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 (eg, 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 glas, of rubber or plastic. Some are for single use others not. Different types are demonstrated. A sterile nonspermicidal lubricant or petroleum jelly may be is 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

microscope slide before being smeared and allowed to air-dry. Spermac® stain is a rapid stain that offers unique differential qualities. The sperm nucleus stains red, the acrosome, midpeace, 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 differentially with Spermac stain. Evaluation of sperm morphology should be completed microscopically using oil immersion. The normal spermatozoa consist of the acrosomal cap, head, neck, middle piece, and tail. The acrosome is a caplike structure covering slightly more than the anterior half of the head, and the middle piece is approximately 1.5 times the length of the head in normal spermatozoa. Individual spermatozoa should be evaluated for abnormalities arising in the head, middle piece, and tail. Commonly identified abnormalieies include detached heads, knobbed acrosomes, detached acrosomes, proximal and distal cytoplasmic 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 generally should have greater than 70% morphologically normal spermatozoa 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 normal and subnormal. Another investigation found that total numbers of morphologically normal and progressively motile spermatozoa per ejaculate was more important in predicting fertility. Artificial insemination with greater than 250 x 10^6 morphologically normal sperm resulted in a pregnancy rate of approximately 82% in 27 bitches evaluated. **Specific morphologic defects associated with infertility** in the dog include abnormalities of midpiece attachement or ultrastructure, microcephalic spermatozoa, and proximal retained cytoplasmic droplets.

The **concentration** is determined using a special photometer (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 spermatozoa/mL. The total number of sperm in the ejaculate is calculated as volume x concentration. This should be ≥ 200 x 10^6, and closer to 400 x 10^6 in larger dogs. Every dog investigated for infertility 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 presence 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 scrotal 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 failure 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 epididymis 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 determine if the dog is not ejaculating due to aversion to manual collection. Careful palpation and ultrasonographic examination should be performed to detect any abnormality of the epididymides or spermatic cords, such as absence or blockage of the epididymis.