Sperm: Collection, Evaluation and Other Considerations
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Whether you breed your dogs by live cover or artificial insemination, either in person, fresh chilled or frozen, knowing the quality of a stud dog's sperm will give you helpful information and a good indication of whether or not he's going to be able to get a bitch pregnant. Sperm can change dramatically over time. Some illnesses can severely impact sperm quality, particularly if the dog runs a high fever. Generally, it will take 6 weeks after he has recovered for his sperm to regenerate and his potency to return. Other illnesses may have long term effects on sperm quality and fertility. The tick-borne diseases have been linked to poor fertility in both stud dogs and bitches, and this may persist even after the dog has otherwise appeared to have recovered or if it never showed signs of being ill in the first place.

Given that they hang freely away from the dog's body, the testicles are also vulnerable to traumatic injury, and function can also become temporarily or even permanently impaired if they become overheated from sitting on hot concrete or tarmac, or even from too hot a hair dryer. Even without excessive abuse, if a dog is not used at stud every two or three weeks, and very few dogs are, sperm hang around in the extensive plumbing of the testes, and gradually degenerate. Before evaluating semen, and especially before breeding a bitch, it is a really good idea to have a practice run seven to fourteen days before the first breeding, to get rid of aging deformed sperm, and bring on some nice fresh active sperm for the actual breeding. Some dogs masturbate to the point of ejaculation, and if they do this regularly this step won't be necessary, but most dogs don't and need helping out.

The breeding evaluation usually begins with inspection of the external re- productive organs, and this is a good place to start with your dog. Get him used to having his equipment handled from the get-go so that he is happy to let you assist in live breedings, and ready to accept the necessary handling for collection for artificial insemination. Palpate his testicles regularly – I do it when I am grooming – look for signs of discoloration, and gently feel for any abnormal lumps or dramatic changes in size (once he's past puberty). Get to know your dog's testicles when they are healthy. The penis should be extruded from the sheath, to make sure that it can do so easily, and examined for discoloration and other abnormalities. In older dogs rectal palpation of the prostate (in neutered as well as intact animals) is a good idea. Older intact dogs tend to undergo prostatic enlargement, and this can inter- fere with urination and defecation.

The next step in stud evaluation in breeding bulls, stallions, boars and some other species is usually measurement of the circumference of the testicles. In these species this gives you a very good indication of the expected volume of ejaculate. It's something we don't do in dogs, but perhaps with more dogs being kept on "ice" when they are in their reproductive prime or before they die, it may become common place.

In dogs we usually move right along to collecting sperm. Everyone has their preferred method, but for artificial insemination, unless the sperm is going straight into the bitch, using an artificial vagina attached to a plastic collecting tube is usually the preferred method. However, most artificial vaginas are latex, most of which is spermicidal (kills sperm) and cannot be gas sterilized. If reused the vagina needs to be washed, chemically disinfected and then rinsed

several times with distilled water prior to air drying. Semen can as easily be collected using a gloved or bare hand into any warm receptacle, sterile "Whirlpak" bags work well. Hard plastic should be avoided though as this can traumatize the penis and lead to substantial bleeding into the ejaculate. While this does not seem to affect fertility in dogs as it does in horses, it interferes with evaluation and is very uncomfortable for the dog, and will likely put him off the whole experience.

It is usually easiest to obtain an ejaculate in the presence of a bitch in estrus, although this is not essential. Synbiotics Corporation manufactures a synthetic pheromone – methyl paraben or "Eau d'estrus" – that may also be helpful. As the dog sniffs or starts to mount the bitch (if present), the prepuce is slipped back over the bulbus glandis – the large bump towards the root of the penis. The tip of the penis is slipped either into the artificial vagina (lightly lubricated with a small amount of sterile, aqueous, non spermicidal lubricant), the plastic bag or other collecting vessel. It id a nice touch if these are warmed to body temperature, although by no means necessary. Maintaining firm pressure behind the bulbus glandis with a ring of thumb and forefinger, the dog will begin to make pelvic thrusts and ejaculate.

Ejaculation can occur intermittently over a fairly long period, (15 minutes or so), so the collector and handler of the dog (and bitch if one is present) should try to get as comfortable as possible before collection begins. A slightly cloudy pre sperm fraction of about 0.1 to 3.0 mls is produced first. This is followed by the whitish sperm rich fraction the volume of which can range from 0.1 to 6.0 ml. The third fraction of the ejaculate is clear prostatic fluid which has relatively few sperm in it, but can have a volume of 50 mls or more. Usually collection stops when the prostatic fluid starts to appear. (Prostatic fluid makes a good diluent for insemination, and can be frozen and thawed to act in this capacity for frozen or fresh chilled semen.

A volume of 3 to 10 mls is used to inseminate a bitch.) It is the total number of sperm, not the volume, which is important. Sometime during the collection, the dog will usually lift his leg over the arm of the collector, as he would in adopting the rump-to-rump breeding posture of a natural tie (some dogs will even attempt to lie down). There is no problem in letting him turn so his penis extends out under his tail. After collection, observe the dog to make sure his penis returns completely into its sheath. Some dogs develop a condition called paraphimosis, whereby the penis does not retract properly and it is at risk of trauma if left exposed. Applying lubricant may help the dog to retract the penis.

Sperm Evaluation

Normal semen should contain 100-500 million sperm/ml; for a total of 300 million to 2 billion sperm per ejaculate. The higher the number the better the odds are that the bitch will get pregnant. Of these sperm 70% or more should show progressive motility; i.e., be swimming forward in a purposeful and rapid fashion rather than going in circles. A progressive motility of 30-50% is fair, and below 30% is poor. More than 80% of the sperm should show normal morphology. They should look like the little tadpoles in medical texts complete with a head and straight tail, and that little bump behind the head that contains the power (mitochondria) to keep their tails lashing so they can move forward. There is also a cap (acrosome) on the top of the head of the sperm which is necessary for penetrating an ovum. Abnormalities in any part

of the structure affect the ability of the sperm either to reach or penetrate the egg.

Healthy sperm should be pearly white or translucent in color. Yellow semen indicates that urine, which it toxic to sperm, has contaminated the ejaculate. Red discoloration indicates blood is present. This can be the result of trauma, prostatic problems or infection. Normal semen should contain few non sperm cells. Epithelial cells and other debris will likely be high if the dog has not been collected for a while, as will the number of dead sperm cells. Bacterial counts higher than 10,000 bacteria/ml indicate that there is an infection in the reproductive tract – usually the testicles or prostate. Neutrophils – a white blood cell – will also probably be present in the semen sediment. Infection is usually associated with decreased progressive motility as well as decreased numbers of morphologically normal sperm. Culture of bacteria or measurement of the pH of the semen can determine which antibiotic to give the dog.

Artificial insemination

There are many purists who believe that only natural breedings are acceptable. However, the ability to use artificial insemination certainly gives bitch owners a far greater access to stud dogs that might be most compatible with their lines, as well as preventing genetic stagnation. Distance of separation becomes less of an issue, and bitches do not have to be shipped long distances to be bred. Of course, things do go wrong. Shipments go astray, weather prevents the semen leaving or arriving as planned. Not all veterinarians are skilled at preparing semen for shipping, evaluating the optimal time to inseminate a bitch nor do they all have necessary skill to inseminate the bitch. It is always wise to make extensive inquiries at both ends before committing your breeding dreams to the hands of others.

If possible it is a good idea usually for dogs to at least experience natural breedings as well as artificial ones, bitches too. Behavioral problems may show up that might make you think twice about the wisdom of breeding the animal, although many breeding problems do not appear to be inherited. Bitches may be too nervous or aggressive to permit the dog to mount. Dogs may need fairly extensive instruction in breeding 101 – trying to mount the wrong end of the bitch, dig through her to the ground or hurling themselves with such force that she is sent flying, being some of the problems you may have to overcome. Some dogs show little libido. This may be the result of constant correction of sexual behavior, or looking to their owners for guidance in every step they take. If a dog was attacked or hurt during a previous breeding he will likely remember that too. Some dogs become so excited they become fully erect prior to penetrating the bitch's vagina. In this case full penetration isn't possible and an outside tie is usually less likely to result in pregnancy.

Once semen has been collected, inspected for gross abnormalities and a small sample examined microscopically the sample must be extended for shipment or freezing or inseminated into the bitch as soon as possible. Without extenders the sperm would not survive shipment or freezing. The extenders increase the volume. There are many different formulas. None works in all situations and some experimentation may be needed to find the best one for a particular dog. Having said that, not all dogs' sperm will ship or freeze well. Cooling and freezing semen damages cell membranes resulting in a loss of motility and general viability – this is known as cold shock. Most domestic species have high concentrations of polyunsaturated fatty acids

(PUFAs) in their semen, but the amount can be variable, especially of DHA – an omega 3 fatty acid, and DPA - an omega 6 fatty acid. These variations influence susceptibility to cold shock. In horses a low DHA:DPA ratio is associated with increased susceptibility to cold shock and decreased fertility. A recent study showed adding a DHA nutraceutical to stallions' diets increased sperm concentration to 1.8 times that of unsupplemented stallions and improved motility in fresh, chilled and frozen sperm. As far as I know, similar research has not been performed in dogs. A good extender has to have the right pH; the right osmolarity – so that cells don't gain or lose fluid; it must nourish and provide energy for the cells; and it needs to protect the cells through the freezing, thawing and rewarming phases.

Usually the total volume of ejaculate is used for insemination. Fertility decreases sharply with insemination of less than 50 million live sperm, and there will be a fair amount of attrition with time, chilling, or freezing and rewarming. Optimal extension rates for dogs have not been established, but work with horses suggests 1:1 to 1:6 (semen:extender by volume) should be used for transporting sperm. Most opt for a rate of 1:2. The semen should be cooled at a rate of 0.3ºC/minute to maintain optimal motility - this is the rate at which commercial Equitainers used for shipping horse se- men, and by some for dog semen, cool down. The Equitainer also insulates the sperm against changes of external temperature extremely well. Commercial canine extenders (Fresh Express from Synbiotics) or equine extenders appear to work equally well. Skimmed milk can also be used if it is first heated to 95ºC for ten minutes in a double boiler and then cooled to 37ºC for use. The heating denatures a spermicidal protein in the milk. The extender and sperm should be at the same temperature and the extender added slowly.

If you intend to use fresh chilled or frozen semen, samples should be evaluated for longevity. The sample should be divided, and then samples should be carefully warmed to room temperature and reevaluated for motility and morphology after various time periods. This will give you a good idea of how well your dog's sperm will survive shipping or freezing. However, as things can al- ways change, a drop of the sample should always be evaluated before the bitch is inseminated so that the status at the time of receipt is known. If all the sperm are dead, the bitch owner may want to institute plan B – if there is one.

It is recommended that sperm be collected no more often than once every 48 hours. Daily collection results in very low sperm concentrations after five to seven days. Registries – as well as some parent breed clubs – regulate who can perform artificial insemination – both collection and insemination steps. There are also regulations for importing sperm from another country, and within some countries. Check before you decide to go this route.