Embryo and Oocyte Vitrification

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Intracellular Ice is the Enemy of Cryopreservation

- Equilibrium or “slow-freeze” methods reduce intracellular water available for ice formation
- Non-equilibrium or “vitrification” (also, “fast-freeze”) methods avoid ice formation

Vitrification

- *Extremely* rapid cooling rates result in formation of a solid without forming ice crystals.
  - As temperature plunges rapidly, viscosity increases to form a super-cooled, highly viscous liquid; below the glass transition temperature (Tg) it converts to an amorphous solid
  - Essentially, water molecules stop in place before they can arrange themselves into crystals

Vitrification Parameters

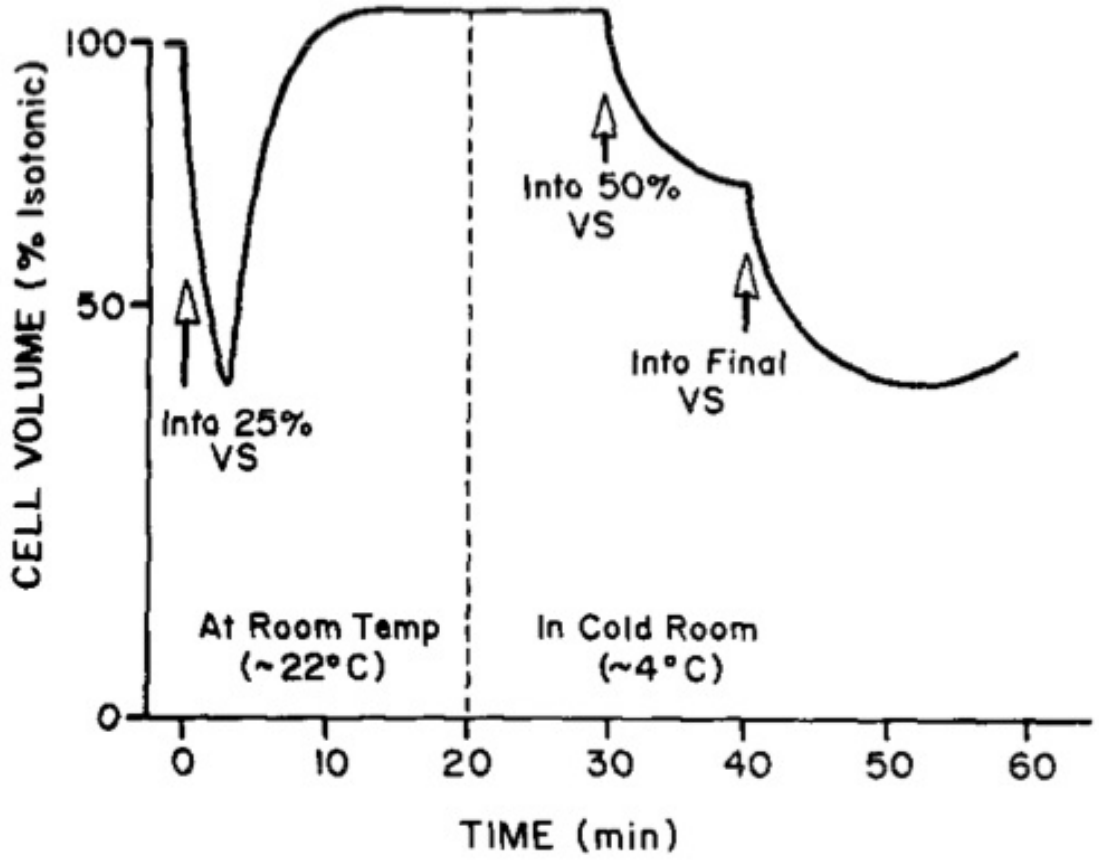
- Fast cooling rates
  - Liquid nitrogen plunge
  - Liquid nitrogen slush
- Inclusion of solutes as CPAs
  - Increases viscosity, lowers Tg
  - Typically on the order of ~5M
- Small volumes increase heat transfer rate
  - Straws and vials
  - Also surface systems, e.g. EM grids, Cryotop, Cryoloop etc.
  - Closed-volume systems, e.g. OPS and CPS systems

The Methodology—Simplicity Itself

- Stepwise equilibration of embryos/oocytes with CPA
- Plunge into liquid nitrogen
- Store

Notice: No seeding. No controlled rate freezer.

Stepwise Equilibration



Rall, W. F. W. (1987). Factors affecting the survival of mouse embryos cryopreserved by vitrification. Cryobiology, 24(5), 387–402

Two Methods

	Leibo	Nakao et al
Dilute CPA	2 M Ethylene Glycol	1 M DMSO
Concentrated CPA	8 M Ethylene Glycol	2 M DMSO 1 M Acetamide 3 M Propylene Glycol
Storage	Straws	V-bottom cryo vial

Liebo, S Rapid Cooling Method for Mouse Zygotes. JAX Cryo Course Manual

Nakao, K., Nakagata, N., & Katsuki, M. (1997). Simple and efficient vitrification procedure for cryopreservation of mouse embryos. Experimental animals / Japanese Association for Laboratory Animal Science, 46(3), 231–234 See also http://card.medic.kumamoto-u.ac.jp/card/english/sigen/manual/ebvitri.html

Oocytes, A Special Case

- Oocytes do poorly in equilibrium methods
  - Low permeability of the oocyte cortex to water & CPAs likely cause
- Vitrification methods are preferred for this stage

If It Is So Easy, Why Doesn't Everybody Do It?

- Possible toxic effects of CPAs
- Formation of intracellular ice during *warming*
  - Between glass transition and freezing temperature, crystallization (crystal growth) can occur
  - Limit time sample resides between these temperatures, i.e., warm as rapidly as possible
  - This effect makes careful sample handling of utmost importance for security

The Way Forward: Improving the Methodology

- Is it necessary?
  - Lots of material is available, so we can afford to take some losses
  - The systems in use seem to work pretty well
- Adaptations from human oocyte vitrification
- Adoption/adaptation of alternative technologies
- Application of improved basic cryobiology