5E Learning Cycle Lesson Plan

1. Title: Module 4A ONPRC Saturday Academy Cryopreservation & Fertility Preservation Laboratory

2. Overview and Statement of Purpose: The cryopreservation lab is designed to teach students one of the cutting edge research procedures in oncofertility and to explore how it can be used to preserve the fertility in female cancer patients.

3. Grade Level and Objective(s): High School lab activity on fertility preservation

4. Student Background Knowledge: Students should know that oncofertility is a new approach in medicine for working with cancer patients under 40 years old and encompasses comprehensive medical approaches to preserving fertility in these patients before their cancer treatment begins. Vitrification (fast rate freezing) in liquid nitrogen is a way to preserve ovarian tissue and eggs for later use by the cancer survivor. Nitrogen goes from the gas phase to the liquid phase at -196^{o}C (-321^{o}F) and from the liquid phase to the solid phase at -210^{o}C (-346^{o}F). As a liquid, it causes rapid freezing of the tissue on contact. Vitrification using a cryoprotective agent, like glycerol, protects the cell since ice crystals won't form inside or outside the cell when exposed to liquid nitrogen. Ice crystal formation inside a cell will destroy the cell due to the expansion of water as it freezes. Vitrified liquids appear clear, like glass, which is what *vitrified* means in Latin. Slow rate freezing can be done as well with no ice crystals forming inside the cell but the results are not as close to being like fresh tissues as vitrified tissues are since ice crystals do form outside the cell.

5. 21st Century Skills

A. Please list 21st Century Skills targeted by this lesson.

- Communication
- Collaboration
- Critical Thinking and Problem Solving

6. Essential Knowledge (For teacher/instructor) Teachers should know how to safely handle liquid nitrogen which includes use of clothing that covers the skin, closed-toe shoes, face shield or goggles, and thermal gloves rated for use with liquid nitrogen.

7. List of Materials: Distilled water, glycerol, small test tubes, test tube racks both large and small, test tube holders, 5 ml syringes (without needles), pipettes, sharpies, pencils, handouts, liquid nitrogen, goggles, and thermal gloves.

8. Safety Considerations: Liquid nitrogen is very cold and freezes living tissue on contact causing the sensation and look of a bad burn. Avoid contact with skin and eyes.

9. Detailed Lesson Plan

A. ENGAGE - Engagement with transition question, challenge, or problem

i. What will you do to engage the students? Students enjoy learning about cryopreservation, especially as it occurs in nature. A good way to engage them would be a discussion of frogs, which use sugar, and beetles, which use glycerol, as a type of antifreeze in their blood to protect them from freezing to death during the winter.
ii. How will you connect to students' everyday lives? Ask the students, "What would happen to a person who falls through ice on a pond"" What happens if a person is outside too long in the winter?"
iii. What questions will you ask? (Provide sample answers.) Do people have a sugar or glycerol type of antifreeze in their blood to protect them in the winter? How do you know? If not, what do people do to protect themselves during the winter?

B. **EXPLORE** – Student-centered exploration of the topic/content

i. How will you set up this exploration? Students will do the calculations determining how much water and glycerol (the cryoprotective substance) would be needed to make up 3.0 ml of 0%, 20%, 40%, 60%, and 80% solutions of glycerol dissolved in water. Then the solutions would then be made by the students. Mix each solution well by using a pipette to pull the solutions into and out of the pipette. Begin with 0% and proceed to 80% one test tube at a time.
ii. What data will students gather or guided inquiry will student complete?
After preparing the solutions, students would put on safety goggles and use a thermal glove and a test tube holder to lower each test tube into liquid nitrogen for 30 seconds. Be sure not to let the glove dip into the liquid nitrogen.
iii. How will you help students generate their own questions? The data table will guide the first two questions. Which test tubes formed ice crystals and which remained clear (no ice crystals)? Which tests vitrified and which didn't? Why did the students get those results? How could vitrification be applied to a real-life situation?
iv. What questions will you ask as a means of formative assessment? (Provide sample answers.)

SAMPLE QUESTION(s): Why do you take three measurements? *(In order to be as accurate as possible. By taking three different measurements, one can see that the results are repeatable.).*

What are the controlled variables? What are the experimental variables? *(Controlled= 0% solution, same vat of liquid nitrogen used each time, same method each time. Experimental = the 20%, 40%, 60%, and 80% solutions used)*

How could you make the data gathering process more accurate? *(Have the same person measure glycerol and water in the syringes, mix the solutions with the pipette, lower the test tubes in the liquid nitrogen, and time the 30 seconds intervals)*

What type of people might be interested in this experiment? *(Physicians, including fertility specialists, cancer survivors and their parents, and researchers)*

How does this experiment connect to real life? *(Preserve oocytes and sperm cells in cancer patients prior to chemotherapy and radiation therapy to give the possibility of having children later.)*

C. **EXPLAIN** – Students generate explanations for what they observe; conduct further research

i. How will you get students to share their data? Each group would compare the 5 test tubes to see if results are the same as that of other groups.
ii. What guiding questions will you ask? (Provide sample answers.)

SAMPLE QUESTION(s): Explain why some of the test tubes vitrified and others didn't. *(The concentration of water/glycerol affects how much or how little ice can form when placed in liquid nitrogen. Glycerol forms hydrogen bonds with water thus interfering with the hydrogen bonding between water molecules which form as water cools as part of ice formation.)*

After finding the solution(s) where vitrification takes place, is there a particular concentration where vitrification occurs better than at others? *(Students will see that there is no vitrification of the 5 solutions except at 60% and 80%. Students could test 1% solutions lower than 60% to see if they get better results. Glycerol can be toxic to cells so using the least amount of glycerol/water mix for vitrification would be the best. Researchers have found a 53% glycerol/water solution gives the best result for vitrification of tissue.)*

How will vitrification benefit the cell as freezing occurs? *(Since no ice forms in the cell, the cell will survive the freezing and thawing process.)*

iii. What ideas will you try to develop? Vitrification cryopreservation is a useful tool for preserving reproductive cells for future use. What are other tissues that might benefit from cryopreservation?
iv. What terminology will you introduce and how will you relate this to the data? Ideas relating to cancer and to cancer treatment and how fertility is affected would be added as well as the normal structure of the ovary and how it looks using vitrification and slow rate freezing methods.

D. **ELABORATE –** Students apply the knowledge they have learned and engage in activities that extend their learning

i. What will students do in the elaborate phase? Endangered species can be helped by cryopreservation of their oocytes and sperm cells for use in later fertilization.
ii. What concepts will you have them apply? What is an endangered species and why are they endangered? How will cryopreservation help these species? Would this method help the beetle and frog that have the sugar or glycerol type of antifreeze in their blood?
iii. How is this different but related to what came before? Before the process was applied to humans and now it is being applied to other species.
iv. What guiding questions will you ask? (Provide sample answers.)

SAMPLE QUESTION(s): Explain why cryopreservation is useful in helping endangered species. *(Being able to successfully freeze the oocytes and sperm cells from endangered species might allow us to use them later to create more members of the species and perhaps provide greater genetic diversity.)*

How have species become endangered? *(Species number can dwindle due to catastrophic events, as in the case of the cheetah, but much of the decline in species numbers has been caused by the increase in the human population and its overuse of resources. This would include overharvesting some species for food, clothing, etc. and decreasing the amount of habitat available for their survival.)*

E. **EVALUATE** - Monitor student understanding

i. What formative assessments will you use and when in the lesson will you use them? By asking the students questions before, during, and after the lab activity, the teacher would be able to assess understanding and misunderstanding of the ideas presented.
ii. What specifically will you look for in these? The teacher would be looking mainly for misunderstanding of ideas so they could be corrected.
iii. What summative assessment will you use? The teacher would have the students write a short essay on cryopreservation by vitrification and applications.
iv. What specifically will you look for in this? The teacher would look for clear understanding of definitions and applications.
v. Provide a sample response to the summative assessment. Vitrification is a method of cryopreservation in which rapid rate freezing can protect living cells because by adding a cryoprotectant, such as glycerol, ice crystals do not form inside or outside the cell which prevents damage to the cell. Vitrification can be used to freeze oocytes and sperm cells from cancer patients prior to chemotherapy and radiation therapy and from endangered species to be used later in the fertility process.

10. **Modifications**

A. **How would you modify this lesson to meet the needs of students with ADD/ADHD?** These students would need more supervision to make sure they are careful around the liquid nitrogen.
B. **How would you modify this lesson to meet the needs of learning disabled students?** These students would need more supervision to make sure they are careful around the liquid nitrogen. They might also need extra help with the calculations done before making the solutions.
C. **How would you modify this lesson to meet the needs of ELL students?** These students would need more supervision to make sure they are careful around the liquid nitrogen.

11. **Sources**

Glycerol can be ordered from most chemical supply companies, such as Fisher Scientific and Flinn Scientific. Liquid nitrogen can be obtained at most universities, colleges, and junior colleges.