Pork Industry Handbook: Semen Collection Evaluation and Processing
Michigan State University Extension Service

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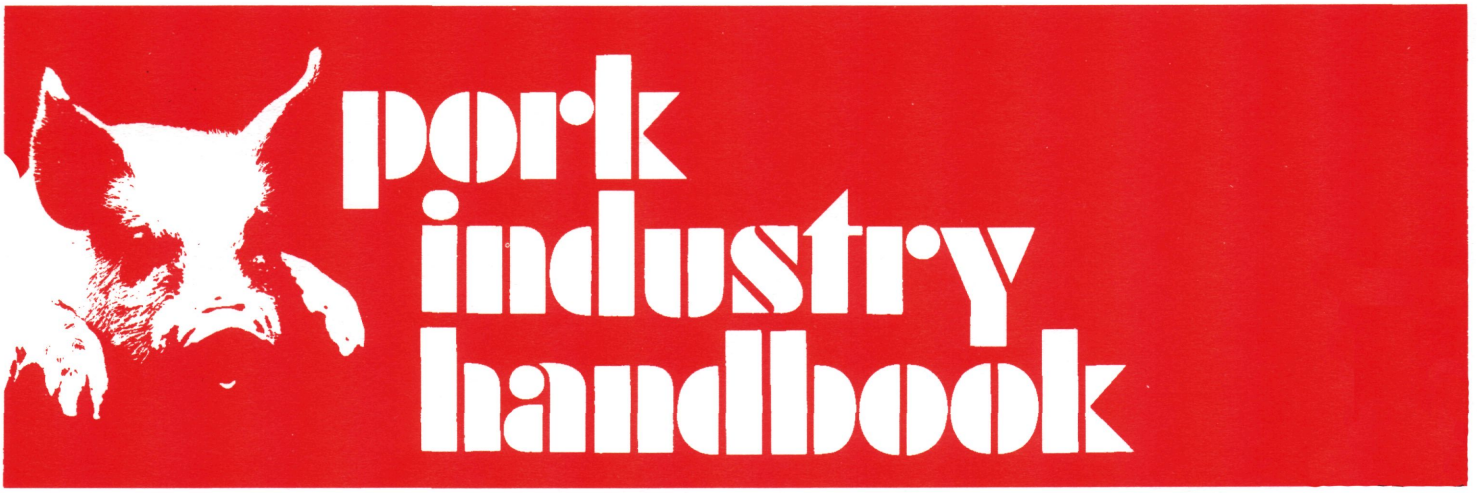

Michigan State University Extension.

Semen Collection, Evaluation, and Processing in the Boar

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Semen Collection

Boars generally show an interest in mounting stationary objects. Therefore, an estrous female is not required when collecting semen to be used for artificial insemination (AI). Adjustable height mounting dummies can easily be made or purchased from a supplier of AI equipment. Basic requirements for a good mounting dummy include appropriate height for mounting and straddling of the boar's forequarters, structural stability, and durability. Good footing around the dummy is essential to aid the boar in mounting and thrusting, and in the semen collection process. Rubber matting material with openings is a popular choice because it provides for good footing, resiliency to constant use, non-absorbency, and ease of cleaning between uses.

A separate semen collection area is incorporated into the design of commercial boar studs and most on-farm studs. The semen collection pen (Figure 1) should have at least two or three of the perimeter walls constructed of 2-inch diameter galvanized pipe. The pipe should be 36 to 42 inches in height and placed at 11- to 12-inch intervals on center, thus a 9- to 10-inch space is provided between pipes. These perimeter pipe walls are a safety feature that allow the handler ease and availability of sites to enter or exit the collection area without opening a gate or scaling a wall, but still hold the boar within the pen area. The collection pen and its surrounding area should be void of distractions that may divert the focus of the boar away from the collection dummy. It may be useful to position the dummy within the pen in such a fashion that boar movement is limited around the dummy and to aid the handler and/or collector in directing the boar to the dummy. This can be done by placing the dummy in the corner of the pen or attaching it to a wall. The recommended

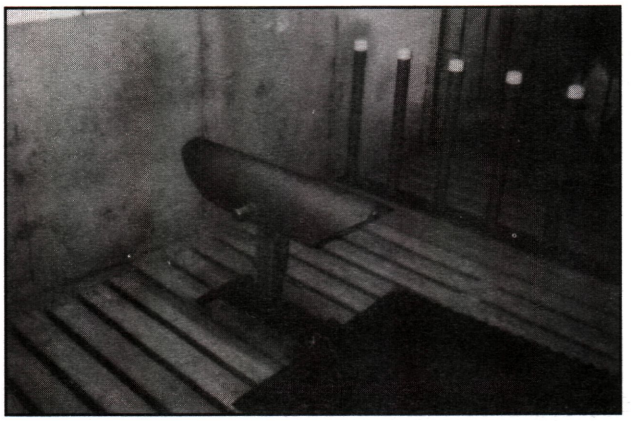
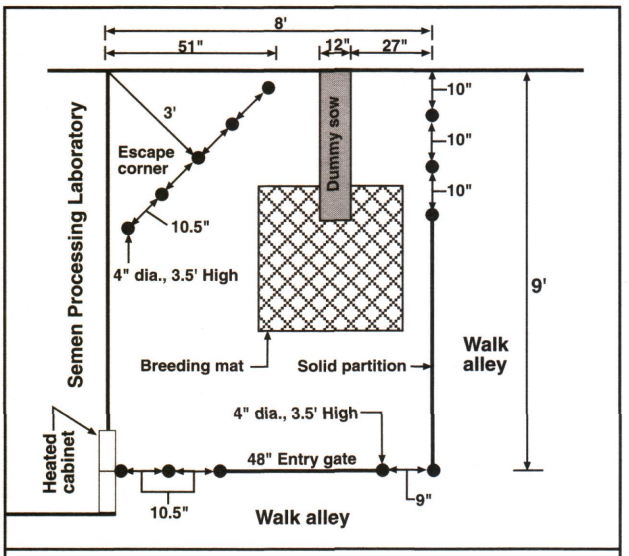


Figure 1. A semen collection pen demonstrating properly constructed perimeter pipe walls, good placement of the mounting dummy, and use of rubber matting to provide for good footing for the mounting boar.

width of the collection pen is 6 to 8 feet, and the recommended length is 8 to 9 feet. When using a diagonal escape corner(s) on one side of the pen, a width of 8 feet is recommended (Figure 2). A smaller collection pen other than those described is helpful when training young boars to mount a dummy.

Sufficient time should be allocated for collecting semen, primarily so that personnel do not feel compelled to hurry the boar to mount beyond his comfort level. Introduce the boar to the collection area and let him investigate. Once the boar identifies and investigates the dummy, he should readily mount. After the boar has become interested and mounted the dummy, approach the boar from the rear. Semen should be collected using sanitary techniques to



Notes:

1. **If young boars walk between the escape pipe, place larger diameter pipe over the smaller pipe until boars get larger.**
2. **A pig board is kept in the escape corner.**

Figure 2. Schematic of an on-farm semen collection pen.

Table 1. Minimum Contamination Techniques for Preparing and Collecting Semen.

1. Periodically trim hair from the preputial opening.
2. If needed, clean preputial opening and surrounding area with a single-use disposable wipe (i.e., diaper wipe).
3. Aggressively evacuate preputial fluids from the prepuce manually prior to grasping the penis for semen collection.
4. Have the semen collector wear disposable vinyl gloves or use an evaporative hand cleanser between boars to minimize contamination of semen and reduce risk of cross-contamination.
5. Hold penis perpendicular to the boar to minimize the chance of preputial fluids to run down the penis and into the semen collection vessel.
6. Allow the first few jets of an ejaculate (i.e., pre-sperm fraction which contains urethral flushings/urine) to go on the ground rather than into the semen collection vessel.
7. Dispose of rubber band and filter/gauze before passing collected semen through to the laboratory for processing.

minimize contamination (Table 1). With two vinyl gloves on your hand, gently reach around to the boar's prepuce and massage the penis through the prepuce. This helps to evacuate preputial fluids and aids in stimulating pelvic thrusting. The boar will then start to thrust and extend his penis out of the sheath. Remove the outer vinyl glove from your hand. Once the boar extends his penis out of the prepuce, the corkscrew-shaped penile tip should be grasped with the fingers with uniform pressure applied (Figure 3). Some boars require a substantial amount of digital pressure, whereas, other boars require minimal digital pressure. The boar is only responsive to pressure applied around the corkscrew-shaped tip of the penis. Pressure applied elsewhere on the penis will elicit a negative response causing most boars to dismount. With the proper pressure applied, the boar will extend his penis and cease thrusting. After a brief pause, the boar will start to ejaculate.

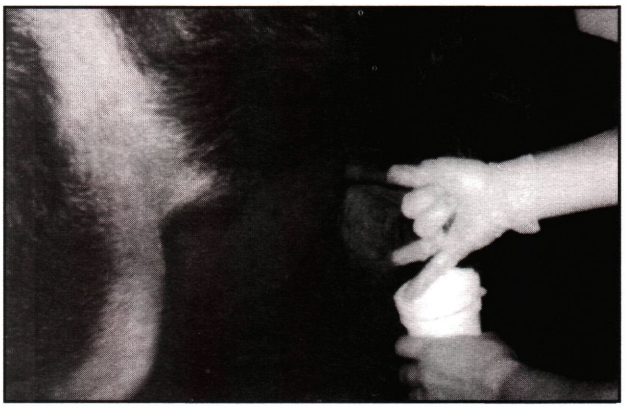


Figure 3. Application of pressure to the spiral portion of the penis for the collection of porcine semen.

A pre-warmed (38°C/100°F) insulated thermos or styrofoam cup is a convenient and economical semen collection vessel. The first few jets of an ejaculate function to flush out the urethra and should be allowed to go on the ground. These jets are usually emitted while the boar is still thrusting. After thrusting has ceased, fluid and gel components are ejaculated by the boar. The gel fraction should be filtered out of the ejaculate during collection using gauze or a mesh filter which has been placed over the mouth of the thermos/cup. Separation of the gel from the fluid during ejaculation is important because the gel coagulates into a semisolid mass which interferes with harvest of the spermatozoa, semen evaluation, and processing. Actual time of ejaculation in the boar can vary considerably. A minimum of 5 to 7 minutes is usually necessary for a boar to complete ejaculation. Ejaculate volume can be quite large, sometimes exceeding 400 ml, and is dependent upon such things as boar age, size, collection technique, and collection frequency.

Attention should be made to protect boar spermatozoa from external insults during and after the collection process. Chemical (e.g., latex gloves, water, soap residues, alcohol, etc.), light (i.e., sun, ultraviolet), and temperature (hot or cold) insults are detrimental to sperm cells and should be avoided. As a general rule, anything which may come into contact with boar semen should be clean and dry. Single use, disposable products are preferred to minimize the risk of exposure to sperm-killing compounds and to eliminate the chance of cross-contamination between boars. When collecting and handling semen, it is important that semen only come into contact with materials/extenders that are at similar temperatures with the semen. Drastic temperature fluctuation is detrimental to sperm quality.

Semen Evaluation

Good quality boar semen is essential to obtaining satisfactory fertility rates. Standard tests currently used to evaluate boar semen quality include sperm motility, morphology, and concentration. When used individually, these standard tests have limited usefulness in actually determining the fertilizing potential of an ejaculate. These tests do, however, have the ability to identify ejaculates of overtly poor quality. Minimum semen quality values for fresh, unextended boar semen processed and used for AI are indicated in Table 2.

Table 2. Minimum values of fresh boar semen processed and used for AI.[1]

Semen Variable	Value
1) Appearance	Milky to creamy consistency
2) Color	gray-white to white in color
3) Total Sperm Numbers	>15 x 10^9 sperm/ejaculate
4) Gross Motility (Unextended)	≥ 70 %
5) Abnormal Morphology	≤20%*
-cytoplasmic droplets[#]	≤15%

[1] Althouse, GC. Compend Contin Educ Pract Vet 19(3):400-404, 1997.
* the 20% maximum includes cytoplasmic droplets
[#] includes both proximal and distal cytoplasmic droplets

Most commercial studs evaluate all ejaculates processed through their facilities. For some on-farm AI laboratories, these same routine semen evaluations tend to be impractical because of limitations of equipment, skilled labor, or time. At a minimum, it is recommended that initial ejaculates on all new herd AI boars be examined by a trained individual to critically assess the boar's semen quality. Subsequently, a monthly screening of semen quality from all AI boars should be done during their use. Routine examination of AI boar semen quality is very important because it's impact on herd reproductive efficiency is increased many fold when compared to natural mating. This examination is insurance against a reproductive catastrophe. The costs from using poor quality semen become quite high when considering its effect on herd farrowing rate, litter size, non-productive days, and inventory of sows and gilts. A record of semen quality should be kept on each boar (Table 3).

Estimation of Sperm Motility

Visual assessment of the percentage of motile sperm by light microscopy is still the preferred method. Accuracy of this technique is largely dependent upon the technician's experience and natural ability. Sample preparation (i.e., dilution rate, type of diluent, temperature) must be standardized to reduce laboratory error and variation among examinations. To estimate motility, a small drop of fresh semen is placed on a warmed (37°C/99°F) microscope slide overlaid with a coverslip. When viewed under a microscope, the sample should be thin enough to visualize individual sperm motility. If individual spermatozoa cannot be seen, a small drop of extender (same temperature as the semen) can be dropped on the sample before overlaying with a coverslip. Sperm motility is then estimated to the nearest 5% by viewing groups of sperm in at least 4 different fields on the slide at 200 or 400X; these readings are then averaged. Only ejaculates with at least 70% gross motility should be used for further processing. This is especially important because sperm motility and viability normally decrease during storage. If ejaculates are used shortly after collection, samples exhibiting at least 60% motility can be used.

Examination of Sperm Morphology

Several stains are commercially available and are essential for examining boar sperm morphology using dry mounted slides. Stains accentuate the outline of the sperm when using a light microscope, allowing for easier visualization by the observer. Higher resolution and more expensive phase contrast or differential interference contrast microscopes have internal components that generate their own contrast. Consequently, wet mount samples can be used for morphological estimation.

To make a stained slide, equal volumes (e.g., 10 µl) of stain and sample are adjacently applied to a microscope slide. The drops are mixed together using the edge of a second slide. The edge of the second slide is used to draw the mixture across the flat slide to produce a thin layer which is allowed to air dry (Figure 4). Under oil immersion, a minimum of 100 sperm are then assessed and catagorized into one of 3 categories: 1) normal sperm (Figure 5), 2) sperm with abnormal heads, and 3) sperm with abnormal tails (including cytoplasmic droplets). If a large number of sperm in an ejaculate are morphologically abnormal, it indicates that a disruption of some type occurred during the development or maturation of the sperm or that the semen was improperly handled. Ejaculates of the general boar population usually exhibit less than 20% abnormal sperm. Therefore, ejaculates accepted for AI use usually contain greater than 80% normal sperm cells.

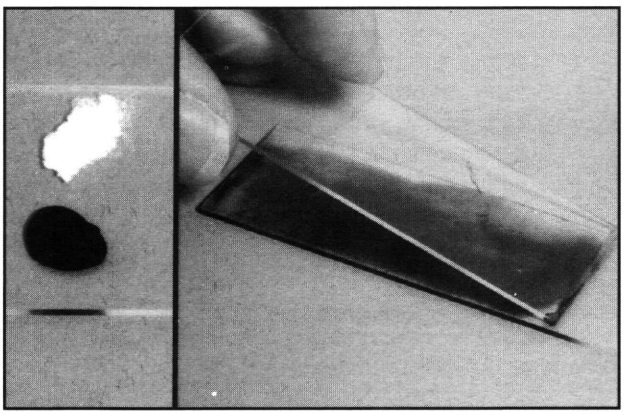


Figure 4. The edge of a microscope slide is used to thinly spread the mixed semen/stain drop across the surface of another slide. The slide is then allowed to air dry, and then sperm morphology is assessed using a microscope.

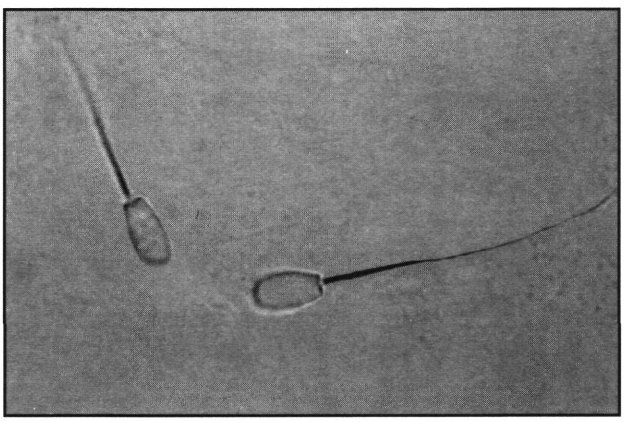


Figure 5. A photomicrograph of normal boar spermatozoa.

Determination of Sperm Concentration

The most common way of estimating sperm concentration in gel-free boar semen is by measuring the degree of sample opacity. Sample opacity is estimated most commonly using a photometer, an instrument that measures the percentage of transmittance or absorbency of light through a sample. In boar semen, sample opacity is dependent upon the number of sperm cells and other ejaculate components which interfere with the movement of light through the sample. Boar semen is normally too opaque for light to pass readily through it. Therefore, a small sample of boar semen is usually diluted into an isotonic solution before taking a measurement. The photometric measurement is then converted into sperm numbers/ml either internally by the photometer or by the producer using a conversion chart which accompanies the instrument. For this photometric measurement to be relatively accurate, it is necessary that the instrument be calibrated specifically for boar semen. Because of inherent differences between instruments, photometric conversion charts are not interchangeable between instruments. Periodic recalibration of the instrument is necessary to maintain accurate readings. Inaccuracies in photometric measurements can occur if readings fall outside the optimum operating range of the equipment, human error (e.g., incorrect dilutions, improper warm-up time, solution mishandling), and innate differences among boar ejaculates. It is important that manufacturer recommendations be followed on the use of the instrument for determining sperm concentration in boar semen.

Another method of directly determining sperm concentration in boar semen is by using a counting chamber (e.g., hemacytometer). The surface of the counting chamber is etched to outline a defined surface area. After diluting a portion of semen to a 1:200 ratio, a very small portion of this mixture is transferred onto the counting chamber. Avoid overfilling! After allowing 5 minutes for sperm to settle onto the surface of the chamber, the number of sperm are counted within the defined surface area using a microscope at 200X to 400X. A minimum of 5 large (80 small) squares are counted in the center grid on each side of the hemacytometer (Figure 6). Only sperm heads touching the top and left lines of the large square are included in the count, while those touching the bottom or right lines are not counted. Tails touching any of the lines are not counted. The two counts are then averaged. If the two counts vary more than 10% of each other, prepare the hemacytometer again and count the two sides until within a 10% variation. The number of sperm cells (N) are then determined by averaging the four counts. This number (N) is then inserted into the formula supplied by the distributor of the counting chamber to determine the number of sperm cells per milliliter of semen. The time and tediousness involved with hemacytometric counts make them impractical for most AI laboratories. Thus, photometric analysis remains the most commonly used technique for determining sperm concentration per milliliter of gel-free ejaculate.

Total Sperm Numbers

Total sperm numbers are calculated by multiplying the total volume of the gel-free ejaculate (ml) times the sperm concentration per ml. Ejaculate volume is measured with a warm graduated measuring cylinder or by weighing the ejaculate, assuming that 1 gram (weight) is equal to 1 ml (volume).

Semen Processing

Semen extender. Most porcine semen extenders come packaged in a powdered form. When buying powdered extenders in bulk, they should be broken down and re-packaged in tightly sealed containers that will make the desired volume of liquid extender. If not mixed in the powdered extender, preservative antibiotics should be added the day the powdered extender is reconstituted with water. Purchased extenders should have production dates, be kept in a frost-free refrigerator, and be used within six months of purchase.

Preparing extender. Extender powder is reconstituted with Type I or II water and incubated at 37°C/99°F in a water bath for a minimum of 1 hour to allow for temperature and pH equilibration. To prevent contamination, it is best to prepare liquid extender in a plastic, single-use, disposable bag.

Extending semen. Total numbers of sperm per dose of semen tend to range from 2 to 6 billion (sperm concentration of 25 to 80 x 10^6 cells/ml). A dose of semen should contain at least 60 ml and no more than 120 ml total volume; 65 to 85 ml being the most common volumes for a dose of extended porcine semen. The final dilution rate of sperm into extender should be dependent upon initial ejaculate quality, extender type, and anticipated duration of storage time. Some facilities employ an arbitrary extension ratio of 1 part semen (sperm-rich fraction) to 7 to 11 parts extender when storing and using semen within 24 to 72 hours. Optimum extension ratios for each type of extender have yet to be established by the industry; therefore, this current practice remains questionable. If boar semen is to be extended by the volume ratio method, a conservative dilution of 1 part semen (whole ejaculate) to 4 parts extender should be followed, with the extended product used within 24 hours of extension. Problems that can occur when using the volume ratio method are: 1) semen is underdiluted, allowing for exhaustion of available energy substrates and buffers over a shorter period of time, and 2) semen is overdiluted, potentially causing reduced sperm viability and fertility. In addition, the optimum number of doses of semen is not obtained; therefore, an economic and genetic loss occurs because the use of sperm cells is not maximized.

The freshly collected semen and extender should be at similar temperatures for mixing. The mixing of semen and extender can be accomplished by adding either semen into the extender or visa versa. Semen is diluted with extender using either a 1-(i.e.,add all of the calculated volume of extender at one time) or 2-(i.e., adding one-half the calculated volume of extender to semen, allowing it to equilibrate for 5 to 10 minutes, then adding the remaining extender to achieve final volume) step technique. Since the one-step process is easier and less time consuming, it is the method preferred by many laboratories.

Pooling semen. Mixing or pooling semen from different boars is a popular technique for processing semen for AI. The benefits of pooling semen include 1) increasing processing efficiency in the laboratory by allowing for a

Table 3. Record of semen quality for fresh (neat) semen.

Boar identification:_____ **Breed of Boar:**_____

Age of Boar:_____ **(as of:**_____**)**
Months **Date**

Date of Examination						
Gel-free volume, mL						
Abnormal Color						
Abnormal Odor						
Motility, %						
Concentration per mL						
Normal sperm, %						
Proximal droplets, %						
Distal droplets, %						
Abnormal midpiece, %						
Tail bent at midpiece with droplet, %						
Tail bent at midpiece, %						
Entire tail coiled under sperm head, %						
Entire tail coiled around sperm head, %						
Tail loops under the head and extends from midpiece, %						
Short/twin tails, %						
Tail without head, %						
Head without tail, %						
Giant, small, pyriform, elongated, or twin heads, %						
Loosened acrosome, %						
Lost acrosome, %						
"Knobbed", bent-over acrosome, %						
Loosened neck, %						

large number of boar ejaculates to be processed simultaneously rather than individually, and 2) a means to reduce or even eliminate inherent differences in fertility between boars. For pooling semen, freshly collected ejaculates are examined for semen quality to eliminate overtly poor ejaculates. The ejaculate is then added to a set amount of extender (i.e., 500 ml), diluted 1:1 with an extender, or fully extended. When pooling semen, all liquids (i.e., raw semen, extender) should be at similar temperatures. The number of boar ejaculates to be pooled should not exceed the volume capacity able to be processed at one time; 3 to 6 pooled boar ejaculates are sufficient in most production systems. After adding the final ejaculate to the pool, the entire sample is diluted to its final calculated volume with the remaining extender or fully extended samples are mixed together. The pooled, fully extended sample is then further processed/handled/stored using standard protocols.

Packaging semen: Choices for the packaging of the extended semen product are in either bags, bottles, or tubes. Currently, there does not appear to be any distinct advantages between the different packaging systems with respect to either sperm longevity or fertility. A variety of manual to fully automated systems are available for packaging boar semen.

Depending on the extender, extended and packaged porcine semen should be stored at 14°C to 18°C (57°F to 64°F). Storage temperatures above 18°C for short periods of time (< 24 hours) appear to not appreciably affect overt semen quality. These higher temperatures, however, are conducive to increased utilization of extender products and bacterial growth. Conversely, if extended semen is exposed to storage temperatures at or below 10°C (50°F), irreversible damage to the sperm cell may occur in most of the popular extender's used today. Stored semen should be gently agitated/rotated at least twice a day to resuspend settled sperm cells in the extender.

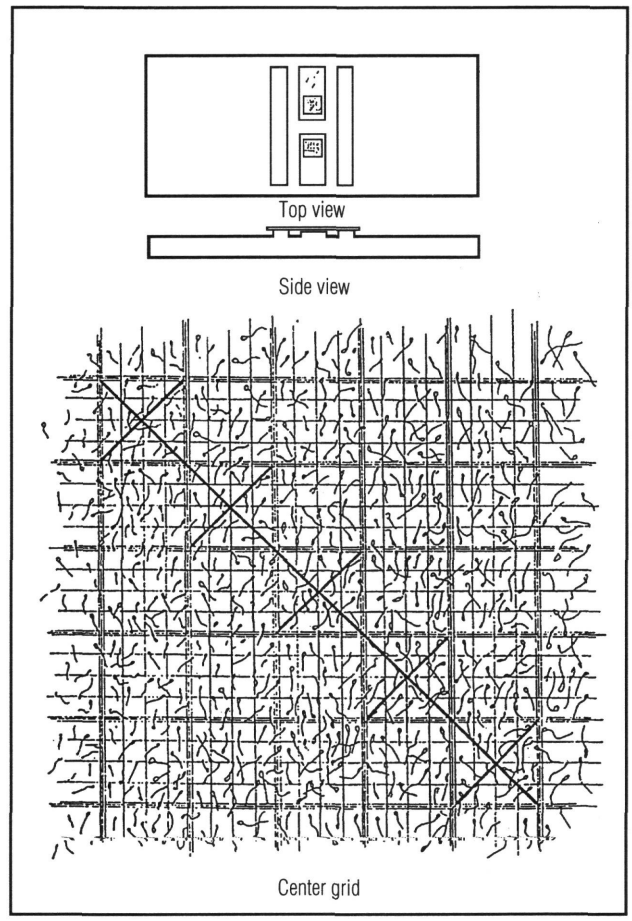


Figure 6. Diagram of both top and side views of a hemacytometer, and the center grid area where sperm are counted in order to estimate sperm concentration.

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