Name: _____

CASE

Activity 6.2.2 Evaluating Viability

Purpose

Producers who utilize artificial insemination and embryo transfer take several precautions to ensure the fertility, viability, and quality of future offspring. When breeding, producers monitor females carefully for signs of estrus and often synchronize females to time the estrus cycle. Producers collect and extend semen by adding sterile nutrients and solution. The semen is then refrigerated or frozen, and shipped around the world.

The process of semen collection and distribution is complex. Mishandling of the semen can cause viability problems. Many semen samples are evaluated for quality at various points throughout the process. While no tests can completely ensure viability and fertilization, there are evaluations that demonstrate definite problems.

The most common evaluations of semen include sperm motility, morphology, dead-alive percentages, and overall concentration of sperm in the semen. Undesirable sperm cells are present in nearly all semen. Quality is determined by low percentages of abnormal and dead sperm and a high concentration of sperm in the semen.

Sperm motility is a subjective measure of the percentage of progressively motile or forward moving sperm. Often evaluators will find sperm swimming in circles or not moving; these sperm have a very low chance of fertilizing an egg. Progressively motile sperm will swim in a relatively straight line and may disappear quickly from the field of view on your microscope.

To determine the percentage of dead versus alive cells and to observe the morphology of sperm, scientists stain and count sperm cells. Cells that absorb stain at the base of the head are dead, whereas cells that do not absorb stain are alive. After staining, scientists observe and count abnormalities in the cell structure using the same sample. Remember a cell does not have to be dead to be abnormal and many dead cells may be normal in their appearance.

Concentration is determined by counting the number of sperm cells in a given volume. Typically, the semen is further diluted to perform the count, as the concentration of cells in an undiluted sample may be too high to count. It would be difficult to see each cell. Thus, the sample is diluted, the concentration determined, and a mathematical formula is used to calculate the actual concentration of the semen. Use your laboratory skills to evaluate the quality of semen samples.

Materials

Per class:

- Sample of boar semen
- Eosin-aniline blue stain
- Hemocytometer solution
- Sodium citrate buffer
- Water bath
- Slide warmer

Per pair of students:

- Sample of bull semen
- Microscope
- 6 slides
- 4 cover slips
- 6 pipets
- 2 ruled microscope slides
- 6 small wooden sticks
- 2 petri dishes

Per student:

- 2 pair disposable gloves
- Pencil
- *Agriscience Notebook*

Procedure

Working with your partner, you will prepare and examine a series of slides to determine the motility, morphology, and concentration of two samples of commercially available semen. Take turns preparing and analyzing slides. Prepare your slides with care and caution, but work quickly as sperm cannot survive for long periods at room temperature.

Part One – Motility Evaluation

1. Place one drop of sodium citrate buffer on a clean, warm slide.
2. Using one small wooden stick, place a very small amount of semen on the drop of buffer.
3. Cover the semen-buffer drop with a warm cover slip.
4. Examine the drop with the low magnification setting on your microscope. You should see movement throughout the specimen.
5. Increase the magnification of your microscope to 40x.
6. Observe the sperm for movement and estimate the percentage of motile sperm. Record the results in Table 3 of *Activity 6.2.2 Student Worksheet.*
7. Work quickly as heat, light, and cold affects motility.
8. When finished, set slide aside for later cleanup and move on to Part Two.

Part Two – Dead/Alive Evaluation

1. Place one drop of eosin-aniline stain approximately one-third from the end of a clean, warm slide.
2. Use a wooden stick to place a small amount of semen into the stain and mix using two or three circular motions.
3. Hold a second slide at approximately a 45° angle and slowly push the second slide through the stain and across the first slide while pressing firmly down.
4. Dry the stain quickly by placing on a warming plate or waving it back and forth in the air.
5. Examine the slide under the microscope, starting at 40x magnification and increasing to 100x. Dead cells will have absorbed stain at the base of the head whereas alive cells will have excluded the stain.
6. Count at least 100 sperm and record the number of dead versus alive cells in Table 1 of *Activity 6.2.2 Student Worksheet*.
7. Calculate the percent stained or dead and record on Table 3.

Part Three– Morphology Evaluation

1. Using the slide prepared in Part Two, examine the cells for abnormalities.
2. Observe a minimum of 100 sperm cells, recording abnormal cells in Table 1.
3. Abnormalities include:
    - Misshapen head
    - Bent or misshapen midpiece

- Tail abnormalities
- Droplets

4. Calculate the percentage of abnormal sperm and record in Table 3 on your worksheet.
5. Set slide aside for later cleanup and move on to Part Four.

Part Four – Sperm Concentration

1. Dilute the sperm cells.
    - Using a pipet, place nine drops of hemocytometer solution in your petri dish.
    - Using a clean pipet, place one drop of semen into the solution and mix with a wooden stick. This produces a 1:10 dilution.
    - Using the first pipet, place nine drops of hemocytometer solution in the other half of your petri dish.
    - Add one drop of the diluted semen from the first dish.
    - Mix again. This produces a 1:100 dilution.
2. Using a clean pipet, transfer approximately three drops of your dilution onto the ruled portion of the ruled microscope slide, covering the area evenly.
3. Cover with a cover slip without applying excess pressure.
4. Allow the dilution to settle for a moment.
5. Observe under the microscope, increasing your power carefully to avoid harming or pressing down on the surface of the slide.
6. When you reach 100x, magnification, count the number of sperm cells in ten of the ruled boxes on the slide. Select the squares at random, do not duplicate or use connected squares. Count each sperm cell that is within the cell or touching the top or left side of the square.
7. Calculate the average number of sperm per cell by adding the count of the ten squares together and dividing by ten.
    - $Avg\ sperm\ cells\ per\ square = (Sum\ of\ 1\ thru\ 10)/10$
8. Calculate the concentration of sperm cells per milliliter of diluted semen.
    - Formula – $\frac{Avg.\#\ sperm/square}{0.0004ml/sqaure} = \frac{x\ sperm}{1ml}$
    - $x = sperm\ cells/ml\ of\ diluted\ semen$
9. Calculate the concentration of sperm cells in the undiluted sample.
    - Formula – $Concentration\ of\ diluted\ semen/ml \times 100\ (dilution\ rate)$
10. Record all calculations in Table 2 and Table 3 of *Activity 6.2.2 Student Worksheet*.

Part Five– Boar Semen Evaluation

1. Repeat Part One through Part Four using the sample of boar semen provided by your teacher. Record your results on *Activity 6.2.2 Student Worksheet*.
2. Clean up your work area as instructed by your instructor.

Conclusion

1. Why is it important to determine sperm motility first?

2. What physical differences in bull and boar semen did you observe?

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Activity 6.2.2 Student Worksheet

Directions: Record your results from the slides you have prepared in the tables below.

Table 1. ***Data***

Bull Semen Data				Boar Semen Data			
Dead Cells	**Alive Cells**	**Normal Cells**	**Abnormal Cells**	**Dead Cells**	**Alive Cells**	**Normal Cells**	**Abnormal Cells**

Table 2. ***Determining Semen Concentration***

Formulas	**Bull Semen Sample**	**Boar Semen Sample**
Sperm/ml (diluted concentration)		
$\frac{Avg.\#\ sperm/square}{0.0004ml/sqaure} = \frac{x\ sperm}{1ml}$		
Sperm/ml (semen concentration)		
$Concentration\ of\ diluted\ semen/ml \times 100\ (dilution\ rate)$		

Table 3. ***Results***

	% Motile	**% Dead**	**% Alive**	**% Abnormal**	**Concentration/ml**
Bull Semen					
Boar Semen					