IMAGE

European Project n° 677353

IMAGE
Innovative Management of Animal Genetic Resources

Breakthrough to improve the reproductive capacity of gene bank material

WP n°3

Blesbois E, Liptoï K, Woelders H, Bernal-Juarez B, Nandi S, Thelie A, Varkonyi E, Govoroun M, Guignot F, Mc Grew M, Santiago-Moreno J

IMAGE

Objectives of the WP3

► ***improve the efficiency of the reproductive cells/tissues to be stored in the cryobanks for the conservation of domestic animal genetic resources. Different reproductive cells and tissues are under study in different major production species.***

IMAGE

Activities

- **Task 3.1: Semen cryopreservation** (J. Santiago-Moreno) . INIA/WR/INRA

- **Task 3.2: New approaches to better predict reproductive success of cryopreserved semen** (E. Blesbois). INRA/INIA/PTP/IDELE

- **Task 3.3 : further development and implementation of gonad transfer in mammals and birds** (H. Woelders). WR/HaGK

- **Task 3.4: PGCs methodology in poultry** (M.Mc Grew). UED/INRA/HaGK

- **Task 3.5: New development in pig embryo cryopreservation** (F. Guignot). INRA/WR

T3.1 Semen cryopreservation

T3.1

► Two species : the chicken and the ram

The chicken

Despite many studies, results of fertility after semen cryopreservation are still highly variables

► Need to study the origins of the variations

► Need to evaluate the environnemental effect

► Need to improve the cryopreservation methodology

T3.1

Examples of results of chicken sperm cryopreservation

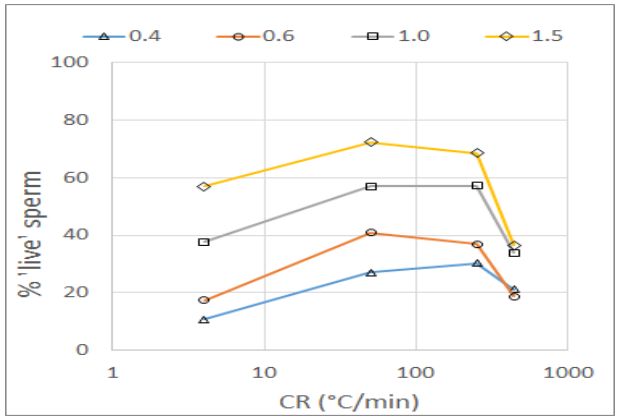
The amino acid composition of seminal plasma differs between different chicken breeds, with consequences on semen freezing. Amino acids such as Valine are involved.

Removal of seminal plasma from the cryopreservation medium reduces the variability of the success of semen freezing

Standardization of the conditions of use of sperm cryopreservation (dil, n sperm/AI, length of storage, adapt to specific field condit,)

Outdoor system, with one group given daily access to a grazing area containing plant species that typically grow on uncultivated Mediterranean land.

Cryoprotectant concentration and cooling rate (DMA 0,4 to 1.5M)



Free range breeding with access to grazing area improves the sperm quality but not its ability to cryopreservation

Santiago-Moreno et al, Poult Sc. 2018; Plos One 2019; Thelie et al, Poult Sci 2018, Thananurak et al., Poult. Sci. 2019

Semen cryopreservation

T3.1

The ram

- AI with fresh semen is not invasive **BUT**
  AI with frozen semen most often need laparotomy due to low sperm quality

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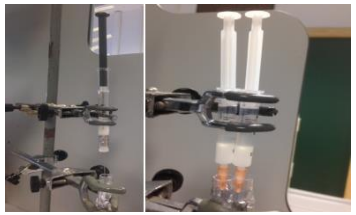
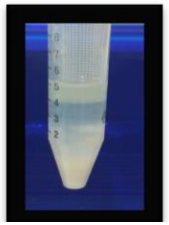
Problem of frozen-thawed sperm quality

⇩

Improvement of sperm selection for cryopreservation.
Comparison of:



Sephadex G-15®



BoviPure®

Percoll®

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⇩

Higher sperm quality after the use of Spehadex filtration

Galarza et al., 2018

T3.2-Semen quality evaluation

T3.2

In the chicken

- Validation of proteins, candidate marker of fertility
- Development of sperm DNA fragmentation evaluation (Tunel assay)

In bovines

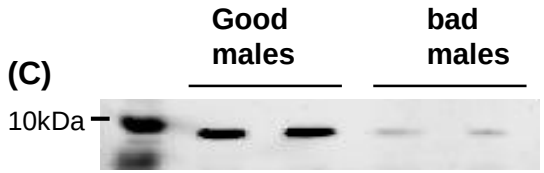
- Validation of miRNA expression in relationship with sperm quality (in due course)

Validation and role of a protein candidate marker of fertility, CLTI example

T3.2

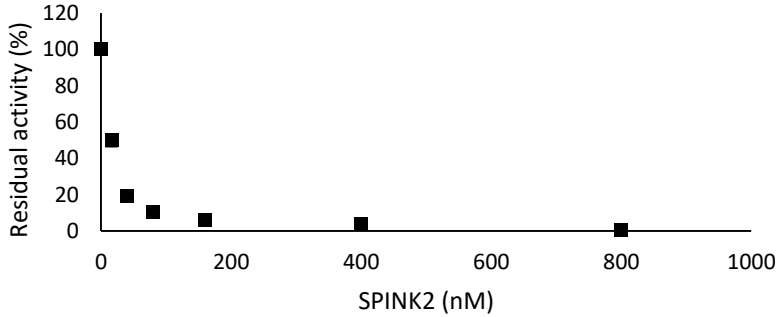
CLTI: a candidate marker of fertility identified in seminal plasma by proteomic ICM-MS andTop down identification approaches (Labas and al., 2015)

We found that CLTI is **SPINK2**,
a protease inhibitor



► **SPINK2 is present in lower amounts in seminal plasma of low fertility males, in highly different chicken breeds (free range, meat, lay lines)**

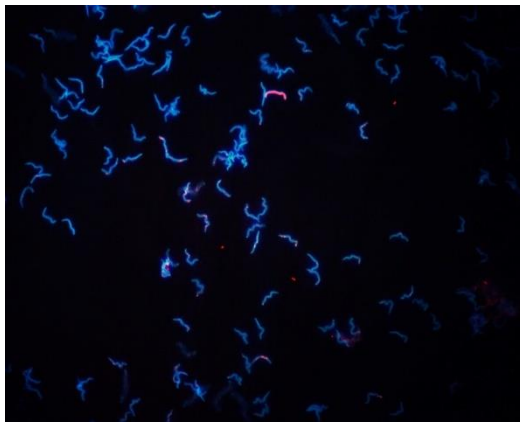
► **SPINK2 is a specific inhibitor of acrosin (a key acrosome enzyme of fertilization)**

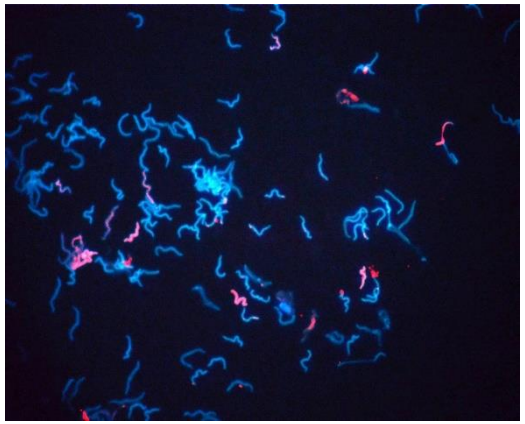


Thelie et al, Mol Rep Dev 2019

T3.2

DNA: sperm DNA fragmentation (tunel assay) differs between breeds





Examples

Breed	% fragmented DNA
Black Red Andaluza	5.2
Birchen Leonesa	15.7
White-Faced Spanish	23.0

Santiago-Moreno et al, Plos One 2019

Task 3.3 : further development and implementation of gonad transfer in mammals and birds

T3.3

In the chicken

► Embryo cryopreservation impossible. Search of alternatives. One of them is the gonadic tissues conservation and transfer in host animals expected to express the genome of the donor.

► In development in Europe (Liptoi et al., 2013)

►Needs improvements, and search of compatible donors and host

In mammals and chickens

► Many ethical and regulation concerns that need to be clarified for gonad and cell transfer

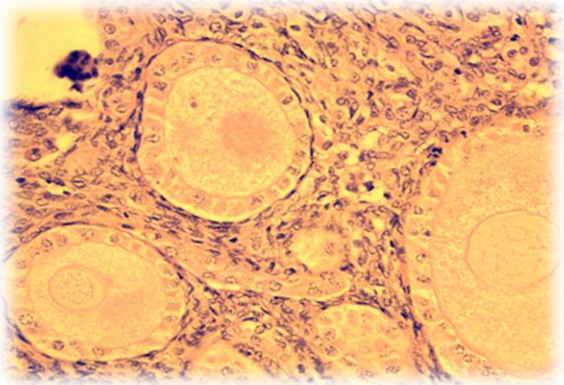
Gonad cryopreservation and transfer in the chicken

T3.3

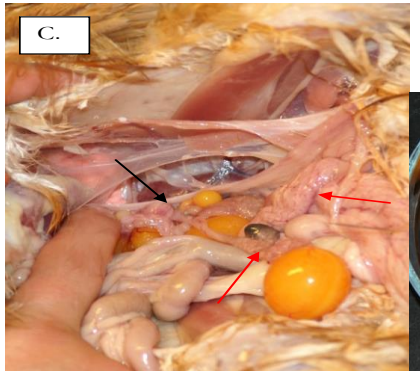
2) Cryopreservation vitrification

3) Grafting 1 day old chick

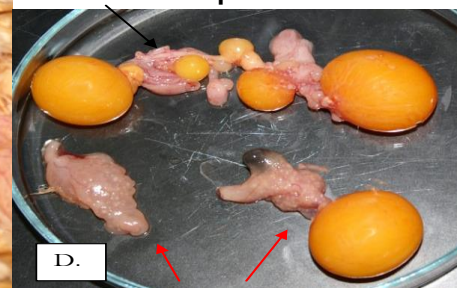
4) Success seen at 8 wks old. Female example



1) Gonad taken in 1 day old chick



At 16 wks old. Female example



Liptoï et al., Anim. Reprod. Sci, 2013; Liptoï et al, Proc. Eur. Congr, WPSA 2018; Buda et al., H. Vet. J. 2019

Suitables donors/recipient breeds

T3.3

Successfill results but the success depends mainly on the pairing of the breeds and is less affected by various chemical treatments

RECIPIENTS

White Leghorn

Novogen White

DONORS

Yellow Hungarian

Partridge-color Hungarian

Speckled Hungarian

Black and Speckled Transylvanian Naked Neck

Regulation Gonad and cell transfer

T3.3

Review pertinent regulation: Gonadal tissue and cells (PGCs)

- Animal procedures involved are generally **not allowed** according to EU directives and/or national laws.
- Use of PGCs to produce chimaera is considered ‘GM’ in NL

But

- National regulation to allow specific animal procedures is possible
  - For a ‘good cause’, e.g. conservation of genetic diversity.
  - Or for a ‘commercial’ cause, e.g. conservation of breeding lines

  - Balancing interests: Importance genetic diversity, or economic interests industry, versus protection of animals, and ethical principles.

Task 3.4: PGCs methodology in poultry

T3.4

- Promising alternative to semen methodology in birds

- In IMAGE:

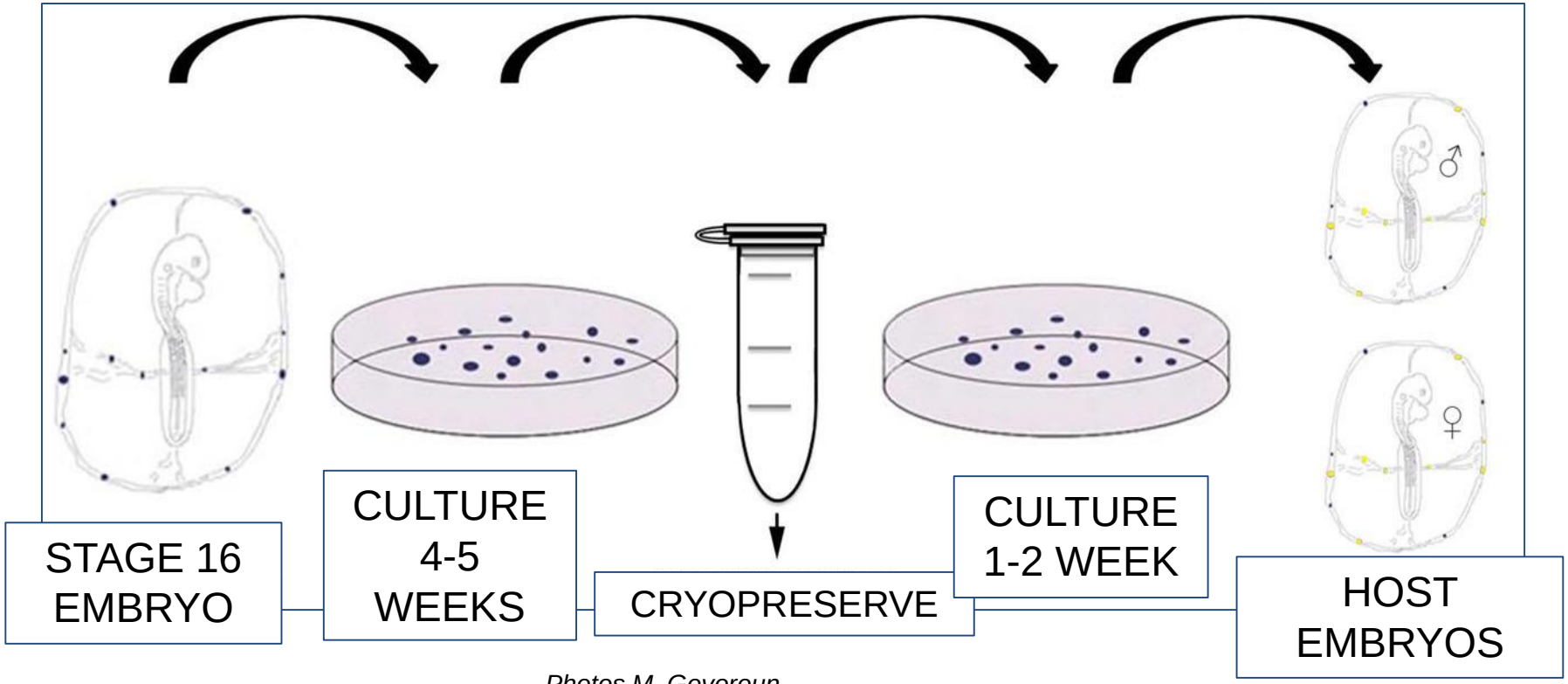
Develop PGC freezing protocols and long term cultures. Include PGCs collections in germ plasm cryobanks and show their efficiency to transfer donor genomes to host animals and their progeny.

Feasability of use of interspecific guinea fowl × chicken sterile host (in due course)

IMAGE

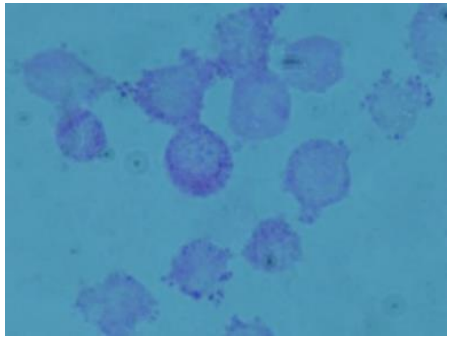
T3.4

Diagram M. Mc Grew

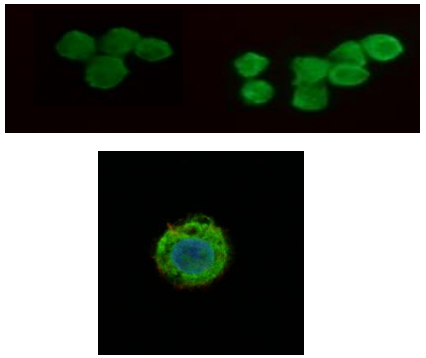


Photos M. Govoroun

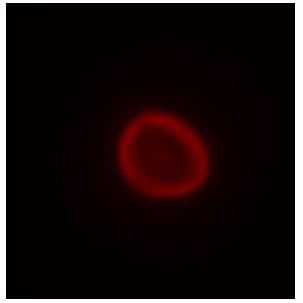
PGCs with different dyes:



Periodic Acid Schiff (PAS)



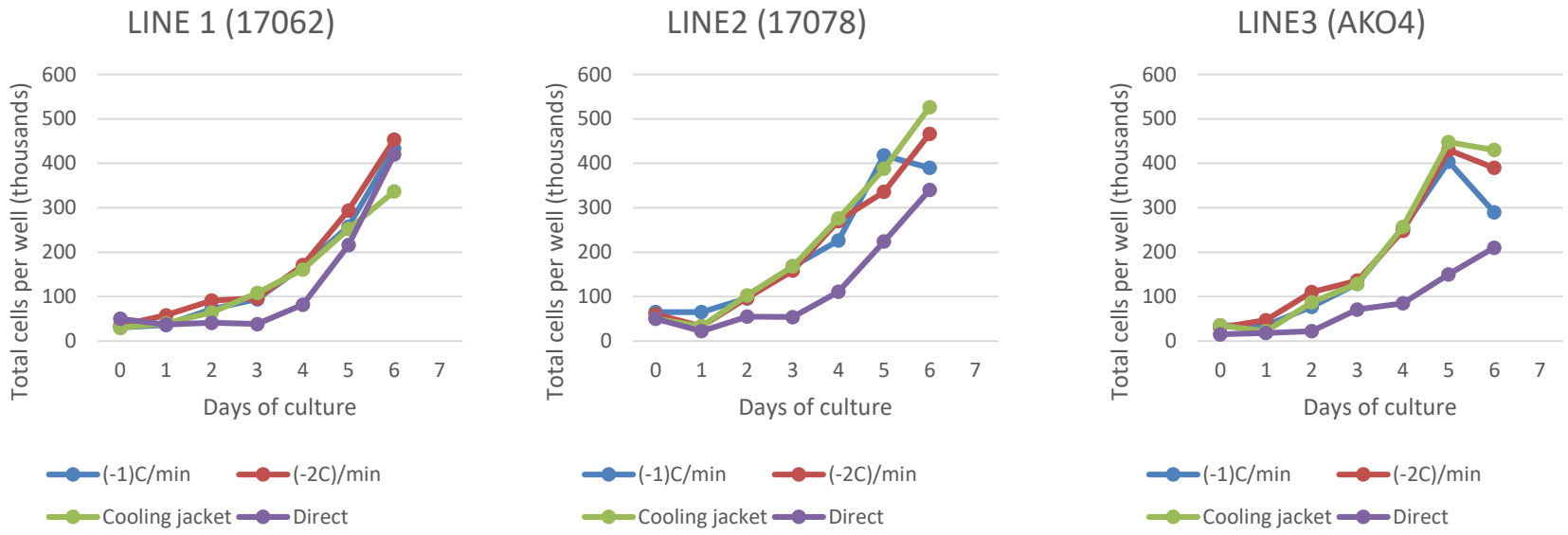
Vasa (CVH), confocal



SSEA1 (confocal)

Example on PGCs cryopreservation methodology test

T3.4



PGCs support quite well the cryopreservation process. An example: their multiplication is only marginaly affected by the freezing curve.

The methods of PGCs culture and cryopreservation allowed in IMAGE to the biobanking of two indigenous chicken genome, the « Noire du Berry » (France) and the « Pure Partidge Colour » in Hungary, and to the restoration of their genomes in host animals

Diagram E. Patakiné

Results – Partridge colour Hungarian

Creating germ line chimeras

52 injections
in 5 trials

CRYOBANK

No. of adult Black Transsylvanian Naked-neck recipients:
24 (13 roosters, 11 hens)

A male and a female PGC line were selected for injection

X

Test-cross with the donor Partridge colour Hungarian breed

Regenerating the donor breed

No. of germ line chimeras: 4 (16.6 %)
No. of donor derived hatchlings: 17 (5 %)

T3.5

Task 3.5: New development in pig embryo cryopreservation

► too highly variable results to be applied in the field

►Aim of the Task: evaluate new vitrification process and increase the knowledge of embryo quality parameters

☞ **An experimental approach that analyze the impact of vitrification / thawing on porcine embryo transcriptome by RNA –sequencing**

☞ **A mathematical simulation of osmotic events in vitrification protocols, to identify and understand potential causes of damage in vitrification protocols and means to prevent them**

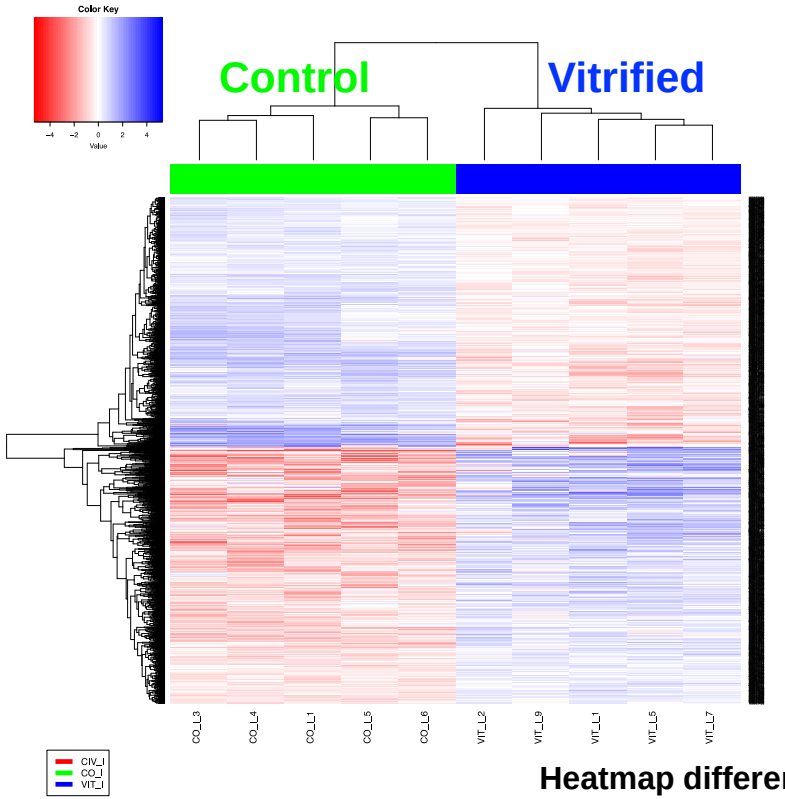
Impact of pig embryo vitrification on RNA expression

T3.5

Results of RNA analysis:

Comparison between Control & Vitrified embryos

EdgeR_result:
Total DEG 1901



Transcripts downregulated in vitrified embryos

Transcripts upregulated in vitrified embryos

Heatmap differential genes (DEG) (FDR cut-off 0.001)

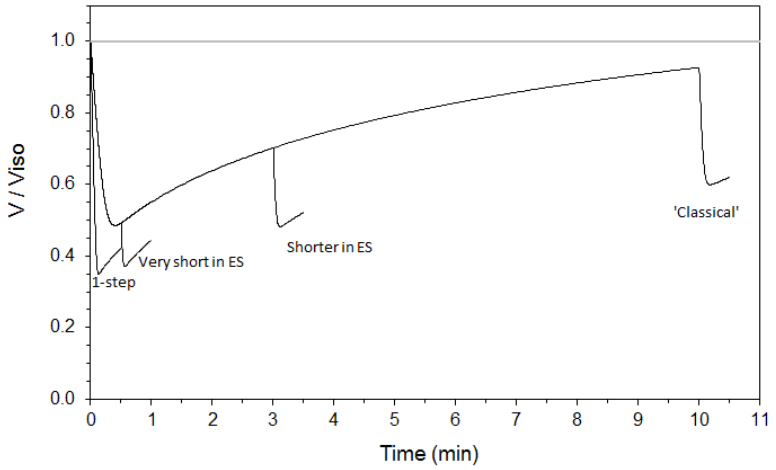
Alminana et al, Spring Conference, Lindau, 2019

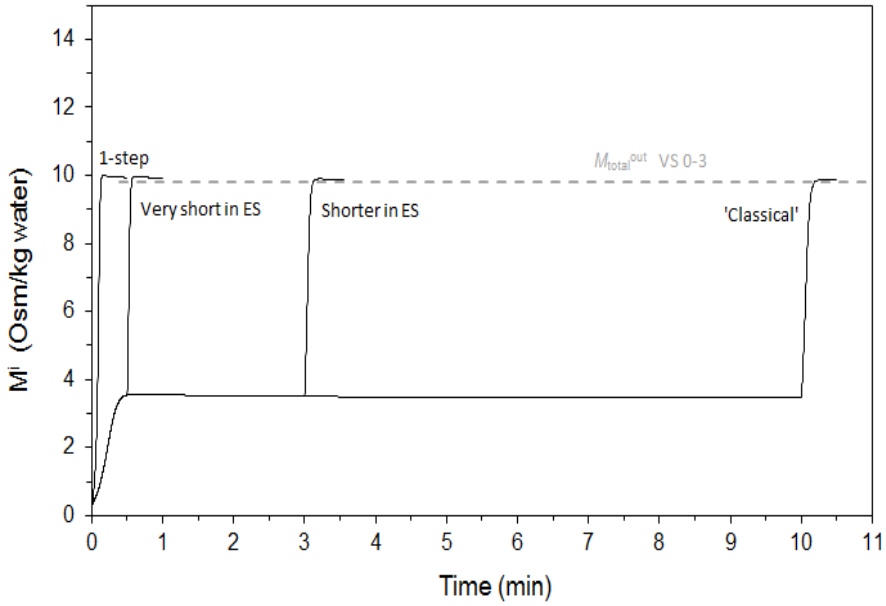
Vitrification pig embryos

T3.5

Mathematical simulations

- Prevent toxic effects cryoprotectants
- Short exposure to cryoprotectants is possible





Woelders et al., Cryobiology 85, 2018

Conclusion

- **The WP3 of Image is still in progress**
- **Already many results allow to improvements in the reproductive biotechnologies of the conservation of genetic resources, and in the evaluation of the quality of the cells/tissues to be conserved**
- **These progress allow to increase the quality and scope of European ex-situ collections**
- **Ethical issues are important in order to choose the best routes for reproductive collections**