FEATURE ARTICLE

New Approaches in Cancer Biology Can Inform the Biology Curriculum

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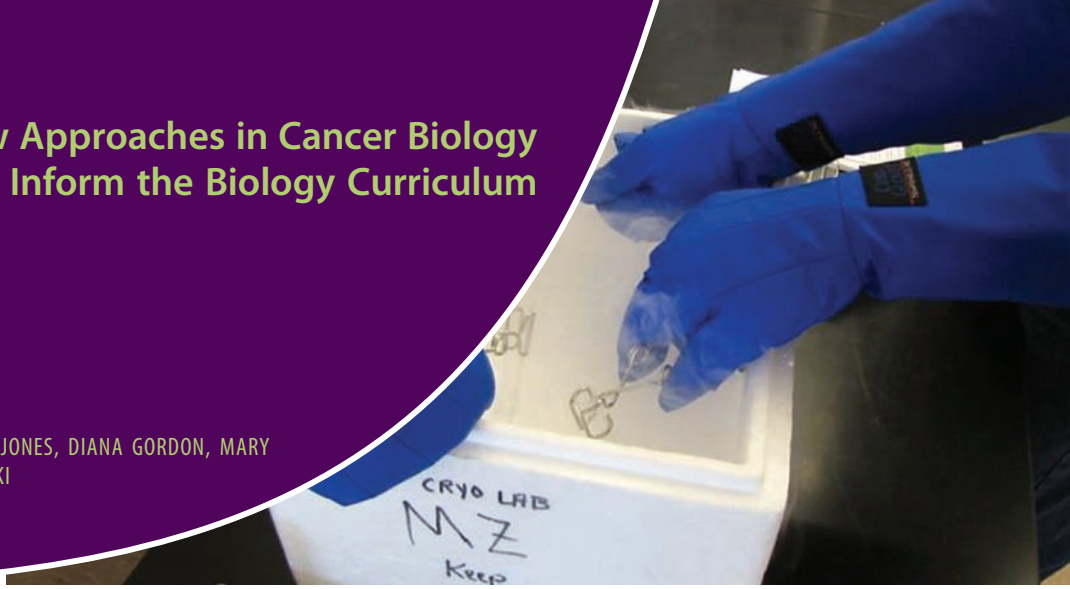

Abstract

Students tend to be very interested in medical issues that affect them and their friends and family. Using cancer as a hook, the ART of Reproductive Medicine: Oncofertility curriculum (free, online, and NIH sponsored) has been developed to supplement the teaching of basic biological concepts and to connect biology and biomedical research. This approach allows integration of up-to-date information on cancer and cancer treatment, cell division, male and female reproductive anatomy and physiology, cryopreservation, fertility preservation, stem cells, ethics, and epigenetics into an existing biology curriculum. Many of the topics covered in the curriculum relate to other scientific disciplines, such as the latest developments in stem cell research including tissue bioengineering and gene therapy for inherited mitochondrial disease, how epigenetics occurs chemically to affect gene expression or suppression and how it can be passed down through the generations, and the variety of biomedical careers students could pursue. The labs are designed to be open-ended and inquiry-based, and extensions to the experiments are provided so that students can explore questions further. Case studies and ethical dilemmas are provided to encourage thoughtful discussion. In addition, each chapter of the curriculum includes links to scientific papers, additional resources on each topic, and NGSS alignment.

Key Words: *curriculum; biomedical research; medicine; cancer; oncofertility; reproduction; cryopreservation; bioengineering; ethics.*

A New Approach in Medicine

New more targeted treatments for cancer have improved the five-year survival rate for all adult cancers combined (now about 68%) and for all childhood cancers combined (now about 81%) (NIH, 2010). This has resulted in a growing population of cancer survivors who, because of the toxicity of the cancer treatment, are infertile. Recognizing the frustration, anger, and disappointment of these survivors, a new medical specialty was born. *Oncofertility*, a term coined in 2006 by Teresa K. Woodruff, Ph.D., a reproductive endocrinologist at Northwestern University, encompasses comprehensive medical approaches to preserving fertility in these patients before their cancer treatments begin (Woodruff et al., 2007).

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Today oncofertility specialists provide counseling for cancer patients under 40 years old about how eggs and sperm can be harvested and stored for the future *prior* to beginning cancer treatments. In addition to giving cancer patients options to increase their likelihood of having children, this approach gives them hope that they will survive their cancer to have children.

Preserving fertility is somewhat more complex in females than in males due to the fact that females are born with all of the follicles containing oocytes (eggs) that they will have for life. Figure 1 shows the damage that radiation and some chemotherapy treatments can cause in the ovary. The result is an individual who has premature ovarian failure, menopause-like symptoms, and infertility. Premature ovarian failure means that all of the follicle-containing oocytes are destroyed by the treatment.

Before cancer treatment begins, a female adult cancer patient in her child-bearing years would likely want to meet with a fertility specialist and explore fertility options. Options include taking fertility drugs and then collecting oocytes for *in vitro* fertilization (IVF). If there is a male partner/spouse, the oocytes could be combined with sperm to form embryos that can be cryopreserved (frozen) and then thawed and transferred into the uterus to establish a pregnancy when the patient has recovered. Patients also can opt to cryopreserve only oocytes, and some may choose to cryopreserve both oocytes and embryos. If the female is prepubertal or an adolescent, the only option is to have ovarian tissue removed and cryopreserved. Later, the tissue can be transplanted back to the patient

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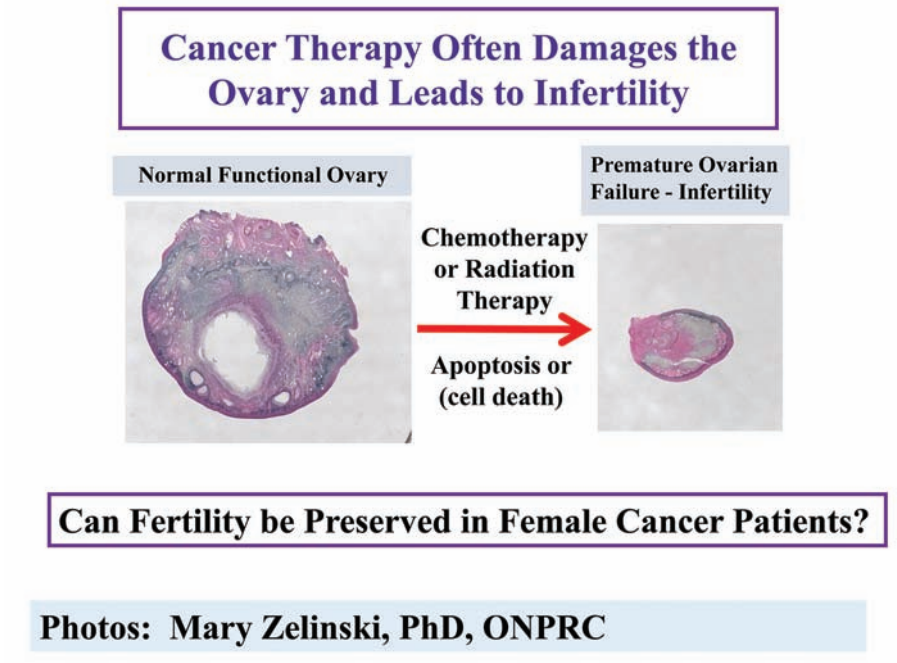

Figure 1. (Left) Cross-section through a normal rhesus monkey ovary. Cells have been stained with hematoxylin and eosin to depict nuclei and cytoplasm, respectively. This ovary has many follicles (small and large circles) that contain oocytes located in the outer cortex of the ovary. (Right) Ovary is from the same animal as the normal ovary, but it was collected after exposure to X-irradiation at a dose similar to human patients. The ovary has no follicles, is much smaller in size, and contains damaged blood vessels.

as an autograft. The transplanted ovarian tissue resumes its function and can produce mature oocytes, which could be collected, fertilized *in vitro*, and grown into embryos for subsequent transfer into the uterus.

Males are born with spermatogonial stem cells in the testes which will continue to make sperm for the lifetime of the male once he reaches puberty. Radiation therapy and some chemotherapy drugs can cause infertility by destroying *both sperm and stem cells* thus preventing *future sperm* formation.

As with female cancer patients, male adult cancer patients would likely want to meet with a fertility specialist and look at fertility options before starting cancer treatment. If there is a female partner/spouse, the sperm could be collected and combined with her oocytes to form embryos and cryopreserved. Later, after the patient has recovered, the embryo(s) would be thawed and transferred to the uterus of his partner. If there is not a current partner, sperm can be collected and cryopreserved prior to cancer treatment. After the patient has recovered, the sperm can be thawed and inserted into the cytoplasm of an oocyte from his future female partner/spouse by intracytoplasmic sperm injection (ICSI). The resulting embryos could then be transplanted into the uterus of his partner. For prepubertal males, the only option is to collect and cryopreserve testicular tissue until an autograft can be done. Later, sperm can be collected and, following ICSI or IVF, could lead to an offspring.

○ Teaching Basic Biological Concepts using Biomedical Research as an Organizing Theme

Through outreach grants sponsored by the National Institutes of Health, the free, on-line *ART of Reproductive Medicine: Oncofertility* curriculum was developed to supplement the teaching of basic biological concepts and to connect biology and biomedical research.

The *ART of Reproductive Medicine: Oncofertility* curriculum is designed to enhance current biology courses rather than to replace them. The curriculum is most appropriate for advanced biology courses in high school and introductory biology and anatomy/physiology courses in both junior colleges and four-year institutions. However, some of the lessons could be used in middle school and high school general biology courses, such as those lessons focusing on cell division and cancer prevention. Although all of the lessons are related to the topics of oncofertility and infertility, each one can be used as a stand-alone unit or used out of sequence. Each chapter of the curriculum includes a Teacher Background Section, a PowerPoint slide show for the students, and instructions for supporting activities (Table 1).

The following basic biology learning objectives are met with this curriculum:

1. **Develop an interest in the field of biology and a broad knowledge base to understand, connect, and synthesize ideas.** Students tend to be very interested in medical issues that affect them and their friends and family. Using cancer as a hook, this approach allows integration of up-to-date information on cancer and cancer treatment, cell division, male and female reproductive anatomy and physiology, cryopreservation, fertility preservation, stem cells, ethics, and epigenetics into an existing biology curriculum.
2. **Make connections to other scientific disciplines and to society at large.** Many of the topics covered in the curriculum relate to other scientific disciplines, such as the latest developments in stem cell research, including tissue bioengineering and gene therapy for inherited mitochondrial disease, how epigenetics occurs chemically to affect gene expression or suppression and how it can be passed down through the generations, and the variety of biomedical careers students could pursue. The topic of oncofertility relates to society at large since students likely will know someone, or have read or heard about someone, who has been affected by cancer and cancer treatments and perhaps by infertility.

Table 1. Chapter titles of the ART of Reproductive Medicine curriculum (ONPRC, 2018, http://www.ohsu.edu/xd/research/centers-institutes/onprc/public-outreach/ART-of-Reproductive-Medicine.cfm).

Chapter 1.	Introduction to Oncofertililty
Chapter 2.	Mitosis
Chapter 3.	Cancer
Chapter 4.	Cancer Treatment and the Effect on Reproduction
Chapter 5.	Meiosis
Chapter 6.	Female and Male Reproductive System
Chapter 7.	Pregnancy
Chapter 8.	Cryobiology
Chapter 9.	Fertility Preservation Options for Cancer Patients Under 40 Years Old
Chapter 10.	Stem Cells
Chapter 11.	Ethical Frameworks
Chapter 12.	Epigenetics
Chapter 13.	Student Case Study and Poster Presentations
Chapter 14.	Biomedical Careers
Chapter 15.	Additional Teacher Resources

Table 2. Next Generation Science Standards (NGSS) alignment by chapter in the ART of Reproductive Medicine curriculum.

Chapter 2.	Inheritance and Variation of Traits: HS-LS1-4
Chapter 3.	Structure, Function, and Information Processing: MS-LS1-1, 1-2; HS-LS1-1, 1-2 Inheritance and Variation of Traits: HS-LS1-4; HS-LS3-2
Chapter 4.	Structure and Properties of Matter: HS-PS1-8
Chapter 5.	Inheritance and Variation of Traits: HS-LS3-1, 3-2, 3-3
Chapter 6.	Structure and Function: HS-LS1-2, 1-3 Natural Selection and Evolution: HS-LS4-2
Chapter 7.	Structure and Function: HS-LS1-2 Inheritance and Variation of Traits: HS-LS1-4, 3-1, 3-2 Natural Selection and Evolution: HS-LS4-2
Chapter 8.	Structure and Properties of Matter: MS-PS1-1; HS-PS1-1
Chapter 9.	Natural Selection and Adaptations: MS-LS4-2; HS-LS4-2
Chapter 10.	Structure and Function: HS-LS1-2 Inheritance and Variation of Traits: HS-LS1-4, 3-1, 3-2 Natural Selection and Evolution: HS-LS4-2
Chapter 11.	Inheritance and Variation of Traits: HS-LS3-2 Natural Selection and Evolution: HS-LS4-2
Chapter 12.	Inheritance and Variation of Traits: HS-LS3-1, 3-2 Natural Selection and Evolution: HS-LS4-2

3. **Develop proficient skills in problem-solving, lab techniques, quantitative analysis, and communication through engaging in the process of science.** The labs are designed to be open-ended and inquiry-based. Students work in pairs or small groups to conduct the experiments; extensions to the experiments are provided so that students can explore questions further, if time permits. Case studies and ethical dilemmas are provided to encourage thoughtful discussion. Teachers may choose to have their students make oral presentations ("grand rounds") and/or poster presentations in their roles as "physicians."

In addition, each chapter of the curriculum includes links to scientific papers and additional resources on each topic. Alignment to the NGSS is provided at the end of each chapter, summarized in Table 2.

Chapter 8 in the *ART of Reproductive Medicine: Oncofertility* curriculum is "Cryobiology". Cryopreserving (freezing) reproductive cells and tissue is a good way to save them while the cancer patient is undergoing treatment. There are two types of cryopreservation, slow-rate freezing and vitrification. The main goal in cryopreserving living cells is to prevent cell death and damage by keeping ice from forming both inside and outside the cell. Water expands as ice crystals form when it changes from a liquid to a solid, and both of these effects damage living cells. The principle underlying cryopreservation is to remove water from cells and replace the water with anti-freeze, or cryoprotectants. Slow-rate freezing allows the cell to freeze without forming ice inside the cell, but it does not prevent ice from forming on the outside of the cell, so damage to the cell may occur during thawing. Vitrification is a new method of cryopreservation in which ice does not form on the inside or outside of the cell; it is the method currently used to cryopreserve oocytes and embryos. When tissues are vitrified, the tissues and fluid surrounding them appear clear like glass, while the fluid surrounding slow-rate frozen tissues will appear icy or white. Vitrification of ovarian tissue for transplantation to restore fertility to female cancer patients is currently the subject of intense research.

One of the hands-on activities in the *ART of Reproductive Medicine: Oncofertility*

Table 3. Sample inquiry questions for cryobiology lab in the ART of Reproductive Medicine.

Sample Inquiry Questions Illustrating the Inquiry-Based Nature of the Cryobiology Activity	
1.	If arctic beetles produce glycerol and wood frogs produce glucose as antifreeze in the winter to survive, do other animals survive the winter in this way?
2.	Do people produce glucose or glycerol as antifreeze in their blood to protect them in the winter? How do you know? If not, how do people protect themselves during the winter?
3.	Which test tubes formed ice crystals and which remained clear (no ice crystals)? Which test tubes vitrified and which didn't? Explain at the molecular level why some of the test tubes vitrified and others didn't.
4.	What are the controlled variables? Which are the experimental variables?
5.	How could you make the data gathering process more accurate?
6.	After finding the solution(s) where vitrification takes place, is there a lower percentage of glycerol where vitrification occurs? What experiments could be done to find out?
7.	How will vitrification benefit the cell as cooling and warming occur?
8.	What type of people might be interested in this experiment?
9.	How could vitrification be applied to a real-life situation?
10.	What is an endangered species and why is it endangered? How would cryopreservation help endangered species?

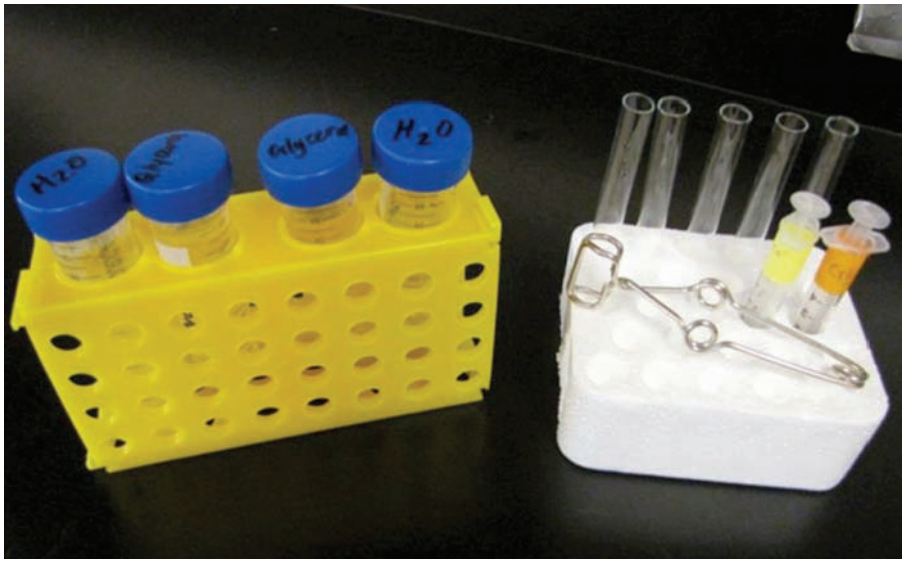

Figure 2. Cryobiology lab equipment: 50 mL of water, 50 mL of glycerol, 5 small test tubes, 5 mL needleless syringes for measuring water and for measuring glycerol, and a long-handled test tube holder. Also necessary but not pictured are a black Sharpie and a 5 mL plastic bulb pipette for each student, a timer, thermal gloves, and a cooler of liquid nitrogen (or dry ice).

curriculum from the chapter on cryobiology includes determining optimal cryoprotectant solutions for vitrification (see Table 3). Students are asked to prepare aqueous solutions of 0, 20, 40, 60, and 80 percent glycerol in test tubes, and then place them in racks (Figure 2), which will be placed on dry ice in a closed styrofoam cooler for 30 minutes. (Liquid nitrogen can be used, in which case the time each test tube is held in liquid nitrogen is reduced to 30 seconds (Figure 3). The students will find that the 0, 20, and 40 percent tubes will be icy (which could be damaging to tissues since they contain too much water) (Figure 4), whereas the 60 and 80 percent tubes appear clear, showing they were vitrified (no ice formation) (Figure 5). For a group of 24 students working in pairs, this lab takes 1 hour, plus 30 minutes to discuss background information on cryopreservation and vitrification. In addition to alignment to NGSS, the 5E Learning Cycle Lesson Plan for this activity is included in the curriculum. (Note: No actual tissue is used in this lab activity.)

Another hands-on activity emphasizes a bioengineering approach to fertility preservation. This activity, which explores alginate gel as a biomaterial that can be used to maintain three-dimensional structures of follicles and their enclosed eggs (oocytes) as they grow *in vitro*, can be found in Chapter 9, "Fertility Preservation Options for Cancer Patients under 40 Years Old" (see Table 4). As frozen oocytes within their follicles are thawed, they need a structure within which the follicles can continue to grow and mature. Follicles are three-dimensional (3-D) spheres and will collapse if not given a scaffold; alginate is a natural choice of a biomaterial for this scaffold. It is derived from large brown algae, such as *Nereocystis* (e.g., bullwhip kelp). A follicle with an oocyte in it can be placed into a small bead of alginate gel (Figure 6).When 1.0 percent and 2.0 percent solutions of liquid sodium alginate (colored with red and blue food coloring, respectively) are then exposed to calcium salt solutions, such as calcium chloride, the monomers of alginate covalently crosslink to form a gelatinous hydrogel. In spherical form, it will structurally support the follicle in 3-D while it continues to grow and mature. Students investigate which percent solution of alginate and which techniques of combining the alginate and salt solutions work

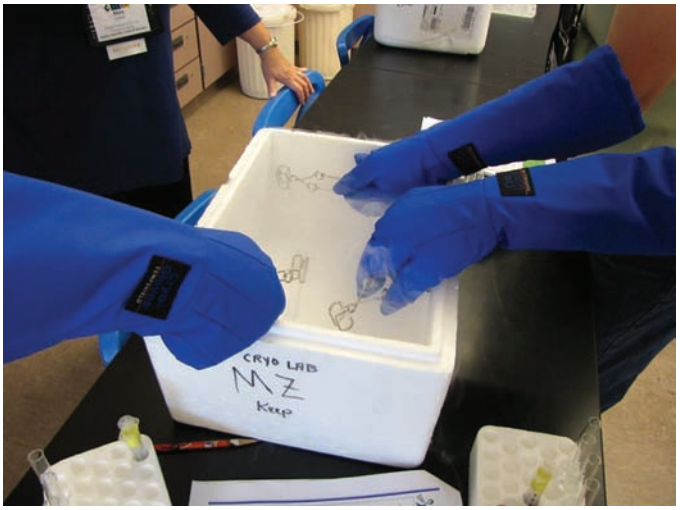

Figure 3. Test tubes are placed in liquid nitrogen for 30 seconds. As an alternative, test tubes in a rack may be placed in a cooler lined with dry ice for 30 minutes. Thermal gloves are required when using liquid nitrogen and strongly suggested when using dry ice.

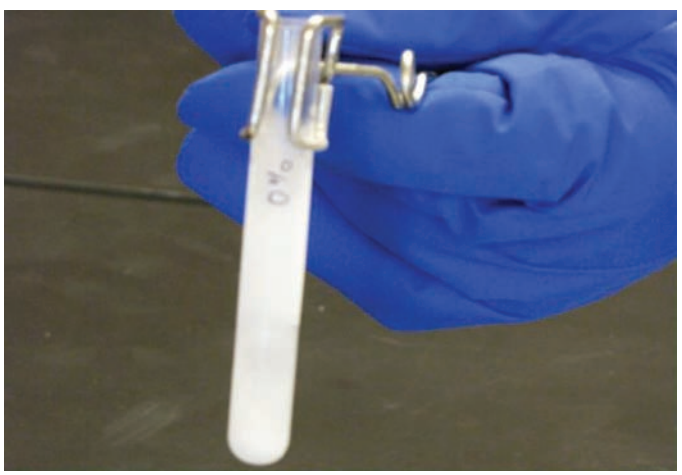

Figure 4. Test tube of 0% glycerol (100% water). As predicted, it is solid ice.

the best for forming spheres (rather than long wormlike structures or other misshapen structures) (Figure 7). For a group of 24 students working in pairs, this lab takes 1 hour, plus 30 minutes to discuss background information on alginate gel formation and applications. In addition to alignment to NGSS, the 5E Learning Cycle Lesson Plan for this activity is included in the curriculum. (Note: No actual tissue is used in this lab activity; all materials used are non-toxic.)

○ Assessment

The development of the *ART of Reproductive Medicine: Oncofertility* curriculum was supported by a National Institutes of Health (NIH), National Centers for Translational Research in Reproduction and Infertility (NCTRI) grant awarded to the Oregon National

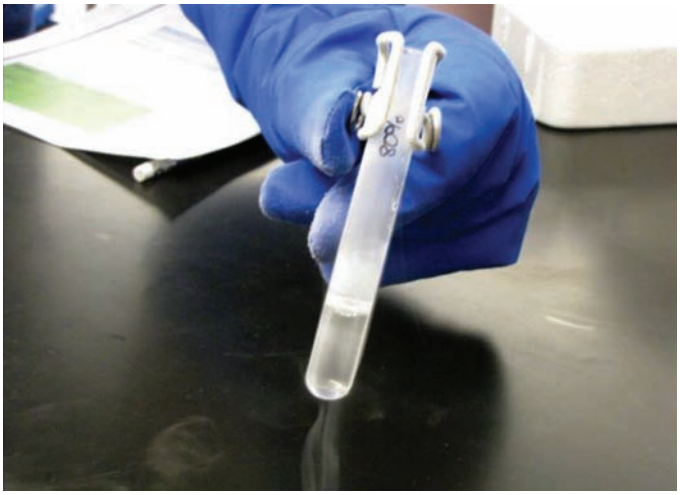

Figure 5. Test tube with 80% glycerol (20% water). Notice that the vitrified solid layer is as clear as glass and there is no ice present.

Figure 6. Lab set-up for alginate lab. Each styrofoam test tube holder contains a tube of 0.5% (not shown), 1.0% (red) and 2.0% (blue) solutions of liquid sodium alginate, a tube of calcium chloride solution, 3 plastic pipettes of different diameter openings, needleless syringe, pair of forceps, 10 mL plastic pipette with pipette pump to transfer calcium chloride, cup of rinse water, small Petri dish per student, small capped vial per student, a pecan (size of human ovary), and pistachio (size of rhesus monkey ovary).

Primate Research Center's Division of Reproduction and Developmental Sciences. A pre-/post-test evaluation instrument was designed to assess changes in student understanding of reproductive anatomy, endocrinology, and physiology, cancer biology, oncofertility, and ethics after participating in a four-day Saturday Academy Oncofertility course covering many of the lessons from this curriculum. An independent evaluator was hired to assess the results. The test consists of 18 multiple-choice questions and 9

Figure 7. Student transferring alginate spheres from the Petri dish to a vial. Alginate spheres in the research lab would be used to provide a 3-dimensional framework in which oocytes could grow.

Table 4. Sample inquiry questions for alginate lab in the ART of Reproductive Medicine.

Sample Inquiry Questions Illustrating the Inquiry-Based Nature of the Alginate Activity	
1.	What is the nature of gelatin used to make jello?
2.	What are some foods that include the gel form of algae (seaweed)? What is the purpose of adding these gels to foods?
3.	What are other uses of the gel form of algae (think science labs)?
4.	What are the controlled variables? Which are the experimental variables?
5.	Which solution formed the smallest and roundest spheres? What would your recommendation be for someone trying the same experiment in terms of % solution, distance, dropper diameter, and technique of delivery?
6.	How could you make the data gathering process more accurate? Why do you take three measurements? Why do you measure from the same spot?
7.	What type of people might be interested in this experiment?
8.	How does this experiment connect to real life?
9.	What solution would you recommend to a research scientist who was using calcium alginate gel to grow follicles and why?
10.	How will the vitrified spherical gel benefit the oocyte in the follicle as it warms?

open-ended questions and can be found in Chapter 15, "Additional Teacher Resources" (see Table 5 for examples).

Institutional Review Board (IRB) approval was obtained from Oregon Health & Science University (OHSU) to collect data from students who attended the Saturday Academy Oncofertility course in 2014 and 2016 (the course was not offered in 2015). The mean score in 2014 was 21.1 on the pre-test and 27.3 on the post-test, and in 2016 was 25.3 on the pre-test and 31.9 on the post-test. Results of a *t*-test showed a statistically significant knowledge gain over time in 2014, $t(6) = 2.76$, $p < 0.05$, and in 2016, $t(9) = 6.13$, $p < 0.05$.

○ Conclusions

The *ART of Reproductive Medicine: Oncofertility* curriculum is an ideal way to teach reproductive anatomy, physiology, and medicine from a clinical and scientific view. It engages students in an area that is personal to them, and results in measurable increases in their scientific understanding of cell biology and the human reproductive system. The curriculum includes information on up-to-date patient treatments and cutting-edge research. We have presented this curriculum and shared lab activities with numerous student groups and at several professional educator conferences. In every case, students and teachers have been eager to learn more. Interestingly, we find this curriculum empowers students to ask questions about their own reproductive health in a respectful and safe environment; students sometime even stayed after class to continue their conversations with our presenters. After Jeri Janowsky, Ph.D., Executive Director of Saturday Academy in Portland, Oregon, accompanied a group of high school students to an all-day "Club Med" summer Saturday Academy class, she stated:

> What was fantastic about the experience our students had were the step-by-step discussion of discovery by a scientist and the importance [of that discovery] in women's lives. This then tied to the students' laboratory experience in which they experimented with the process of freezing cells or vitrification. In most traditional classroom science "experiments," the "right" answer is known and

Table 5. Sample of questions in the Pre-Test and Post-Test.

1.	What is Oncofertility? a) Study of new ways to prevent cancer of the reproductive organs. b) The treatment of people with genetically based infertility. c) Comprehensive medical approaches to preserve fertility for cancer patients. d) Entirely new ways to treat cancer, without using harmful radiation.
2.	Cancer is best defined as: a) An inherited disease. b) Widespread cell death. c) An infection that destroys tissue in organs. d) Uncontrolled cell growth.
3.	A 22 year old woman is going to be treated for lung cancer. Would any of the following therapies be likely to affect her ability to become pregnant? a) Chemotherapy. b) Radiation. c) Surgery.
4.	What does radiation do? Select all that apply. a) Radiation causes damage to cells that may cause them to grow uncontrollably into cancer. b) Radiation kills cancer cells. c) Radiation kills non-cancer (normal) cells.
5.	Open-ended: When Linda was 16 years old, she was diagnosed with lung cancer. In order to preserve her fertility, one of her ovaries was removed and frozen for later use. Unfortunately, she died of her cancer a year later. Linda was an only child and her parents want to use her eggs to have a grandchild. List what you consider to be the ethical issues surrounding this decision. List as many as you can.

the goal is to get that answer. During the oncofertility experiment the students learned hands-on that science is the process of discovery of the unknown. And they loved that! [quoted with permission]

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