Canine Semen – What you Really Need to Know

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Canine Semen collection

The most common semen collection technique is by digital manipulation of the penis with an artificial vagina (AV) comprised of a latex rubber cone attached to a 15 ml calibrated plastic centrifuge tube (Reproduction Resources, Hebson, IL 800-331-0195)

Most experienced dogs will ejaculate with simple hand stimulation, whereas in-experienced or younger dogs may require a teaser bitch in heat. Some dogs don't care if the teaser bitch is in heat or not – they just like a company!

If you are using a teaser bitch not familiar (A) with the male give them some time to 'get-to-know' each other. After about 15 minutes, the bitch and the stud in the picture (B) seem to be comfortable with each other and the bitch is raising her tail and showing an anxious look on her face, ---- probably saying 'what is he waiting for!'

A.

B.

The semen collector, if right handed, kneels at the dog's left side and holds the AV with top folded...

...in the left hand and uses the right hand to move the prepuce up and down the shaft of the penis.

Left-handed collectors may reverse the sides described here.

At this time, many dogs will begin to thrust the penis in to the cone, and the collector should then move the AV above the engorging bulbus glandis and keep firm, steady pressure around as much of the penis as possible to maintain the erection.

If the male doesn't begin to thrust, the AV should be worked up on the penis in an up and down massaging motion to stimulate erection and ejaculation.

Most dogs ejaculate the presperm (clear) and sperm-rich fraction (cloudy) of semen during the time of most rapid pelvic thrusting and then dismount and lift one hind leg as though trying to step over the bitch and achieve the tie.

At this time, the penis is rotated at about 180 degrees and is directed caudally while still maintaining tight pressure around the bulbus glandis until the third prostatic fraction of semen is ejaculated.

The prostatic fluid is ejaculated after a short pause. At the same time anal contractions can be seen and the collector can feel a rhythmic pulsation of the penile urethra.

After few ml of the prostatic fraction are collected, the AV may be removed.

Let the dog ejaculate the rest of the prostatic fluid, which may take between 5 to 15 minutes.

The teaser bitch (if used) may be removed from the collection area.

Allow some time for the dog to lose his erection and retract the penis back into the prepuce before leaving the clinic.

Paraphymosis, which is the inability to retract the penis into the prepuce because of constriction of the preputial orifice, has been reported following semen collection.

Semen Evaluation

Pre-sperm fraction Sperm-rich fraction

The color of normal semen should be milky white.

A red or pinkish ejaculate may be indicative of a prostate problem.

A yellowish color can indicate urine contamination or less commonly, pus or other foreign debris, originating from the prostate or epididymides.

Semen volume can be read from the calibrated collection tube.

Semen volume varies with the breed, size of the dog and how much prostatic fluid has been collected. However, it is important to note the semen volume because for BSE results you need to record the sperm number per ml or per ejaculate based upon the semen volume.

There is no benefit in collecting the entire 3rd fraction (prostate fluid) for breeding soundness examination (BSE) results because it varies between dogs and the length of collection time. You can easily spend 20-30 minutes 'on your knees' to collect all the prostatic fluid!

Sperm motility should be evaluated within a few minutes of semen collection. Place a drop of semen on a warm (37 degrees C) microscope slide, place a glass cover slip over the drop and examine under 100-200X magnification.

Estimate the percentage of motile sperm, those moving in a progressive forward manner. Sperm moving in circles or wiggling in one spot can indicate chemical or cold shock. These sperm may be **mobile** but are not **motile**.

I look at 4 to 5 different microscopic fields and make a 'mental' average of the fields asking myself if half of the sperm are motile and go from there to make my estimate. Even though sperm motility is a subjective test, it is a quick functional test of semen quality.

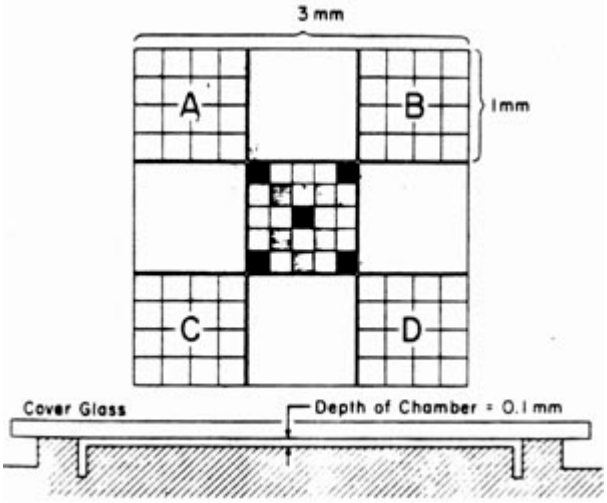
Expensive automated semen analyzers and computer-based equipment are available to assess sperm motility.

About 80% or higher sperm motility is expected from a normal dog ejaculate. Low sperm motility may be an indication of:

- incomplete ejaculation
- infectious diseases involving the reproductive system or
- too much heat exposure to the scrotum (e.g. fever).

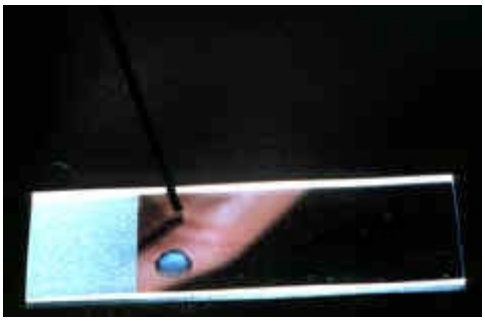
Sperm concentration or sperm numbers are more important to count in the whole ejaculate (total volume) than per milliliter of the semen, because total semen volume is going to depend upon how much prostate fluid you have collected. Again, expensive automated semen analyzers are also available for this purpose, but the most common technique used in veterinary practices is to count sperm using a hemocytometer.

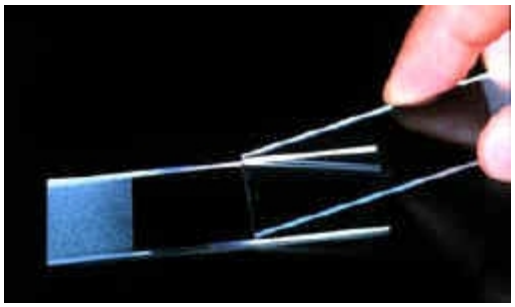
A WBC Unopette system (Becton Dickson, Rutherford, NJ) is used.



The semen sample is mixed gently and drawn into the capillary pipette, which holds 20ul. Place the contents of the capillary pipette into the container of diluent according to the kit instructions. The diluted semen is mixed and is loaded into the 2 counting chambers of the hemocytometer and the sperm are counted under the microscope.

Sperm morphology (normal and abnormal sperm) is assessed by examination of the stained slide of the semen. I use eosin-nigrosin stain (SFT office, 530 Church St., Suite 700, Nashville, TN 37219). One drop of semen and a similar sized drop of stain are placed on one end of a glass slide and the drops are mixed gently with a wooden applicator stick.





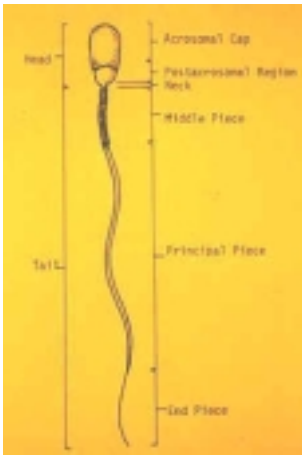
A second slide is used as a spreader slide to draw out a thin film similar to a blood smear.

The smear is air-dried. 100 sperm are examined using the oil-immersion (1000X) objective. The smear must be thin enough to visualize individual sperm. This cannot be accomplished if the sperm are crowded in-groups.

Some clinicians prefer Wright's Giemsa stain (Diff-Quik, American Scientific products, Columbia, MD), as used for preparing a vaginal cytology sample of a bitch. The semen smear is made as for a blood smear and allowed to air dry. Immerse the slide into each of the three solutions 5-7 times each. The slide is then rinsed with tape water and allowed to dry before examination under microscope.

For long-term preservation of the sperm, especially for acrosomal examination, buffered formal saline (BFS) is used. A few drops of semen are added to a small amount of BFS, and sperm morphology is assessed using a phase contrast microscope.

Normal sperm



One hundred sperm cells are examined. Each sperm is classified as either:

1. normal sperm

2. showing a primary abnormality

    all head abnormalities
    proximally coiled tails
    proximal cytoplasmic droplets

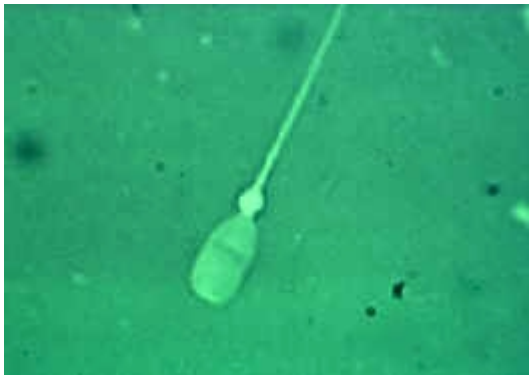
3. showing a secondary abnormality

    detached heads
    bent tail
    distal cytoplasmic droplets

Primary abnormalities represent abnormalities that originated during spermatogenesis in the testes. Secondary abnormalities occur during epididymal transport and as a result of sample handling.

Primary abnormalities should not exceed 20%; however, pregnancies have been achieved when the abnormalities have exceeded this number. Most normal dogs, which ejaculate regularly, would have >200 million total sperm in an ejaculate, of which over 80% are normal

A. Proximal cytoplasmic droplet



B. Distal cytoplasmic droplet

All sperm when released from the testes have proximal cytoplasmic droplets (A) at the neck of the sperm. Once transported to the epididymis and during epididymal maturation the cytoplasmic droplet moves to the distal part of the midpiece (B).

The sperm ejaculated should not have a cytoplasmic droplet. The presence of proximal cytoplasmic droplets in ejaculated sperm is a primary abnormality and distal cytoplasmic droplets a secondary abnormality.

Characteristics of Normal Canine Semen

Parameter	Unfractioned Ejaculate	Seminal Fraction		
		1	2	3
Color	Opaque to white	clear	milky-white	clear
Volume (ml)	~ 30	0.1-2	0.1-4	1-25+
pH	6.1-7.0	6.2-6.4		
Sperm motility	>80%			
Morphologically normal sperm	>80%			