Using Caffeine To Hyperactivate Bull Sperm

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The introduction of reproduction utilizing only the usual array of textbook graphs and diagrams can take an otherwise fascinating subject, particularly to high school and college-age students, and reduce it to sheer boredom. This exercise gives students the opportunity to see live, swimming mammalian sperm and to watch how they respond to a trigger that prepares them for fertilization in the same way that they are prepared to fertilize eggs inside the female. The trigger we use in this experiment is caffeine, a chemical familiar to students. While it is not the physiological trigger (which is unknown at present), it produces the same effect. Although students are taught that cells respond in many ways to different molecules, they rarely get a chance to observe these responses or to see how cells might respond to a chemical that is familiar to them. Here is a chance to actually see a cell respond to something that a student might drink every day.

This exercise will reinforce students' understanding of mammalian reproductive processes by enabling students to see live sperm. In addition, the use of caffeine to produce a change in sperm that is normally undergone just before fertilization can foster an interesting discussion about how caffeine might affect sperm in the men and women who drink it, including a discussion of how caffeine could get from the digestive tract to the reproductive tract via the circulatory system. This experiment includes controls that show the importance of using proper controls in designing experiments.

Background

In different species of mammals, males deposit sperm into the vagina (e.g., primates and cattle) or directly into the uterus (e.g., pigs). Human sperm are deposited into the vagina right at the entrance to the cervix, where thousands quickly enter. This saves the sperm from being killed in the vagina. The pH of human vaginal fluid is highly acidic, which serves to kill bacteria and other potentially infectious microbes; however, the acid can also immobilize sperm that fail to enter the cervix quickly.

Sperm are able to swim through the cervix by following grooves in the wall. Although the cervix is filled with mucus, the mucus is very watery during the fertile period of the month, particularly in the grooves, and sperm can easily swim through the grooves to reach the uterine cavity.

Sperm that enter the uterus may be helped along towards the oviduct (fallopian tube) by peristaltic contractions of muscle in the uterine walls. When sperm reach the entrance to the oviduct, they must squeeze through the opening to get inside. Only vigorously motile, well-shaped sperm can make it through. Sperm may be stored in the oviduct for a few days (or a few months in hibernating bats) until ovulation occurs and the egg enters the oviduct.

When a human sperm is first deposited in the vagina, its flagellum (tail) generates low symmetrical waves that propel it forward in a straight path. This helps the sperm to pass through the cervix and uterus, and to enter into the oviduct. When the time of ovulation nears, the sperm in the oviduct are triggered to hyperactivate. The waves generated by the flagellum increase in height, but the beat becomes asymmetrical (reviewed in Suarez & Pacey, 2006).

If hyperactivated sperm are put on a microscope slide, this asymmetrical beating causes sperm to swim rapidly in zigzags or circles (Figure 1)–not the sort of behavior one would expect from a sperm that is about to fertilize an egg! A microscope slide, however, is not the same as the inside of the oviduct. In the oviduct, the sperm must swim through a narrow tunnel filled with a dense mucous secretion and lined by walls of multiple soft folds. Hyperactivation helps the sperm to move out of pockets formed by the folds, and to pass through the mucus. The egg

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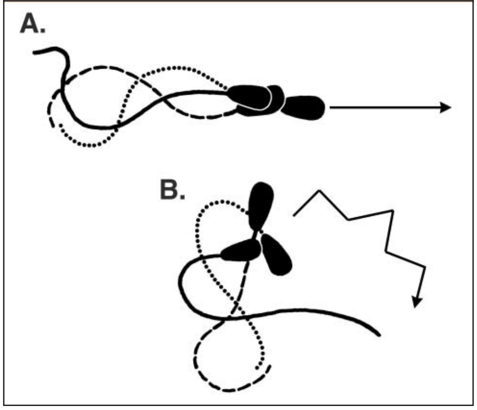
itself (oocyte) is embedded in a mass of cumulus cells and their viscous, elastic secretion (Figure 2). Immediately around the oocyte is an elastic protein shell called the zona pellucida. Thus, sperm must penetrate viscous and elastic substances in order to fertilize an egg. When artificial viscous and elastic substances are added to hyperactivated sperm on a microscope slide, it slows them down; however, they straighten out and penetrate it much more effectively than sperm that have not been hyperactivated (Suarez et al., 1991; Suarez & Dai, 1992). Also, if eggs are added to sperm in a dish, only sperm that are hyperactivated can penetrate the zona pellucida surrounding each oocyte (Stauss et al., 1995; Ren et al., 2001). These experiments indicate that hyperactivation helps sperm to reach the egg and fertilize it.

Figure 1.
Swimming patterns of pre-hyperactivated (A) and hyperactivated (B) bull sperm.



Some cases of human male infertility have been linked to poor hyperactivation of sperm (Chan et al., 2001). Recently, a mutant strain of mice was developed in which the sperm cannot hyperactivate. The mutant males are completely infertile, demonstrating that hyperactivation is absolutely required for fertilization (Carlson et al., 2003).

Sperm hyperactivate when exposed to a trigger that raises calcium levels in the flagellum (Suarez et al., 1993). The natural trigger that hyperactivates sperm in the oviducts is still unknown, but sperm can be hyperactivated by drugs that are known to increase intracellular calcium. One such drug is caffeine (Ho & Suarez, 2001). Caffeine can raise calcium levels by opening calcium channels in the plasma membrane of the sperm flagellum.

For this classroom activity, students will use caffeinated drinks or even pure caffeine to hyperactivate bull sperm. Bull sperm behave quite similarly to human sperm, thus they serve as an excellent model to use for these experiments. The main difference between bull sperm and human sperm is that bull sperm have paddle-shaped heads, while human sperm have conical heads.

This activity would ideally be undertaken during a unit on reproduction. Students should be familiar with female reproductive anatomy, sperm structure, fertilization, and microscope use. It could also fit nicely into a unit on cells, where the focus would be on the effect of caffeine on cells, or into a unit on human body systems, where students could consider the connections between the digestive system, circulatory system, and reproductive system that would allow some of the imbibed caffeine to reach sperm.

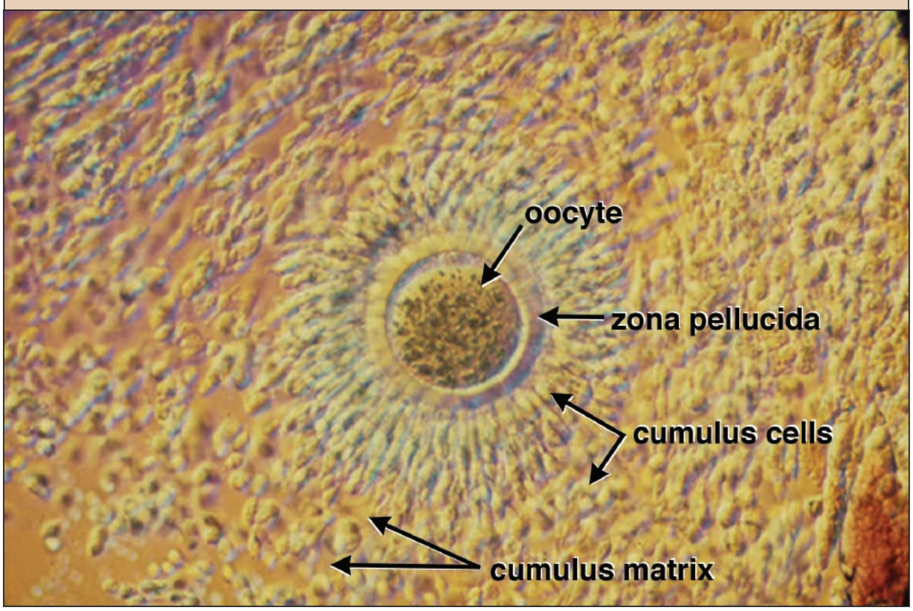
Introducing Students to the Experiment

Review female anatomy and the process of fertilization with students. Then, discuss the following concepts and questions.

1. How do sperm get to the egg? What organs do they pass through? What is it like inside these organs from the perspective of a sperm?
2. How does a sperm swim? What propels it forward?
3. What happens during fertilization?

A PowerPoint presentation on the subject of sperm movement through the female reproductive tract can be obtained from Suarez (sss7@cornell.edu).

Figure 2.
An egg from a rabbit, showing the oocyte, zona pellucida, and cumulus mass containing cells and viscoelastic matrix. The oocyte is approximately 100 microns in diameter.



Overview of Experiment

Working in pairs, students dilute bull sperm in a medium made of non-fat dry milk. The students then observe the sperm under a microscope to see their linear, progressive swimming movements. Next, students add coffee to a sample of sperm and see that it hyperactivates some and kills others, probably because organic chemicals in coffee other than caffeine are toxic to sperm. As a control, students treat sperm with decaffeinated coffee. Students can also treat sperm with a solution of pure caffeine, which should hyperactivate nearly all of them without killing any.

Materials

- Bull semen (West Hill RD-501, $24.00 at www.westhillbio.com). Order to arrive the day before the experiment and keep in the refrigerator.
- Carnation Instant Nonfat Dry Milk (we know that Carnation works; we cannot guarantee that another brand will)
- Eppendorf tubes (1.5 ml size) or plastic or glass test tubes
- distilled water
- hot plate or some device for keeping sperm and liquids at body temperature
- 1 M NaOH for adjusting pH and pH meter or pH paper for measuring pH
- thermometer for checking temperature of solutions
- plastic pipets
- microscope slides and cover slips (if they are to be re-used, clean thoroughly)
- microscope with 40X objective
- caffeinated drinks (coffee, tea, soda) and matching decaffeinated drinks to use as negative controls. Drinks high in caffeine work best and most coffees contain more caffeine than most teas. Mountain Dew contains more caffeine than colas.
- If using ground coffee: filter paper & funnel
- Pure caffeine can be purchased from Flinn Scientific (www.flinnsci.com), catalog number C0344.

Methods

1. Milk medium for sperm can be prepared one day ahead and stored in a refrigerator.

   Dissolve 9.58 grams of Carnation Instant Nonfat Dry Milk in distilled water and bring volume up to 100 ml. Increase the pH to 8.3 by adding 1 M NaOH drop-wise.

2. Coffee and other caffeinated drinks can be prepared one day ahead and stored in refrigerator.

   Coffee: Dissolve 1 tsp Folgers Instant Coffee to 30 ml of milk medium. Add 1 M NaOH drop-wise to pH 8.3. Do the same with decaffeinated coffee as a negative control. The higher the caffeine content, the better the results. A strong ground coffee, such as expresso, can be prepared by placing 1 tbsp ground coffee in a filter paper in a funnel. Pour boiling milk solution on the coffee. Collect filtrate and re-filter through the coffee a second time. Readjust pH to 8.3. For a negative control this can be done with a decaffeinated version of the coffee.

   Tea: Bring 30 ml of milk medium to a boil. Remove from heat and immediately add a bag of strong black tea. Allow it to brew for five minutes. Remove tea bag and readjust to pH 8.3. For a negative control this can be done with a decaffeinated version of the tea.

   Soda: Allow 30 ml of cola, Mountain Dew, or other caffeinated soda to go flat. Add 1 M NaOH to bring pH to 8.3.

3. Make a 40 mM solution of pure caffeine by dissolving 0.077 grams caffeine in 10 ml of milk medium.

4. Just before adding to sperm, warm milk and test solutions to 37° C (98.6° F). Also warm all tubes, pipets, slides, and coverslips.

5. To prepare sperm, add about 0.1 ml of the semen to 0.9 ml of pre-warmed milk medium in a tube. The remaining semen should be kept cooled for use later in the day or the next day. Once the sperm have been diluted in the warm milk medium for the experiment, they should be kept at 37° C in an incubator, on a hot plate, or in a warm water bath.

6. Stir up the sperm very gently by flicking the bottom of the tube. Using a plastic pipet, place a small drop of the sperm suspension onto a microscope slide. Gently cover with a coverslip.

7. Place the slide on the microscope stage and focus on the sperm using the 40X objective. The sperm are 70 microns long, so they are barely visible under a 10X objective. If the microscope is equipped with a substage diaphragm in addition to the field diaphragm, the sperm will be easier to see if the diaphragm is closed down to increase depth of field.

8. Describe the swimming patterns of the sperm and estimate by eye the percentage of sperm that are motile.

9. Place a small drop of warm coffee solution on a clean slide. Immediately add a small drop of sperm suspension into the drop of coffee solution and top with a coverslip.

10. Describe the swimming patterns of the sperm and estimate by eye the percentage of sperm that are motile.

11. Repeat Steps 9-10 using decaffeinated coffee. Compare the effect on sperm with that of caffeinated coffee.

12. If time permits, the effects of tea or caffeinated soft drinks can be compared with those of coffee (Are they as effective as coffee? Do they kill more or fewer sperm?). Repeat Steps 9-10 and use decaffeinated controls if available. Different groups of students could test different drinks.

13. If pure caffeine is to be used, which is highly recommended, repeat Steps 9-10 using the solution of caffeine in milk medium. For the negative control, use milk medium without caffeine.

Precautions

1. Sperm are delicate. It is important that they are kept warm for the duration of the experiment and handled gently. Those that will be used at a later time must be kept cool, according to the instructions provided with the package.

2. Whenever transferring sperm, students should pipet smoothly and slowly to avoid killing them.

3. Sperm are diluted in milk rather than in water in order to approximate the fluids encountered by sperm in the female reproductive tract. Adding plain water to sperm will kill them immediately.

4. Any container used for sperm, medium, or test substances should be very clean, rinsed free of any soap residue using tap water, given a thorough final rinse in distilled water, and then completely air dried before use.

5. Make sure that students are able to make wet mounts and focus a microscope using 40X objectives well before the start of the experiment. If they take more than a few minutes trying to find sperm because they are unfamiliar with using the microscope, the sperm may slow down or die.
6. If pure caffeine is used, note that it can be toxic to people if ingested in amounts greater than a few hundred milligrams.

Results

Prior to treatment with coffee or decaffeinated coffee, the sperm should be seen swimming in straight lines (Figure 1). After treatment with coffee, about half of the sperm die, due to other organic chemicals in the coffee. About half of the sperm that remain motile should be hyperactivating, which means they will swim rapidly in circles or show a zigzag movement pattern. While these patterns may not seem useful for getting to the egg, it should be pointed out that hyperactivated sperm can penetrate thick elastic substances around the egg. Other caffeinated and decaffeinated drinks will produce similar effects and the strength of the hyperactivation response will depend on the amount of caffeine present in the drink.

Treatment of sperm with decaffeinated drinks serves as a negative control to demonstrate that it is the caffeine in the drinks that causes hyperactivation, while the other ingredients kill sperm. The same percentage of sperm should be killed as those treated with caffeinated drink; however, the sperm remaining motile will continue swimming in straight lines.

Treatment with pure caffeine will hyperactivate most of the sperm without killing any of them. A movie of fully hyperactivated bull sperm can be seen online as a supplemental file to a published scientific paper by Ignotz and Suarez (2005). The Web address is: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15878888&query_hl=1&itool=pubmed_docsum.

Discussion

In class, the following questions could be discussed:

1. How did you describe the swimming patterns of the sperm?
2. How did the swimming patterns change when caffeine was added?
3. What were the experimental controls? What was the purpose of the controls? Were these the best possible controls?
4. What could be the purpose of these different swimming patterns?
5. If a woman drank a strong cup of coffee while sperm were in her oviduct, what route would the caffeine take to reach the sperm?
6. What could happen if a man drank a strong cup of coffee?

During the discussion, students should be reminded that caffeine is a drug that simulates whatever it is that actually hyperactivates sperm during the natural fertilization process. The natural trigger for hyperactivation is yet to be discovered. Realistically, very little caffeine from a cup of coffee would make it to the sperm in the reproductive tract. The other organic chemicals in the drinks that kill sperm are unlikely to kill sperm in the body, because most will be broken down by digestive enzymes. Caffeine is a small molecule that is not quickly destroyed by digestive enzymes.

During the class discussion or in the lab write-up, students could be given the opportunity to design their own experiment. If students have seen other swimming microorganisms under the microscope in the past, they could compare the anatomy and behavior of those organisms to the swimming of sperm.

Acknowledgment

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APPENDIX. Handout

Give Your Bull Sperm a Pick-Me-Up!

Introduction

In this lab you and a partner will examine bull sperm and see how caffeine can affect it. What does caffeine do to you when you ingest it? What might it do to sperm? Before reading the lab, answer the following questions:

1. Describe the path taken by sperm through the mammalian female reproductive tract to reach the egg (including humans). What organs do the sperm pass through?

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   ____

2. What do you think will happen when caffeine is added to sperm?

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3. How could caffeine get from the digestive system into the reproductive system?

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Materials

bull semen
hot plate, incubator, or warm water bath
thermometer
plastic tubes
plastic pipets
slides and cover slips
microscope with 40X objective
milk medium for sperm
caffeinated coffee
decaffeinated coffee

Procedure

1. Warm milk and all test solutions to 37° C (98.6° F).
2. Add 0.1 ml semen to 0.9 ml of pre-warmed milk medium in a tube. *The sperm will work best if it is kept warm, treated gently, and protected from light as much as possible. Perform all tests as soon as possible after warming sperm.* Return the unused portion to the refrigerator without allowing it to warm up.
3. Using a plastic pipet, place a small drop of the sperm suspension onto a microscope slide. Gently cover with a coverslip.
4. Quickly place the slide under a microscope and try to find sperm using the 40X objective. If you have trouble finding sperm, go to the edge of the coverslip and focus on the glass edge of the coverslip. This will put you at the right focal length for seeing sperm. Use the table below to record your observations.
5. Place a small drop of warm coffee-in-milk solution on a slide and immediately add a small drop of sperm suspension into this drop. Gently cover with a coverslip.
6. Quickly examine the sperm using the 40X objective on your microscope. Note your observations in the table below.
7. Repeat Steps 5 and 6 using decaffeinated coffee. Compare the effect on sperm with that of caffeinated coffee and write your observations in the table.
8. Repeat Steps 5 and 6 using other caffeinated and decaffeinated drinks or a solution of pure caffeine.

Treatment	Approximate percentage of sperm that are moving	Swimming patterns of moving sperm
milk medium alone		
coffee		
decaffeinated coffee		
caffeine		

Discussion

After competing the experiment, answer the following questions

1. What effect did coffee have on sperm? How do you know it was the caffeine in the coffee that had this effect?

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2. What was the control in this experiment?

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3. Was it an appropriate control? If not, what would be a better control?

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   ____

4. What ingredients in the drinks might have killed sperm?

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   ____

5. Why was the sperm diluted in milk? What might happen if it was diluted in water?

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6. Describe an experiment that you would do to find out if drinking caffeine can really affect sperm movement in an animal.

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