Collection and evaluation of the semen in the dog

Konrad Blendinger

Med Vet, Hofheim (D)

Semen collection is performed with the dog on good footing (e.g., a rug) rather than on a slippery surface or table. Care should be taken to intimidate the dog as little as possible; lifting the dog to an examination table, giving injections, etc., should be performed after the semen is collected. Semen may be collected in the absence of a bitch (although sperm numbers may be lower), but the presence of a bitch is preferable. The pheromone methyl paraben may be helpful for collection in the absence of a bitch; some veterinarians freeze swabs of estrous bitch urine for this purpose but the reaction of male dogs is variable. There are several types of reservoirs to collect the semen in. Some made of glass, of rubber or plastic. Some are for single use others not. Different types are demonstrated. A sterile nonspermicidal lubricant or petroleum jelly may be used.

COLLECTION PROCEDURE

With the gloved hand (using a non-latex glove), initial friction movements are performed and the penile sheath is gently pulled back behind the bulbus as soon as penile erection starts. Then constant pressure is maintained caudal to the bulbus with the fingers building a ring at this level, and erection and eventually ejaculation should be achieved. Some manipulations may be helpful as scratching the dog’s chest, stimulation of the perineal area, careful touching the glans penis and speaking encouragingly.

If a bitch in estrus is available, the penis should be deviated as the dog mounts. The first (clear) fraction and the second (sperm-rich, cloudy) fraction should be collected. After these fractions are ejaculated, close inspection of the collection tube should demonstrate that clear fluid is starting to layer on the cloudy second fraction; at this point, the collection may be stopped. The dog may continue to ejaculate prostatic fluid for up to 10 min before the erection subsides. The sheath should be examined after the penis is retracted to ensure that the penis is situated normally within the sheath and that no hair is caught within the sheath. Residual protrusion may occur if the sheath rolls inward as the penis retracts.

Semen evaluation consists of determination of appearance, volume, concentration, motility, and percent morphologically normal sperm. Yellow, brown, or red samples may indicate the presence of blood or urine in the ejaculate. The volume is variable, depending on how much prostatic fluid was collected and the size of the dog; it ranges from <2 to >20 mL but is typically ~5 mL.

Motility of individual spermatozoa should be assessed as quickly as possible after obtaining a semen sample. A drop of semen should be placed on a clean slide and microscopically examined at x200 to x400 for progressive forward motion of individual spermatozoa and presence of sperm agglutination. Canine spermatozoa are resistant to cold shock, so the slide need not be warmed. Highly concentrated samples can be diluted with autologous prostatic fluid, phosphate-buffered saline, 2.9% sodium citrate solution, or a semen extender. A normal semen sample should have greater than 70% of the spermatozoa exhibiting vigorous forward motility. Individual spermatozoa should be carefully assessed for type of movement. Spermatozoa that are moving in small circles or that have side-to-side motion without forward progression are not normal. The percentage of actively motile spermatozoa may be altered by exposure of the semen to extremes in temperature, acidic diluents, water, urine, pus, blood, or lubricants. The first ejaculate from a dog following a prolonged period of sexual rest may contain a greater percentage of old and dead sperm that have been stored in the epididymis. This results in a decreased percentage of actively motile sperm. Semen samples obtained on subsequent days should be more normal. Rarely, hypomotile or nonmotile viable spermatozoa may also be seen with Kartagener’s syndrome, an immotile cilia syndrome with autosomal recessive mode of inheritance. In the dog this syndrome is characterized by respiratory tract disease, male sterility, situs inversus, deafness, and hydrocephalus.

SPERM MORPHOLOGY

Smears of the undiluted ejaculate are examined microscopically for structural abnormalities of the spermatozoa. A small drop of fresh, undiluted semen can be placed on a slide and covered with a large coverslip. This spreads the fluid out into a thin film, allowing accurate evaluation of individual sperm without stains. This evaluation is best performed using phase contrast microscopy.

Alternatively semen can be smeared evenly on a glass slide in a manner similar to that of blood; the smear is then air-dried, fixed, and stained. The rapid three step Giemsa-Wright stain technique (e.g., Harleco Hemacolor, Diff Quick, Camco Stain Pack) is quick, effective, and readily available in most practices. These stains do not stain the acrosomal area of sperm. India ink and eosin-nigrosin are background stains that outline the sperm rather than stain the sperm directly. For the latter, a drop of eosin-nigrosin stain and a drop of semen are gently mixed on a warmed slide.
microscope slide before being smeared and allowed to air-
dry. Spermac® stain is a rapid stain that offers unique dif-
erential qualities. The sperm nucleus stains red, the acro-
some, midpiece, and tail stains green; and the equivocal
region of the acrosome stains pale green. Spermac stain can
be used on extended semen, since constituents such as egg
yolk, seum, and milk commonly included in extenders do
not interfere with the staining. Leukocytes do not stain dif-
erentially with Spermac stain. Evaluation of sperm mor-
phology should be completed microscopically using oil
immersion. The normal spermatooza consist of the acrosom
cap, head, neck, middle piece, and tail. The acrosome is a
caplike structure covering slightly more than the anteri-
or half of the head, and the middle piece is approximately
1.5 times the length of the head in normal spermatooza.
Individual spermatooza should be evaluated for abnormali-
ties arising in the head, middle piece, and tail. Commonly
identified abnormalities include detached heads, knobbled
acrosomes, detached acrosomes, proximal and distal cyto-
plasmic droplets, reflex (i.e., bent) midpiece, bent tails, tails
tightly coiled over the midpiece, and proximally coiled
tails. Abnormalities may be further classified into primary
and secondary abnormalities. Primary abnormalities are
believed to represent abnormalities in spermatogenesis (i.e.,
within the testes), whereas secondary abnormalities are
non-specific and may arise during transit through the duct
system (i.e. within the epididymis), during handling of the
semen or following infection, trauma, or fever.

Historically, it has been suggested that normal males gen-
early should have greater than 70% morphologically normal
spermatooza and that primary and secondary abnormalities
should constitute less than 10% and 20% of the defective
sperm, respectively. A more recent investigation uses 60%
normal sperm morphology as the cutoff point between nor-
mal and subnormal. Another investigation found that total
numbers of morphologically normal and progressively
motile spermatooza per ejaculate was more important in pre-
dicting fertility. Artificial insemination with greater than 250
x 10^6 morphologically normal sperm resulted in a pregnan-
ty rate of approximately 82% in 27 bitches evaluated. Specific
morphologic defects associated with infertility in the
dog include abnormalities of midpiece attachment or ultra-
structure, microcephalic spermatooza, and proximal retained
cytoplasmic droplets.

The concentration is determined using a special pho-
tometer (SpermaCue®, Minitube) or a hemocytometer. To do
this, the sperm is diluted at 1:100, and the number of sperm
in the large central square (made up of 25 smaller squares)
on the hemocytometer is counted. The number of sperm
counted x 10^6 is the concentration of spermatooza/mL. The
total number of sperm in the ejaculate is calculated as vol-
ume x concentration. This should be ≥ 200 x 10^6, and closer
to 400 x 10^6 in larger dogs. Every dog investigated for infer-
tility should be screened for Brucella canis.

Sperm quality may be normal or abnormal, or no sperm
may be seen in the ejaculate. Infertility is rare in dogs with
a normal sperm evaluation and, if seen, the history should be
reviewed for mismanagement or bitch infertility. The pres-
ence of WBC or RBC in the ejaculate suggests inflammation
of the tract, most commonly prostatitis; culture of prostatic
fluid and appropriate treatment may help fertility. If sperm
quality is abnormal, the history should again be reviewed
to determine if the dog has been sick recently or has received
any drugs, especially anabolic steroids. Other recognized
causes of abnormal sperm quality include inflammation of
the scrotum or other factors that may be causing a high scro-
tal temperature, testicular neoplasia (ultrasonography of the
testicles is recommended because many neoplasms of the
testes are not palpable), trauma to the area of the scrotum, or
brucellosis. However, most cases of low sperm quality in
dogs are idiopathic.

The dog’s pituitary status can be investigated but is usually
unrevealing. Luteinizing hormone and follicle-stimulating
hormone are typically normal to high in dogs with abnormal
semen quality because the degenerating testes are not able to
provide the feedback mechanism to the pituitary. Because
abnormal sperm quality may be induced by a recent transient
disease or exposure to toxins, and spermatogenesis might
resume, collections should be repeated about every 3 mo for
~1 yr before a definitive prognosis for breeding can be given.

Azoospermia is relatively common in dogs. It may be due
to failure of the dog’s testicles to produce sperm, or to fail-
ure of the sperm to exit the testicles because of epididymal
blockage or incomplete ejaculation. The ejaculate may be
tested for the presence of alkaline phosphatase, which is
secreted by the epididymis. A high value (>5,000 IU/L [very
high in comparison with blood]) indicates fluid from the epi-
didymis was collected. High alkaline phosphatase values in
sperm-free fluid suggest that the testes are not producing
sperm or that sperm transit is blocked between the testes and
epididymides. Low values suggest epididymal blockage or
failure of ejaculation; semen collections should be repeated,
using a strong stimulus such as a bitch in estrus. The urinary
bladder should be catheterized to determine if retrograde
ejaculation is occurring; swab samples of the vagina of a
bitch after natural mating may also be performed to deter-
mine if the dog is not ejaculating due to aversion to manual
collection. Careful palpation and ultrasonographic examina-
tion should be performed to detect any abnormality of the
epididymides or spermatic cords, such as absence or block-
age of the epididymis.