

## Guidelines for using the canine breeding soundness evaluation form\*

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### Introduction

The canine breeding soundness examination (BSE) is a minimum data base with which to evaluate the reproductive capability of a dog. Interpretation of the results of parameters evaluated remains a challenge to the veterinarian, particularly in the area of infertility. For example, the threshold between fertility and infertility may change for an individual dog when there is an increase in numbers of breedings required over a short period of time depleting the number of normal sperm cells available.

An example of a BSE form is provided (Figure 1). Use of this form or one similar to it promotes consistency. The form provides an area for identifying the animal properly to ensure accuracy of medical records. The date of the examination is important in serial examinations of an individual; fertility parameters may change over time. Since the spermatogenic cycle in the dog is approximately two months, subsequent examinations, if needed, are usually performed at two month intervals. The animal's age is of particular importance due to the associated increased incidence of reproductive problems in the aged dog or the transient infertility in the prepubertal dog.

Breeding history of the dog should be obtained if available. Many dogs are presented for examination after fertility problems have been encountered; this should be noted in the record as the reason for the evaluation. It is helpful to know when the last successful breeding took place in order to assess the chronological progression of any problems. *Brucella canis* testing is advisable in all canine reproductive examinations. Due to the problem of false positive results in many screening tests, it is helpful to know which test was used.<sup>1</sup> Pedigree of the animal may be potentially helpful when researching the possibility of hereditary problems or the early loss of fertility as a familial problem.

A thorough physical examination should be performed as a part of the examination for breeding soundness in the dog. The physical condition, weight, and chronic health problems may adversely affect a given dog's reproductive capabilities. Obesity, skin disease, and endocrine disorders are just some examples of common conditions found in the dog that could adversely influence the dog's fertility. The penis and prepuce should be examined for inflammation or abnormality. The spermatic cord and scrotum should be examined for any problems including those that might interfere with the thermoregulation of the testes. The prostate gland is the only accessory sex gland in the dog and is responsible for the volume of the canine ejaculate. Because disorders of the canine prostate gland are common (benign hyperplasia with or without overlying infection, with associated signs of hematuria, hemospermia, etc.), digital examination of the

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prostate per rectum with concurrent abdominal palpation should be performed for determination of prostate size, symmetry and texture, and for assessment of pain. The epididymides should be palpated and any abnormalities noted. The tail of each epididymis should be located on the caudal pole of each horizontally positioned testis. The body and head of the epididymides can be palpated along the dorsal and anterior aspects of the testes. The testes should be examined and total scrotal width measured with calipers. Although testicular size varies widely between breeds, size should be recorded for purposes of comparison at subsequent examinations. Softening of the testes may indicate testicular degeneration, hypoplasia, atrophy, or abnormal development. Hardness of the testes may indicate testicular fibrosis, neoplasia, inflammation, or the presence of a prosthesis. An appreciation of normal testicular consistency and practice are necessary to pick up subtle changes.

### **Semen collection**

As general rule, five days of sexual rest should be allowed prior to semen collection and evaluation.<sup>2</sup> Conversely, if an animal has not ejaculated within ten days, collecting a second ejaculate may be necessary to provide a reliable indication of the dog's spermiogram. The adverse effects of sperm cell ageing may be seen in the first ejaculate obtained following prolonged sexual rest with increased numbers of secondary abnormalities such as detached heads and distal cytoplasmic droplets.<sup>3</sup> The number of these abnormalities should be dramatically reduced in a second ejaculate if, indeed, they are due to aging.

When presented for semen collection, the animal needs to acquaint himself with the collection area and the personnel involved so as to be as comfortable and relaxed as possible. He should be allowed the opportunity to urinate and defecate prior to the examination. In some dogs, urination immediately prior to collection will cause urine contamination of the ejaculate (urospermia), which may affect semen quality. The area used for semen collection should have good surface traction to prevent slipping. It is best to keep distractions to a minimum to maintain the dog's concentration. It may even be necessary to exclude the owner; sometimes the owner's presence may be a detrimental influence on the collection procedure. Conversely, the owner's presence may be beneficial to the collection procedure for certain dogs. Dogs' likes and dislikes should be noted in the record for future reference.

The most commonly used method for collection of semen from the dog is digital manipulation. A director cone may or may not be used (Figure 2).<sup>4,5</sup> Latex director cones, thoroughly rinsed with hot water and dried between uses, may be used multiple times. Disposable polyethylene director cones also are available. Digital manipulation with a director cone has the advantage of preventing loss of any part of the ejaculate during collection (Figure 3). The disadvantage is increased contamination of the ejaculate (microbial and particulate) from the penile movements within the director cone. The director cone or collection tube should be vented to prevent a vacuum within the cone. This vacuum may impede semen flow or forcibly dislodge the collection tube during semen collection. Any lubrication used in the director cone must not come in contact with the semen due to potential spermicidal activity.<sup>6</sup> Finally, it may be difficult to separate the three fractions if so desired using a director cone.

Digital manipulation without a director cone has the advantage of requiring the least amount of equipment. It is also a relatively simple procedure and contamination of the semen is held to a minimum. The primary disadvantage of this technique is the potential for losing the sperm-rich fraction due to the vigorous thrusting some dogs exhibit during ejaculation. Care also must be taken to prevent damage to the tip of the penis during collection due to any thrusting activity by the dog against the collection tube. The collection tube can be either glass or plastic. In either method, graduated polypropylene plastic conical tubes have the advantage of immediate volume determination (Figure 2).

Semen can be collected from a dog with or without the presence of a bitch. Many male dogs can be collected without the presence of a bitch using digital manipulation alone. The

ejaculate collected without a bitch present is usually adequate to assess a dog's breeding soundness. If the presence of a bitch is necessary, it is desirable but not necessary that she be in proestrus or estrus. The benefits of utilizing a bitch during semen collection include better responsiveness of the dog to collection and a higher total number of sperm in the ejaculate.<sup>7,8</sup> If an estrual bitch is unavailable, frozen-thawed vaginal swabs from a disease-free estrual bitch can be used through their direct application to the perineal area of a tractable bitch. With either method, the dog should be allowed to sniff the bitch's genital area before and during semen collection. If no teaser bitch is available, administration of 0.1 mg/kg of prostaglandin F2alpha 15 minutes prior to collection also has been shown to increase total number of spermatozoa in the ejaculate.<sup>8</sup>

Prior to collection, the collector may wish to wipe the preputial orifice and penile tip with a moistened cotton pledget to reduce ejaculate contamination with preputial discharge. Grasp the penis and prepuce behind (proximal to) the bulbus glandis (bulbus) in a tourniquet-like manner with a thumb and forefinger maintaining moderate but consistent digital pressure (Figure 4). By doing this, the collector is trying to mimic the copulatory lock, or tie. Using the other three available fingers, apply pulsating pressure directly on the bulbus. The dog usually responds readily to this manipulation by initiating the erection process and may begin thrusting movements. Some dogs may require stimulation of the penis and bulbus through the prepuce prior to this simulated tie. Once a partial erection is initiated, retract the prepuce behind the bulbus by placing the free hand at the point of attachment of the prepuce to the abdomen and directing the prepuce caudally to expose the bulbus if so desired (Figure 5). A dog may experience pain or give an incomplete ejaculate if erection occurs while within the prepuce. Conversely, some dogs maintain erection far longer than necessary if the prepuce is retracted behind the bulbus. Either method is acceptable as long as the ejaculation is complete and detumescence and replacement of the penis into the prepuce is ensured before the dog is kenneled.

During the thrusting movements, maximal erection is gained and the first of the three fractions of the ejaculate is emitted. The volume of this first fraction (F1) is variable. This fraction may be difficult to separate from the second, sperm-rich, fraction (F2). Practically, F1 and F2 are often collected together to prevent losing the sperm-rich fraction. Fraction 1 is clear to slightly cloudy. The dog will cease thrusting, almost abruptly, and will usually attempt to "tie" by lifting his leg over the arm of the collector, thus directing his penis backwards between his rear legs; not all dogs will attempt this. After a brief pause, the dog emits F2, which can be identified by its milky-white color. Fraction 2 also varies in volume. After emitting F2, the dog begins to emit the third fraction (F3) originating from the prostate gland. Fraction 3 can be distinguished from the sperm-rich fraction by the change in appearance from cloudy to clear. Pulsations can be felt in the urethra and observed as contractions of the anal sphincter as F3 is ejaculated. The collector can stop collecting F3 once adequate dilution of the sperm-rich fraction has occurred to permit appropriate microscopic analysis. It is advisable to keep the exposed penis protected from damage by restraint of the dog until detumescence has occurred. This can be encouraged by the use of a water-soluble lubricant on the penis itself, which also helps to alleviate any drying of the penile mucosa. Special attention should be afforded to long-haired breeds where hair may become entangled around the penis during detumescence and retraction of the penis into the prepuce.

### **Semen evaluation**

The semen must be protected from external insults during and after the collection process. Temperature, chemical, and mechanical insults to the sperm cells should be avoided. To prevent temperature insults, all equipment coming into contact with the ejaculate should be at room temperature. Attempts should be made not to shock the sperm cells with equipment and temperatures that are either too hot or too cold. Chemical damage (latex gloves and director cones, water, detergent residues, bacteriostatic lubricants, etc.) should be avoided.<sup>9,10</sup> Finally, any

aspiration of the ejaculate into syringes or pipettes should be done slowly to avoid mechanical to the cells. Normal values for semen evaluation in dogs are described in Table 1.<sup>7,11-13</sup>

#### Color

Fraction 1 should be clear to slightly cloudy. Fraction 2 should be some variant of milky-white and homogenous. If F2 is clear or only slightly cloudy, this may indicate decreased sperm numbers or complete azoospermia. Yellow discoloration of F2 may indicate urine contamination or presence of a purulent exudate. Red or brown discoloration of F2 indicates presence of fresh or hemolyzed blood; this may be indicative of either reproductive tract disease or trauma at collection. Fraction 3 should be clear. Red or brown color of F3 strongly suggests the presence of a disease process such as prostatic disease, trauma or injury, or neoplasia of the reproductive tract.

#### Volume

Measure the volume of the collected ejaculate before removing semen for anything else. This volume will be needed to calculate total number of spermatozoa in the ejaculate. The volume of F1 and F2 can be read directly from the calibrated tube into which it was collected or measured in a syringe after removal from another sort of collection vessel. The volumes vary greatly between individuals and among breeds. The volume of the separate F3 need not be recorded as this is even more variable depending on collection technique and duration, and because this fraction contains no spermatozoa.

#### pH

To measure pH, a drop of semen should be applied to the pH paper; do not immerse the paper into the semen sample. Normal values range from 6.5-7.0. Changes in pH may indicate that prostatic infection is present, and may guide the choice of antibiotic therapy.<sup>14</sup>

#### Motility

Motility should be measured as soon as possible after collection. Place a drop of semen on a slide and examine at 100-200x magnification. The evaluator may or may not wish to compress the drop with a cover slip; either technique is appropriate as long as the evaluator is consistent. A conventional light microscope can be used with a decreased light setting. Assessment also can be performed, with excellent results, on a phase contrast microscope. Very concentrated samples may need to be diluted to allow for visualization of individual spermatozoa. Diluents used include the dog's own prostatic fluid (F3), phosphate-buffered saline or 2.9% sodium citrate. Motility parameters that should be assessed are total motility (% of spermatozoa that are moving), progressive motility (% of spermatozoa that are moving in a straight line), and speed of motility (slow/medium/fast). Commonly accepted values are greater than 70% total motility, greater than 80% progressive motility for the sperm that are motile, and greater than 80% with a speed rating of "fast".

#### Concentration

Concentration of spermatozoa is measured on the collected ejaculate containing F2. The WBC dilution system (BD Unopette™ reservoir #365854, Becton-Dickinson, Franklin Lake, NJ) may be used. Draw semen up into the 20 microliter pipette and dispense it into the 2 ml diluent container according to kit instructions; the diluted solution can be used immediately. Dispense the solution into both chambers of a hemacytometer. The number of sperm in the center 1 mm square (the square that fills the field using the 10x objective) equals the number of million sperm per milliliter in the ejaculate. For quality control, count both sides of the hemacytometer. If the two values are not within 10%, reload the hemacytometer and count again.

### Total sperm number of spermatozoa in the ejaculate

The total number of sperm in the ejaculate is calculated as the concentration (millions of spermatozoa per ml) times the volume (ml per ejaculate). A commonly accepted normal value is greater than 300 million spermatozoa in the ejaculate. Large breed dogs have a higher total number of spermatozoa per ejaculate than do small breed dogs due to the larger testicular mass.

### Morphology

Morphology is most often assessed by staining the semen sample and observing the cells under 1000x magnification (oil immersion). Morphology may be assessed without staining using a phase contrast microscope after fixing a sample with formol-buffered saline at a dilution of 1:9. Different sample preparations cause artifacts that will be seen as morphologic abnormalities; percentage of morphologically normal spermatozoa should be fairly consistent regardless of method.<sup>15</sup> The two most common stains used are a rapid Wright's Giemsa stain (Dif-Quik™, Baxter Healthcare, Miami, FL) and the eosin-nigrosin stain (Morphology Stain, Lane Manufacturing, Denver, CO)

To stain with a rapid Wright's Giemsa stain, place one drop of semen on a slide and smear out as for a blood smear. Allow to air dry. Immerse the slide into each of the three solutions in the same order as used for any type of cytology, allowing the slide to sit in each solution for five minutes. Rinse the slide completely and let it air dry before evaluation under oil immersion.

To stain with eosin-nigrosin, place one drop of semen and a similar-sized drop of stain on one end of a glass slide. Gently mix the two together with a pusher slide, and draw out a thin film similar to a blood smear. Allow to air dry as quickly as possible; placing the slide on a warming plate (37 °C) and blowing on the slide will hasten the drying process and help to prevent staining artifacts.<sup>16</sup>

Spermatozoa stained with rapid Wright's Giemsa stain appear purple on a clear background. Normal spermatozoa stained with eosin-nigrosin stain appear white against a black or violet background. Eosin-nigrosin stain is taken up by spermatozoa that have abnormal plasma membranes and so appear pink against a dark background. It might be assumed that such spermatozoa are non-viable but no significant correlation between "live-dead" ratio and fertility has been described in dogs.

Under oil-immersion, examine and count at least 100 spermatozoa. The number of normal spermatozoa in 100 is the percentage morphologically normal spermatozoa. Total normal for the sample is calculated by multiplying the total number of spermatozoa in the ejaculate by percentage morphologically normal. Commonly accepted values are greater than 80% morphologically normal spermatozoa and greater than 200 million total normal spermatozoa in an ejaculate. Abnormal spermatozoa may be classified as having primary defects (those that occur during spermatogenesis, including defects in head shape, bent midpiece, persistent proximal cytoplasmic droplet, and doubling of any portion of the spermatozoon) or secondary defects (those that occur during epididymal maturation or slide preparation, including detached heads, persistent distal cytoplasmic droplets and bent tails). Correlation between specific defects and fertility in dogs is poorly defined.

### Cytology

To assess cytology, place a drop of undiluted semen on a glass slide, drawn out as for a blood smear, and allowed to air dry. Immerse the slide into the three solutions of a rapid Wright's Giemsa stain as for any cytologic preparation. The presence of white blood cells (WBCs), red blood cells (RBCs), epithelial cells, and bacteria is noted with a designation of 0 to 4+ to indicate relative amounts (Table 2).

Fraction 3 is evaluated separately; presence of RBCs or WBCs may indicate prostate disease. To evaluate a fairly large acellular sample, centrifuge the semen and process the pellet as

described above. A fair number of epithelial cells may be observed, especially after a period of sexual rest. The presence of occasional WBCs and bacteria is normal. If infection of the reproductive tract is suspected based on history and physical examination findings, culture of seminal fluid should be performed even if there does not appear to be a large number of WBCs in the sample.<sup>17</sup>

Table 1. Normal seminal characteristics for the dog.<sup>7,11-13</sup>

	Unfractionated ejaculate	F1	F2	F3
Color	Opaque to white	Clear	Milky-white	Clear
Volume (ml)	Variable	0.25-3.0	0.4-3.0	1-30
pH	6.5-7.0	---	---	6.5-7.0
Concentration (million/ml)	65 (10-200)	---	---	---
Total number of sperm in ejaculate (million)	>300	---	---	---
Total motility (%)	>70	---	---	---
Progressive motility (%)	>80	---	---	---
Velocity (speed)	Fast	---	---	---
Normal morphology (%)	>80	---	---	---


Table 2. Quantification of cells other than spermatozoa in canine semen

Designation	Number of cells per high power field (hpf)
0	<1
1+	1-3
2+	4-6
3+	7-10
4+	>10

## References

1. Johnson C, Jacobs J, Walker R: Diagnosis and control of *Brucella canis* in kennel situations. Proc Soc for Therio 1991; p. 236-239.
2. Foote RH: The influence of frequency of semen collection, fractionation of the ejaculate, and dilution rate on the survival of stored dog sperm. Cornell Vet 1964; 54:89-97.
3. Christiansen IJ: Andrology of the normal male. In: Christiansen IJ, editor. Reproduction in the dog and cat. Philadelphia: Baillere Tindall; 1984. p. 99-107.
4. Pineda MH: A simple method for collection of semen from dogs. Canine Pract 1977;4:14-18.
5. Hopkins SM, Evans LE: Artificial insemination. In: McDonald LE, Pineda MH, editors. Veterinary endocrinology and reproduction, 4th ed., Philadelphia: Lea and Febiger; 1989. p. 366-367.
6. Froman DP, Amann RP: Inhibition of motility of bovine, canine, and equine spermatozoa by artificial vagina lubricants. Theriogenology 1983; 20:357-362.
7. Boucher JH, Foote RH, Kirk RW: The evaluation of semen quality in the dog and the effects of frequency of ejaculation upon semen quality, libido, and depletion of sperm numbers. Cornell Vet 1958;48:67-86.
8. Root Kustritz MV, Hess M: Effect of administration of prostaglandin F2alpha or presence of an estrous teaser bitch on characteristics of the canine ejaculate. Theriogenology 2007;67:255-258.
9. England GCW, Allen E: Factors affecting the viability of canine spermatozoa. I. Potential influences during processing for artificial insemination. Theriogenology 1992;37:363-371.
10. Althouse GC, Ko JCH, Hopkins SM, et al: Effect of latex examination and vinyl examination gloves on canine spermatozoal motility. J Am Vet Med Assoc 1991;199:227-229.

11. Harrop AE: The physiology of reproduction in the dog and bitch. In: Harrop AE, editor. Reproduction in the dog. Baltimore: Williams and Wilkins Co., 1960. p. 64-77.
12. Bartlett DJ: Studies on dog semen. II. Biochemical characteristics. J Reprod Fertil 1962;3:190-205.
13. Olar TT, Amann RP, Pickett BW: Relationships among testicular size, daily production and output of spermatozoa, and extragonadal sperm reserves in the dog. Biol Reprod 1983;29:1114-1120.
14. Olson PNS: Disorders of the canine prostate gland. Proc Soc for Therio 1984. p. 46-60.
15. Root MV, Johnston SD, Olson PN, et al: Effect of stains and investigators on assessment of morphology of canine spermatozoa. J Am Anim Hosp Assoc 1998;34:348-352.
16. Shaffer HE, Almquist JO: Vital staining of bovine spermatozoa with a eosin-aniline blue staining mixture. J Dairy Sci 1948;3:677-678.
17. Root Kustritz MV, Johnston SD, Olson PN, et al: Relationship between inflammatory cytology of canine seminal fluid and significant aerobic, anaerobic or mycoplasma cultures of seminal fluid: 95 cases (1987-2000). Theriogenology 2005;64:1333-1339.



**CANINE BREEDING SOUNDNESS EVALUATION**  
 Guidelines Established by the Society for Theriogenology  
 PO Box 3065, Montgomery AL 36109 • (334) 395-4666

AKC Reg #/Other ID # \_\_\_\_\_ Exam Date: \_\_\_\_\_  
 Call Name: \_\_\_\_\_ Registered Name: \_\_\_\_\_  
 Client Name: \_\_\_\_\_ Address: \_\_\_\_\_  
 Breed: \_\_\_\_\_ Color: \_\_\_\_\_ Date of Birth: \_\_\_\_\_

**HISTORY**  
 Reason for evaluation: \_\_\_\_\_  
 Date of last litter: \_\_\_\_\_ Brucellosis test: positive/negative Date of test: \_\_\_\_\_ Test used: \_\_\_\_\_  
 Infertile relatives: \_\_\_\_\_

**PHYSICAL EXAMINATION**  
 Body condition: \_\_\_\_\_ Weight: \_\_\_\_\_ pounds/kilograms  
 Pertinent health problems: \_\_\_\_\_  
 Penis/Prepuce: \_\_\_\_\_ Scrotum: \_\_\_\_\_  
 Prostate: \_\_\_\_\_  
 Epididymides: (R) \_\_\_\_\_ (L) \_\_\_\_\_  
 Testes Width: (R) \_\_\_\_\_ (L) \_\_\_\_\_ Spermatic cords: \_\_\_\_\_  
 Testes Consistency: (R) \_\_\_\_\_ (L) \_\_\_\_\_ Masses/fluid/pain/other: \_\_\_\_\_

**SEMEN COLLECTION & EVALUATION**  
 Libido/Ease of collection: Poor / Fair / Good / Excellent Teaser bitch present: Yes/No Stage of cycle: \_\_\_\_\_

	Color	Volume (ml)	Concentration (sperm/ml)	Total Sperm/Ejaculate
Fraction 1	_____	_____	_____	_____
Fraction 2	_____	_____	_____	_____
Fraction 3	_____	_____	_____	_____

Total Motile Spermatozoa: \_\_\_\_\_% Progressively Motile Spermatozoa: \_\_\_\_\_% Speed/velocity of motility: 0-5 \_\_\_\_\_

**Morphology:** Stain(s) utilized: \_\_\_\_\_  
 % Normal: \_\_\_\_\_ Number Cells Counted: 100/200  
 Head defects (%): \_\_\_\_\_  
 Midpiece defects (%): \_\_\_\_\_  
 Tail defects (%): \_\_\_\_\_  
 Other defects (%): \_\_\_\_\_

**Longevity:** Extender(s) used: \_\_\_\_\_ Diluent rate: \_\_\_\_\_  
 Motility: 24 hours \_\_\_\_\_ 48 hours \_\_\_\_\_

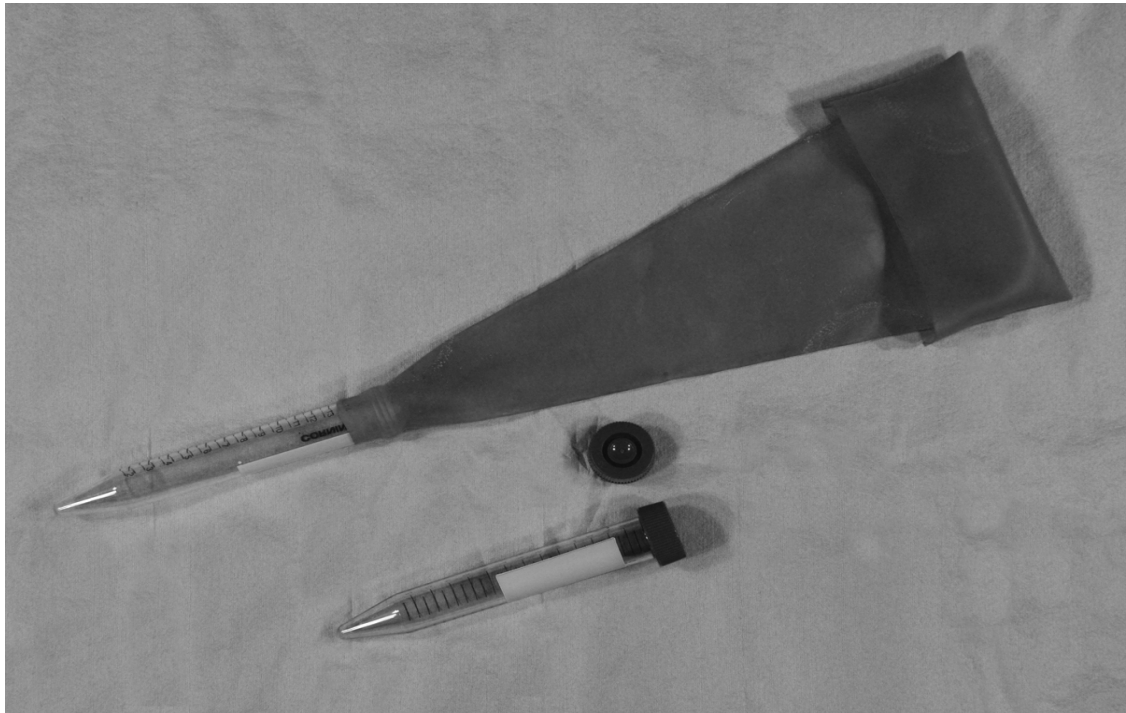
**Cytology:** Fraction(s) evaluated: \_\_\_\_\_ Stain(s) utilized: \_\_\_\_\_  
 Presence of RBC, WBC, Epithelial cells, Bacteria, Germ cells (0 – 4+): \_\_\_\_\_

**CONCLUSIONS / COMMENTS / INTERPRETATION**

Signed: \_\_\_\_\_ Clinic Name: \_\_\_\_\_  
 (Member – Society for Theriogenology) Address: \_\_\_\_\_

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**Figure 1. Sample canine BSE form. Available at the Society for Theriogenology website.**



**Figure 2. Equipment used in the manual collection of canine semen: director cone (collecting funnel) and graduated polypropylene conical tubes.**



**Figure 3. Manual collection of canine semen using a director cone; penis directed backwards.**





**Figure 4. Manual collection of canine semen without a director cone; bulbus glandis retained within the prepuce; penis directed backwards.**



**Figure 5. Manual collection of canine semen without a director cone; prepuce retracted caudally to expose the bulbus glandis; penis directed backwards.**

