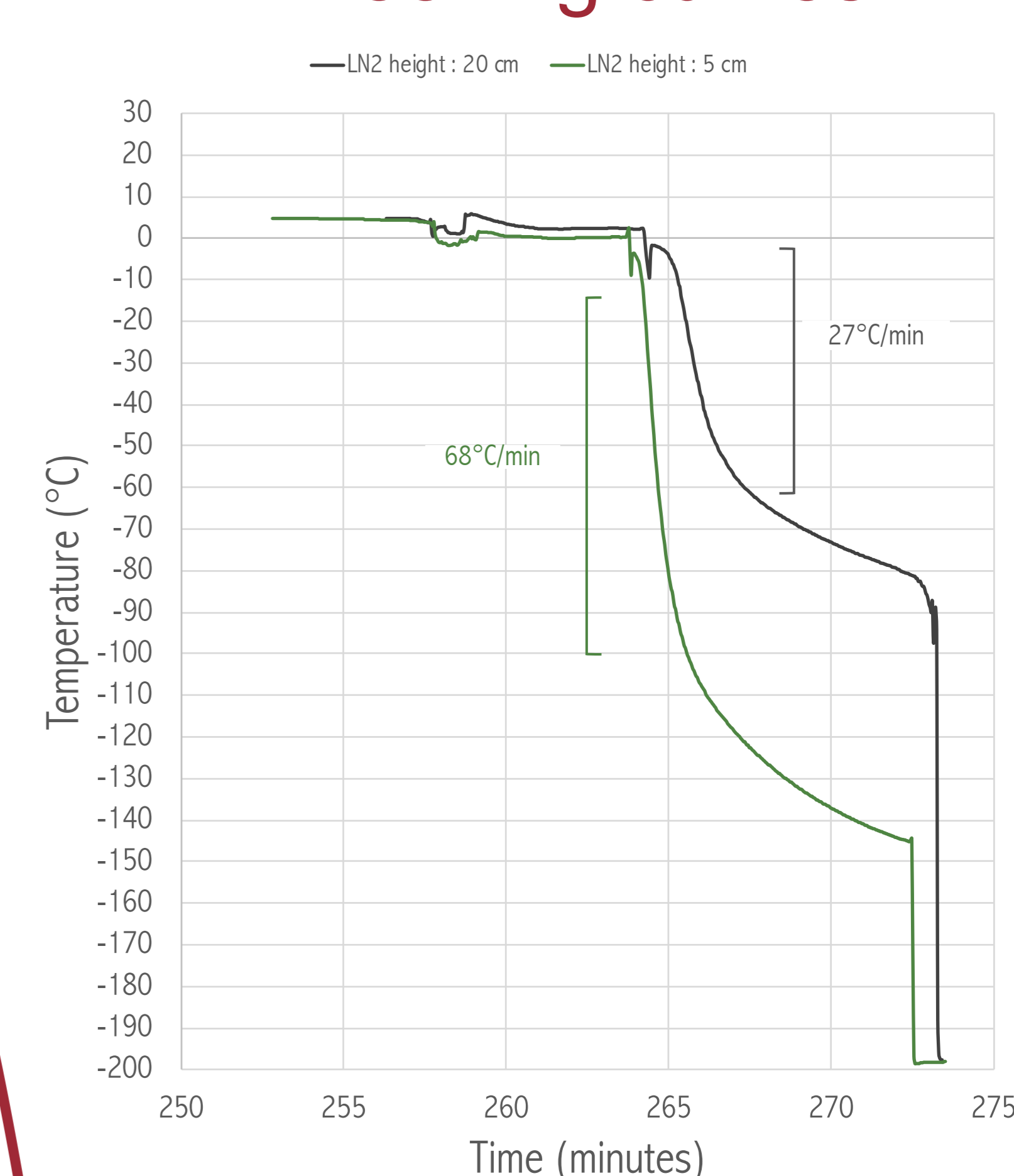
in vivo assessment of frozen sperm



AI were performed with straws frozen at a LN2 height of 5 cm, from three rams and 3 sessions of sperm collection.

The cooling rates: no significant effect (except on the membrane integrity)
Media: significant differences
EYM showed the highest values. TCFG-CRYO3 and TCFG were not significantly different.

Freezing curves



No significant difference was observed. However, the negative control TCFG showed the best parturition rate with 74% of delivered ewes.

Thermodynamic properties of the media

Table 2. Thermodynamic characteristics of the freezing media.

Medium	T _m (°C)	ΔH _{max} (J/g)	T _{c2.5°C/min} (°C)	ΔH _(-50°C/min) (J/g)	T _{c50°C/min} (°C)
EYM	-1.27 ± 0.16	-215.54 ± 5.70	-18.66 ± 2.33	-197.94 ± 0.91	-20.72 ± 1.21
TCFG-CRYO3	-1.44 ± 0.34	-223.14 ± 5.45	-15.42 ± 1.87	-217.74 ± 4.77	-16.84 ± 1.29

Results are presented as the means ± standard deviation of 3 replicates of each medium. T_c(2.5°C/min): Crystallization temperature at a cooling rate of 2.5°C/min; T_c(50°C/min): Crystallization temperature at a cooling rate of 50°C/min; ΔH_{max}: maximum crystallization enthalpy variation; ΔH_(-50°C/min): crystallization enthalpy variation at a cooling rate of 50°C/min.

The two media are quite similar in terms of thermodynamic properties. Surprisingly, TCFG-CRYO3 was found to show more variability than EYM.

3

CONCLUSION

This study showed that the product, CRYO3, cannot replace egg yolk and milk in freezing extenders. Moreover, we showed that laparoscopic inseminations allowed a 74% parturition rate thanks to an easy and inexpensive medium composed only of a Tris buffer and glycerol. This medium, although could not be used in a large scale, remains an option for international transport or long-term storage of genetic diversity.