

# ONPRC Module 4A Saturday Academy Cryopreservation & Fertility Preservation Laboratory

<u>Main Question</u>	<u>Laboratory Questions</u>
<p>What is “cryopreservation” and how can we use it to preserve the fertility in female cancer patients?</p>	<ul style="list-style-type: none"> <li>• What are the main causes for cell or tissue damages cryopreservation?</li> <li>• What are the 2 main techniques used in cryopreservation to minimize freezing-induced damages?</li> <li>• What are cryopreservation agents and how do they work?</li> <li>• How can we preserve fertility in female cancer patients using cryopreservation?</li> </ul>

## What is cryopreservation?

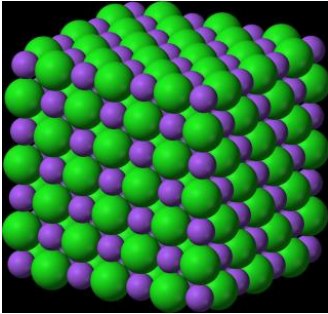
Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures (usually the temperature of liquid nitrogen,  $-196^{\circ}\text{C}$ ). At these low temperatures, any biological activity, including the biochemical reactions, that would lead to cell death is effectively stopped.



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# Cryopreservation Vocabulary

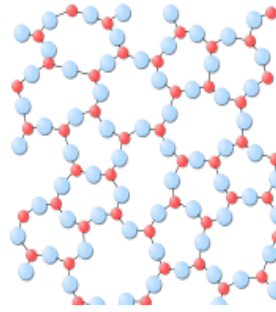
## Crystalline structure:



A solid composed of molecules or atoms arranged in a very specific and orderly way that displays perfect symmetry.

<http://upload.wikimedia.org/wikipedia/commons/e/e9/Sodium-chloride-3D-ionic.png> Both Figures-Public Domain

## Amorphous state (glass transition):



A solid in which there is no specific order of the atoms. Usually resulted from rapid cooling (before the molecule has enough time to organize into an orderly structure (crystal).

<http://en.wikipedia.org/wiki/Glass#mediaviewer/File:Silica.svg>

**Viscous:** Thickness of a certain fluid. Water is "thin", having a lower viscosity, while honey is "thick", having a higher viscosity.

**Cryoprotective agents (CPAs):** A substance that is used to protect biological tissue from freezing damage (damage due to ice formation). CPAs are usually very thick and have high viscosity.

**Dehydration:** The removal of water from a cell, tissue or organism.

**Liquid Nitrogen:** Nitrogen in a liquid state at a very low temperature (-196°C). It can cause rapid freezing on contact with living tissue, which may lead to frostbite (use with caution! Always use goggles and thick gloves).

**Vitrification:** The transition of a substance into a glass (amorphous state). Cells in a solution of water and CPAs can be vitrified by rapid freezing in liquid nitrogen.

**Devitrification:** The growth of ice crystals during rewarming of a vitrified solution.

**Supercooled liquid:** Liquid at normal freezing temperature without ice formation.

**Slow rate freeze:** Freezing of biological samples using programmable steps at a very slow declined rate of temperature.

**Seeding:** Seeding is the process of inducing ice crystal formation outside the cell during slow rate freeze.



<http://www.biopoliticaltimes.org/img/original/egg%20freezing.jpg>

# Cryopreservation Laboratory Experiment

## Vitrification Experiment

- Label 1 round bottom plastic tube with “0%.”
- Label 4 round bottom glass tubes with “20%, 40%, 60%, 80%.” Place all labeled tubes in a rack.
- Add 3ml water to a round bottom plastic tube labeled “0%” glycerol.
- Make up 20, 40, 60, and 80% glycerol in water for a total volume of 3ml (First do the calculation (table below) and try to figure out how much glycerol and water you will need to make up each solution.

	0%	20%	40%	60%	80%
Glycerol (Gly) (ml)	0.0				
Water (ml)	3.0				
Total Volume (ml)	3.0	3.0	3.0	3.0	3.0

- Mix each solution thoroughly with a transfer pipette (pipette the solution up and down 20 times).
- Set the timer for 30 seconds. Wear safety goggles and thick gloves and hold one tube (starting with the lowest %) with forceps. Submerge the tube into liquid nitrogen for 30 seconds. Make sure that all the solution is submerged below the surface of liquid nitrogen.
- At 30 seconds, carefully take the tube out of liquid nitrogen and observe the solution. Ask yourself, “is the solution vitrified?” How are you tell? **Record your results in the table below.**
- Repeat the last 2 steps for all your tubes, if time.

## Results

Glycerol	0%	20%	40%	60%	80%
Evidence of Ice? Yes or No					
Vitrified? Yes or No					

Students Notes or Questions:

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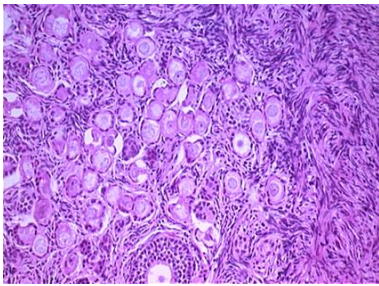
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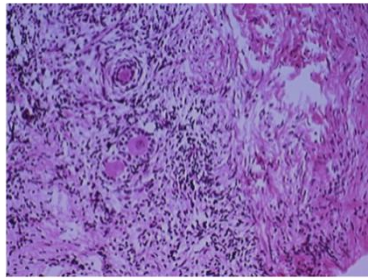
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# Cryopreservation Laboratory Experiment

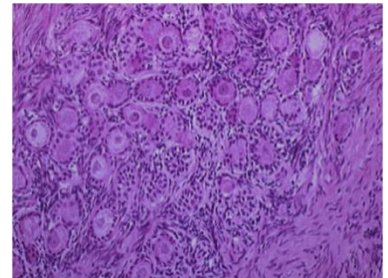
1. How can you tell that a solution is vitrified successfully?
2. What is the percentage of glycerol (CPA) needed for successful vitrification?
3. Give an example of cryopreservation in nature.



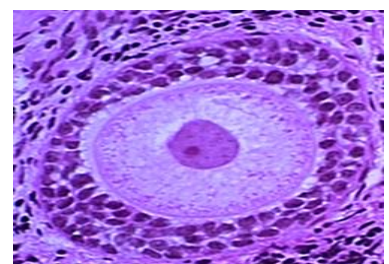
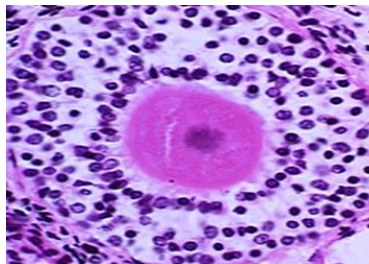
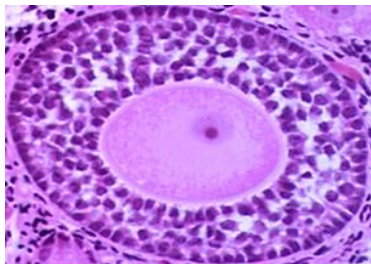
Fresh Tissue



Tissue After Slow Freeze



Tissue After Vitrification



H & E Staining – Top photos - low magnification of ovarian follicles;  
Lower photos - high magnification of ovarian follicles.

4. Describe the difference in the morphology of cryopreserved ovarian cortex compared to fresh ovarian cortex – what do you notice about follicles, oocytes, stromal tissue?
5. Why is cryopreservation important for fertility preservation?

