Identifying etiologies of subfertility in stud dogs

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Introduction

Subfertility can be defined as sporadic success in a male's ability to sire litters or semen parameters that are substantially below expected normal. Subfertility can be congenital or acquired. Dogs with acquired conditions have a history of previous normal fertility. However, if breeding is not attempted until later in life, it may not be possible to determine if fertility problems are congenital or acquired. If a cause of subfertility is identified, appropriate management and intervention may help to improve fertility in a particular stud dog. Unfortunately, in more than 50% of cases, a definitive cause may not be determined.[1] In such cases, empirical treatment (e.g., dietary supplements and hormonal treatment) may be beneficial.

Laboratory evaluation of semen

Accurate semen analysis for diagnosis of subfertility requires knowledge of the appropriate tools and tests to use, and evaluation of as many parameters as possible. Semen evaluations have traditionally been performed manually; however, more recently computer-assisted sperm analysis (CASA) systems have become popular.[2] Advantages of CASA systems are their ability to rapidly and objectively analyze semen parameters without the influence of human variability.[3] A major disadvantage is their inability to accurately detect sperm morphological abnormalities.

Motility evaluation

Manual semen motility evaluation is performed by placing a drop of raw semen on a warmed slide with a coverslip and estimating the percent motile sperm as viewed under 100 - 400 x bright field microscopy. If semen concentration is high, dilution with physiologic saline allows evaluation of individual sperm movement and provides a more accurate estimate of progressive motility. CASA systems assess total and progressive sperm motility by obtaining multiple digital images of a field of sperm in rapid succession, identifying individual sperm, and tracking those sperm across frames.[3] Anecdotally, manual evaluation results in higher estimation of percent motile sperm compared to CASA evaluation. Subfertile dogs frequently have lower total and progressive motility compared to dogs exhibiting normal fertility.[1]

Viability evaluation

Some newer CASA systems can evaluate sperm viability, defined as membrane integrity, by fluorescent labeling. This is an important advancement as viability may correlate better than progressive motility to fertility.[4] Live:dead ratio of a sample stained with eosin-nigrosin and identification of membrane intact sperm using a hypoosmotic swelling test are manual methods of evaluating viability that do not require special equipment. The Nucleocounter SP-100 (ChemoMetec, Allerod, Denmark) has lower variability in evaluating sperm membrane integrity compared to human-analyzed tests.[5]

Total sperm count

Manual evaluation of an ejaculate concentration is performed using a Neubauer hemocytometer and a standard dilution, thereby allowing calculation of total sperm count. The reader is referred to literature[6,7] for details. CASA systems also analyze semen samples at a standard dilution, allowing the system to calculate concentration of a raw sample. Other semen analyzers are commercially available that are not part of a CASA system. These can be photometers or densimeters that measure concentration of a semen sample based on transmittance of light through the sample as compared to a standard buffer. All photometers and densimeters are calibrated to a certain concentration range; therefore, samples outside this range must be diluted for accurate measurement. Extraneous particles in the sample (e.g., white blood cells or undissolved components of semen extender) may falsely elevate the measured sample concentration. Additionally, the parameters by which a CASA system identifies and analyzes sperm vary by species, instrument, and settings. Differences in the technical settings of these instruments can influence the results.[8] This is an important consideration when comparing results among laboratories and in fresh versus chilled or cryopreserved semen containing egg yolk extender. Correct dilution in any methodology is vital to obtain an accurate result. When comparing multiple samples to each other, it is valuable to have analyses performed by the same person to reduce the risk of human variability. CASA systems have lower variability than human-derived (e.g., hemocytometer), measurements of semen concentration.[9,10] The Nucleocounter SP-100 has been more accurate than traditional CASA systems or densimeters because it excludes background debris from measurement.[3] For this reason, it is also useful for measuring sperm concentration in semen extended with egg yolk.

Morphology evaluation

Sperm morphology is perhaps one of the most important albeit most commonly misused tests in semen analysis. Morphology is correlated with fertility in multiple species, including dogs, bulls, and humans.[1,11-14] Accurate evaluation requires appropriate stains, quality bright field, phase-contrast, or

differential interference contrast microscopy, and knowledge and experience in identifying sperm abnormalities. Canine semen morphology is commonly evaluated with bright field microscopy using an eosin-nigrosin stain or a modified Giemsa stain.[15] Phase-contrast microscopy uses light to produce a high contrast image of transparent sperm, eliminating the need for stain. A minimum of 200 sperm should be counted, and percentage of normal and abnormal sperm calculated.[7] Some CASA systems have the ability to evaluate morphology; however, this is generally limited to crude analysis of the sperm head and tail coiling, and accuracy is highly dependent on the software settings of a particular instrument.[3] Results of CASA morphology evaluations are not comparable to evaluation by a trained human.[3,16,17]

There are several systems for categorizing sperm abnormalities. In the more common classification system, primary abnormalities are considered those that develop during spermatogenesis or spermiogenesis and are caused by pathologic processes in the seminal epithelium, whereas secondary abnormalities are considered those that originate during transport through or storage in the epididymis, and tertiary abnormalities are those caused after ejaculation by rough handling or environmental conditions.[12] Another system classifies sperm with major abnormalities as those with severe aberrations that are generally believed to be incapable of fertilization, and minor abnormalities as those that are less likely to cause changes in fertility.[11,12] This creates some confusion as there is often not detailed knowledge of the effect on fertility of specific morphologic abnormalities in the dog. A system of classifying morphologic abnormalities as compensable or noncompensable, depending on whether increasing sperm dosage has an impact on fertility or not, could be useful for determining insemination doses.[12] However, similar to major and minor classification, there is limited information on which abnormalities are compensable versus noncompensable in the dog. A newer morphological classification system groups defects based on their location on the sperm cell; head, midpiece, or tail.[12] This has also not been correlated to fertility; however, it may be beneficial in tracking a dog's morphological changes over time as the categories are more descriptive.

Determining etiology of subfertility

Many insults to the spermatogenic cycle result in oligo-astheno-teratospermia. Specific deviations from a normal spermiogram, combined with a complete history of breeding and husbandry, can help narrow down possible etiologies and guide toward appropriate further diagnostic tests.

Timing and variety of morphologic abnormalities observed in an ejaculate may help to determine the time and type of insult to spermatogenesis. For example, increased scrotal temperature in bulls caused sperm mitochondrial defects ~ 14 days after insult, and nuclear vacuoles ~ 24 days after insult.[12] Spermatocytes undergoing meiosis and cells undergoing spermiogenesis are most susceptible to damage.[11]

Determination of serum hormone concentrations may be useful to further characterize infertility. Male dogs with at least 12 months duration of subfertility had lower basal testosterone concentrations than dogs of normal fertility.[18] Increased follicle stimulating hormone (FSH) can be a marker of primary testicular failure.[6,19] This occurs due to decreased negative feedback from inhibin that is normally produced by Sertoli cells.[6] Increased FSH in the face of declining semen quality or azoospermia carries a poor prognosis because testicular changes are likely irreversible.[20] These tests should be performed by a laboratory using assays validated for dogs.

Determination of alkaline phosphatase concentrations in semen can be used to diagnose ductus deferens patency. Nearly all alkaline phosphatase present in a normal ejaculate is contributed by the second (sperm rich) fraction.[21] The cutoff of 5,000 U/L is generally used to indicate a complete ejaculate. Low alkaline phosphatase in an azoospermic sample can indicate bilateral epididymal obstruction or incomplete ejaculation.[21] High alkaline phosphatase concentrations in an azoospermic ejaculate indicate failure of spermatogenesis and carries a poor prognosis for future fertility.

Anti-Müllerian hormone (AMH) is produced in the male exclusively by Sertoli cells. Determination of AMH concentrations in dogs can be used for determining gonadectomy status,[22] and diagnosing testicular atrophy and Sertoli cell tumor.[23-25]

Ultrasonography is an important tool in evaluation of stud dog fertility. It has been used for some time in evaluation of prostatic disease and testicular tumors. More recently, B-mode and Doppler ultrasonography findings have been correlated to current fertility in the dog.[18,25] Increased echogenicity of testis was associated with fewer morphologically normal sperm.[25] Conversely, hypoechoic testis was associated with poor morphology.[27] It is possible that both increased and decreased echogenicity may represent changes in the testicular architecture that lead to decreased semen quality. Rate of blood flow through the testicular artery is positively correlated to current semen quality, likely because it represents a marker for the rate of spermatogenesis.[18,28] Testicular size and total sperm output are positively correlated with body weight in dog; however, among dogs of comparable body weight, testicular volume, as measured by ultrasonography, has not been a reliable indicator of fertility.[6,18]

Conclusion

Tests discussed should provide a reasonably complete picture of the nature of subfertility. Is it primarily due to oligospermia, teratozoospermia, or to prostatic or testicular abnormalities? Understanding the anatomic and physiologic processes involved in sperm production can help pinpoint the stage at which it went wrong. Furthermore, breeding history can help narrow down the timeframe at which subfertility may have started, allowing the clinician and owner to investigate potential insults to spermatogenesis that occurred at that time. Hormonal tests (e.g., FSH and AMH) can help determine the prognosis for return to fertility. A logical approach to diagnose and determine the etiology of subfertility, starting with an accurate semen evaluation, provides the best chance to correct or manage a subfertile stud dog.

Conflict of Interest

None to declare.

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