Aviagen™ Turkeys

Technical Bulletin

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MEASURING THE NUMBER OF SPERM CELLS INSEMINATED USING PACKED CELL VOLUME

One of the major factors influencing fertility is the number of spermatozoa in the area of the germinal disc at ovulation (when the ovum is released from the ovary). Studies of the holes made in the perivitelline membrane at the time of ovulation indicate that at least 50 spermatozoa need to be at the germinal disc to attain a fertility of 90%.

Unfortunately, most of the spermatozoa inseminated do not make it to the ovum at the time of ovulation. *Table 1: Fate of the Sperm Cell in the Oviduct*, gives us an idea of how many spermatozoa must be inseminated in order for 50 to arrive at the germinal disc.

Table 1. FATE OF THE SPERM CELL IN THE OVIDUCT

Area of the Oviduct	Number of Spermatozoa
No. of Spermatozoa Inseminated	100,000,000
Storage Sites	1,249,000
Infundibulum	16,700
Ovum	4,300
Germinal Disc	50

Source: Wishart, et.al.

If we inseminate 100 million sperm cells into the hen we can expect 50 of those sperm cells to be present when the ovum is released from the ovary. We need 50 sperm cells there every day until the next insemination.

How many spermatozoa do we need to inseminate to get 90% fertility for 1 week? *Figure 1: Sperm Number and Duration of Fertility* looks at this question.

In this experiment, one insemination of 7.2 million spermatozoa gave one day of fertility. Where as an insemination of 360 million spermatozoa gave 9 days of fertility above 90%.

The Perivitelline and Duration of Fertility experiments demonstrate one of the most important facts of insemination: **Sperm number plays a major role in fertility.** The ability to measure the sperm number is important to maximizing fertility in any insemination program. In addition, knowing the sperm number is a vital part of resolving fertility problems when they occur. This tech bulletin explains how to measure sperm number using a procedure called packed cell volume (PCV) or spermatocrit.

Equipment Needed

- Micro-hematocrit capillary tubes
- Clay[1]
- IEC Model MB Micro-hematocrit centrifuge (see **Figure 2**) with a 24 place rotor[2]
- PCV reader or PCV card reader

[1] Tinted clay makes it easier to differentiate between the clay plug and the semen.

[2] The IEC Model MB centrifuge spins at 11,600 to 14,000 rpm. With the 24 place rotor (cat# 275), it exerts a force of 13,200 to 14,000g. Using a centrifuge with different specifications will give an inaccurate reading when used with the formulas in this technical bulletin.

Figure 1. SPERM NUMBER AND DURATION OF FERTILITY

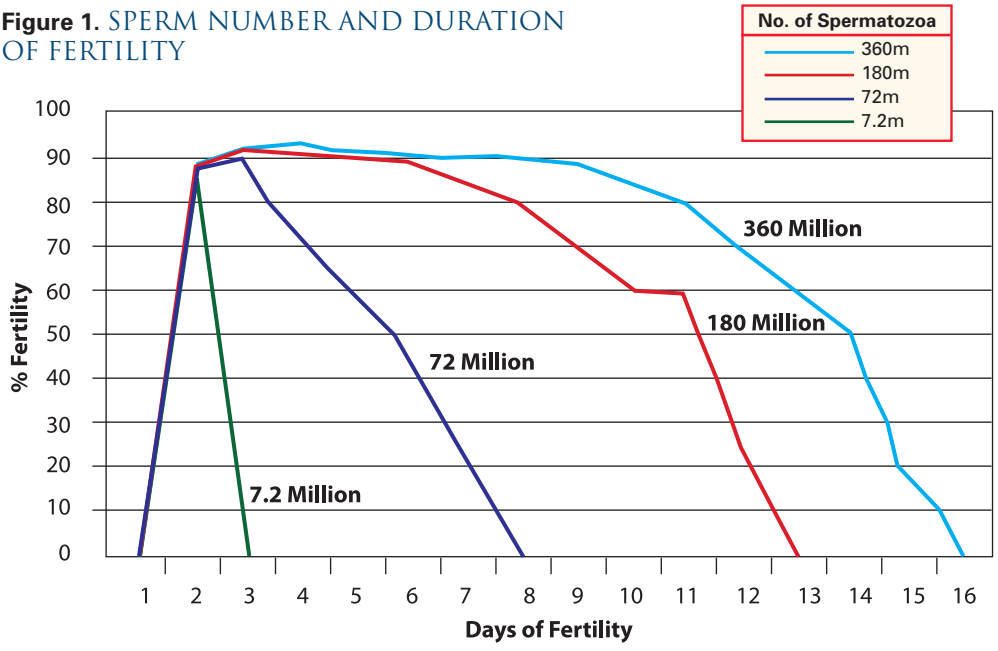

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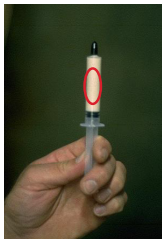
Figure 2.
IEC Model MB centrifuge

Sampling

- Always measure semen that is "hen ready"; that is, measure the semen after the addition of extender and just prior to use.
- The semen should be clean and free of contaminants. Semen contaminated with feces, dirt, urates, blood or lymph can give a false high reading. Fewer sperm cells actually will be inseminated than indicated by the PCV.
- Avoid taking the semen sample from the tip of the syringe or edge of the vial. This can give a false reading. It is best to take the sample from the center of the syringe or vial (see **Figure 3**).
- Taking at least two samples from the same vial will reduce the chances of losing data in the event one of the samples is lost or destroyed.

Figure 3.
Take the semen from the center.



Centrifuging the Semen

1. Fill the capillary tube approximately ¾ full with "hen ready" semen.

2. Plug one end of capillary tube with clay.

3. Place the tube in one of the slots in the centrifuge head. Make sure the end of the tube, which has been plugged with clay, is against the gasket around the outer edge of the centrifuge head.

4. Place the metal top on the centrifuge head and screw it down firmly.

5. Close the centrifuge lid and turn the centrifuge on for 10 minutes. *Spinning the semen for less than 10 minutes will result in an inaccurate reading when used with the formulas in this technical bulletin.*

6. After 10 minutes a bell will sound. Bring the centrifuge to a complete stop by activating the brake switch.

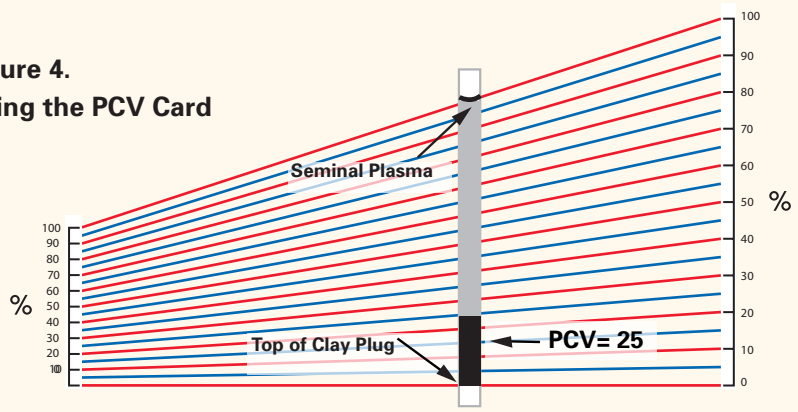
7. Open the centrifuge, unscrew the metal lid and take out the capillary tube. There should be an air space at the top of the tube, an area of clear fluid (seminal plasma), and an area of "packed" sperm cells next to the clay plug.

Reading the Packed Cell Volume

1. Position the centrifuged tube on the reader – place the capillary tube on the card reader so the top of the clay plug is on the line marked 0 (zero) and the meniscus (bottom of the air bubble at the top of the seminal plasma) is at the 100 line. **The tube must be aligned straight up and down on the card reader for correct reading.**

2. The PCV is then read by observing which line passes across the top of the packed sperm cells (see **Figure 4**).

Figure 4.
Using the PCV Card



Calculating the Number of Sperm Cells Inseminated from the Packed Cell Volume (PCV)

1. Using the PCV from Step 2 of *Reading the Packed Cell Volume,* calculate the number of sperm cells in 1 cc of semen using the formula:

(O.296 x PCV) + 1.3 = billions of cells/cc of semen
ex. (O.296 x 25) + 1.3 = 8.7 billion cells/cc of semen

2. To calculate the number of sperm cells inseminated, divide the "billions of cells/cc" from Step 1 by the "number of hens inseminated per cc of semen."

billions of cells/cc ÷ number of hens inseminated/cc = millions of sperm cells inseminated
ex. 8.7 billion cells/cc ÷ 20 hens inseminated = 435 million sperm cells

Using the Number of Sperm Cells Inseminated to Maximize Fertility

- **Measure each vial of semen that is milked during the insemination.** Duplicate packed cell readings from the same vial should agree within one PCV unit. If the difference is greater, it is an indication that the sample was not well mixed.

- **The values from all the PCV readings from one insemination should agree within four PCV units.** A larger spread could indicate problems in semen mixing, adding semen extender, tom milking and tom management.

- **All hens in the flock should receive more than the minimum live sperm numbers indicated in *Table 2*.** If this is not the case, the dose size should be increased or the concentration of the semen should be increased by reducing the amount of extender used.

Table 2. MINIMUM SPERM CELL REQUIREMENTS

Week of Production	Minimun Live Viable Cells Required (Millions)
0 – 2	360
3 – 10	320
11 – 16	345
17 – 22	370
23 – 28	395

- **Measure the PCV of a sample of semen from the toms at the last premilking to determine the dosage for the first insemination.** Extend the semen from this premilking as if it were to be used for insemination then measure the pack cell volume as discussed above. Use this PCV to estimate how many hens to inseminate per cc of "hen ready" semen at first insemination.

Table 3. PCV DOSE CHART

Maximum Number of Hens to Inseminate Per CC of Semen

PCV	Week of Production 0-2 (wks)	3-10 (wks)	11-16 (wks)	17-22 (wks)	23-28 (wks)
8	10	11	11	10	9
9	11	12	11	11	10
10	12	13	12	12	11
11	13	14	13	12	12
12	14	15	14	13	12
13	14	16	15	14	13
14	15	17	16	15	14
15	16	18	17	16	15
16	17	19	17	16	15
17	18	20	18	17	16
18	18	21	19	18	17
19	19	22	20	19	18
20	20	23	21	20	18
21	21	23	22	20	19
22	22	24	23	21	20
23	23	25	23	22	21
24	23	26	24	23	21
25	24	27	25	24	22
26	25	28	26	24	23
27	26	29	27	25	24
28	27	30	28	26	24
29	27	31	29	27	25
30	28	32	30	28	26

NOTE: Number of Hens to Inseminate is based on results from an IEC Model MB micro-hematocrit centifuge with a 24 place capillary tube head at 13,000g and on semen with 85% or better live normal spermatozoa.

- **Use the lowest PCV from each insemination to determine how many hens per cc to inseminate at the next insemination.** Should the PCV unexpectedly drop during an insemination, the number of hens per cc should be adjusted downward per PCV Dose Chart (**Table 3**) recommendations.

In summary, the number of spermatozoa inseminated into each hen can have a major influence on fertility. The number of sperm cells inseminated can be measured using the Packed Cell Volume (PCV). By monitoring the PCV and the sperm cells inseminated fertility can be improved. If you would like additional assistance in measuring sperm numbers, please contact your Aviagen Turkeys representative.

References:

Wishart, G., Robertson, L. and Wood, L; Sperm:Egg Interaction in Turkeys: Mechanisms and Practical Applications; 18th Technical Turkey Conference organized by Turkeys; 20th and 21st April 1995.

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