Addressing subfertility in stud dogs

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Introduction

Addressing subfertility in dogs starts with identification and correction of causative factors. Fertility history may be incomplete, as owners may not present their dog to a theriogenologist until a problem is perceived, or may not attempt breeding until a male is several years old, in which case his fertility as a young dog is unknown. Components (motility, total sperm number, or specific morphological abnormalities) of the spermiogram that are affected, signalment of the dog, and breeding history may suggest where the problem originates. However, in over 50% of cases the underlying cause may not be known. In these cases, management of living conditions, overall health, and diet should be optimized in attempt to improve semen quality.

Correction of underlying factors

Normal spermatogenesis occurs at temperatures 3 - 5 degrees Celsius lower than body temperature. Increased scrotal temperature is known to cause temporary testicular dysfunction and sperm abnormalities in multiple species. Generally, minimal to no change is noticed in the first 10 days after heat insult because sperm ejaculated during that time were nearly mature and in transit through the epididymis when hyperthermia occurred.[1] Depending on the intensity and duration of the insult, sperm abnormalities may be observed for the length of 1 or more spermatogenic cycles (62 days in the dog). Anecdotally, some individual dogs seem more susceptible to heat stress than others. Frostbite and contact with irritating disinfectants or rough kennel surfaces can be a source of heat stress secondary to scrotal dermatitis. Infection, in the reproductive tract and systemic, may affect sperm quality through increased scrotal temperature, by direct action of bacteria on sperm, or by induction of autoimmune reaction after breakdown of the blood-testis barrier. Obesity can also increase scrotal temperature. Management of living conditions and general health to reduce the risk of heat stress may be beneficial to some dogs. This can include limiting breedings, or planning frozen semen collections, if appropriate, to the cooler months of the year.

Several bacteria can cause infections in the testes, epididymides, and prostate that lead to altered semen quality. Many of these are opportunistic infections by normal flora (e.g., mycoplasma and ureaplasma) that makes causation difficult to prove. Demonstration of organisms by culture or PCR, exclusion of other causative factors, and response to treatment are suggestive of infection mediated subfertility.[2] In addition to treating the suspected cause of infection, one should investigate what host factors allowed an opportunistic infection to occur. Brucella organisms are not normal flora and should be tested for in all cases of unexplained subfertility or pregnancy loss. *Brucella canis* (*B. canis*) and *Brucella suis (B. suis)* have been isolated in dogs. *B. suis* requires additional testing for smooth strains as it will not be detected by the *B. canis* rough strain assays.[3] There is no evidence that viruses act directly on male fertility; however, they can be transmitted through semen and cause pregnancy loss in females.[4]

Prostatitis due to benign prostatic hypertrophy/hyperplasia (BPH) or bacterial infection can alter semen pH and negatively affect sperm motility. Although red blood cells are not directly toxic to sperm, substantial hemospermia may physically block sperm motility and does affect frozen semen success.[5] BPH most commonly affects older males that may also have age-related changes in sperm quality.

Any alteration in the hypothalamic-pituitary-gonad axis can affect spermatogenesis. Typically, cases of hypopituitarism, hypothalamic or pituitary tumor, or adrenal disease would have primary clinical signs other than subfertility that aid in diagnosis. Idiopathic decreases in FSH or LH production will suppress spermatogenesis, usually without other systemic signs. Although advanced hormone testing aids in diagnosis, they are not widely available. Furthermore, assays validated for dogs must be used. Testicular tumors can cause direct destruction of testicular tissue, inflammation and increased scrotal temperature, and excess hormone secretion that suppresses spermatogenesis. These may cause subfertility before a tumor is noticeable on physical examination.[5]

Anatomical defects may be congenital, such as testicular hypoplasia, segmental aplasia of the epididymis, or vas deferens agenesis, and thus would present with a history of never being fertile. These can be an accident of development or caused by a genetic abnormality such as 79XXY.[5] Comparatively, dogs with acquired defects such as spermatocele or sperm granuloma may have a history of previous successful breedings.

History of pharmaceutical use is an important part of a fertility evaluation. Steroids and medications with steroid-like action, such as some antifungals (ketoconazole), may inhibit testosterone synthesis and spermatogenesis.[6,7] Antineoplastic agents, antacids (cimetidine), behavioral medications (amitriptyline), NSAIDS (naproxen, ibuprofen), and sulfasalazine are some of the medications that may negatively affect sperm quality.[5,8-10]

Sperm quality may also be poor due to age (too old or too young), season, or idiopathic testicular degeneration. These may be presumed after exclusion of other causes.

Dietary supplements

Veterinarians and dog owners have used several dietary supplements in attempts to improve semen quality in stud dogs. Data correlating use of specific supplements to improve semen quality is sparse and correlations to fertility are nearly absent. There is more evidence for beneficial effects of dietary supplements in humans, laboratory animals, and production animals; however, it is unknown whether or not these effects carry over to dogs. All dietary supplements should be used cautiously, especially when beneficial effects are not identified, as harmful side effects are possible.

Supplements with potential benefits

Improvement in semen parameters was noticed in dogs after treatment with various combinations of vitamin E, selenium, zinc, and n-3 polyunsaturated fatty acids (PUFA). [11-14] Parameters that improved include semen volume, semen concentration, total sperm number per ejaculate, total motility, progressive motility, functional membrane integrity measured by hypoosmotic swelling test, structural membrane integrity as evaluated by eosin-nigrosin staining (described in some publications as live:dead ratio or percent vitality), and percent morphologically normal sperm.[11-14] These supplements may help improve sperm quality by acting as antioxidants. Zinc is a cofactor of superoxide dismutase and selenium is a cofactor of glutathione peroxidase, and have important roles in protecting sperm against oxidative damage. Zinc concentrations are also high in seminal plasma of dogs with normal fertility, which may promote sperm membrane stability, sperm chromatin integrity, and have a role in capacitation and the acrosome reaction.[12] Vitamin E is a nonenzymatic antioxidant that decreases lipid peroxidation, thereby decreasing oxidative stress to sperm. Vitamin E treatment corrected some sperm abnormalities induced by stress.[15] Sperm plasma membrane contains high concentrations of PUFA that makes it particularly sensitive to oxidative stress. PUFA, vitamin E, and selenium treatment increased motility, and was associated with a decrease in flagellar defects[14] and improved flagellar motion.[13] PUFA treatment in rams accelerated spermatogenesis, a cause for increased total sperm number.[13] Beneficial effects were observed after treatment with folic acid in combination with selenium, vitamin E, and PUFA.[11,12] However, there was not enough evidence to determine effects of folic acid alone on sperm quality.

Some supplements do have potential to cause harm if used excessively or in high doses. Excessive selenium supplementation actually increased sperm damage.[16] Risk of oxidative damage within the testis was increased by PUFA supplementation, possibly due to lack of co-supplementation with vitamin E or other antioxidants.[13,16] Toxic doses of zinc caused hemolytic anemia in dogs.[17] Excessive concentrations of vitamin E, folic acid, and selenium may decrease sperm motility in humans and poultry.[11]

Carnitine is involved in fatty acid transport into sperm that affects sperm motility, and it may also have a role in sperm maturation as an antioxidant and free radical scavenger. There is some evidence that carnitine may improve total sperm count, total motility, and percent morphologically normal sperm in humans and laboratory species[20,21] and hasten recovery from experimentally induced testicular damage in mice.[22-24] No such specific studies have been performed in dogs. Carnitine content is high in meat and milk, therefore dogs fed with high animal protein diet may not need supplement. There are no documented toxicity studies in dogs.

Glucosamine and glycosaminoglycans, most commonly chondroitin sulfate, have been anecdotally reported to improve semen quality in multiple species. It is a component of several nutritional supplements marketed for male dogs and recommended by some veterinarians.[18] However, there is no documentation of beneficial effects and no known adverse effects of supplementation.[19]

Supplements without benefit or with potential harmful effects

There are limited controlled studies on the use of saw palmetto (*Serenoa repens*) in dogs and data regarding use by men with BPH are conflicting. There was no effect on prostatic size, testosterone concentration, or semen parameters.[25] The largest meta-analysis in humans suggested that it may improve urologic symptoms (e.g., urine flow and residual volume after voiding); however, there was no evidence to indicate that it reduced prostate size.[26,27] Hematuria and hemospermia are the most common clinical signs of BPH in dogs; they do not typically have stranguria and incomplete voiding similar to men. There is likely no substantial benefit to the use of saw palmetto in dogs with BPH.

Cottonseed meal may be used as a protein source in livestock feed and is an ingredient in some supplements marketed for reproductive health in dogs. Gossypol is a phenolic toxin that is a natural component of cotton plants. Gossypol is known to disrupt spermatogenesis and reduce libido in bulls.[28] Reproductive effects have not been documented in dogs; however, there are multiple reports of death in dogs that were fed or accidentally ingested toxic doses of cottonseed meal.[29,30]

Phytoestrogens are nonsteroidal plant compounds that have estrogen-like activity in mammals. Flaxseed, soybeans, and legumes are common ingredients in dog foods and supplements that contain phytoestrogens. Dogs fed a diet with high soybean content for 1 year had alterations in endocrine function.[31] Coumestrol, a phytoestrogen, present in soybeans, legumes, alfalfa, and clover, has been investigated as a possible contraceptive in male dogs.[32,33] Reproductive and other health effects of phytoestrogens may vary with individual phytoestrogens, sex, dose and duration of exposure, and timing of exposure during reproductive development.[34]

Hormonal treatments

Gonadotrophin releasing hormone (GnRH) causes the pituitary gland to release luteinizing hormone and follicle stimulating hormone that in turn cause testis to produce testosterone. GnRH antagonists treatment reduced fertility by disrupting spermatogenesis, resulting in decreased sperm number and quality.[35,36] Treatment with a GnRH analogue may increase serum testosterone concentrations, sperm num-

ber, motility, viability, and normal morphology in dogs with certain disorders.[37,38] Initial treatment is typically followed by weekly injections; however, no specific dosing regimen has been validated. Prolonged exposure to GnRH results in suppression of fertility in male dogs.[39]

Prostaglandin $F_{2\alpha}$ treatment 15 minutes prior to semen collection, especially when combined with the presence of a teaser bitch in estrus, can increase the number of sperm in the ejaculate.[18] This protocol does not affect the number of sperm produced in the testes; rather, it acts on the contractile tissue in the cauda epididymis to encourage ejaculation of mature sperm stored there.

Frequency of sperm collection

Frequency of semen collection can be used to the benefit or detriment of total sperm number and semen quality. In dogs collected every 2 - 3½ days, the total number of sperm obtained per collection did not change. When collection frequency increased to once or twice daily, the number of sperm per collection decreased rapidly, presumably until daily sperm output was reached. The amount of sperm obtained over a given interval did not substantially change, regardless of collection frequency, i.e. daily collections for 7 days resulted in the same total number of sperm as 2 semi-weekly collections.[40] Two collections in 1 day produced slightly more total sperm than 1 collection; however, when repeated on subsequent days, sperm number dropped dramatically.[41] There is no evidence of significant change in semen motility or percent morphologically normal sperm with increased frequency of collection in normospermic dogs.[40,41] This suggested that in dogs with normal fertility, there is no benefit to a 'clean out' collection frequently requested by owners. However, in asthenoteratospermic dogs, it was assumed that increased collection frequency stimulated secretory function of the epididymal epithelial cells, and to normalize pH and sodium:potassium ratio in semen. This may help maintain acceptable semen quality in these dogs.[42]

Assisted reproductive technologies

Assisted reproductive technologies (e.g., intracytoplasmic sperm injection) that improve conception of poor-quality sperm in other species are not currently available in canine theriogenology. However, sperm separation by density-gradient centrifugation may be useful. This process separates viable, motile sperm from nonmotile sperm. It also removes certain contaminants (e.g., red blood cells).[43] Multiple commercial products are available. Several of these increased the percentage of progressively motile sperm, sperm viability, membrane integrity, and in vitro oocyte penetration rate of treated ejaculates.[44-46] Although it is not likely indicated for the majority of dogs, sperm separation may increase breeding success in certain cases of subfertility and when epididymal sperm are used.[46]

Conclusion

Cause of subfertility in a dog is identified in 50% of cases or fewer. In cases where the cause is known, the chance of restoring fertility may be as low as 10%.[5] Anatomical defects, dietary imbalances, and spermatotoxic pharmaceuticals are potential causes of subfertility. The underlying etiology should be corrected, if possible. In cases where the etiology is not known or not rectifiable, management through dietary supplements, hormone treatments, and individually tailored breeding management strategies may help improve chances of fertility. Owners should be cautioned that these dogs will likely never be reliable producers. The physical, financial, and emotional costs of pursuing breeding attempts should be weighed against the stud dog's potential genetic contribution to the population.

Conflict of Interest

None to declare.

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