REPRODUCTIVE REVOLUTIONS
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Fertility Evaluation in the Stud Dog

The normal male dog attains puberty at approximately 6 – 8 months of age. Sexual maturity is generally attained at 18 – 30 months. Males may successfully breed bitches prior to sexual maturity but they will not attain maximal fertility or daily sperm output until mature. The normal male can breed once every 2 - 5 days and maintain adequate sperm numbers.

A complete fertility evaluation in the male involves history, physical examination, libido determination, semen collection and evaluation, hormonal evaluation, and prostatic examination. The initial database should include a detailed history, a complete physical examination, complete blood count, serum chemistry and urinalysis. History should include travel, diet, past or current illnesses, medications, vaccinations, deworming history and prior laboratory tests. Details of breeding history should be obtained, including the dates of all known matings, type of breeding (natural vs. AI – vaginal, transcervical or surgical; fresh, chilled or frozen semen) and the results of these matings (including pregnancy rates and litter size). Breeding management of each bitch should also be described.

Physical examination of all body systems should be performed with careful examination of the skin, eyes, heart, lungs, abdomen and musculoskeletal system. Following a complete physical examination, a complete reproductive system examination should be performed, including palpation of the scrotal contents, examination of the penis and prepuce and palpation of prostate per rectum. Serology for Brucellosis should be obtained.

The scrotal skin should be evaluated for any thickening, signs of infection or accumulation of fluid within the sac. Both testes should be palpated for size, consistency and presence of any masses. The total scrotal width can be measured with calipers or by ultrasound examination. Testicular volume can be determined by measuring length, width and height of the testes via ultrasound. The epididymides (the tubules in which the sperm mature before being transported to the vas deferens) and spermatic cords (vas deferens and testicular artery and vein) should be palpated for any thickenings, enlargements, pain (due to inflammation or granuloma [sperm plugs]), or missing segments (aplasia). Ultrasound examination can further be used to visualize the testes, epididymides and spermatic cords for masses, signs of inflammation, or abnormal fluid accumulations.

The penis should be evaluated for the presence of a persistent frenulum (remnant of skin securing the tip of the penis to the prepuce which normally breaks down around the time of puberty), signs of inflammation, abnormal discharge, reddening, or the presence of any masses. Rectal palpation of the prostate should be performed (may be completed prior to and/or after semen collection). The prostate should be located within the pelvic canal and should be small and symmetrical. In some cases upward pressure may be applied to the abdomen to push the prostate further backward such that it can be palpated in its entirety. Enlargement (either symmetrical or asymmetrical) of the prostate should instigate further investigation as to the cause. X-rays of the abdomen may help determine the size and location of the prostate if it cannot be palpated completely per rectum. Ultrasound examination of the prostate may reveal masses, cysts (either within or outside the prostate), inflammation, abscesses or generalized enlargement. Prostatic fluid can be evaluated either after semen collection or following prostatic massage or wash (if the male cannot be successfully collected).

Semen collection should be performed in the presence of an teaser bitch in heat (estrus) whenever possible. If a teaser bitch is not available, some males can be stimulated to erection using estrus bitch vaginal swabs or urine, or commercially available pheromone, applied to cotton balls or the vulva of a non-cycling bitch. Some males may not require any external stimulus beyond manual massage to attain erection. During collection the male should be observed for ease at which he develops an erection, presence of a normal erection, normal thrusting behavior and normal pulsation associated with ejaculation and prostatic fluid emission. Semen is ejaculated in 3 fractions: 1) pre-sperm – which arises from the prostate and urethral glands, thought to cleanse the urethra of contaminants (urine, bacteria and smegma) prior to ejaculation; 2) sperm-rich – which arises from the epididymal stores and vas deferens; 3) prostatic secretions – which provides volume to the ejaculate, assists in pushing the sperm out of the vagina, through the cervix, and into the uterus, and provide nutrients for the sperm while traveling to the oviducts. Semen should be collected in fractions whenever possible to facilitate evaluation of each portion of the ejaculate. The pre-sperm fraction is clear in color, is usually minimal in volume (less than 5 ml) and is not usually collected. The sperm rich fraction is white – cloudy white in color and usually 0.5 – 4 ml in volume. The prostatic fluid is normally clear in color and may range in volume from 3 – 80 ml. Following collection, it is important to be sure that the erection subsides, that the penis is drawn back into the prepuce and that the prepuce does not roll inwardly when this occurs.

Once the semen is collected, all equipment contacting it should be maintained at 37°C either until insemination or it is properly extended. Routine semen evaluation includes measurement of semen volume, semen motility, semen concentration, evaluation of individual sperm morphology (shape and form), and sometimes determination of the pH of the ejaculate. Semen

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volume is measured in milliliters. There is no minimal accepted semen volume since it depends on how well fractionated the ejaculate is and how much of each of the fractions is collected.

Semen motility is assessed by placing a drop of raw semen on a pre-warmed microscope slide and applying a pre-warmed coverslip. Semen is then examined at high and low magnification (i.e. 10x and 40x). A regular light microscope can be used, but the use of a phase contrast microscope enhances the ability to visualize individual sperm movements. Both total and progressive motility are determined and expressed as a percentage of 100. Total motility is defined as the percent of sperm that are moving, while progressive motility is defined as the percent of sperm that are moving *forward*, progressively. Of the two, progressive motility is most important in determining the number of sperm potentially able to fertilize the oocytes (eggs). During motility assessment, sperm velocity is also assessed. Velocity of forward movement is rated on a scale of 0 – 5 (0 = no movement, 5 = rapid, forward movement). A normal ejaculate contains a minimum of 70% progressively motile sperm.

Semen concentration is measured in millions of spermatozoa per milliliter of semen. Semen concentration and daily sperm output are directly related to testicular volume. So, the larger the testicles, the greater the daily sperm output and total sperm/ejaculate should be. Concentration is typically determined by obtaining a manual count. A small volume of semen (20 microliters) is added to a known volume of formalin based fluid which kills the sperm (to arrest motility and allow for accurate counting) and causes any red blood cells in the ejaculate to be destroyed so they do not interfere with the count. A known volume of this fluid is applied to a counting chamber, called a hemacytometer. Using the counting chamber's grid and a microscope, the number of sperm in a certain area is counted to determine the concentration per milliliter of semen. There are some automated sperm counters available but care must be taken to purchase a unit that is calibrated for dog semen. A normal ejaculate contains a minimum of 200 million spermatozoa/ejaculate. On average acceptable numbers of total sperm/ejaculate will be from 200 – 300 million for toy breeds; from 200 – 500 million for small breeds; from 400 – 800 million for medium breeds; from 500 million – 1.5 billion for large breeds; and from 600 million – 2 billion for giant breeds.

Individual sperm should be examined (for normal shape and structure) after staining the raw semen. The most common stain used is eosin-nigrosin. This stain is a vital stain, which means that it stains the live and dead sperm different colors. The sperm is divided into 3 segments: head, midpiece and tail. The head contains the DNA and has a cap (the acrosome) which contains the enzymes that allow fertilization to occur. The midpiece contains the motor apparatus that propels the sperm. The tail provides the propulsion to move the sperm forward. Defects may be classified in several different fashions: primary vs. secondary defects (primary occurring in the testicles, and secondary occurring during storage, transport or handling); major vs. minor (major affecting the ability of the sperm to fertilize and minor not affecting the ability to fertilize) or compensable and non-compensable (compensable defects can be overcome by providing access of the sperm to the egg and non-compensable defects cannot be overcome by the sheer presence of providing access to the egg). Primary vs. secondary is the most common classification scheme used. A normal ejaculate contains >70% normal sperm.

Sperm may also be examined to assess for the presence of a normal acrosome using special stains. These stains differentially stain the DNA portion of the sperm head a different color than the acrosome. In some cases of infertility, the acrosomes may have already reacted prior to the sperm reaching the oviducts. If this occurs these sperm will be incapable of fertilizing the eggs when they reach the oviducts. The semen may also be stained with Wright – Giemsa stain to assess for the presence of white blood cells or germ cells (immature sperm cells shed by the testicle when testicular degeneration is present). Semen culture may be submitted if high numbers of white blood cells are present in the ejaculate. Culture for aerobic bacteria and Mycoplasma are commonly obtained.

The pH of the prostatic fluid is usually 6.3 – 6.7. Either a combination of fraction 2 and 3 or fraction 3 alone can be tested. Alterations in pH may affect sperm longevity and motility. Increases or decreases in prostatic fluid pH are common with prostatic disease. Increases in pH may occur with use of excessive amounts of lubricant or improper cleaning and disinfection of collection equipment.

When semen will be chilled and shipped for insemination, assessment of sperm longevity may be evaluated (following extension with semen extender), in order to determine the potential success with the use of this type of semen. Semen is collected and extended depending on the semen concentration, the type of extender being used and the type of insemination being performed. Generally, an ejaculate will be extended at a minimum ratio of 2-3:1. Semen should be chilled (slowly) to 4 - 5°C and held for a *minimum* of 48 hours at this temperature. A small sample of the semen is warmed to 37°C at 24 and 48 hours and total motility, progressive motility and velocity are determined. In some cases, motility may be so good at 48 hours to make evaluation at 72 and 96 hours (or longer) indicated.

In certain cases, where fertility issues are evident based on pregnancy rates but are not evident based on normal fertility examination, advanced testing may be dictated. This may include hormonal evaluation, karyotyping, testicular aspiration

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or biopsy, sperm chromatin structure assay, electron microscopy, HOST (hypo-osmotic swelling test) and acrosomal integrity tests.

Hormones that may be evaluated include testosterone, estrogen, prolactin, LH (luteinizing hormone), FSH (follicle stimulating hormone), and thyroid hormones. Dogs with testicular degeneration may have elevated estrogen concentrations and decreased testosterone concentrations. They may also have elevated FSH and LH concentrations. Prolactin concentrations may be increased or decreased depending on normal pituitary (brain) function and feedback from the testes. Thyroid hormones are commonly assessed when faced with infertility problems. There is little direct evidence to substantiate that hypothyroidism *directly* affects reproductive function. However, it is believed that through indirect mechanisms, *chronic* thyroid dysfunction may affect the brain's ability to either produce hormones or respond to hormones released by the testicles in feedback loops and may thereby result in reproductive dysfunction and infertility secondarily (in dogs with overt signs of hypothyroidism).

Males that have decreased fertility without any obvious cause may have genetic defects resulting in testicular hypoplasia or degeneration. Although most of these individuals are sterile, in some cases, one or more litters may have been sired. Evaluation of the DNA of these individuals through karyotyping (chromosome analysis) may indicate a genetic reason for the infertility.

Testicular aspiration or biopsy can be performed on subfertile or infertile males to help differentiate the cause of the infertility. Biopsies or aspirates are generally recommended when sperm production is low or steadily decreasing to help elucidate the cause of the problem and help determine if there is a treatment that will improve the dog's fertility. Biopsies are taken under general anesthesia through a small incision in the scrotum. Aspirates can be obtained with heavy sedation.

HOST or hypo-osmotic swelling test evaluates the integrity of the sperm plasma membrane (the membrane that surrounds the entire sperm cell). The test is performed by placing the sperm in a special solution which results in water being transported across the sperm plasma membrane into the cell to try to equalize the osmotic pressures from the inside to the outside of the sperm. Water will cross the membrane and enter the cell if the membrane is intact and the transport mechanisms are functioning normally. If the membrane is not intact, then the transport mechanism will not function properly and no fluid will enter the cell to equalize the pressure differences. If fluid crosses the membrane, the cell will swell, which results in a bending/curling of the sperm tail.

Sperm chromatin structure assay is a method to assess the DNA content of the sperm head. This assay compares the amount of DNA present in each sperm and how much variation there is between individual sperm cells. Normal dogs have very little variation in the amount of DNA present in each sperm head, while dogs with abnormal sperm may have a wide variation in the DNA content of the sperm.

Electron microscopy is a special microscopic technique that allows for very detailed examination of the entire sperm at very high magnifications. Cross sections and full length sections of individual sperm may be examined to see if there are abnormalities of structure that are beyond that seen by the light microscope. There are 2 types of electron microscopy, transmission and scanning. Transmission EM provides a two dimensional view of the interior of the sperm cell, while scanning EM provides a three dimensional view of the exterior surface of the sperm. Transmission EM is typically more helpful in the assessment of infertility.

Acrosome function is required in order for the sperm to penetrate the egg during fertilization. In some dogs, adequate sperm may reach the oviduct and surround the egg, but the acrosome reaction may not occur normally resulting in failure of fertilization. In these dogs, all other sperm testing may be normal. To assess acrosome function, a drug called calcium ionophore is added to the semen. This substance will induce the acrosome reaction in normal sperm. A fluorescent dye is then added and those sperm whose acrosomes react will take up the dye, while those that don't react, don't take up any stain. This test is still being refined for use in the dog, but holds good promise for future use.

Males should be evaluated for reproductive function prior to their first attempt at breeding, if it has been several months or years between breedings, or if fertility begins to decline or is questionable. In some cases, a routine reproductive examination will suffice, but in others, advanced diagnostics may be required.

Dr. Lopate is board certified in reproduction (Theriogenology). She owns and operates a reproductive specialty practice providing service to companion animals and horses south of Portland, OR. Questions regarding male fertility or other reproductive issues may be directed to Dr. Lopate at (503)537-1123, via email at info@reproductiverevolutions.com or on the web at www.reproductiverevolutions.com.