

# Semen collection in the dog

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## Abstract

This review will discuss semen collection in the dog. Semen samples may be collected from male dogs for the purposes of artificial insemination, cryopreservation or diagnosis. The materials needed for semen collection depend on which method is used and the collector's level of expertise with this procedure. At minimum, two sterile centrifuge tubes or specimen cups can be used to collect semen as it is ejaculated (for the combined first and second fractions and for the third fraction). The most common method for semen collection in the dog is by digital stimulation. Under ideal conditions, this procedure is performed in the presence of an estrous bitch. Initially, the dog's penis is vigorously massaged through the prepuce at the level of the bulbus glandis (caudal-most aspect of the prepuce) until a partial erection develops (initial engorgement of the bulbus glandis). The prepuce is quickly retracted past the bulbus glandis and firm constant pressure is applied to the penis behind the bulbus glandis by squeezing the penis between index finger and thumb. Pelvic thrusting may occur following application of pressure behind the bulbus glandis during the development a "full" erection. The ejaculate is composed of three fractions: first (sperm-poor), second (sperm-rich) and third (prostatic fluid). In addition to digital stimulation of the penis, spermatozoa have been collected from dogs using electroejaculation and pharmacologic methods.

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## 1. Introduction

The indications for collecting semen from a male dog include artificial insemination, cryopreservation or diagnostic purposes. Occasionally, when both the male and female dogs are present and the bitch is at the proper stage of the estrous cycle to accept breeding

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(estrus), artificial insemination is still requested due to presence of vaginal anomalies in the bitch (e.g. narrow vagina (maiden), vaginal–vestibular stricture, vaginal septum, vaginal hyperplasia) or a behavioral incompatibility between the male and female dogs. More commonly though, the male and female dogs are located in distant locations and transportation of the semen is less expensive and less stressful than shipping either the male or female dog. Canine semen is also collected routinely for freezing (cryopreservation), which enables stud dog owners to preserve genetics of their male and allows breeding after the male is no longer fertile, is unavailable for breeding or deceased. Although every sample of semen collected should be evaluated (at least progressive forward motility, total sperm count and morphology) before it is used for artificial insemination or cryopreservation, veterinarians are often asked to collect canine semen for these purposes alone. Older males (>12 years of age), males that have not been used for breeding for several years, males that have a history of infertile breedings (females not whelping after breeding) or small litter sizes (<3 pups in medium-breed bitches, <4 pups in large and giant breed bitches) should have semen evaluated prior to the next breeding. Intact males that present with abnormal preputial discharge (e.g. hemorrhagic), hematuria, blood in the ejaculate or any other signs associated with prostatic disease, should have a semen sample collected, with particular interest in the third fraction (prostatic fluid) of the ejaculate.

The specific method for collecting semen from a male dog depends on what the semen is to be used for. Only the first (pre-sperm) and second (sperm-rich) fractions of the ejaculate are needed for semen used for artificial insemination or cryopreservation. However, all three fractions should be evaluated when males are presented for breeding soundness examinations or overt evidence of reproductive disease. In all cases when fertility is in question, it is important that complete ejaculation (seminal plasma alkaline phosphatase >10,000 U/L in the combined first and second fractions) occurs [1].

## **2. Materials**

The materials needed for semen collection in male dogs depend on which method is used and the collector's level of expertise with this procedure. At minimum, two sterile centrifuge tubes or specimen cups can be used to collect semen as it is ejaculated (one for the combined first and second fractions and the other for the third fraction). Occasionally, copious first fraction (>3 mL) is ejaculated before second fraction is collected. For the purposes of artificial insemination or cryopreservation, a third collection vessel should be available so that copious first fraction can be discarded. For beginners, using a semen collection cone (Fig. 1) with a 15 mL sterile centrifuge tube attached minimizes chances that precious few drops of sperm-rich (second) fraction will be lost during the thrusting phase of semen collection. However, this method is not ideal if cultures need to be obtained from the sample, as the ejaculate will become contaminated with normal bacterial flora from the surface of the penis and prepuce. Lane manufacturing sells these disposable semen collection cones for <US\$ 1.00 each (<http://www.lane-mfg.com/bovineprod.html>). Latex semen collection cones (artificial vaginas) are often used with digital manipulation for semen collection in dogs [2–4]. The main disadvantage to the use of latex semen collection cones is the need for careful cleaning between males including the removal of all



Fig. 1. Disposable semen collection cones manufactured by Lane.

disinfectant residues and water prior to the next use. In addition, Althouse et al. found that contact of dog semen with latex gloves for 1 min has a detrimental effect on sperm motility. Contact with vinyl gloves or talcum powder had minimal effect on sperm motility [5].

The presence of a teaser bitch in estrus will increase the probability that nervous or virgin males will ejaculate when semen collection is attempted (Fig. 2). However, the presence of a submissive bitch at any stage of the estrous cycle or even a castrated male can be useful. Estrous bitch synthetic pheromones are commercially available (methyl *p*-hydroxybenzoate; Aldrich Chemical, Milwaukee, WI, USA) or estrous vaginal secretions from healthy, *Brucella*-negative bitches can be preserved within gauze sponges from earlier examinations in a regular freezer ( $-20^{\circ}\text{C}$ ) for future use. These pheromones can either be presented on a gauze sponge to the male or rubbed over the base of the tail of the teaser animal. Sterile, non-spermicidal water-soluble lubricant (e.g. H-R lubricating jelly) should be applied to the penile mucosa, especially behind the bulbous glandis, following semen collection to prevent inversion of the prepuce.

### 3. Methods

#### 3.1. Traditional method for semen collection in dogs

The most common method for semen collection in the dog is by digital manipulation. Under ideal conditions, this procedure is performed in the presence of an estrous bitch. The lack of an available estrous bitch should not automatically preclude an attempt to collect a semen sample from a dog. The majority of dogs collected by this author have not been in the presence of an estrous bitch. It is imperative that any distractions or procedures that



Fig. 2. Virgin male dog being prepared for semen collection. The presence of a teaser bitch in estrus will increase the probability that nervous or virgin males will ejaculate when semen collection is attempted.

would induce anxiety be eliminated or minimized. Fear and pain will prohibit a dog from attaining a complete erection and ejaculating. For excessively timid males, allowing the male to “play” with the teaser or the owner or the collector prior to collection may improve the quality of the ejaculate.

Initially, the dog’s penis is vigorously massaged through the prepuce at the level of the bulbus glandis (caudal-most aspect of the prepuce) until a partial erection develops (initial engorgement of the bulbus glandis). The step is not necessary for males that present with a partial erection. If the collector is right-handed, the author finds it least awkward to collect the semen from the dog’s left side, holding the dog’s penis with the right hand and the collection container in the left hand. If a teaser bitch is present, the dog may be allowed to mount the teaser bitch. The prepuce is quickly retracted caudally (pushed back) past the bulbus glandis and firm constant pressure is applied to the penis behind the bulbus glandis by squeezing the penis between index finger and thumb (Fig. 3). Back and forth manipulation of the penis at this stage is not necessary and may result in the loss of the erection (detumescence). If the bulbus glandis is too engorged, the preputial opening will not be large enough to allow the prepuce to pass by the bulbus glandis. Should this occur, the procedure should be discontinued until the bulbus glandis engorgement subsides (within a few minutes). Distracting the dog by offering him food or taking him for a brief walk will hasten the detumescence of the bulbus glandis.

Pelvic thrusting (minimal to exaggerated) may occur following application of pressure behind the bulbus glandis during the development a “full” erection. Erection is due to impulses from the nervi erigentes, composed of parasympathetic fibers from the pelvic and



Fig. 3. The collector has retracted the prepuce past the bulbus glandis and is applying firm constant pressure to the penis behind the bulbus glandis by squeezing the penis between index finger and thumb.

sacral nerves. These nerve impulses lead to dilation of the external and internal pudendal arteries to the cavernous body of the penis. Due to contraction of the ischiourethral muscles, venous return from the cavernous body of the penis is prevented. Blood retained in the sinuses of the cavernous tissue causes the bulbus glandis to swell, glans penis to elongate and the pars longa glandis to slide forward over the os penis. Following the development of a complete erection, the male may attempt to “tie” as demonstrated by lifting his leg over the collector’s right arm (if the collector is right-handed; Fig. 4). The erect penis is rotated 180° caudally, maintaining the dorsum of the penis dorsally. The collector should continue applying firm pressure behind the bulbus glandis as well as gently pull the penis caudally away from the dog. The 180° turn is made possible by a twist of the penis just behind the bulbus. The penis is very elastic in this area, and the twisting does not seem to cause any discomfort [6]. The penile bone of the dog prevents occlusion of the urethral opening during the erection and twist. It has been suggested that the rotation causes occlusion of the emissary vein of the glans, thus preventing detumescence.

Ejaculation will begin immediately following the placement of pressure behind the bulbus glandis. Ejaculation is caused by stimulation of the sympathetic nerves of the penis. The semen and the prostatic fluid are expelled by peristaltic contractions in the muscles surrounding the urethra, particularly the bulbocavernosus and ischiocavernosus muscles. The ejaculate is composed of three fractions: first (pre-sperm), second (sperm-rich) and third (prostatic fluid). The characteristics of the ejaculate should be monitored (dripping, cloudy secretion = second fraction; spurting, clear secretion = third fraction). The release of the sperm-rich fraction usually takes <2 min. Collection of the third fraction is only



Fig. 4. This male has achieved a complete erection and the penis has been rotated 180° caudally while maintaining the dorsum of the penis dorsally.

needed if analysis is required. A new collection container should be used for the collection of the third fraction. The semen should be protected from abrupt changes in temperature, undue agitation, water, germicides, and detergents. Canine sperm is not as easily cold shocked as are sperm from other species (e.g. bovine, equine). However, canine sperm can be heat shocked very easily. To prevent harming the sperm, the semen should be maintained between body temperature (37 °C) and room temperature (20 °C). The semen should be evaluated for color, volume, motility, concentration, morphology, etc. However, it is outside the scope of this review to discuss evaluation of the canine ejaculate. It is important to note that the male may be collected immediately following the initial collection without a change in the semen quality in the second ejaculate [7]. England reported that this technique results in the collection of approximately 70% more spermatozoa when combined with the initial ejaculate [7]. It is also important to note that the administration of oxytocin (10 IU i.m.) or prostaglandin F2alpha (2.5 mg i.m.) 10 min before semen collection to three Samoyed dogs (body weight not given) did not increase the number of spermatozoa in the ejaculate [4].

Occasionally, hairs from the abdomen and prepuce can be trapped between the penis and the preputial orifice during detumescence, or the skin on the outer layer of the prepuce may be rolled inward (inverted). This may cause pain to the dog, and he may then require some assistance. Application of a non-spermicidal lubricant around the opening of the prepuce so that it does not stick to the dry mucosa of the penis will prevent the prepuce from becoming inverted. The male usually licks his penis, which then retracts into the prepuce.

### 3.2. Alternative methods for semen collection in dogs

In addition to the method of digital manipulation of the penis, spermatozoa have been collected from male dogs using electroejaculation and pharmacologic methods. The total sperm count ( $381.7 \pm 104.6$  million and  $243.4 \pm 60.5$  million, respectively) and total motile sperm ( $103.9 \pm 30.9$  million and  $78.0 \pm 28.1$  million, respectively) in semen collected from beagle dogs using digital manipulation and electroejaculation with halothane anesthesia (combining both antegrade and retrograde samples) did not differ [8]. However, the sperm motility from either method was significantly lower than previously reported for dogs [9]. In addition, alkaline phosphatase concentrations were not measured.

We performed preliminary investigations on male dogs ( $n = 3$ ) to determine if ejaculation could be induced using a combination of xylazine and imipramine that was reported to be successful in stallions [10]. Dogs were sexually rested for 5 days and administered oral imipramine (0.33 mg/kg) twice daily during that time. On the sixth day, dogs received an intravenous injection of xylazine (0.66 mg/kg) and Whirl-Pak<sup>®</sup> bags (Nasco, Fort Atkinson, WI) were taped over their preputial opening. Dogs were monitored for 1 h following xylazine treatment. Small amounts of urine containing non-motile spermatozoa were collected from each dog (Kutzler and Volkmann, unpublished observations). These results are supported by the observations of Dooley et al. in which spermatozoa were found to flow retrograde into the urinary bladder of dogs during ejaculation or after administration of xylazine (2.2 mg/kg i.m.) to sexually rested dogs [11]. Pilocarpine has previously been used in dogs to induce ejaculation in studies examining the components of prostatic fluid [12,13]. Juniewicz et al. administered 0.7 mg/kg of pilocarpine hydrochloride dissolved in 3 mL of saline intravenously to anesthetized male dogs and found a significant increase in semen volume compared to semen volume collected from males by digital manipulation [14]. However, a comparison of the sperm quality (progressive forward motility, concentration, morphology, etc.) collected using these methods was not reported. In addition, atropine sulfate (0.5 mg/kg) was administered intravenously following prostatic fluid collection to counteract the significant pharmacologic effects of the pilocarpine (intense salivation and lacrimation). Future research is needed to investigate methods for pharmacologically induced ejaculation in fearful or painful dogs that are unable to be collected by digital manipulation and in cases in which electroejaculation is not an option.

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