COST Action FA1205
AQUAGAMETE

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Basic information on the cryopreservation process

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Summary

- Cryopreservation: basics of cryopreservation – cooling of water and aqueous solutions
- Cryopreservation: cooling of live cells, vitrification, the role of cryoprotectants
- Cryopreservation of fish sperm – the role of extenders, cryoprotectants and dilution ratios
- Cryopreservation of fish sperm – methods, straws, cooling rates
- Cryopreservation of fish sperm – storage, thawing
- Cryopreservation of fish sperm – fertilization with cryopreserved sperm
- Commercial application – reasons of failure

Cryopreservation: basics of cryopreservation – cooling of water and aqueous solutions

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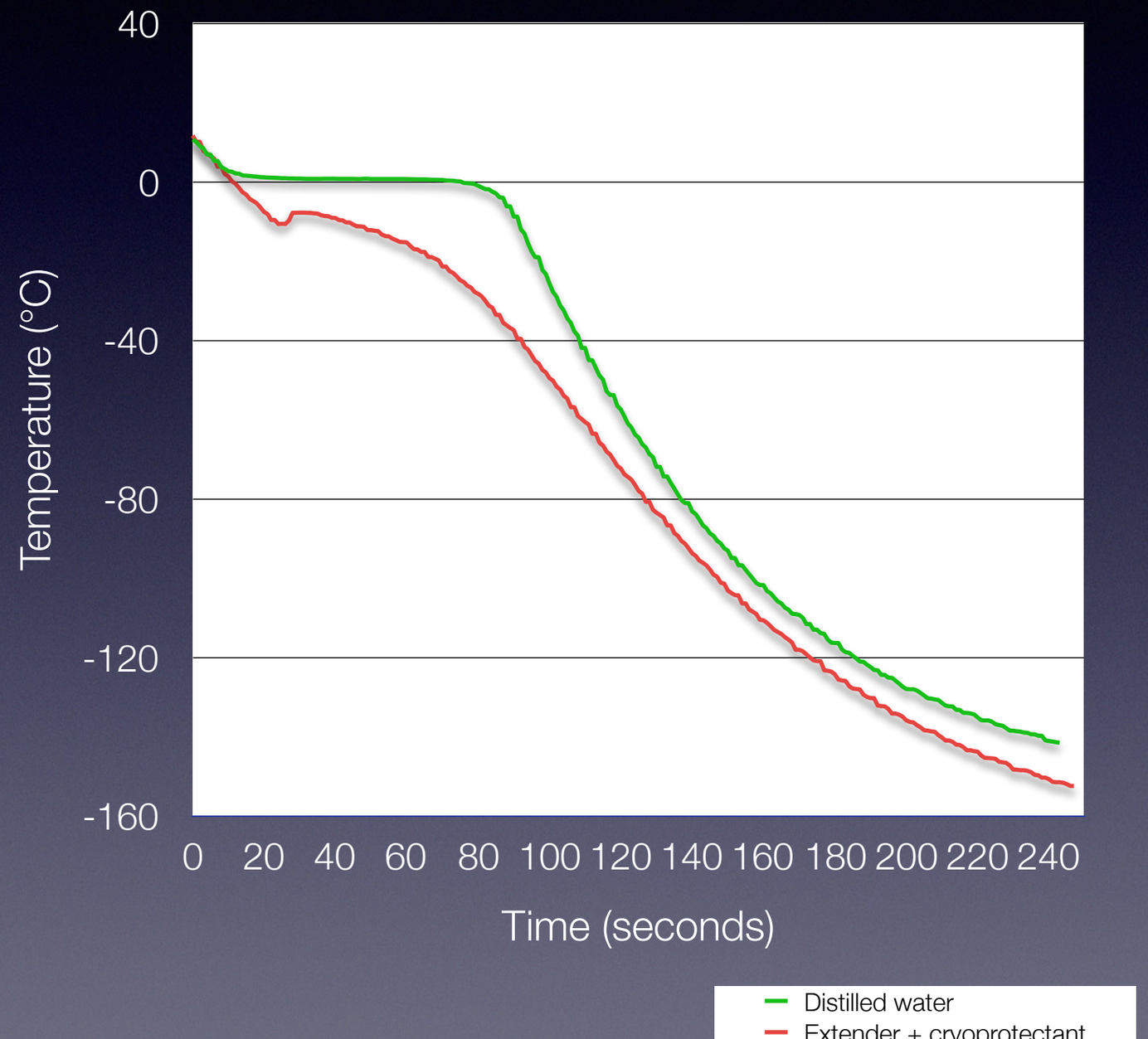
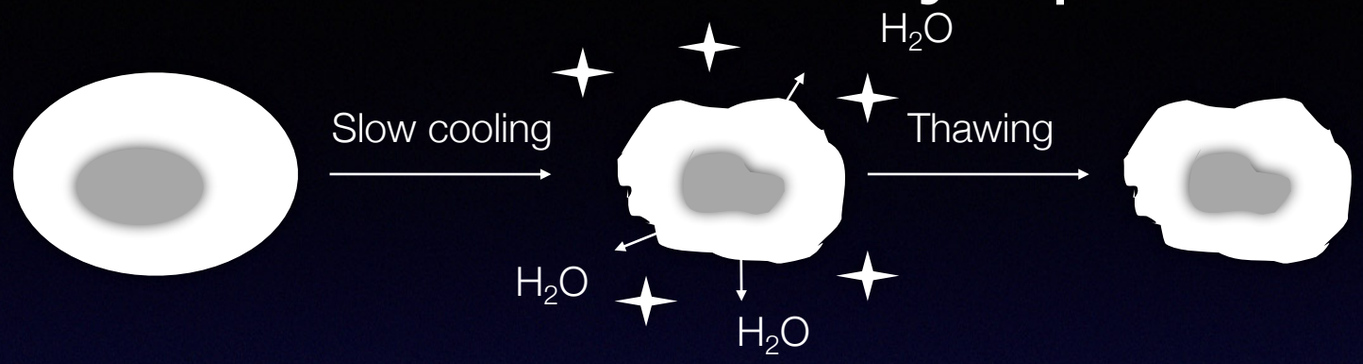
Cryopreservation: basics of cryopreservation – cooling of water and aqueous solutions

- Water supercools below the freezing point
- Ice formation starts along ice nuclei
- Primarily water molecules are incorporated into the ice crystals

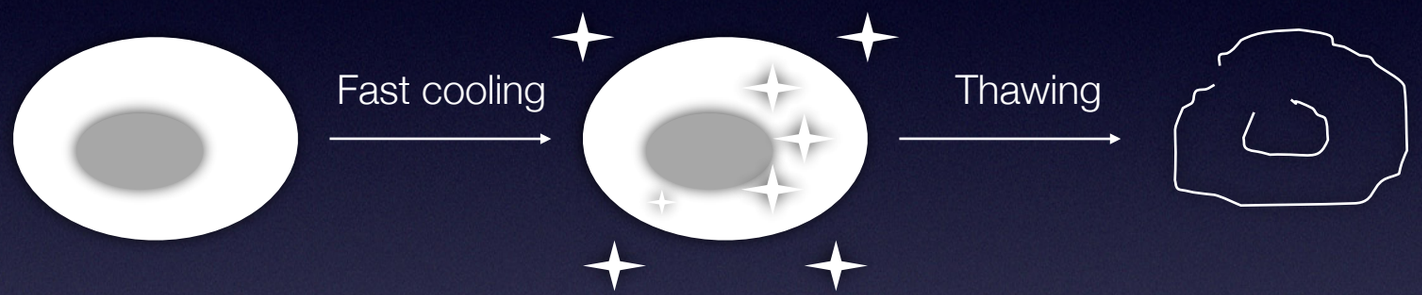


Cryopreservation: cooling of live cells, vitrification, the role of cryoprotectants

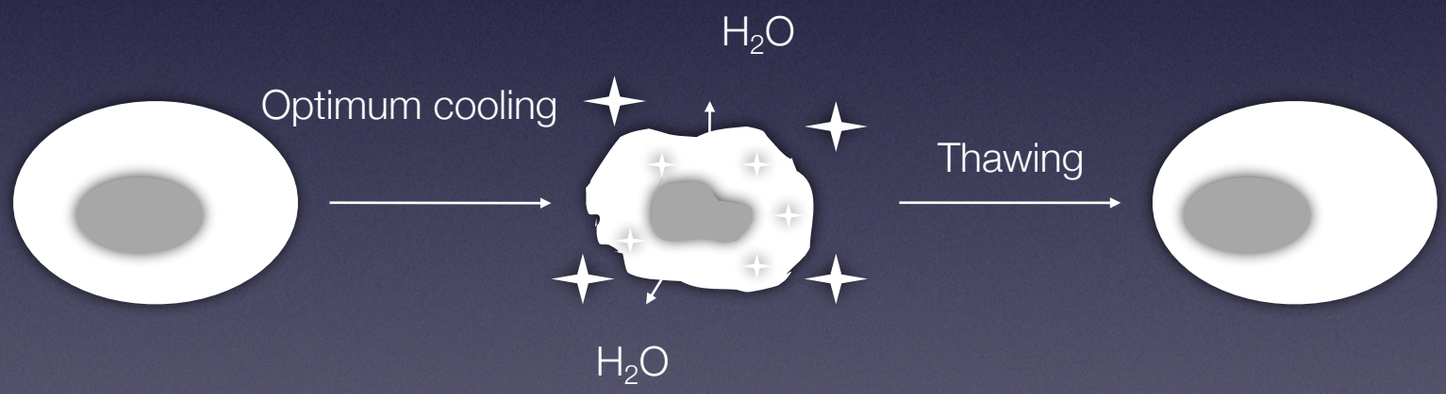
Case 1.



Case 2.



Case 3.



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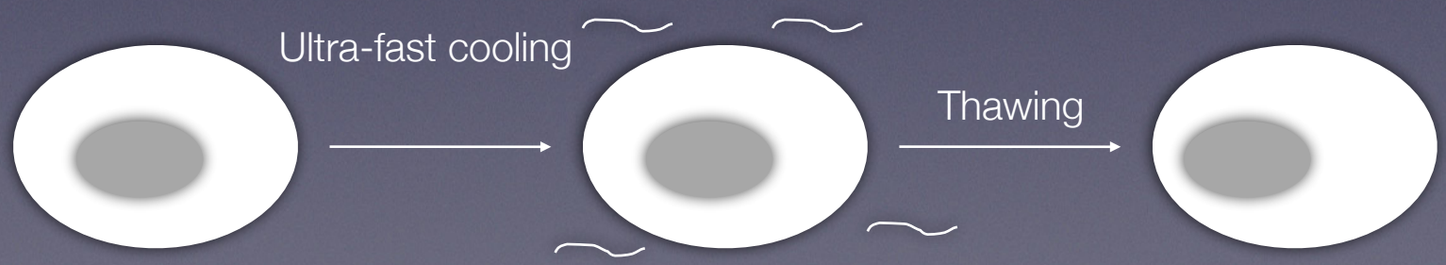

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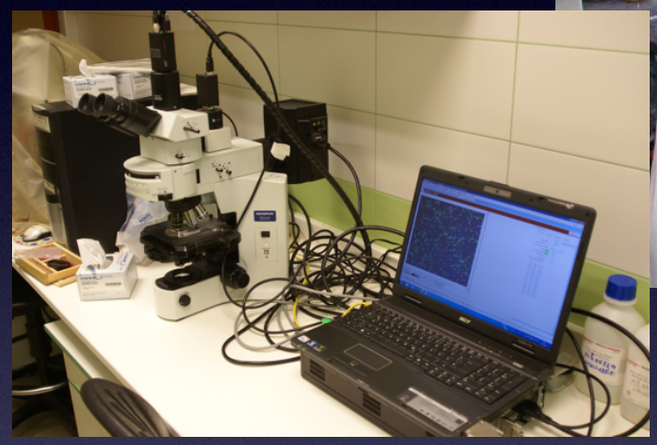
Cryopreservation: cooling of live cells, vitrification, the role of cryoprotectants

- Cryoprotectants
  - —External cryoprotectants
    - Sugars (glucose, fructose, sucrose, trehalose)
    - Polymers (polyvinyl pyrrolidone)
  - —Internal cryoprotectants
    - Alcohols – methanol
    - Polyols – ethylene glycol, propylene glycol, glycerol
    - Others: dimethyl sulfoxide (DMSO), dimethyl acetamide (DMA)
  - —Roles
    - Membrane stabilization
    - Inhibition of ice crystallization
    - Lowering of the freezing point

Cryopreservation of fish sperm – the role of extenders, cryoprotectants and dilution ratios

- Estimation of motility
  - —Visual
  - —CASA
- Extenders – solution of sugars and salts
- Cryoprotectants – most often DMSO or methanol
- Dilution ratios: from 1:1 to 1:9



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Cryopreservation of fish sperm – methods, straws, cooling rates

- Cooling in pellets:
  - —In a block of dry ice
  - —Requires a thawing medium
- Cooling in straws:
  - —Most common technique
  - —Used in most livestock species
- Cryovials
- Glass capillaries

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Cryopreservation of fish sperm – methods, straws, cooling rates

- Cooling in the vapor of liquid nitrogen

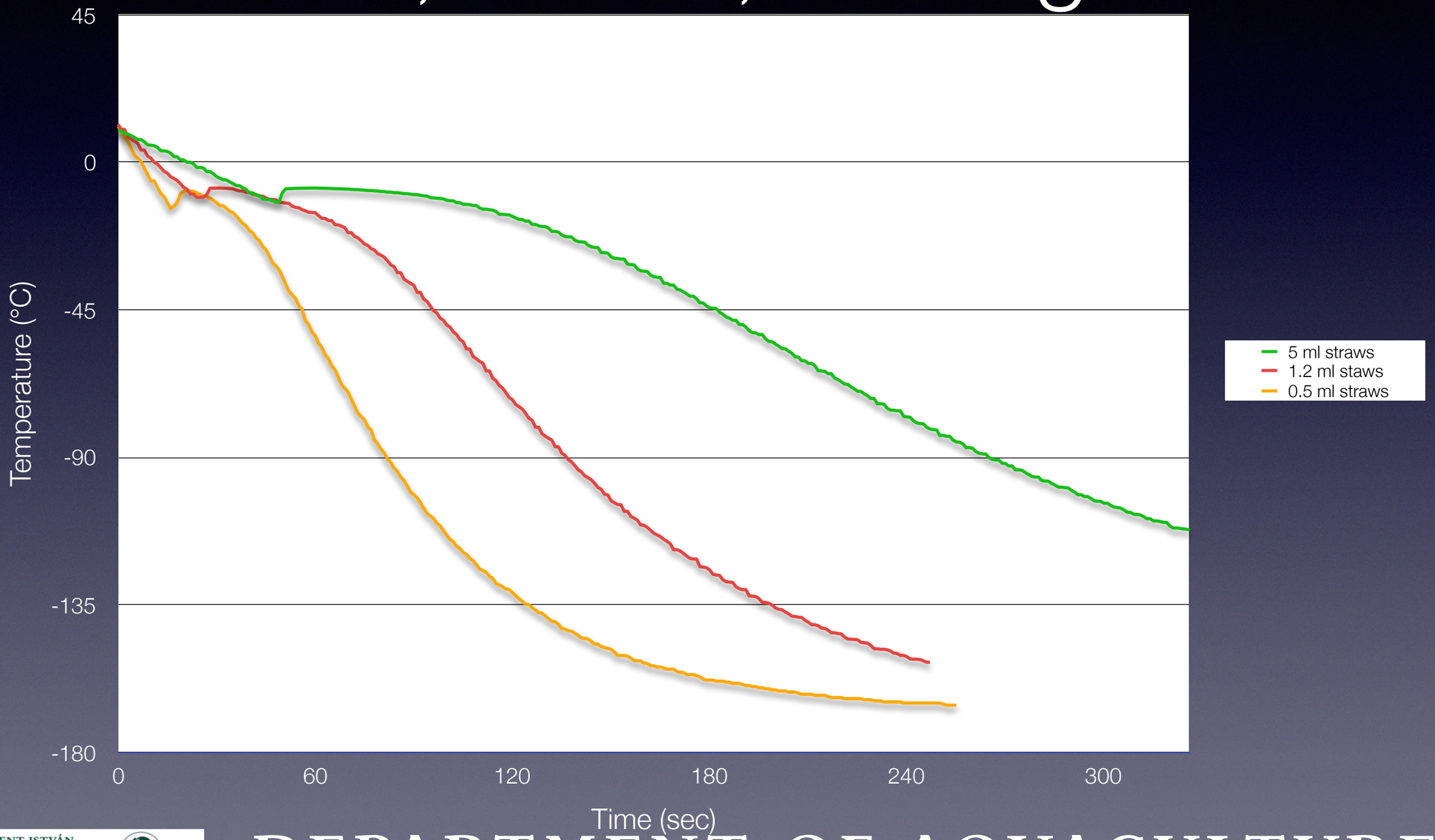
—Styrofoam box

- Simplicity
- Low cost

—Computer-controlled freezer

- Controlled conditions
- More reliable replication

Cryopreservation of fish sperm – methods, straws, cooling rates



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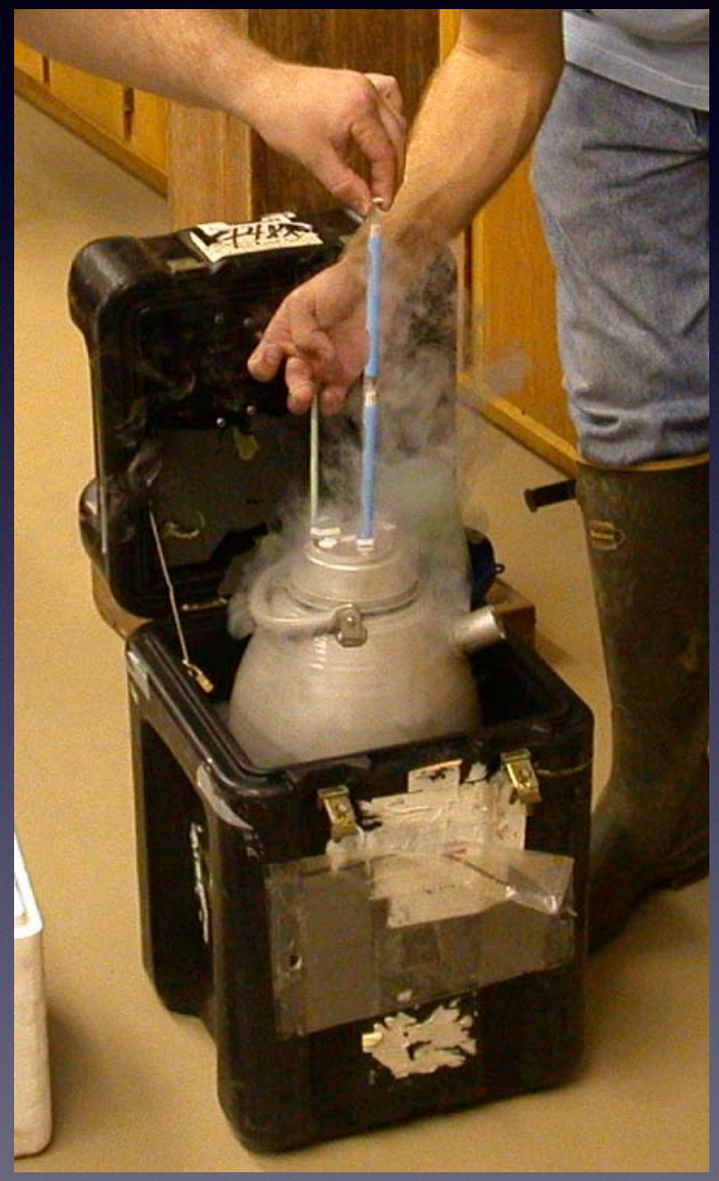
Cryopreservation of fish sperm – storage, thawing

- Storage
  - — Dewars:
    - Large capacity
    - Extended storage time
    - Shipping dewars
  - — Other storage devices
    - Goblets
    - Canes
- Thawing
  - — Typically at 40°C



Cryopreservation of fish sperm – fertilization with cryopreserved sperm

- Determination of post-thaw motility – varies with species
- General rules of fertilization are similar to those with fresh sperm
- Effective sperm:egg ratios start from 5000 spermatozoa to 1 egg
- Research for the prediction of sperm quality without fertilization

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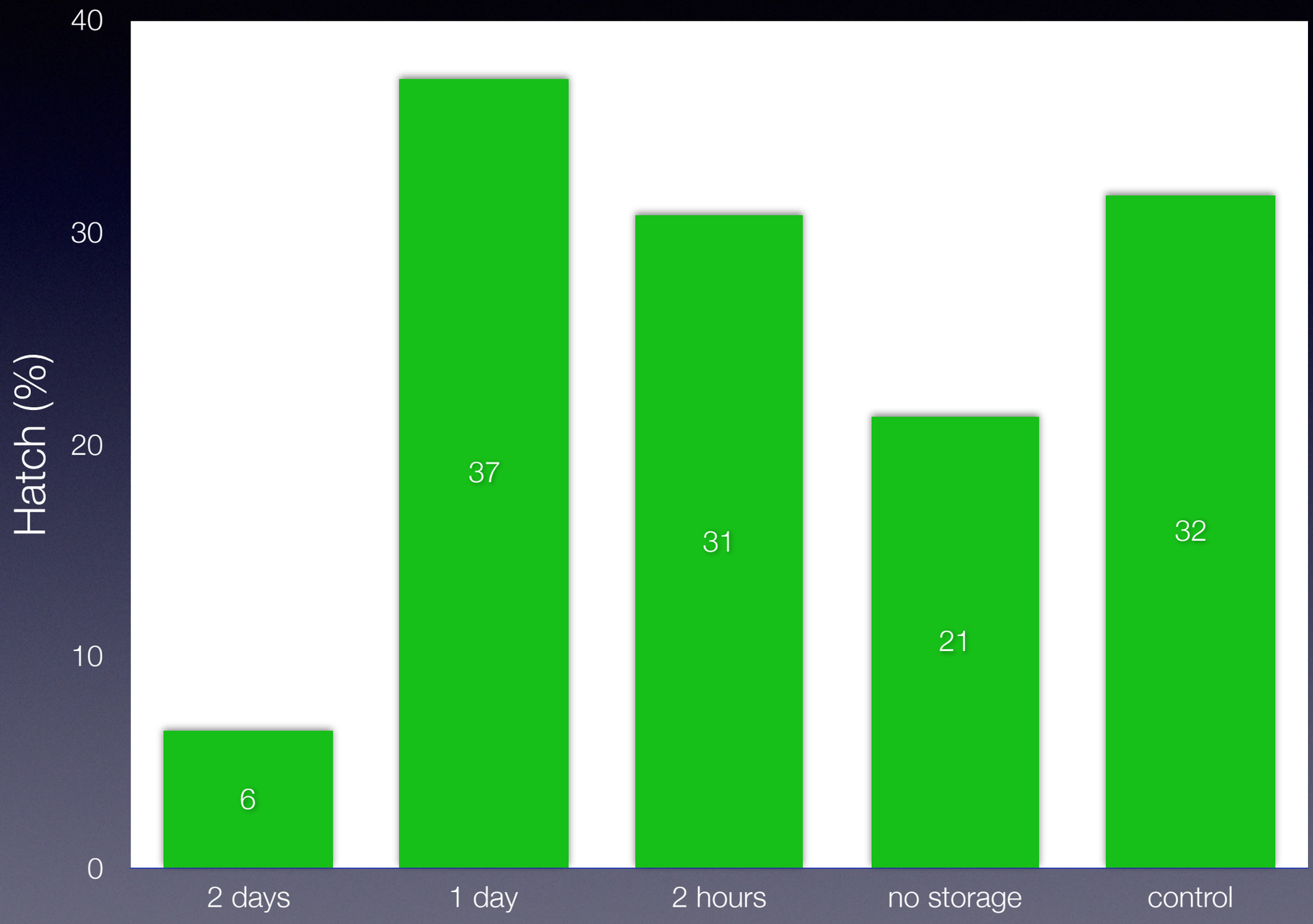
Cryopreservation of fish sperm – fertilization with cryopreserved sperm



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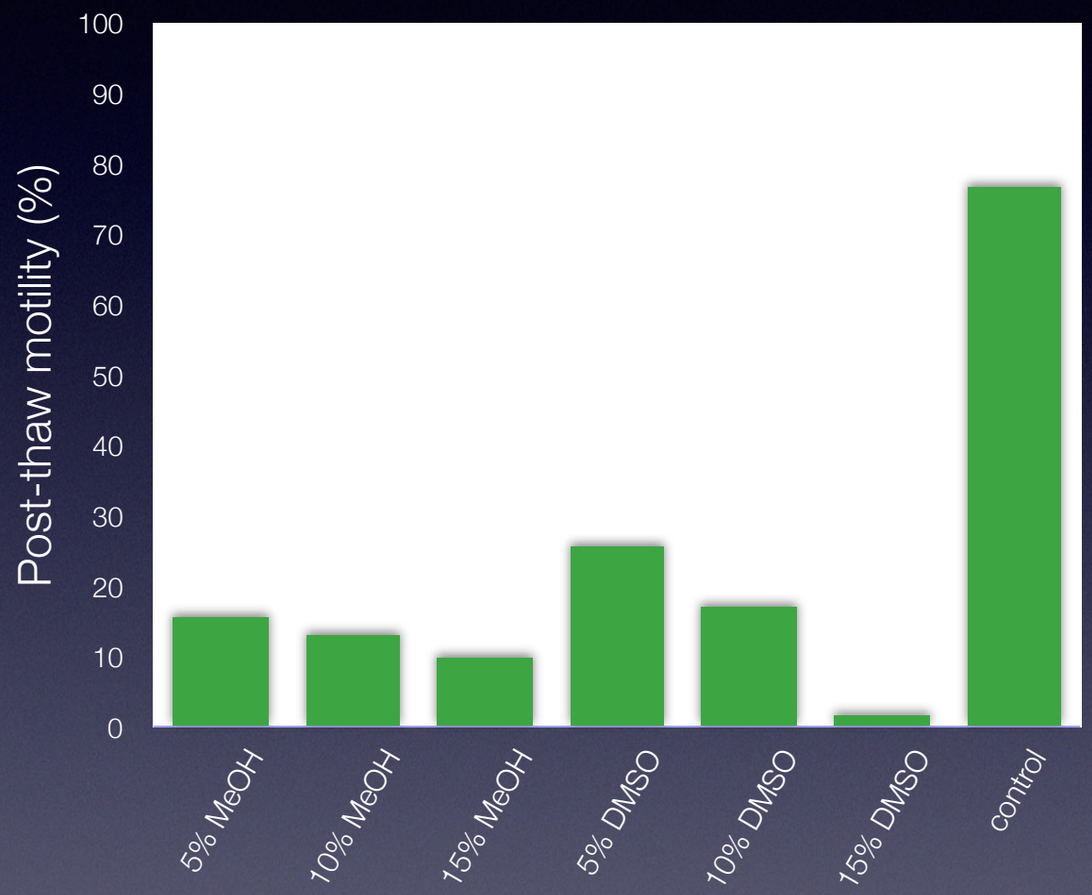
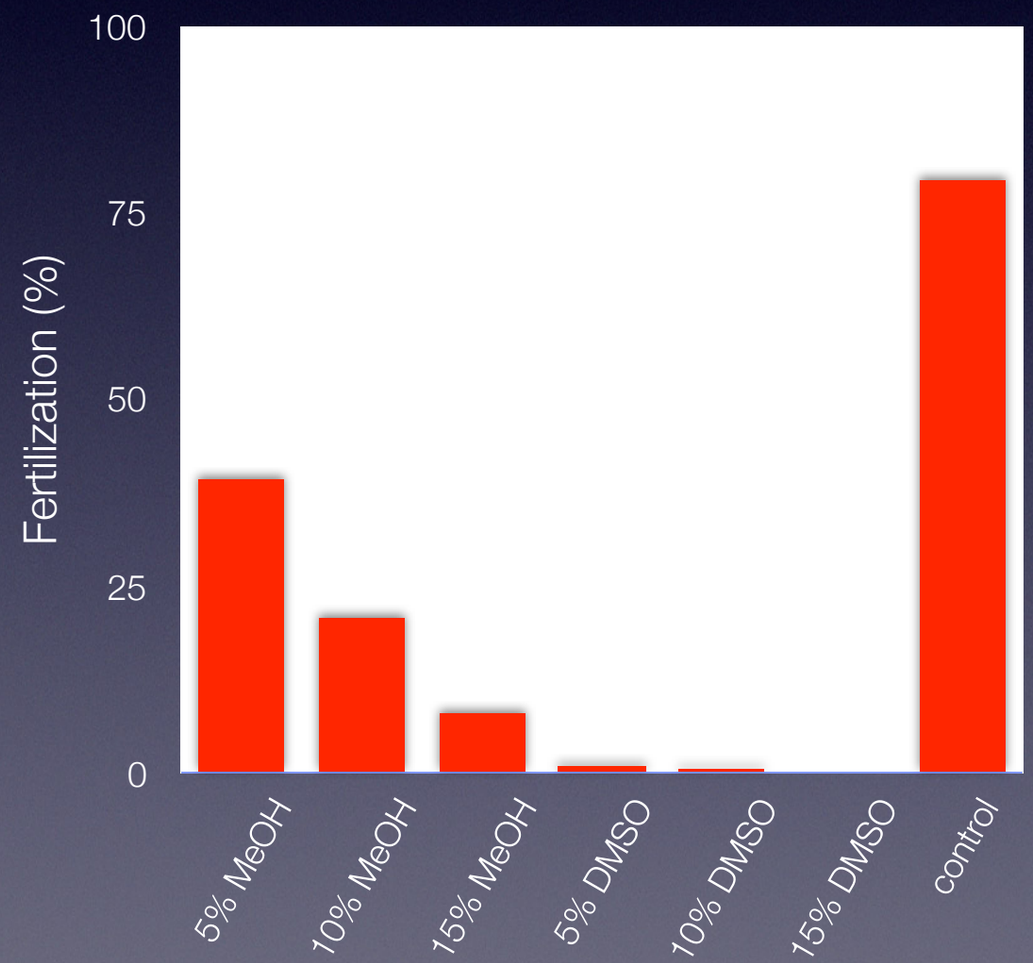
Prediction of sperm quality without fertilization





Motility is not always a good predictor of fertilizing capacity

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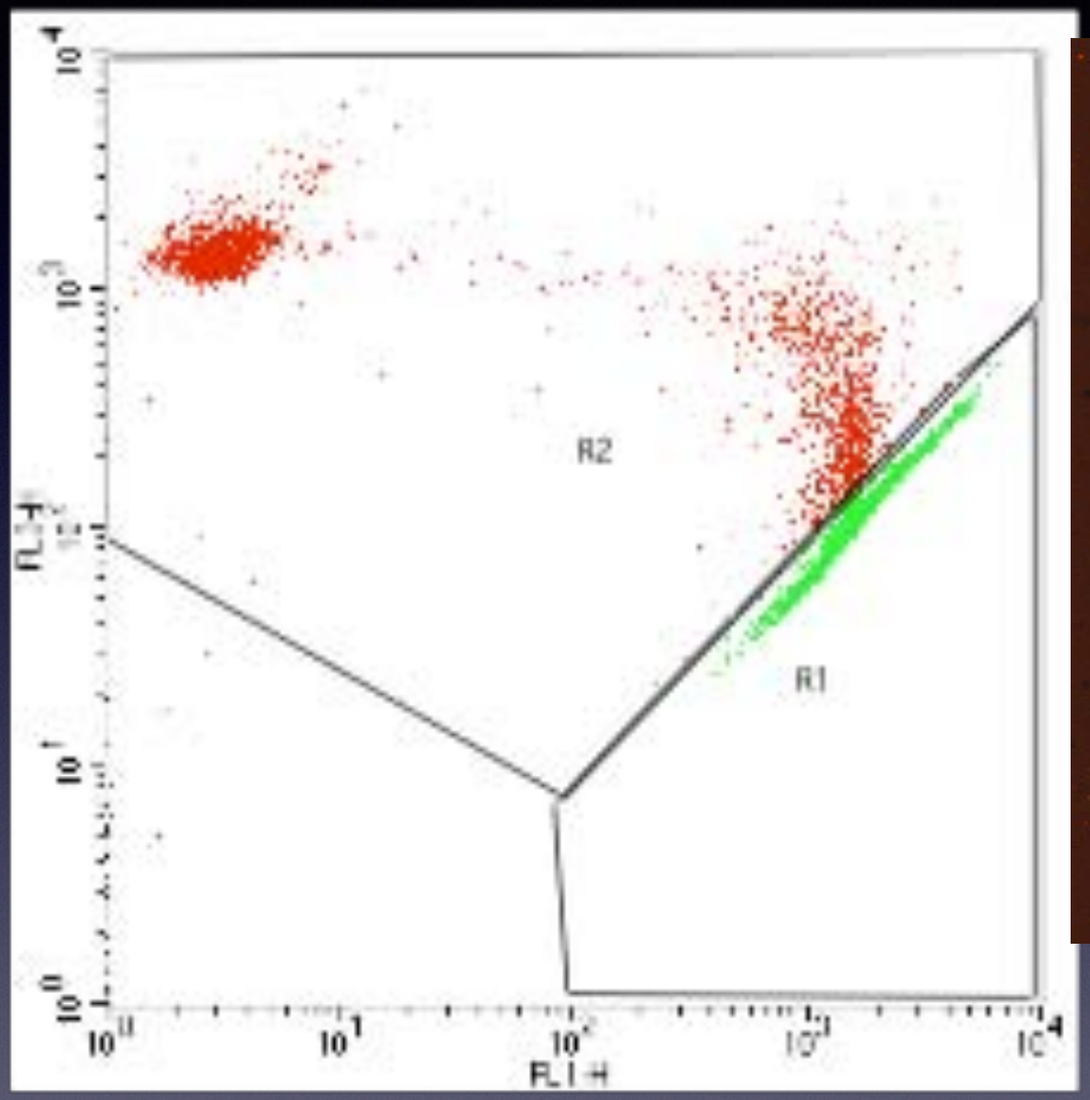
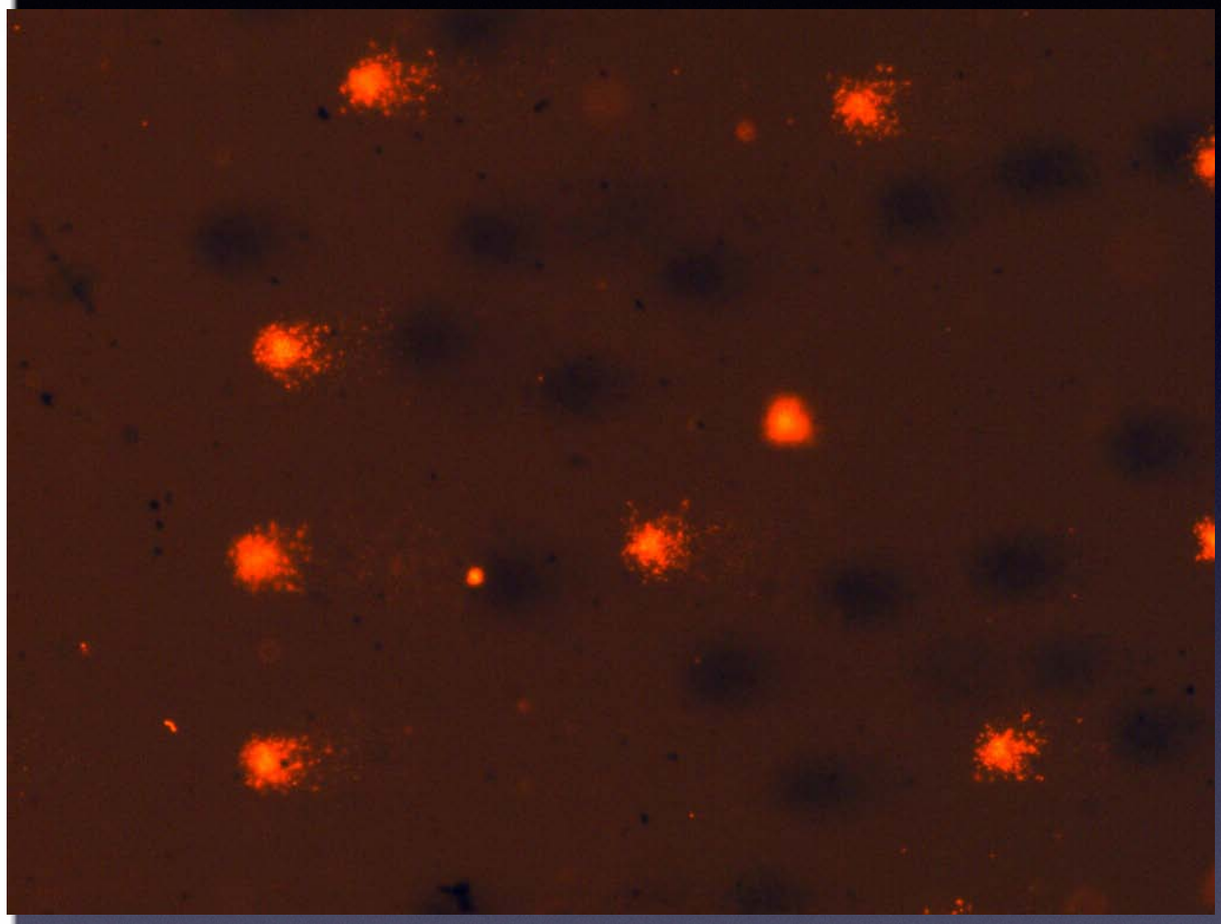
Prediction of sperm quality without fertilization



Viability: live-dead fluoresecent dual staining combined with flow cytometry



Comet-assay: single-cell gel electrophoresis assay

Computer-assisted sperm analysis - CASA

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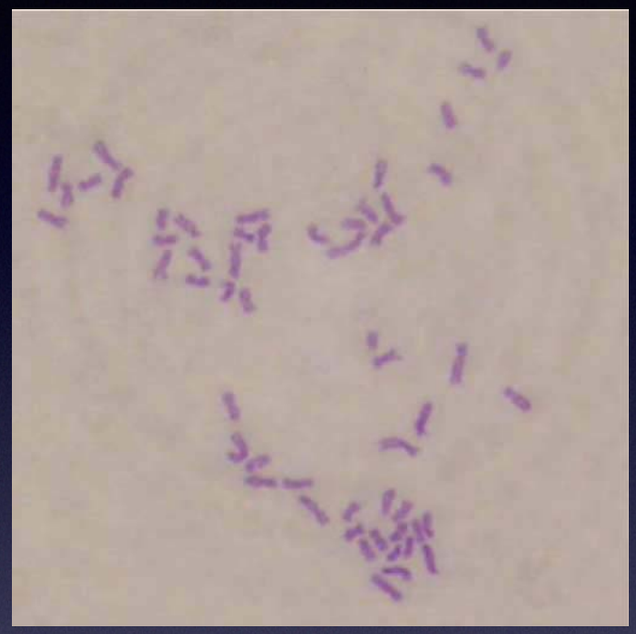
Genetic damage associated with cryopreservation



Cryopreservation of fish eggs and embryos

- Problems:
  - —Egg envelope
  - —Egg activation upon release into a liquid
  - —Structure of fish embryos
  - —Which embryonic stage to use
- Limited success
  - —Vitrification of flounder embryos
  - —Vitrification of carp embryos

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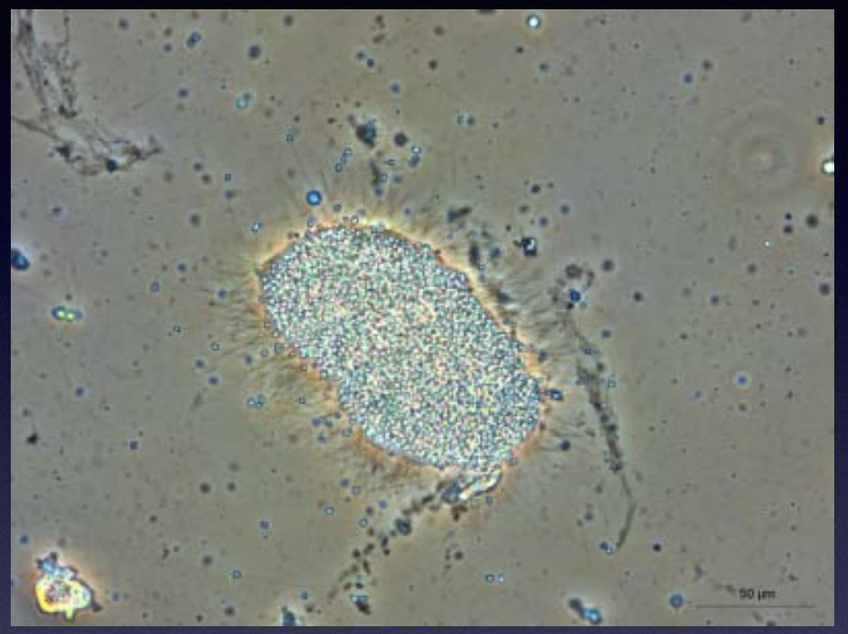
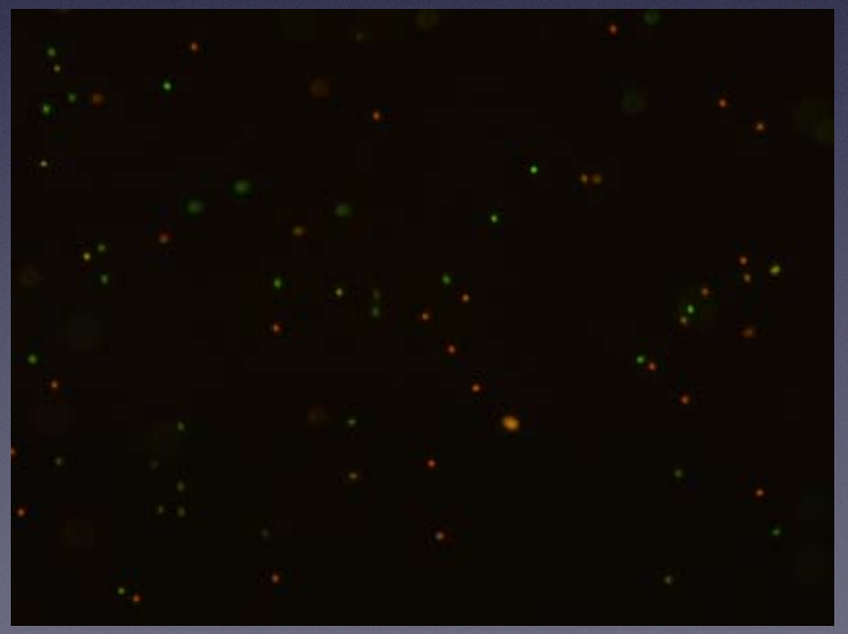
Cryopreservation in other aquatic species

- Cryopreservation of bivalvian sperm
- Differences from fish sperm:
  - —Reduced post-thaw motility
  - —Reduced fertilization
  - —Extender osmolality around 1000 mOsmol/kg





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Cryopreservation in other aquatic species

- Cryopreservation of larvae is possible
- Best larval stages for cryopreservation are trochophores and veligers
- Very slow cooling rates

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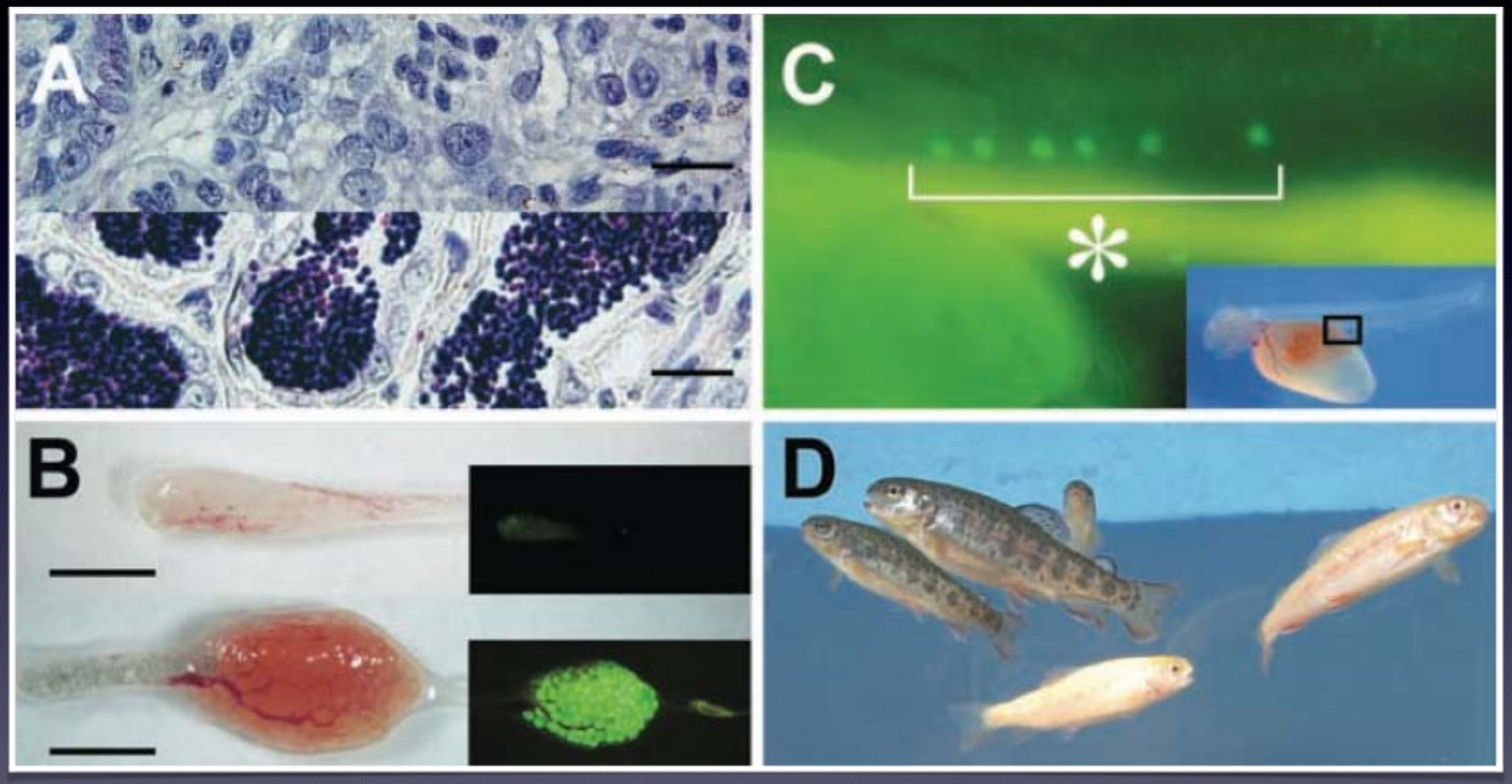
Commercial application – reasons of failure

- Very few cases of commercial application (if any)
- Mostly sperm banks maintained by laboratories and research institutions
- Reasons:
  - Science ahead of industry
  - Adaptation of protocols originally developed for livestock are not suitable for aquaculture
  - Low level of standardization and international cooperation

Further development



Okutsu et al. 2007 Science, 317, 1517.

- Cryopreservation and transplantation of primordial germ cells (PGCs)
- Cryopreservation and transplantation of undifferentiated type A spermatogonia