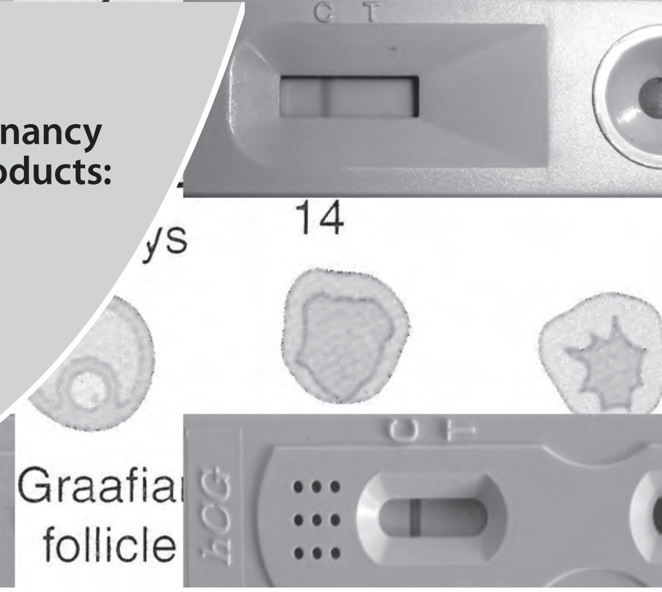
INQUIRY & INVESTIGATION

Exploring Ovulation & Pregnancy Using Over-the-Counter Products: A Novel Guided Inquiry



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Abstract

In this guided inquiry, students explore the complex hormonal regulation of the female reproductive cycle using inexpensive ovulation and pregnancy detection kits that are readily available over the counter. This hands-on activity engages students in the practice of doing science as highlighted by the National Science Education Standards. *The laboratory approach described is an effective alternative or complement to traditional lecture presentations of this challenging topic. The laboratory activities described are appropriate for both college and high school students, as reproductive physiology is covered in a wide variety of curricula.*

Key Words: *Menstrual cycle; ovulation; pregnancy; luteinizing hormone; human chorionic gonadotropin; guided inquiry.*

Reproductive physiology and human development are common topics in biology courses at many levels. Discussions of the hormonal regulation of the female reproductive cycle and the early stages of human development are included in the curricula and textbooks of high school biology courses, anatomy and physiology courses, and introductory biology courses for majors and nonmajors in both 2-year and 4-year colleges. In a survey of high school biology teachers, Finley et al. (1982) identified the hormonal control of reproduction as among the 10 most difficult topics to teach in biology. In a more recent survey, Çimer (2012) concluded that the endocrine system and hormones were among the five topics that students find the most difficult to learn. Our own experience teaching introductory college biology courses for majors and nonmajors confirms that this complicated topic is challenging for students.

Understanding the hormonal regulation of the menstrual cycle is important and has many clinical applications.

○ Regulation of the Female Reproductive Cycle

The regulation of the female reproductive cycle, the menstrual cycle, is complex and controlled by the female sex hormones estrogen (E), progesterone (P), luteinizing hormone (LH), and follicle stimulating hormone (FSH) (see Figure 1). Throughout the cycle these hormone levels rise and fall, initiating changes in the ovaries and uterus (Jones & Lopez, 2006). The average menstrual cycle lasts 28 days, with cycle day 1 (CD 1) marked by the onset of menses. Ovulation typically occurs midway through the menstrual cycle and is triggered by rising LH levels. The LH levels rise for ~36 hours, beginning around CD 11. This is known as the "LH surge." Typically on CD 13, the LH surge rises to a peak, causing resumption of meiosis and ovulation of the Graafian follicle. Following ovulation, the collapsed Graafian follicle forms a yellow glandular body, the corpus luteum. The main secretion of the corpus luteum is P, which stimulates the secretion of uterine glands and prepares the inner lining of the uterus, the endometrium, for pregnancy. If the oocyte is fertilized by a sperm, the resulting blastocyst will implant in the endometrium and the hormone human chorionic gonadotropin (hCG) is secreted by the syncytial trophoblast. The hormone hCG maintains the corpus luteum and the continued secretion of P and E. The cells of the syncytial trophoblast will form the placenta and continue to secrete hCG. During pregnancy menses does not occur, leaving the endometrium intact. If fertilization does not occur, the corpus luteum degenerates, leading to a decline in E and P and the onset of menses.

Understanding the hormonal regulation of the menstrual cycle is important and has many clinical applications, particularly in the field of fertility/infertility services. This knowledge forms a basis for many modes of contraception and for practices utilized in fertility clinics. This understanding is of particular importance because the incidence of infertility and use of assisted reproductive technologies are on the rise in the United States (Centers for Disease Control, 2008, 2009). Disruption or alteration of the hormones that control reproductive function can also affect other organ systems and physiological processes. The significance of these concepts in reproductive physiology has been recognized by science educators. The hormonal regulation of reproduction is included in the curricular guidelines and learning outcomes of the Human Anatomy and Physiology Society (HAPS, 2005), an organization dedicated to enhancing the quality of education in anatomy and

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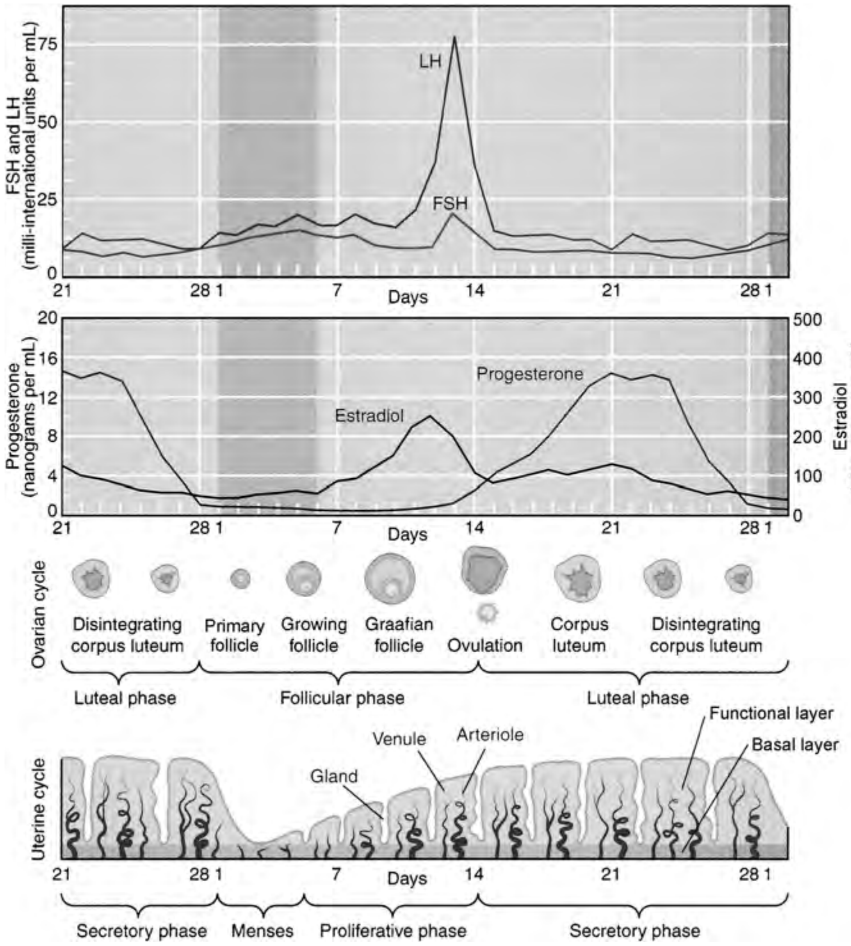


Figure 1. Hormone fluctuations during a 28-day menstrual cycle. Estradiol is the main estrogen. This figure was published in Jones & Lopez (2006, p. 78) and is reproduced here by permission of the copyright holder, Elsevier.

physiology. The proposed *Next Generation Science Standards* (National Research Council, 2012) specifically advocate as a core idea that students understand the form and function of organisms that facilitate life processes, growth, and reproduction. By addressing the clinical applications of reproductive physiology, students will meet the national science education standard that encourages them to view "science in personal and social perspectives" (National Research Council, 1996).

○ The Laboratory Investigation: Guided Inquiry

The complex regulation of the female reproductive cycle is typically taught in a traditional lecture by presenting hormonal changes and their effects on the ovaries and uterus in graphical form similar to Figure 1. These concepts are seldom explored in the laboratory or using an active-learning approach, particularly in an introductory course. As reviewed by Wood (2009), the traditional, passive teaching methods used in most high schools and colleges have significant shortcomings that can be overcome by promoting active learning through hands-on experiences. We propose that this challenging topic can be better addressed through an active-learning technique that uses guided inquiry to engage students and that more closely models how scientists learn about the natural world.

In this laboratory investigation, students use guided inquiry to examine key aspects of reproductive physiology using inexpensive, readily available ovulation and pregnancy kits. The investigation is easy to perform and is adaptable to a variety of levels and settings. The laboratory contains two guided-inquiry scenarios. In scenario 1, the ovulation scenario, the class is provided with six simulated urine samples prepared by the instructor that represent different days of the menstrual cycle (CD 10–CD 15). Using ovulation kits, students determine whether LH is present or absent in the six different urine samples. Students interpret their results to ascertain on which cycle day the LH surge occurs so that they can predict the day of ovulation. In scenario 2, the pregnancy scenario, the students will use a pregnancy kit to test for hCG in an unknown simulated urine sample. Students will interpret their results to determine whether their patient is pregnant.

The guided inquiry is designed to be performed in groups of three or four students, recognizing that students can learn from each other and that discussion and reflection are crucial features of the learning process. The investigation engages students in the practice of science. The guided inquiry begins with an initial observation that asks students to consider their prior knowledge. Students then collaborate in groups to arrive at a group hypothesis. Next, groups are asked to predict the outcomes. The hypothesis and prediction are typically lacking in traditional "cookbook labs" and are critical in making the transformation to inquiry-based science. These steps allow students to think critically and provide the yardstick for students to measure their understanding in future self-assessment. Students then proceed to test their hypothesis by collecting data. This is followed by data analysis and interpretation. Discussion then follows in which students have the opportunity to present their results, assess their understanding, evaluate their original predictions, and revise their hypotheses. The importance of providing students with opportunities to practice scientific inquiry is underscored in the *National Science Education Standards* (National Research Council, 1996, 2012).

Student Profile

This investigation is conducted in Anatomy and Physiology II, an introductory course for health science, nursing, and exercise science majors. This 4-credit course meets for 3 hours of lecture and one 3-hour laboratory each week. Most students typically take the Anatomy and Physiology I and II sequence during their freshman or sophomore year. Approximately 450 students enroll in this course each academic year.

Learning Objectives

After completing the guided inquiry, students will be able to

1. Describe the hormonal control of ovulation and predict how LH levels change during various days of the cycle.

2. Explain the endocrine role of the placenta during pregnancy and compare hCG levels in pregnant versus nonpregnant women.
3. Judge the validity of an ovulation and pregnancy test and analyze and interpret the results.
4. Propose hypotheses, develop predictions, conduct and interpret experiments, and defend conclusions.
5. Present results, evaluate predictions, and revise hypotheses.
6. Apply their understanding of the regulation of the female reproductive cycle to new cases and problems.

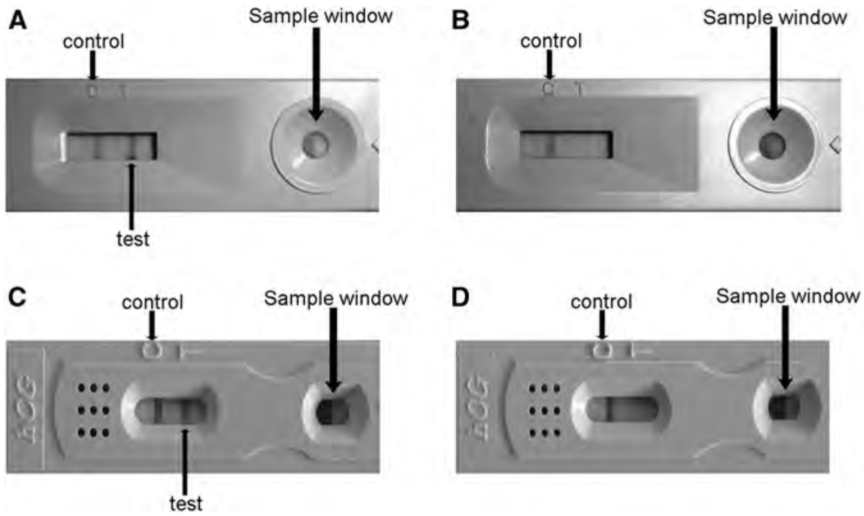


Figure 2. Interpretation of ovulation and pregnancy kits. Ovulation results are shown in panels A and B. (A) The two bands, control and test, indicate a valid test positive for LH. (B) The single control band indicates a valid test negative for LH. Pregnancy results are shown in panels C and D. (C) Two bands, a control and test, indicate a valid test positive for hCG (pregnant). (D) The single control band indicates a valid test negative for hCG (not pregnant).

○ Laboratory Materials & Preparation

Urine Samples

Simulated urine samples were prepared by mixing 200 mL of fresh 0.1% bovine serum albumin with 1 drop of yellow food coloring. All samples were prepared the day prior to the laboratory and stored in the refrigerator, except that hormone stock solutions were stored at –20°C. Before preparing urine samples, verify hormone sensitivity for the pregnancy and ovulation kits to be used in the experiment. The sensitivity of the ovulation and pregnancy kits used in our laboratories were 20 mIU/mL. We have found that hormone concentrations well above the test kit's sensitivity were necessary to achieve positive results. This could be attributed to several factors, such as simulated versus real urine samples, pH, age of LH, etc. For best results, hormones should be added to the simulated urine samples on the day when they will be utilized for the laboratory activity. If simulated urine samples are prepared in advance, it may be necessary to spike the urine with a few microliters of the stock hormone solution prior to use. The instructor should verify a positive result with each urine sample before distributing samples to students.

Hormone	Source
Luteinizing hormone	EMD Millipore, emdmillipore.com Product number: 869003 – 10 µg Cost: $89.00
Human chorionic gonadotropin	EMD Millipore, emdmillipore.com Product number: 230734 – 1 mg Cost: $39.00

Scenario 1

To predict ovulation, LH was purchased from EMD Millipore (869003-10 µg) and reconstituted with 1 mL of sterile, distilled water to obtain a 0.05 IU/µL stock solution. Ten milliliters of simulated urine was added to a urine sample cup and 16 µL (~800 mIU) of the LH stock was added and mixed for a final concentration of 80 mIU/mL. Label this sample cup "CD13." In this scenario, CD13 is the expected day for the LH surge to rise to peak. This sample will yield a positive result when tested with the ovulation kit (Figure 2A). Obtain 5 additional urine sample cups and add 15 mL of simulated urine to each cup. Label the first cup "CD10," the second "CD11," the third "CD12," the fourth "CD14," and the fifth "CD15." These samples will yield a negative result when tested with the ovulation kit (Figure 2B).

Scenario 2

For pregnancy detection, 20 mL of simulated urine was added to a urine-sample collection container. We purchased hCG from EMD Millipore (230734-1 MG) and reconstituted with 1 mL of sterile, distilled water to obtain a 3 IU/µL stock solution. Five microliters of the hCG stock (~15 mIU) was added to the 20 mL of simulated urine and mixed, for a final concentration of 750 mIU hCG/mL. Label the sample cup "pregnancy detection." This sample will yield a positive result when tested with the pregnancy kit (Figure 2C). A negative result (not pregnant) is shown in Figure 2D.

Detection Kits

Both pregnancy and ovulation kits are readily available from a wide variety of sources, including scientific vendors, pharmacies/drug stores, and discount stores. The cost can be as low as $1 per kit. We typically divide each laboratory section into six groups of three or four students. Each group will require one pregnancy kit and one ovulation kit to complete the activity. Extra kits should be purchased to test simulated urine samples and serve as reserves in the event that any kits are invalid. Directions for using the kit are straightforward and are included with the packaging. Most brands of kits contain either a disposable pipette for transferring the urine sample and a testing cassette or a "dipstick" to submerge in a urine sample. Because the directions vary according to the manufacturer, they are not included here. Directions for each kit are distributed to each group.

○ Guided Inquiry Scenario 1: Predicting Ovulation

Note: Scenario 1 of this laboratory activity should be prefaced with an introduction to the female reproductive cycle and its hormonal regulation (Figure 1). The following worksheet is provided to the students for scenario 1:

OBSERVATION: Dr. Stork, chief of the Fertility Department at General Hospital, is counseling her patients, Jack and Jill, a newly married couple. They are ready to start a family and are seeking Dr. Stork's advice as to the best time to conceive a child. Dr. Stork has determined that Jill has a normal 28-day menstrual cycle. How can Dr. Stork help?

HYPOTHESIS: If Jill's menstrual cycles are normal, how can Dr. Stork determine when she will ovulate? Students are directed to discuss this question with their group members, generate a hypothesis, and write it in the box.

GROUP HYPOTHESIS:

PREDICTION: Make a prediction based on your hypothesis. When will Jill's LH level be highest? On which cycle day will Jill ovulate? Students are directed to discuss these questions with their group members and write their predictions in the box.

GROUP PREDICTIONS:	
Highest LH:	CD of ovulation:

Testing Your Hypothesis: The Experiment

Levels of LH can be detected in urine. Dr. Stork has instructed Jill to collect a urine sample between 4:00 and 5:00 pm on cycle days 10, 11, 12, 13, 14, and 15. For each urine sample, an ovulation test will be performed to detect the presence of LH, determine whether the LH surge is occurring, and when LH is at its peak. LH will be detected using commercially available ovulation kits. Follow the instructions included with the kit.

1. Obtain one of Jill's urine samples and record the cycle day. Urine Cycle Day: ______
2. Remove the ovulation test kit from the pouch. Determine whether LH is detectable in your urine sample by following the directions in your ovulation kit.
3. Record your results in Table 1 for each sample. (Note: You will have to obtain data of other cycle days from other groups.) For test validation, indicate whether the control band was present (+) or absent (–). For each urine sample, indicate whether a test band was present (+) or absent (–) and describe the intensity of the test band compared with the control band. Additionally, draw a diagram of the results window in the last column. Place your values in Table 1.

Analysis & Interpretation

Interpret your results as shown in Table 2. Record your interpretations in Table 1.

Table 1. Data table for ovulation test results.

Cycle Day	Control Band (+ or –)	Test Band (+ or –)	Test Band Intensity (Darkness)	Results Window	Interpretation: No LH Surge, LH Surge, or Invalid
CD10				C T	
CD11				C T	
CD12				C T	
CD13				C T	
CD14				C T	
CD15				C T	

Conclusions

Students are directed to discuss the following questions with their group.

1. Were the ovulation tests valid? How do you know?
2. On which cycle day(s) was LH present? How do you know?
3. On which cycle day did the LH surge rise to its peak?
4. On which cycle day will Jill ovulate? How do you know?
5. Consider your original hypothesis. Is it supported by the experimental evidence? Does it need to be modified or discarded?

○ Guided Inquiry Scenario 2: Pregnancy Detection

The steps below are distributed to students on a worksheet.

OBSERVATION: Dr. Stork has determined a conception timeline for Jack and Jill. They follow Dr. Stork's advice and return for a follow-up appointment because Jill's menstrual period is 3 weeks late. Jill has provided Dr. Stork with a urine sample, and it's your job to test the urine and determine whether Jill is pregnant.

HYPOTHESIS: How can Dr. Stork determine whether Jill is pregnant? Discuss this question and place your hypothesis in the box.

GROUP HYPOTHESIS:

PREDICTION: Make a prediction based on your hypothesis. Will hCG be detected in Jill's urine if she is pregnant? If she is not pregnant?

GROUP PREDICTIONS:	Will hCG be present in urine if pregnant?
	Will hCG be present in urine if not pregnant?

Testing Your Hypothesis: The Experiment

1. Obtain the urine sample labeled "pregnancy detection."
2. Remove the pregnancy test kit from the pouch. Determine whether Jill is pregnant by following the directions in the kit.
3. Record your results in Table 3. For test validation, indicate whether the control band was present (+) or absent (–). Indicate whether a test band was present (+) or absent (–) and describe the intensity of the test band compared with the control band. Additionally, draw a diagram of the results window in the last column.

Analysis & Interpretation

Students are directed to discuss the following questions with their group.

1. Is the pregnancy test valid? How do you know?
2. Is Jill pregnant? How do you know?
3. Consider your original hypothesis. Is it supported by the experimental evidence? Does it need to be modified or discarded?

Table 2. Interpreting ovulation test.

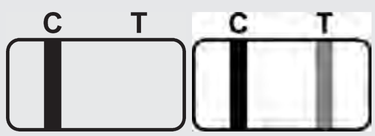
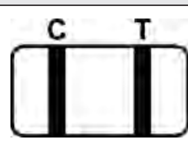
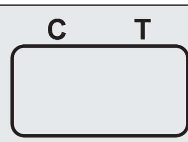
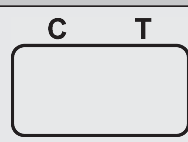
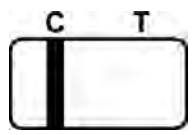
Interpretation	Appearance	Results Window
No LH surge	Only one control band (C) or test band (T) is lighter than control	
LH surge	Two bands are visible, and test band (T) is equal to or darker than control band (C)	
Invalid test	No bands present	

Table 3. Data table for pregnancy test results.

Urine Sample	Control Band (+ or –)	Test Band (+ or –)	Test Band Intensity	Results Window
3 weeks after missed menstruation				

Extending Your Understanding

Jill took an over-the-counter pregnancy test on CD 16. The results are shown below.



1. What hormone is this over-the-counter pregnancy test detecting?
2. Based on these results, is Jill pregnant?
3. How do these results compare to the results you obtained in lab? Explain any discrepancies.

○ Implementation & Application

Student groups can present their results, interpretations, and conclusions to the class. We have found that a video projector is a useful tool for students to project their cassettes and dipsticks and to share their results. Students are asked to examine and re-evaluate their hypotheses. The entire activity takes about 1 hour to complete.

To extend this inquiry and provide opportunities for students to apply their understanding to new case studies and problems, alternative scenarios can be generated. Scenarios in which the length of the menstrual cycle varies from the normal 28 days may be useful for reinforcing the role of LH with respect to ovulation. Additionally, pathology-based scenarios could be presented to demonstrate the effects of altered hormone levels on ovulation and pregnancy. For example, LH could be absent from Jill's urine samples from all cycle days. This could lead to a class discussion of anovulation, its physiological basis, and student groups could then discuss possible treatments for Jill, assuming she wants to become pregnant.

Other variations include providing each student group with a complete set of all simulated urine samples. This would allow the instructor to spike the urine samples differently, such that each group would obtain different ovulation and pregnancy results. Again, each student group would present their findings at the conclusion of the experiments. This approach could be used to introduce the class to cycle variations, each urine series representing a different cycle length. This option would be more expensive, because each group would need six ovulation kits and one pregnancy kit.

○ Assessment of the Guided Inquiry

Students responded positively to this laboratory. During the activities, they remained engaged in the inquiry process and were very interested in the outcomes of the experiments. Class discussions were lively, and these activities generated student questions. In a sample of 99 students, 78% could correctly answer exam questions that required a comprehension of the hormonal regulation of ovulation and the endocrine role of the placenta. Eighty-eight percent of students were able to correctly evaluate the validity of pregnancy and/or ovulation tests and could correctly interpret the results. These assessments demonstrate that the learning objectives were met by most students.

○ Conclusion

This guided inquiry provides a novel approach to teaching a difficult topic. These activities employ active learning and allow students to engage in a meaningful practice of science. They have been a useful addition to our reproduction lab in anatomy and physiology. Traditionally, these reproduction labs have focused on anatomy and these activities provide an easy, inexpensive way to introduce physiology into the study of human reproduction. Moreover, by working in groups with this type of guided inquiry, students develop hypotheses and predictions, perform experiments, and collect and analyze data. Implementation of these activities will advance students' understanding of reproductive physiology, engage them in the process of scientific inquiry, and help prepare them to make better health decisions.

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