Lower intracellular concentration of cryoprotectants in mouse zygotes after vitrification than after slow freezing

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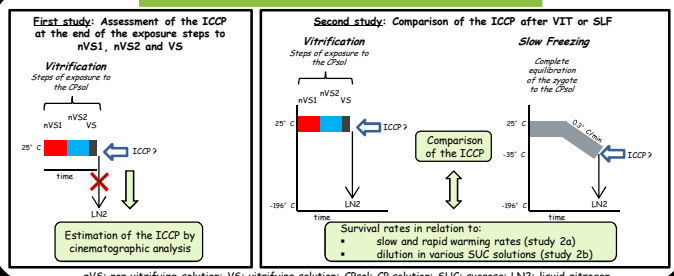
Introduction

Vitrification (VIT) of an aqueous solution is its conversion into a solid glassy state without any ice crystal formation upon very fast cooling. VIT is competing with conventional slow freezing (SLF), that relies on extra cellular crystallization, as a reference cryopreservation technique. This rises hot debates on presumed toxicities resulting from exposure of embryos to high cryoprotectant (CP) concentrations, i.e. 3 to 4 fold those found in SLF.

The aim of our study is to report on the intracellular concentration of cryoprotectants (ICCP) during VIT or SLF of mouse zygotes.

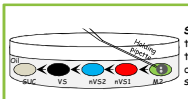
Experimental Design



nVS: non-vitrifying solution; VS: vitrifying solution; CPsol: CP solution; SUC: sucrose; LN2: liquid nitrogen

Results

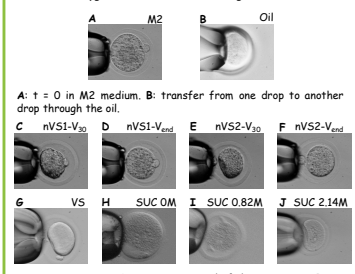
1. First study: Assessment of the ICCP at the end of the exposure steps to nVS1, nVS2 and VS by cellular volume monitoring



Schematic representation of the method used to monitor the change in volume of zygote during exposure to CP and SUC solutions.

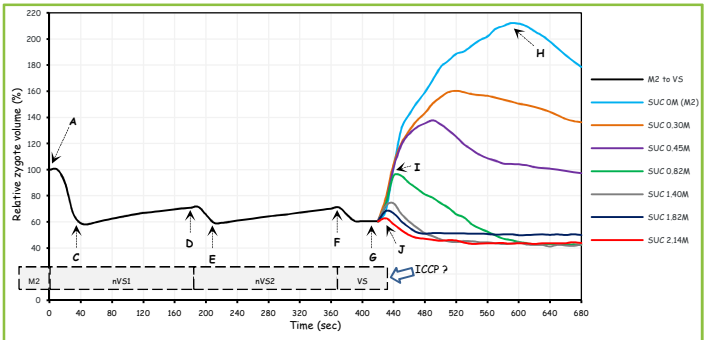
Transfer of one zygote from one 50 µl droplet of CPsol to the other one with the help of a holding pipette in order to maintain it on the same focal plane. Pictures are taken every 2 to 5 seconds.

Pictures of zygotes when immersed through the various CPsol



A: t = 0 in M2 medium. **B**: transfer from one drop to another drop through the oil.

C: t = 30 sec in nVS1, **D**: t = 180 sec: end of the nVS1 step. **E**: t = after 30 sec in nVS2, **F**: t = after 180 sec in nVS2. **G**: after 30 sec in VS. **H – I – J**: pictures of zygotes when they have reached their maximal volume in SUC solutions of 0M, 0.82M and 2.14M, respectively. V_{30} = Volume after 30 sec of exposure to a specific CPsol; V_{end} = Volume at the end of a period of exposure to a specific CPsol.



Cinematographic study: Evolution of the relative zygote volume when immersed in solutions with various concentrations of SUC following the exposure steps to nVS1, nVS2 and VS. Letters on the graph refer to corresponding pictures.

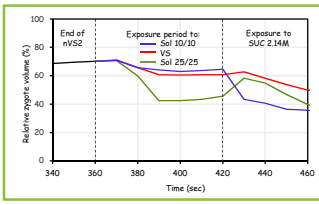
Directly after the exposure to SUC solutions:

-From 0 to 1.82M SUC solutions: huge to weak volume variations

-In 2.14M SUC solution: minimal change in cell volume:

➔OSMOTIC EQUILIBRIUM between intracellular and extracellular compartments

➔During **VIT: ICCP ≈ 2.14M** at the end of the exposure to CPsol



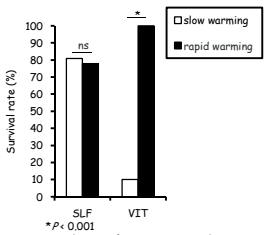
Cinematographic study: Evolution of the relative zygote volume when immersed in 2.14M SUC solution following the exposure steps to nVS1, nVS2 and VS (EG/DMSO = 20/20) or alternative solutions (Sol 10/10 or Sol 25/25).

Upon immersion in the 2.14M SUC solution, after exposure to:

- **Sol 10/10**: dramatic reduction in cell volume
  ➔ **hypo-osmotic situation**
- **Sol 25/25**: increase of the volume
  ➔ **hyper-osmotic situation**
- **VS**: faint volume variation
  ➔ **iso-osmotic situation**

2. Second study: Comparison of survival rates after VIT or SLF in relation to:

a. the warming rate



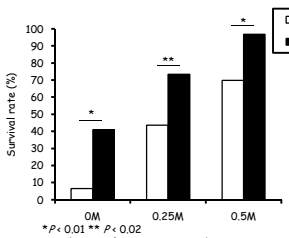
*P< 0.001
3 replicates of 10 zygotes in each group

Survival rates after SLF poorly affected by the warming rate

After VIT, only 10% of survival after slow warming

Higher warming speed after VIT➔ cell lysis reduction

b. dilution in various SUC concentrations (rapid warming)



*P< 0.01 ** P< 0.02
3 replicates of 10 zygotes in each group

Significantly **higher rates of live zygotes after VIT** than after SLF whatever the SUC concentration in the warming solution

Conclusions

During **VIT**, the **ICCP** is **approximately equal to 2.14M** prior to plunging in LN2 and is consequently **3-fold inferior to the CPs concentration in VS** (6.4M). Exposures to two alternative solutions of VS characterized by lower and higher concentrations, lead to hypo-osmotic or hyper-osmotic situations, respectively. This confirms that the iso-osmotic situation observed at 2.14M with VS corresponds approximately to the ICCP.

Zygotes survive to cryopreservation by VIT only if a very high warming rate is used (study 2a). On the contrary, SLF zygotes survive whatever the warming rates thanks to a higher ICCP that inhibits recrystallization. The observation of significantly higher survival rates after VIT than after SLF whatever the SUC concentration in the warming solution in study 2b is in perfect agreement with a more pronounced toxicity of a **higher ICCP in SLF zygotes.**

In conclusion, this study reveals a **lower ICCP in vitrified zygotes than after SLF despite higher concentrations of CPs in the VIT media**. This explains the observed efficiency of VIT despite the previously anticipated high levels of ICCP and their putative toxic effects.

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