ONPRC Module 4C Cryopreservation

Preservation of Endangered Species

Middle School Science

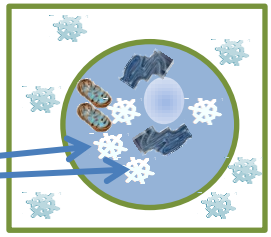
ONPRC

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

Cryopreservation is a process where cells or tissues are preserved by **cooling** to very low temperatures (usually the temperature of liquid nitrogen, -196°C). At this temperature, almost all biological activities, including the ones that would lead to cell death, are stopped. Sometimes biological activity can be restored upon warming.

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

Most of a cell is water. Freezing will lead to the formation of ice crystals in the cell which expand and can burst the cell.



Drawing: Alison Ting, PhD, ONPRC

"Beetle-juice antifreeze": Arctic beetles achieve their protection due to the glycerol produced by their livers. Glycerol is an "antifreeze"; it has high viscosity and reduces ice formation.

"The Living Dead": Wood frogs make increased levels of glucose in cells of vital organs as temperatures approach freezing. A partially frozen frog will stop breathing, and its heart will stop beating. It will appear quite dead. But when the weather warms up, the frog's frozen parts will thaw; its heart and lungs resume activity.

cc BY NC SA

Clip Art: Microsoft

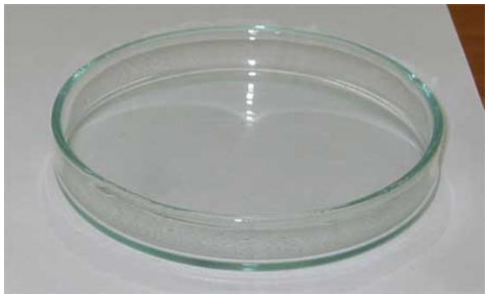
http://www.pbs.org/wgbh/nova/nature/costanzo-cryobiology.html

Vitrification – Super Rapid Freezing

Vitrification is a new technique for freezing oocytes, sperm, and embryos. Through vitrification, ice formation inside and outside the cell is avoided.

Pure water can be vitrified (no ice formation) if cooled at a rate of millions of degrees Celsius per second.

Not Vitrified (Ice crystal)



Vitrified (glass-like)

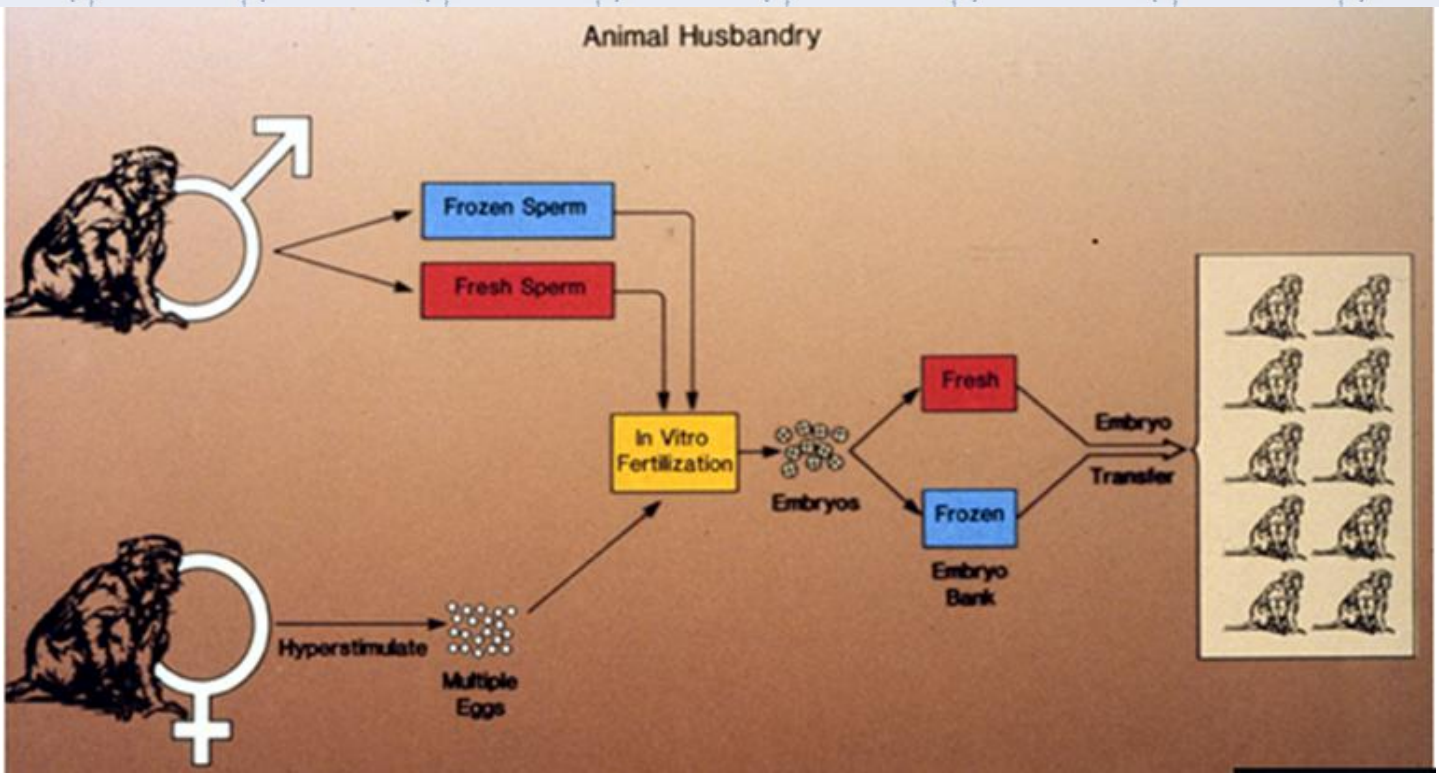
http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm197586.htm

http://en.wikipedia.org/wiki/Glass#mediaviewer/File:Szalka_petriego.jpg http://creativecommons.org/licenses/by-sa/3.0/ No changes were made.

When sucrose is cooled slowly, the result is crystal sugar (or rock candy), but, when cooled rapidly (vitrified), the result is syrupy and airy spun cotton candy.

http://commons.wikimedia.org/wiki/File:Rock_Candy.jpg http://en.wikipedia.org/wiki/GNU_Free_Documentation_License No changes were made.

http://commons.wikimedia.org/wiki/File:Spinning_head_of_the_cotton_candy_maker.jpg http://creativecommons.org/licenses/by-sa/3.0/deed.en No changes were made.



Drawing: Joel Ito, Medical Illustrator, ONPRC

Cryopreservation of sperm and embryos is being used to preserve endangered species. They are stored in liquid nitrogen – "a zoo in a freezer". Sperm can be thawed and used for mating with a female. Embryos can be thawed and transferred to a female for live birth.

Photo: **Timu** - baby lowland gorilla, Courtesy of Cincinnati Zoo

cc BY NC SA

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 with "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					

Student Notes or Questions

_____

_____

_____

cc BY NC SA

Cryopreservation Questions

1. **What is cryopreservation?**

2. **How can you tell that a solution is vitrified successfully?**

3. **What is the percentage of glycerol (CPA) needed for successful vitrification?**

4. **Give an example of cryopreservation in nature.**

5. **Why is cryopreservation important for preservation of endangered species?**

CC BY NC SA

Cheetah
http://nationalzoo.si.edu/SCBI/EndangeredSpecies/Cheetah/

Baby Panda Bao Bao
Photo: Abby Wood, Smithsonian's National Zoo
http://nationalzoo.si.edu/Animals/GiantPandas/PandaUpdates/

Cryobiology helped Zoo scientists breed black-footed ferrets, once thought to be extinct. (*Mehgan Murphy/NZP*)
http://nationalzoo.si.edu/publications/zoogoer/2010/1/FrozenAssets.cfm