

# Basic Principles of Sperm Cryopreservation

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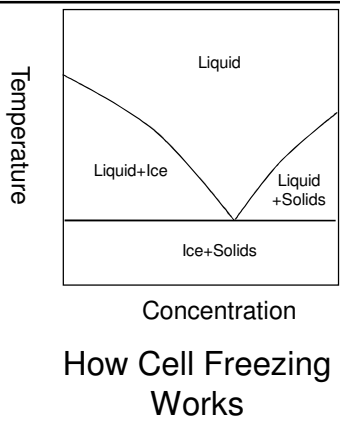
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How Cell Freezing Works

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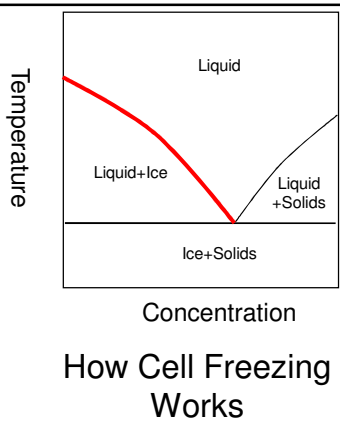
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How Cell Freezing Works

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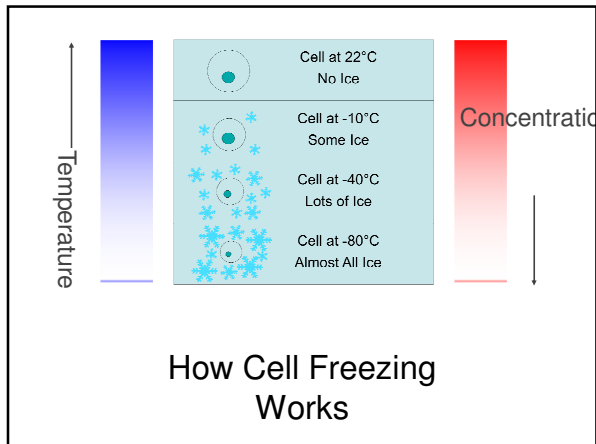
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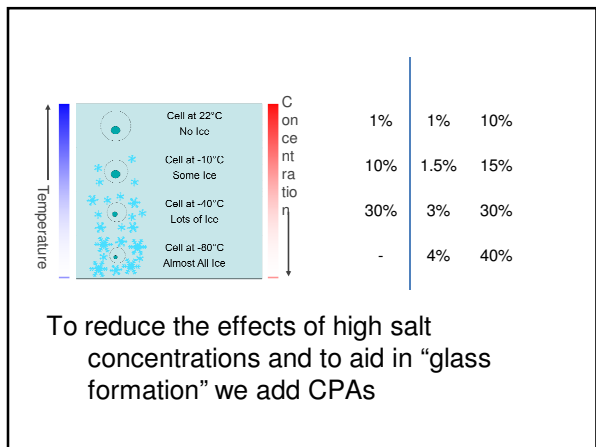
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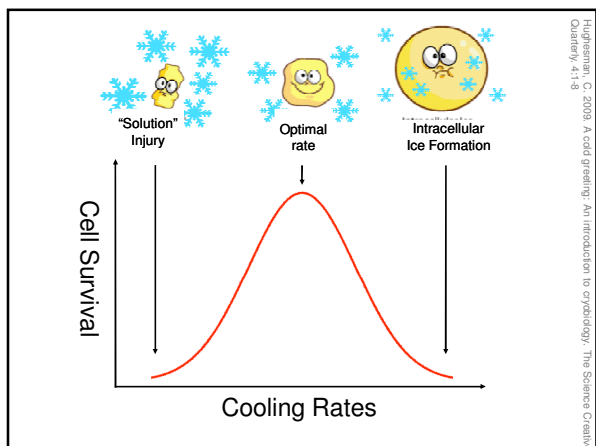
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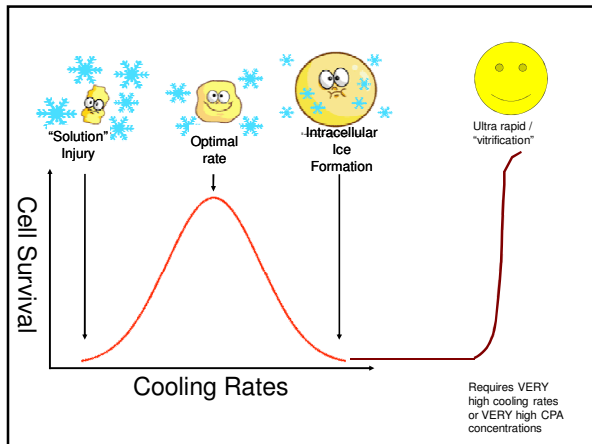
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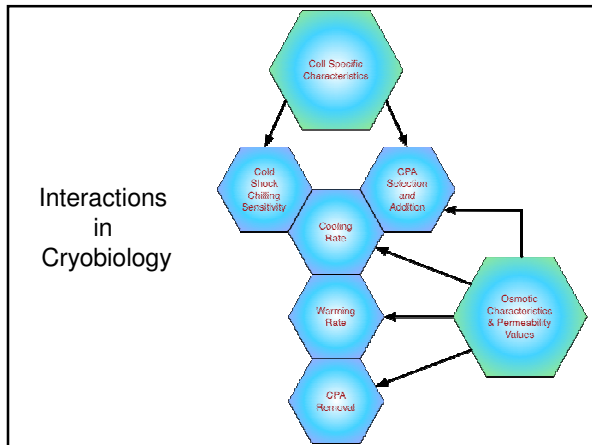
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- ### Cell Specific Characteristics
- Cold shock sensitivity
  - Specific CPA toxicity
  - Intracellular lipid content
  - Cytoskeletal sensitivity

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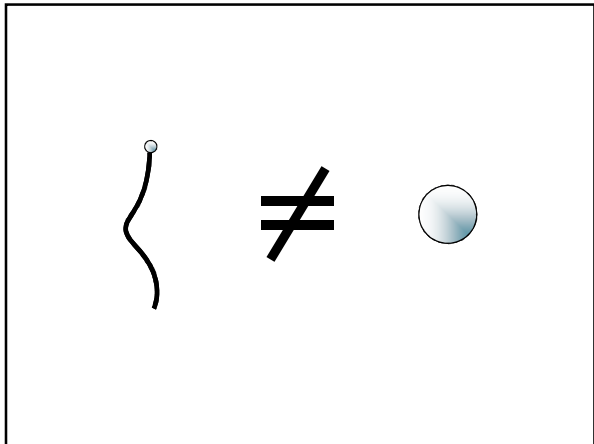
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Osmotic Damage

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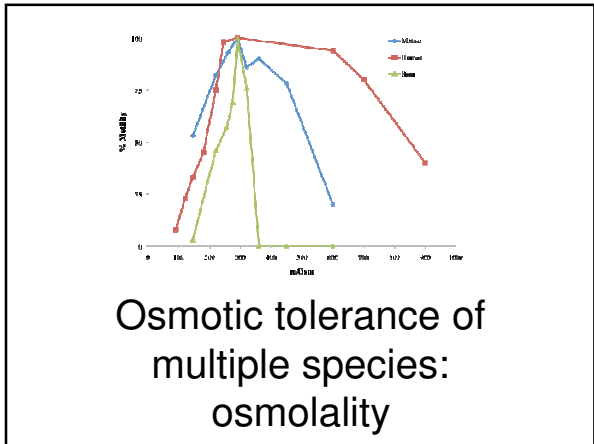
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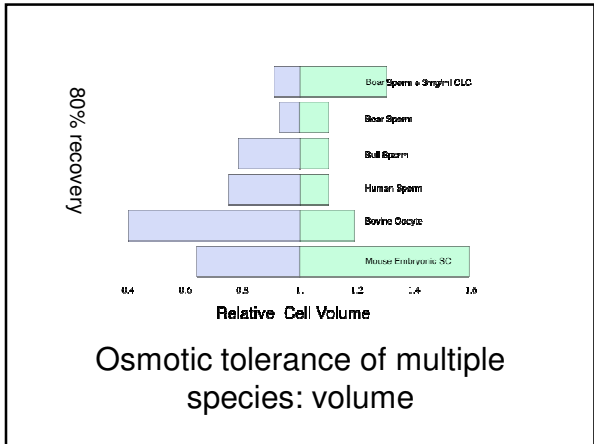
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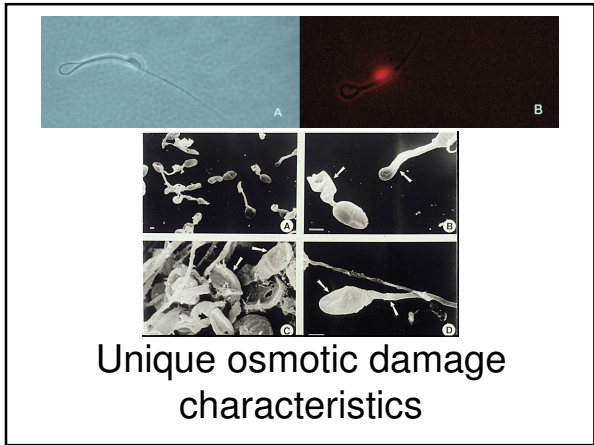
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Unique osmotic damage characteristics

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## Media Choice

- Cryoprotective Agent/Additives
  - Glycerol
    - concentrations used vary from clinic to clinic, ranging from 5% to 10%.
  - Ethylene Glycol
    - Shown to likely be superior but uncommon in practice

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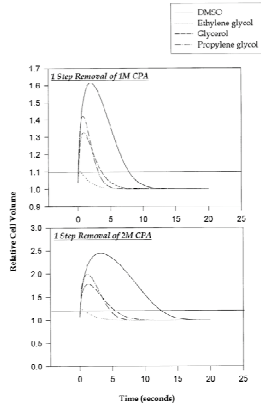
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# Media Choice: CPA Selection




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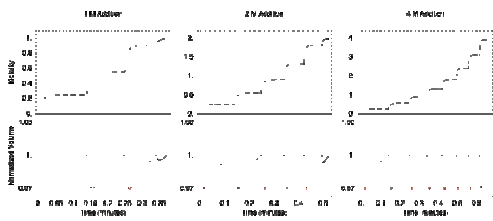
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To remain within osmotic tolerance limits multiple steps are needed

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## Media

- Cryoprotective Additives
  - Chicken egg yolk
    - Enhances membrane fluidity
    - Extends osmotic tolerance
    - Difficult to implement QC
  - Glutamine or Cholesterol-laden-Cyclodextrin an alternative

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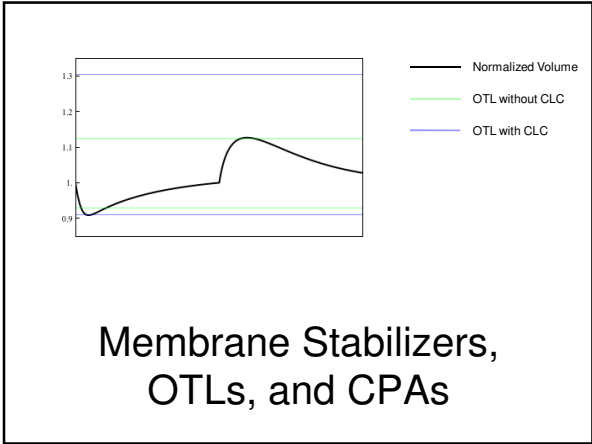
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**Membrane Stabilizers,  
OTLs, and CPAs**

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**Cooling Rates**

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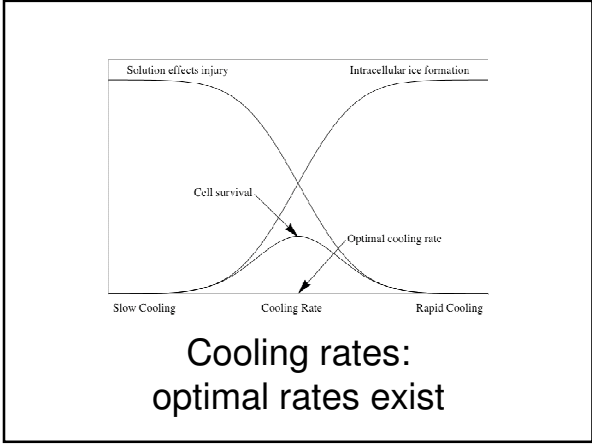
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**Cooling rates:  
optimal rates exist**

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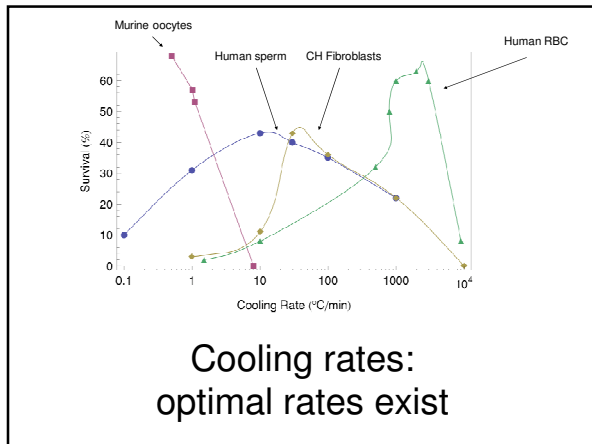
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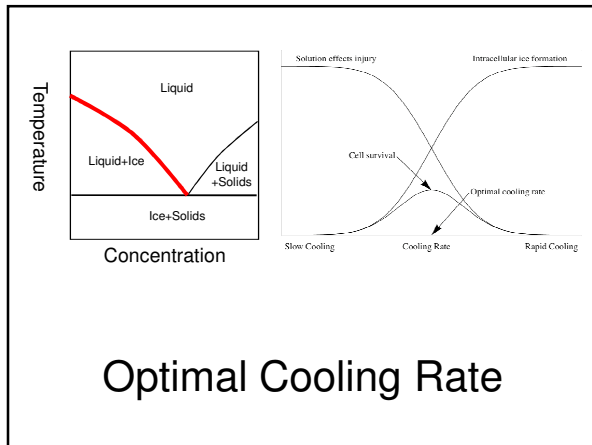
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How do we determine optimal protocols?

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## Measurements

- Relative water content  $-(V_{iso}-V_b)$ 
  - Boyle Van't Hoff
- Water Permeability ( $L_p$ )
  - Temperature dependence of  $L_p$
- Solute Permeability ( $P_s$ )
  - Temperature dependence of  $P_s$

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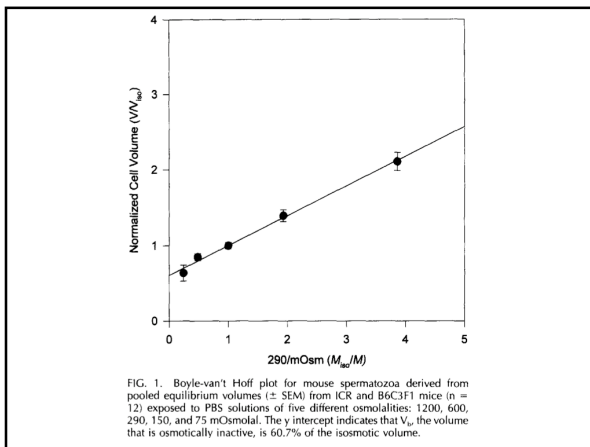
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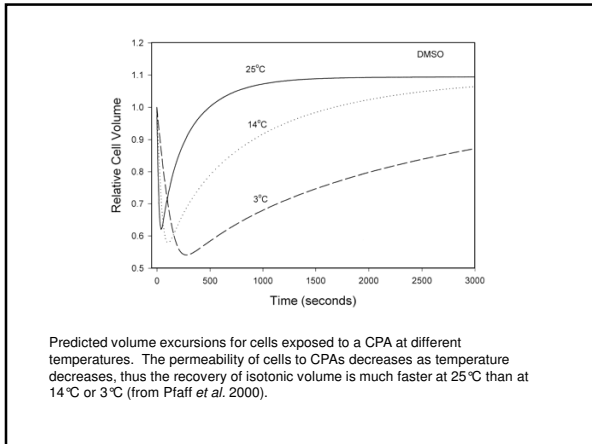
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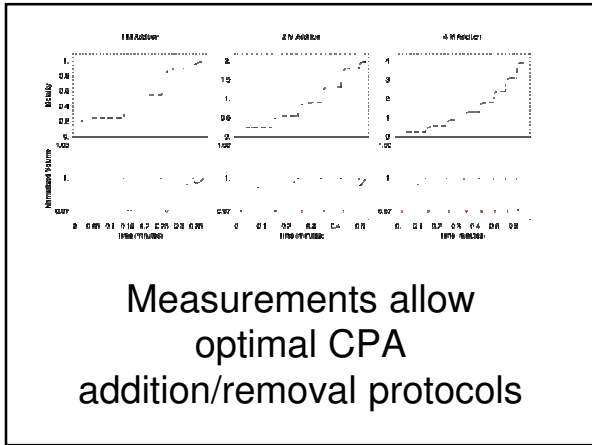
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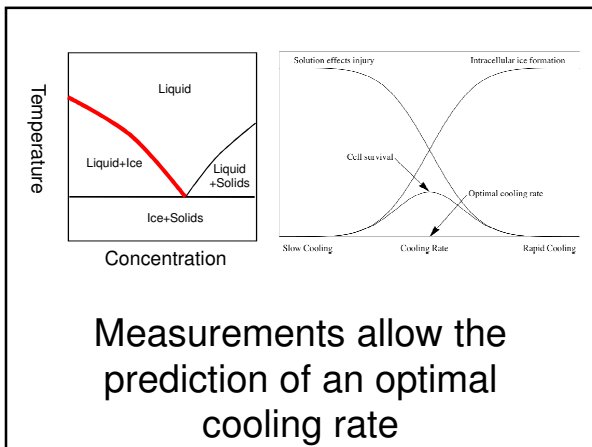
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## Why use an empirical-fundamental approach?

- 4 CPAs
- 4 CPA Addition Protocols
- 4 Cooling rates
- 4 Warming rates
- 4 CPA Removal Protocols
- 4 x 4 experimental design
- $4 \times 4 \times 4 \times 4 \times 4 \times 4 = 16384$  data points with little likelihood of specific results

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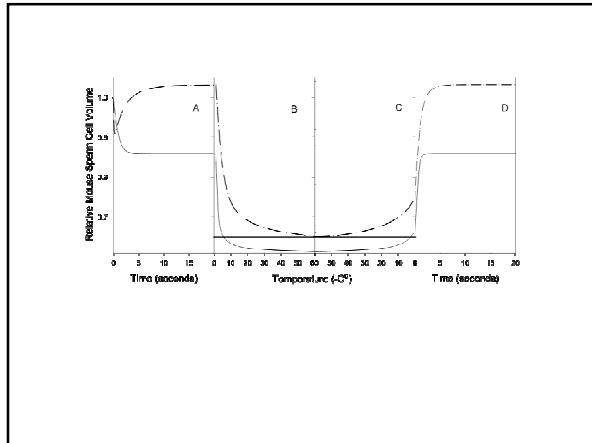
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## Future Directions

- Refined metrics for osmotic tolerance limits
- Continuous and optimal CPA addition and removal
- Nonlinear control of sample temperatures
- Optimization of temperature control

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## Acknowledgments

- ESHRE
- National Research Council-NIST  
associateship
- Steve Mullen

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