Counting Spermatozoa - Part 1

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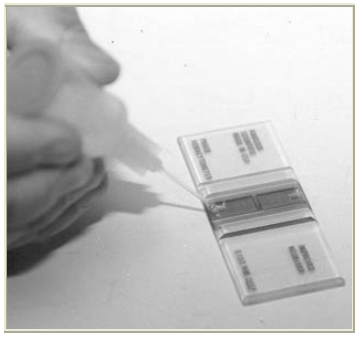
A hemacytometer, originally designed to count blood cells, has been used for many years to count other types of cells. Toward the end of World War II, with the advent of artificial insemination in cattle, it became painfully obvious that other methods of counting spermatozoa had to be developed, because of the length of time it required for an accurate count to be obtained with the hemacytometer.



Since the late 1930's numerous scientific papers have been published describing how to use the hemacytometer to calibrate other instruments to estimate the number of spermatozoa more rapidly in a sample of semen than is possible with the hemacytometer. Consequently, today (2004), the number of spermatozoa are estimated in bull, boar, stallion, chicken, turkey and dog semen using electronic methods. However, the initial calibration of these instruments is done with a hemacytometer. In general, for an accurate calibration it requires 70 normal, clean semen samples ranging in count, per milliliter of semen, from 50 million to 1 billion, depending upon the species. Regardless of species, the important point is that the sperm concentration of the 70 ejaculates must be equally distributed over the range of expected counts regardless of frequency of collection of semen. Unfortunately, it requires 8 to 10 counts of each of the 70 samples to obtain an average count that is highly accurate. Of course a "quick and dirty" estimate can be made with fewer counts, but is not satisfactory for scientific purposes or any other purpose when accuracy is essential.

During the calibration procedure the same samples used for the hemacytometer counts are placed in the electronic instrument to be calibrated. Once the 70 samples have been through the process, a regression equation is "fitted", then the electronic instrument is used to estimate the number of spermatozoa per milliliter of semen. The electronic method provides a quick, simple, repeatable method, provided the semen sample is "clean". The presence of gel in the ejaculates of stallion or boar semen will cause the electronic instrument to estimate a larger number of spermatozoa than are actually present. The same is true of my sample containing extraneous material such as dirt, hair or cells other than spermatozoa. Consequently, it is not uncommon for an appropriately calibrated electronic instrument to overestimate the number of spermatozoa in a series of unselected semen samples, particularly if the electronic instrument in question was calibrated with a hemacytometer with too few samples selected over a narrow range, or if there were a majority of samples within a narrow range. To further complicate matters, the individual counting spermatozoa must have a good, appropriately adjusted phase-contrast microscope and be skilled in utilizing the counting procedure, as well as the instrument.

The method of counting spermatozoa with a hemacytometer is based upon the principle that the hemacytometer is loaded with a constant volume of semen between the cover slip and counting grid, or chamber. Accurate counts can be obtained with this method when used properly.

It is highly unlikely that a breeding farm would consider using a hemacytometer on a routine basis due to:

a) the number of chambers that must be counted for reasonable accuracy,
b) the time required to prepare the sample for counting with the hemacytometer,
c) the time required to count the number of spermatozoa in the appropriate number of squares,
d) procedure for counting the spermatozoa,
e) preparing and counting a sample of semen requires considerable technical skill and equipment.

However, the hemacytometer is very useful in counting semen samples that have been placed in an extender and those that are contaminated with debris.

Counting Spermatozoa - Part 2

Return to to part 1

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In Part 1 five factors were listed that significantly influence the use of a hemacytometer to count the number of spermatozoa in a semen sample. They are as follows:

a) The number of chambers that must be counted for reasonable accuracy.

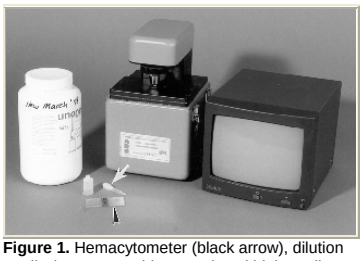

Figure 1. Hemacytometer (black arrow), dilution media (Unopette, white arrow) and high quality, phase-contrast microscope used to determine concentration of spermatozoa.

Considering all the variables associated with hemacytometer counts, it was statistically determined that a minimum of eight chambers should be counted to determine a mean accurate count for a single sample of semen. In the event any one of the eight counts is 10% or more above or below the mean, that count should be eliminated and another count made. Under these circumstances, the accuracy of the hemacytometer is superior, in general, to an electronic method, but the precision (or deviation) is less with the electronic counter, because there are fewer variables in preparing the semen sample for counting. Further, the human (technician) error involved in preparing a sample for electronically counting is relatively minimal compared to preparing a sample for counting in the hemacytometer.

2b) The time required to prepare a sample for counting with the hemacytometer.

Figure 2. Puncturing Unopette reservoir using the pointed shield.

In recent years a system for counting cells with a hemacytometer (Unopette) has been developed (Becton-Dickinson, Rutherford, NJ) that has certainly simplified the older methods, which utilized a white or red blood cell pipette.

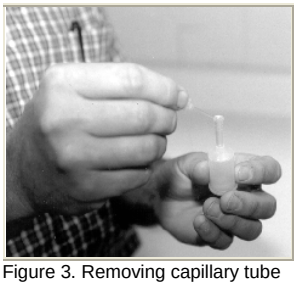

Figure 3. Removing capillary tube from the pointed shield, which will be used to load an appropriate volume of raw semen for an accurate hemacytometer count.

Presented in Figure 1 is the equipment necessary for utilizing this newer method of counting spermatozoa. Once the semen sample has been collected and thoroughly mixed, a sub-sample is taken for ease of sampling. The Unopette is used to puncture the plug of the reservoir with the pointed end of the plastic shield (Figure 2). With the shield in place, and serving as a cap for the reservoir, remove the capillary tube (Figure 3) Submerge the capillary tube in a thoroughly mixed sample of raw semen (Figure 4). Allow semen to fill the capillary tube, then remove it from the sample. Wipe excess semen from the outside of the capillary tube, being careful not to remove any semen from within the capillary tube. Remove cap and lightly squeeze the reservoir between thumb and forefinger. Place pipette into the reservoir and release the pressure (Figure 5), this allows semen to be siphoned into the reservoir. Rinse the capillary tube by gently squeezing the reservoir several times without forcing fluid out of the top. Remove and invert the pipette (Figures 6 and 7).

Figure 4. Loading an appropriate volume of raw semen by capillary action for counting in a hemacytometer.

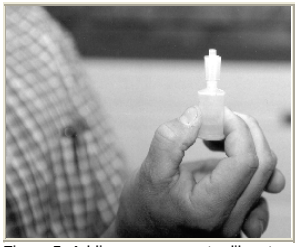

Figure 5. Adding raw semen to diluent reservoir in order to provide proper dilution for an accurate hemacytometer count.

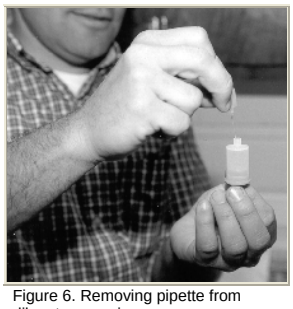

Figure 6. Removing pipette from diluent reservoir

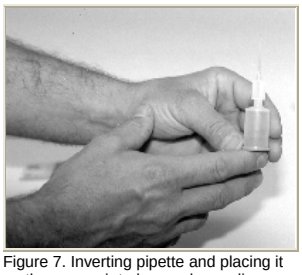

Figure 7. Inverting pipette and placing it on the reservoir to be used as a dispenser in loading the hemacytometer.

Presented in Figure 8 is a schematic drawing of the top and side view of the hemacytometer. There is a grid on each of the two shorter, wider portions, (chambers) of the hemacytometer. The two elongated portions (one on each side of the chambers) are slightly higher than the chambers. Consequently, when the weighted cover slip is placed over the chambers, there is a very specific volume of diluted semen over the grid, which must be used as one of the multiplication factors to calculate the number of spermatozoa per milliliter of semen.

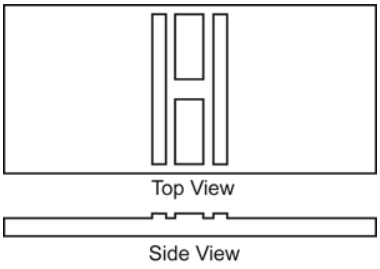

Figure 8. Drawing of the top and side view of a hemacytometer.

c) The time required to count the number of spermatozoa in the appropriate number of squares.

Obviously, the length of time required to prepare and count the sample (including the calculations) will vary from 10 to 20 minutes for the two chambers (one on each side) depending upon the experience of the technician. It must be emphasized that a phase-

transparent cells can be seen in rich contrast to their background. Consequently, without a phase-contrast microscope it is difficult, if not impossible to differentiate some cellular debris from spermatozoa.

d) Procedure for counting the spermatozoa.

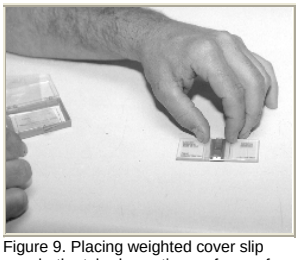
Figure 9. Placing weighted cover slip over both etched counting surfaces of the hemacytometer.

Place the hemacytometer on a flat surface, such as a counter top. Position the special weighted cover slip so that it is centered and cover both counting grids (Figure 9). Load the diluted sample onto the hemacytometer by allowing a single drop of diluted semen to be drawn across the counting grid by capillary action (Figure 10). To assure an accurate count, do not overfill the space between the cover slip and the counting grid. If overfilling occurs, the chamber must be dismantled, cleaned and the procedure repeated. Load both counting chambers on the hemacytometer. Place the loaded hemacytometer on the microscope stage (Figure 11). Locate the counting grid on the monitor. Adjust magnification so that the central block of 25 squares can be observed (Figure 12). Count the spermatozoa in the 25

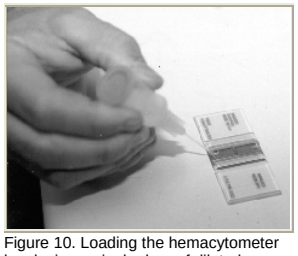
Figure 10. Loading the hemacytometer by placing a single drop of diluted semen in the loading slot of the hemacytometer.

squares on both sides of the hemacytometer. Be sure to count all spermatozoa within a square and only those spermatozoa on or across two of the four borders. This will prevent counting the same spermatozoon twice on two adjoining squares. At a 1:100 dilution, this total number represents millions of spermatozoa ml in this sample of gel-free semen. For a reasonably accurate count, two chambers (25 squares/ chamber) should be counted on four hemacytometers, thus duplicate counts made on each sample of semen. Since utilization of a hemacytometer for estimating sperm numbers can be very time consuming, more rapid procedures would save time, and thus, eventually money.

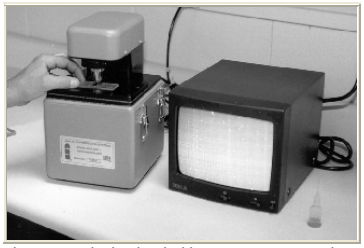
Figure 11. Placing loaded hemacytometer on the stage of a phase-contrast microscope so that the counting grid is visible on the monitor.

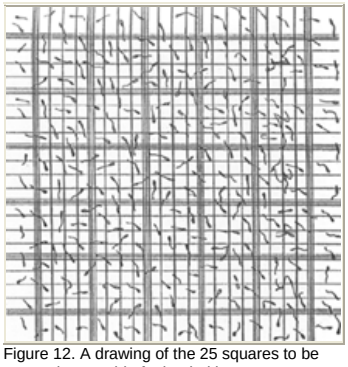
Figure 12. A drawing of the 25 squares to be counted on a grid of a loaded hemacytometer..

Counting is further complicated when semen samples are very dilute or extremely thick, which changes the number of large squares that must be counted for sufficient accuracy.

e) Preparing and counting a sample of semen requires considerable technical skill and equipment. Therefore, very few farms are prepared to make accurate counts with a hemacytometer.

It should be noted that the hemacytometer requires some special care. A dirty or

1. Wash the hemacytometer chamber and cover slip individually with a mild detergent (Ivory). Be careful not to scratch the chamber. Rub the surface gently with a facial tissue, lens paper or fingertip.

2. Rinse well in warm water.

3. Rinse in distilled or deionized water.

4. Rinse in alcohol or acetone to dry or dry with lens paper.

5. Store in a dust-free location with the cover slip and hemacytometer wrapped separately in tissue.