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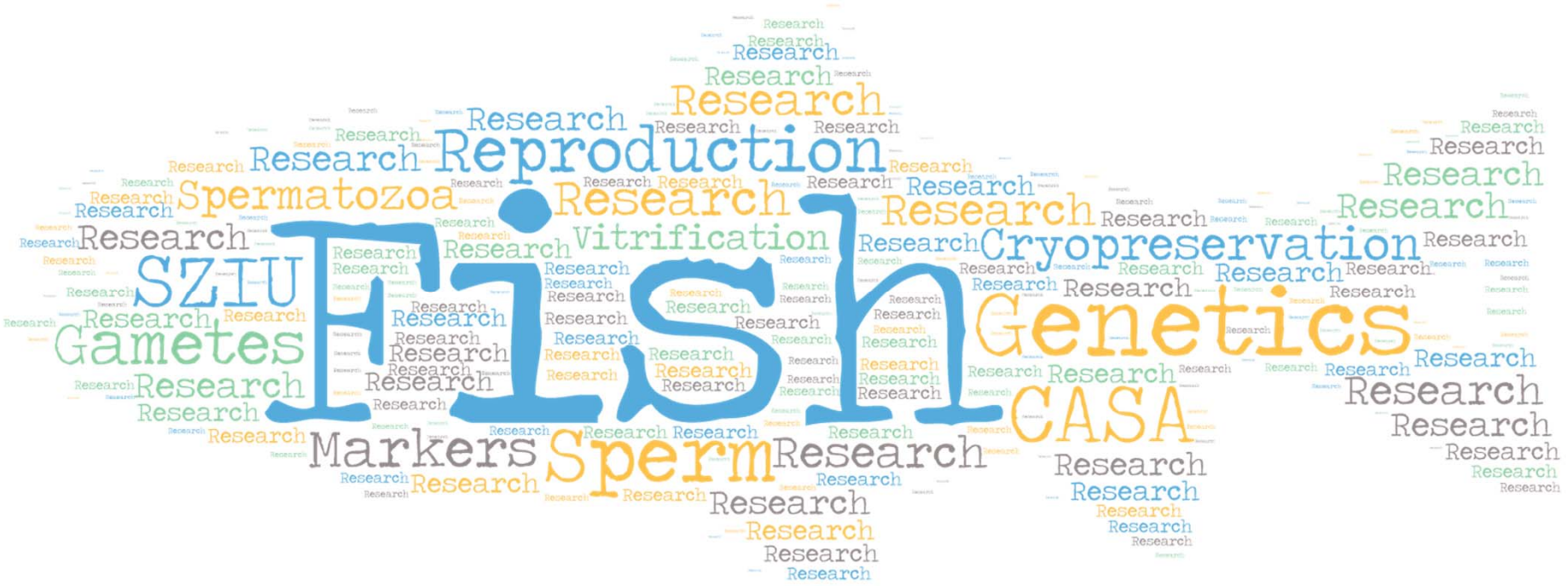

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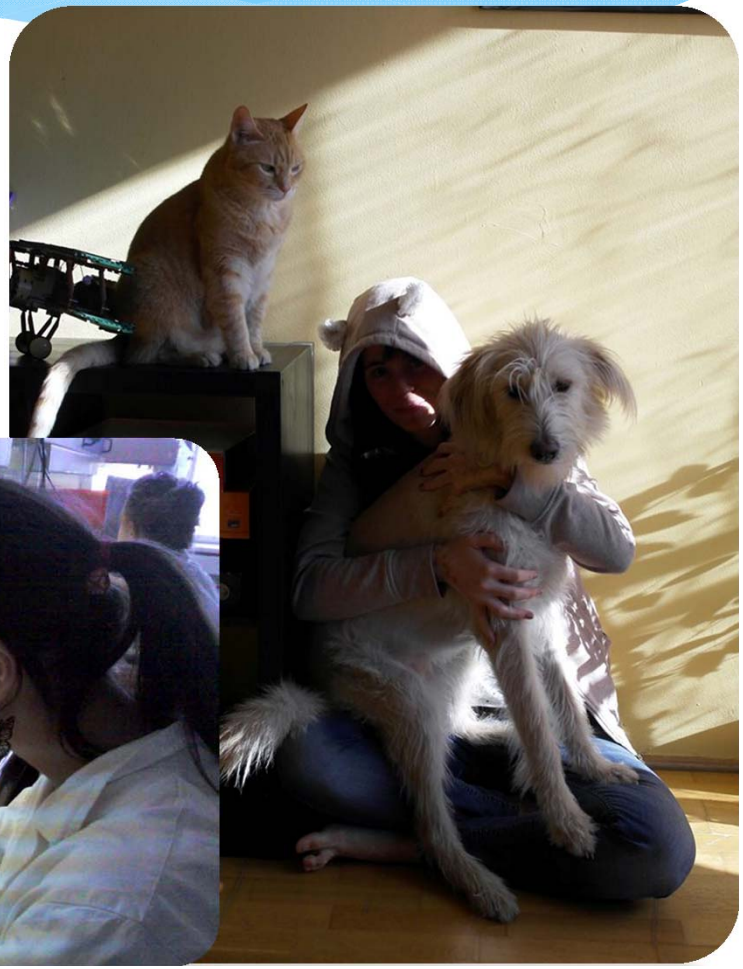
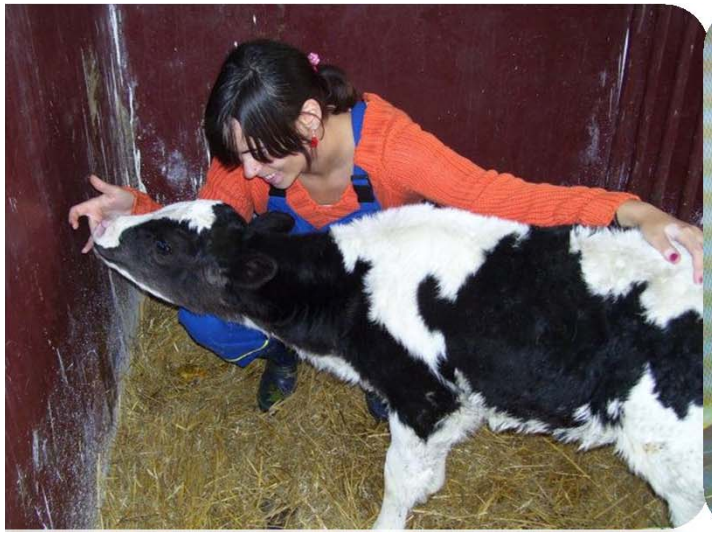
Studies & animals

* Animal Husbandry B.Sc.
* Animal Biotechnology M.Sc.
* Ph.D: Vitrification of fish sperm





Hungary in Europe

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BUDAPEST


SZIGET
ISLAND OF FREEDOM

Lake Balaton: the largest shallow lake in Central Europe

QUACULTURE

My PhD topic: Vitrification of fish sperm

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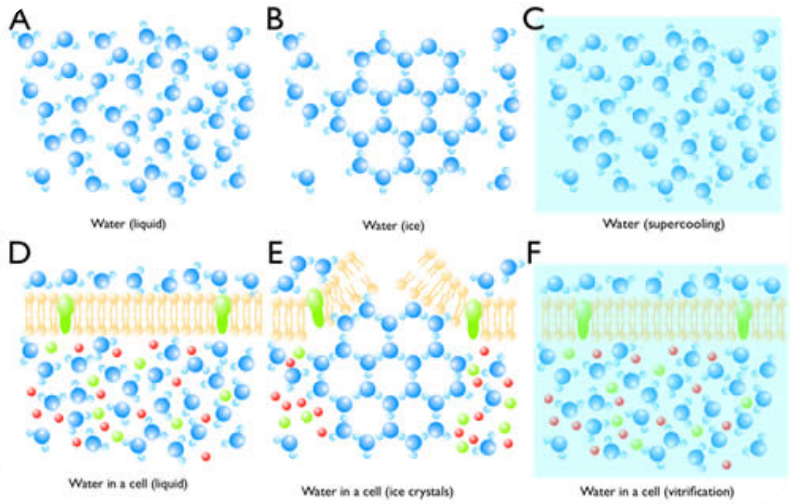
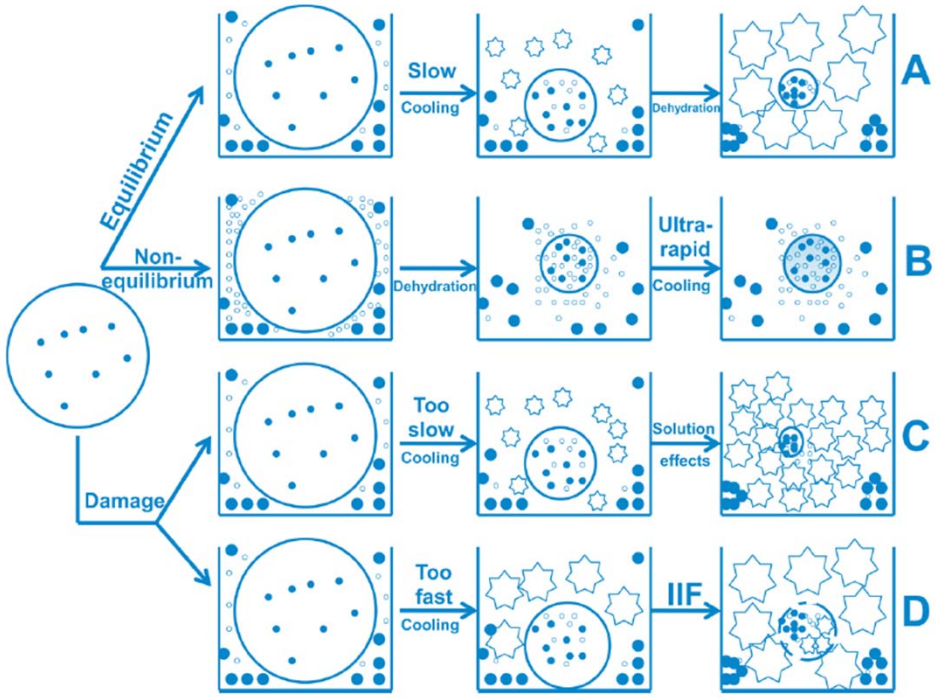
What is vitrification?

* Solidification of a liquid into an amorphous/glassy state
* Without creation of harmful ice cristals
* Can be attained at very fast cooling rates (10^6-10^{10} ° C/s)
* Is commonly used for long-term storage of embryos, oocytes and tissues



Vitrification vs. conventional cryopreservation

* Vitrification alters from cryopreservation:
  * Cryoprotectant % ↑
  * Cooling rate ↑
  * Warming rate ↑
  * Exposure time ↓
  * Sample volume ↓
  * Cooling device



Cuevas-Uribe, 2011

Vitrification of fish sperm

* 2011:
  * Green swordtail *(Xiphophorus hellerii)* – **7% motility** (Cuevas-Uribe et al)
  * Channel catfish *(Ictalurus punctatus)* - **25% fertilization** (Cuevas-Uribe et al)
* 2013:
  * Rainbow trout *(Onchorynchus mykiss)* – **31,0% fertilization** (Figueroa et al)
* 2015:
  * Atlantic salmon *(Salmo salar)* – **44,1% motility, 46,2% fertilization** (Figueroa et al)


Cryobiology 63 (2011) 186–197

Contents lists available at SciVerse ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Production of channel catfish with sperm cryopreserved by rapid non-equilibrium cooling ☆

Rafael Cuevas-Uribe [a], S.P. Leibo [b], Jonathan Daly [a], Terrence R. Tiersch [a,*]

[a] Aquaculture Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, 2410 Ben Hur Road, Baton Rouge, LA 70803, USA
[b] Department of Biological Sciences, University of New Orleans, Audubon Center for Research of Endangered Species, 2000 Lakeshore Drive, New Orleans, LA 70148, USA


Theriogenology 83 (2015) 238–245

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of seminal plasma on Atlantic salmon (*Salmo salar*) sperm vitrification

E. Figueroa [a,b,*], O. Merino [c], J. Risopatrón [c], V. Isachenko [d], R. Sánchez [c], B. Effer [a], E. Isachenko [d], J.G. Farías [b], I. Valdebenito [e]

[a] School of Aquaculture, Catholic University of Temuco, Faculty of Natural Resources, Temuco, Chile
[b] Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias, Universidad de la Frontera, Temuco, Chile
[c] Department of Basic Sciences, Faculty of Medicine, La Frontera University and BIOREN–Center for Biotechnology in Reproduction, La Frontera University, Temuco, Chile
[d] Department of Obstetrics and Gynecology, University of Cologne, Köln, Germany


Aquaculture 372–375 (2013) 119–126

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Spermatozoa vitrification of sex-reversed rainbow trout (*Oncorhynchus mykiss*): Effect of seminal plasma on physiological parameters

E. Figueroa [a,*], J. Risopatrón [a], R. Sánchez [a], E. Isachenko [c], O. Merino [a], V. Isachenko [c], I. Valdebenito [b]

[a] BIOREN – Center for Biotechnology in Reproduction, La Frontera University, Temuco, Chile
[b] School of Aquaculture, Catholic University of Temuco, Temuco, Chile
[c] Department of Obstetrics and Gynecology, University Maternal Hospital Ulm, Prittwitzstrasse 43, 89075 Ulm, Germany

Experimental species

- European eel *(Anguilla anguilla)*
- Grayling *(Thymallus thymallus)*
- Eurasian perch *(Perca fluviatilis)*
- Tench *(Tinca tinca)*
- Common carp *(Cyprinus carpio)*
- Zebrafish *(Danio rerio)*
- Goldfish *(Carassius auratus)*

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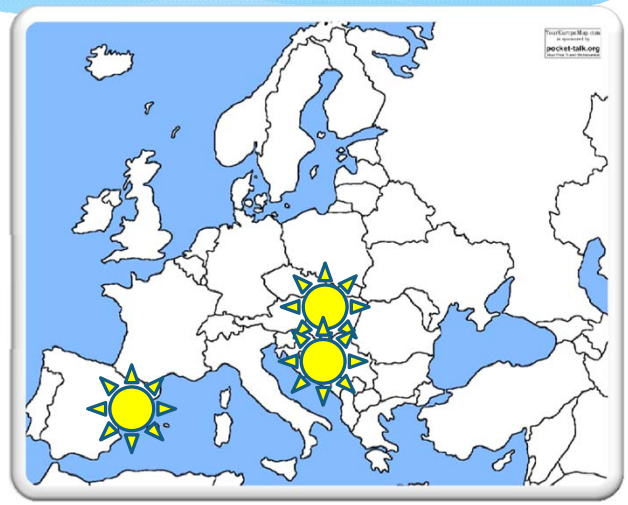

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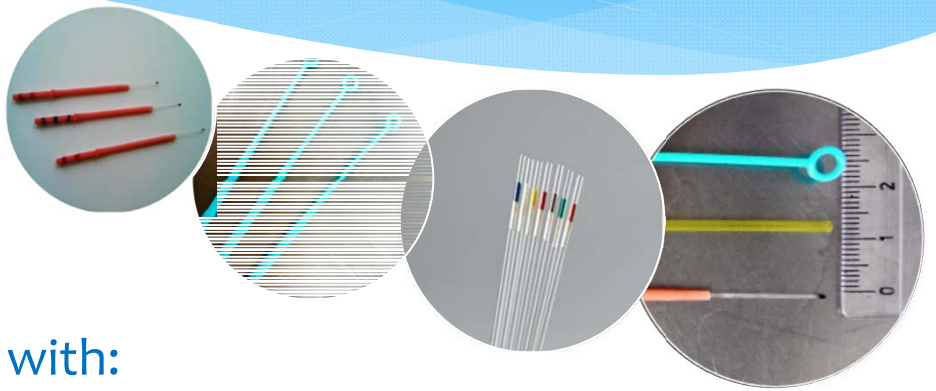
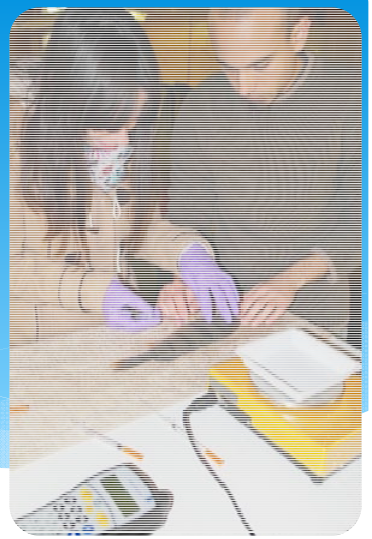
Experimental designs I.

* Cooling devices
  * Cryotop – 2.5 µl
  * Inoculating loop – 10 µl
  * Straw - 250 µl
* Dilution ratios (1:1 – 1:100)
* Cooling media – supplemented with:
  * Sugars – to **increase the osmolality** of the solution
  * Proteins – to **increase the viscosity** of the solution
* Cryoprotectants: high concentration is required (30-50%)
  * Methanol
  * Propylene-glycol
  * Methoxyethanol

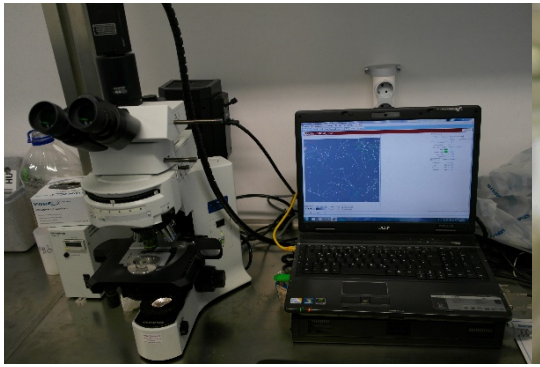


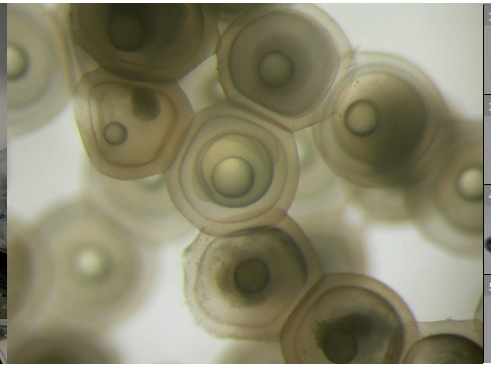
Sperm suspension was plunged directly into liquid nitrogen without pre-cooling in its vapour.

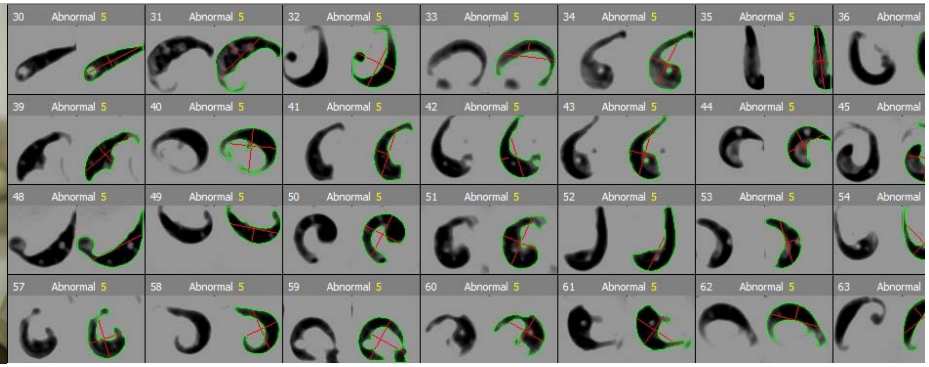
Experimental designs II.



* Evaluation of the efficiency of the vitrification protocols
  * **Progressive motility: CASA** (computer-assisted sperm analysis)
  * **Fertilisation** test
  * **ASMA** (computer automated sperm head morphomtery analysis)







Experimental designs III.

Hormonal injection

Sperm collection

CASA analysis of the fresh samples

Sperm dilution (extender + cryopro-tectants)

Vitrification on Cryotops

Egg collection (hCG, 500 IU/g fish)

Fertilization and incubation

Counting fertilization rates

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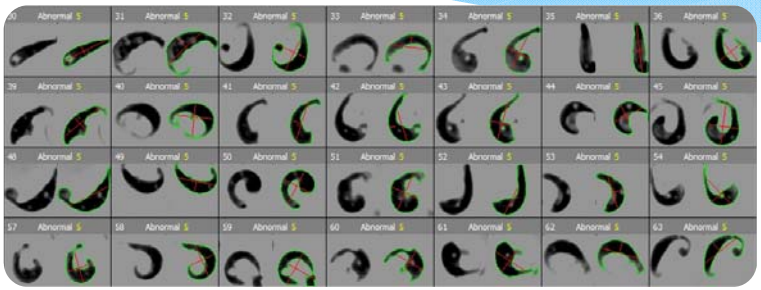
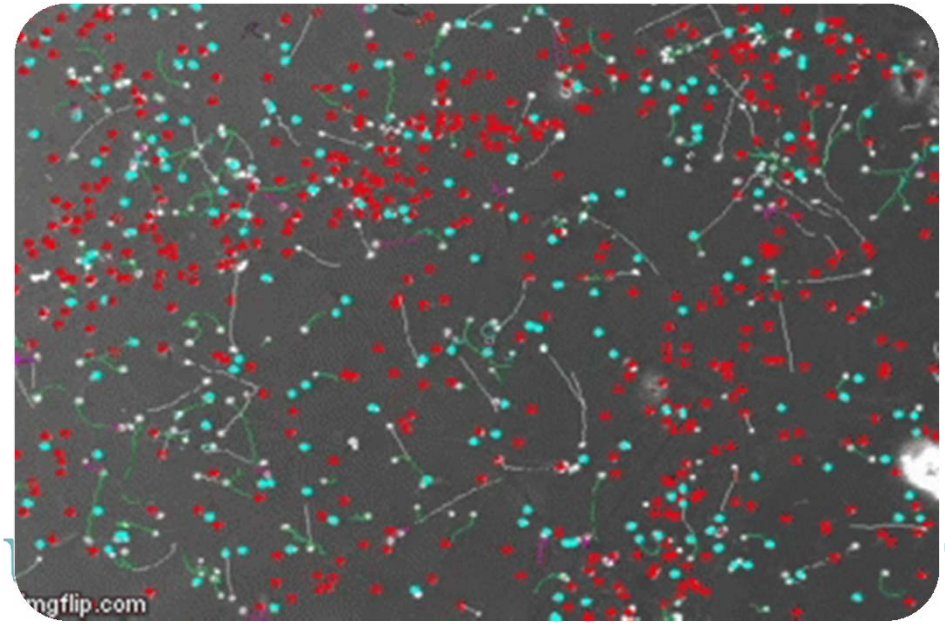
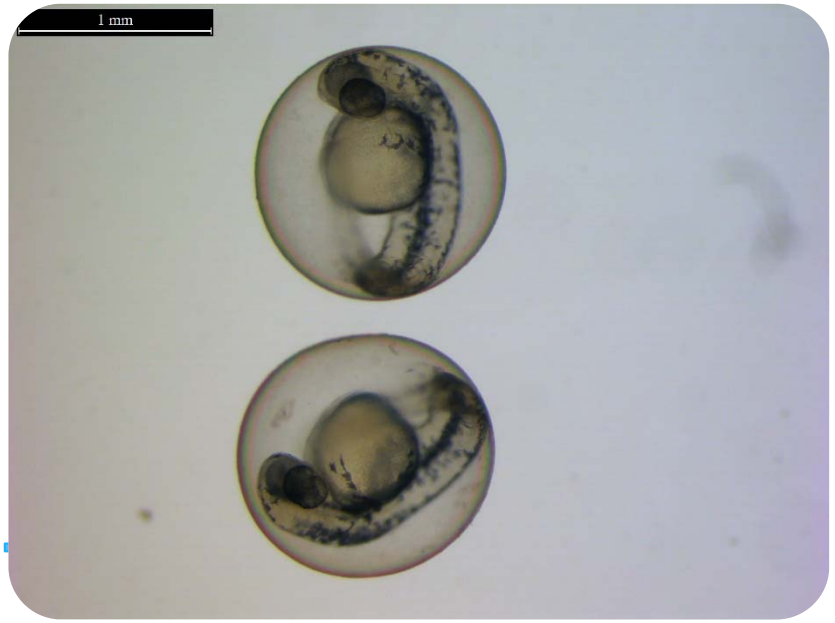
Results



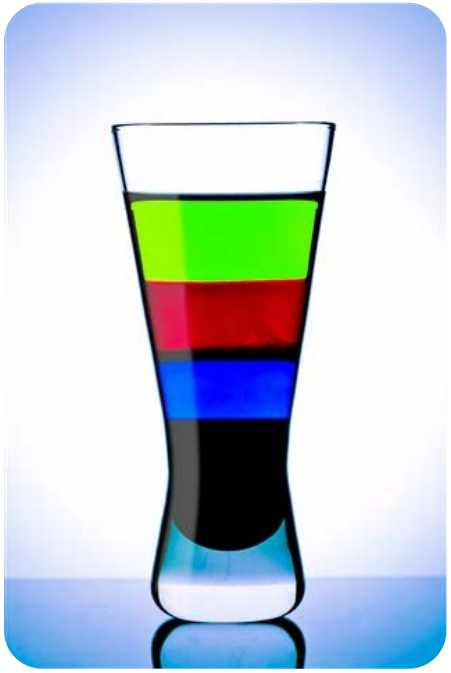




Conclusion

* Small volumes (2-10 µl)
* Species specific media and dilution ratio

* Vitrification media should contain:
  * **Proteins** (carp seminal plasma, FBS, BSA)
  * **Mixture of cryoprotectants** (2-3 CPs, 30-40%*)
  * **Sugars: trehalose** – high efficiency
  * **Non-activating dilution media**



*above 40%: toxic, below 30%: ice formation is not entirely inhibited

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