Canine autoimmune orchitis
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Abstract
Autoimmune orchitis is defined as autoimmune inflammation of the testis with or without the presence of anti-sperm antibodies and will result in infertility in both males and females. It is a rarely described disorder in dogs that may lead to permanent infertility. However, non-invasive diagnostic tools has been lacking and thus, this condition may be under-diagnosed. Clinical signs include infertility and sperm abnormalities. Diagnostics include a thorough workup for infertility including history, general and reproductive tract-specific physical examination, blood work, urinalysis, and ultrasound. Antisperm antibodies may be present, but their significance is still disputed. Testicular biopsies reveal lymphocytic infiltrates progressing from the straight tubules to the rete testes and the efferent ducts eventually resulting in necrosis and the absence of spermatogenesis. If no underlying causes for the autoimmune orchitis are discovered and testicular atrophy has not occurred, treatment with immune suppressive agents have been suggested. If anti-sperm antibodies are present and the patient still produces some sperm, direct surgical intrauterine insemination should be considered to increase fertility rates.

Key words: Autoimmune orchitis, orchitis, canine, testis, infertility, antisperm antibodies

Introduction
Autoimmune orchitis is defined as autoimmune inflammation of the testis with or without the presence of anti-sperm antibodies and will result in infertility in both males. Autoimmune orchitis can be classified as either primary or secondary depending on its cause. In primary autoimmune orchitis, antisperm antibodies are antibodies against testicular tissues, which are present in the absence of systemic disease. Secondary autoimmune orchitis is generally associated with systemic autoimmune disease. Dogs and mink are the only species in which spontaneous autoimmune orchitis has been shown to occur. It can be induced in guinea pigs and rabbits by vasectomy. A disease similar to autoimmune orchitis has been described in mice after thymectomy shortly after birth. It can also be induced in mice and other species by injection of homologous sperm or testis antigen. Histologic changes similar to those found in autoimmune orchitis have been described in humans and have been associated with infertility in some cases. In humans, most cases of autoimmune orchitis are due to either trauma in the broadest sense to testes or vas deferens (torsion, obstruction, vasectomy) or systemic immune mediated disease (lupus, chronic rheumatic disease).

Early studies of autoimmune orchitis in dogs were performed in a beagle colony with lymphocytic thyroiditis and concurrent orchitis. Dogs were observed to become infertile during the first two years of stud service. Initially, the dogs were shown to be normospermic, then oligospermic, and finally, aspermic. This study suggests a genetic predisposition, as the more severe the changes were, the more closely these dogs were related. Dogs in the same study without lymphocytic thyroiditis or orchitis were not related to the affected dogs. Further evidence of a genetic component was demonstrated in a study of two related dogs (sire and son) that had the same testicular pathology and autoantibodies. Interestingly, these serum autoantibodies did not bind to the testes of other dogs. Deposition of IgG and C3 was demonstrated around interstitial capillaries in the testes and occasionally on sperm tails in the related dogs.

The incidence of autoimmune orchitis in dogs is not known, perhaps because testicular biopsies are not often performed and the detection of antisperm antibodies is not commonly done. In humans, immune responses within the testes have not been well studied either. However, the incidence of serum and/or seminal fluid antisperm antibodies in primary autoimmune orchitis has been estimated to be between 7 and 12% of infertile men and women.
Clinical signs
In dogs with spontaneous or induced autoimmune orchitis, decreased fertility or infertility was the most common complaint. In a few affected beagles, the testes were palpably slightly smaller than in unaffected dogs. Semen evaluation in affected dogs may reveal oligospermia, teratozoospermia, azoospermia, aspermia or sperm agglutination.

Diagnostics
Even if autoimmune orchitis is suspected, a complete workup for infertility should be performed. A complete history is imperative and should begin with general information such as vaccination history, previous illnesses, trauma, current and previous medications, supplements, and environmental information such as number of dogs and other animals in the household and use of the dog (field trial or hunting dog, show dog, etc.). Information specific to reproductive health should also be obtained, such as previous or current episodes of potential hyperthermia to the testes or scrotal swelling or dermatitis. Breeding history also provides information as to the degree of infertility. The age at which the first attempts at breeding were undertaken should be assessed as well as the number of matings performed, the number of litters sired, timing of the bitch used, and if the stud in question was bred to a proven female. Results of recent brucellosis testing should also be reviewed.

The physical examination should include a general examination followed by the male reproductive examination. Libido and semen evaluations are performed before uncomfortable things such as prostatic examination are done. Briefly, the testes should be examined for size, shape, and consistency. The head, body, and tail of the epididymides should be carefully palpated and the prostate is examined for symmetry, size, and pain. Testes, epididymides, and prostate can all be evaluated by ultrasonography. Laboratory tests include a complete blood cell count, a serum biochemistry screen, urinalysis, antisperm antibodies, and complete semen evaluation. Alkaline phosphatase may be measured in the ejaculate to assess if the collection is complete. While bacteria to some degree are always present in the ejaculate regardless of fertility status, culture and sensitivity may be considered if there are clinical signs of infection or if neutrophilia and toxic neutrophils are present in the ejaculate. If autoimmune orchitis is suspected, testicular cytology or biopsy may be considered. The advantage of cytology is that the procedure is quick, simple, and leaves minimal damage. On the other hand, the disadvantage is a low yield of cells and progression of spermatogenesis cannot be evaluated. In general, Leydig cells cannot be assessed nor can the architecture of the testicular tissue. Side effects of a needle biopsy may include minor hemorrhage, minimal scrotal swelling and erythema, cellular degeneration and necrosis, and in one case testicular atrophy was noted. In most cases, the lesions remained focal and did not have any effect on sperm quality, testicular size, or testosterone concentrations. Testicular biopsies can be performed either by surgically removing a wedge or by using a triggered biopsy instrument, such as a needle punch biopsy device. The advantages are detailed assessment of the testicular tissues including progression of spermatogenesis and architecture of the tissue. Side effects include hemorrhage, maturation arrest, and focal fibrosis and the development of sperm granulomas. The presence of antisperm antibodies have been documented after testicular biopsy. However, these antibodies disappeared rapidly after biopsy and no significant long-term changes in sperm quality and motility were detected. In humans, immune responses after biopsy have been shown to be minimal and of no clinical significance.

Pathophysiology
Experiments were performed to characterize antibody responses to sperm in dogs that were chronically infected with B. canis. The results revealed sperm agglutinating antibodies both in serum and in seminal fluid that were highest in those dogs infected for four to six months. The chronically infected dogs were skin tested with sperm extracts and showed delayed type hypersensitivity reactions. The most severe reactions were seen in those dogs with testicular atrophy. Semen evaluation demonstrated teratozoospermia and sperm adhering to clusters of inflammatory cells. In this model, it is thought that
the pathogens engulfed by macrophages are able to breach the sperm blood barrier thus presenting sperm cells to the immune system resulting in autoantibody production.8

Studies in guinea pigs revealed that injecting sperm homogenates along with Freund’s complete adjuvant induces antisperm antibodies and results in the development of orchitis. Similar studies in BALB mice and in dogs using Freund’s incomplete adjuvant did not result in a similar outcome. It was subsequently shown that when BALB mice were immunized with sperm homogenates and injected with \textit{Bordetella pertussis} subcutaneously, orchitis could be induced. Based on these studies, dogs received a single intratesticular injection of Bacillus Calmette-Guerin, which resulted in temporary azoospermia within three to six weeks that lasted from six weeks to almost one year. Fertility was assessed by breeding the dogs during the phase of azoospermia and after recovery. No puppies were produced during the azoospermic period but after recovery all dogs sired healthy litters. Interestingly, no antisperm antibodies were detected during any of the azoospermic phases or after recovery. In humans, autoantibodies are often induced upon restoration of vasectomy, but these antibodies rarely result in reduction of fertility. In general, many of the studies show that recovery is likely if there is incomplete degeneration of the germinal epithelium, indicating that aspermatogenic orchitis may not lead to irreversible infertility.9

Experimental studies in mice have shown that cell mediated immunity in autoimmune orchitis is conferred through CD $^4$ cells. Antibody mediated responses were evidenced by the presence of antibodies on testicular germ cells outside of the blood testis barrier, which was often present well before cellular infiltration of the testes. Other studies in mice and rabbits have shown immune complexes of testicular antigens and autoantibodies within the testes. In these studies, the progression of orchitis was followed histologically. Initially, focal lymphocytic infiltrates were seen around the straight tubules. Next mild inflammatory infiltrates were noted within the rete testis and/or the efferent ducts. Occasionally, mild dilation of the seminiferous tubules was observed. Eventually, the changes became moderate to severe over time. Final changes included severe inflammation of the testis with necrosis, dilation of the seminiferous tubules and a complete lack of spermatogenesis. These changes were not just localized to the testes but also to the epididymides and the vas deferens, which exhibited a similar progressive pattern of change. In this study, administering antibodies against TNF alpha reduced pathology in the testes to more focal type lesions demonstrating the importance of TNF alpha in the development of orchitis. Epididymitis was also completely inhibited by the administration of antibodies against TNF alpha. In addition, experimental studies of autoimmune orchitis in mice have shown that TNF alpha is produced both in testicular lymphocytes and macrophages leading to progression of disease.4

Further studies have shown that TNF alpha, its type I receptor, IL-1 alpha, IL-1 beta, and its receptor are normally expressed in the male gonads. These cytokines have been shown to play an important role in maintaining testicular health. Increasing concentrations of TNF alpha are present in pro-inflammatory responses, immune regulatory responses, and finally apoptosis. In experimental models of autoimmune orchitis, the increased concentrations of TNF alpha result in up regulation of its type I receptor. Sertoli cells and testicular macrophages produce IL-1 alpha. Increased concentrations of IL-1 alpha result in inflammation and induction of other cytokines and enzymes. Cytokines and enzymes induced include TNF alpha and nitric oxide synthase, which themselves have pro-inflammatory properties. Studies indicated that this increased expression of TNF alpha may lead to increased permeability of the endothelium allowing for extravasation of monocytes into the testicular tissues and activation of T cells and macrophages. The macrophages then in turn produce more TNF alpha leading to progression of orchitis.15

Treatment
Currently, there is no treatment for autoimmune orchitis. If secondary autoimmune orchitis is suspected, then immune suppressants such as glucocorticoids could be considered. One would have to carefully consider the doses and the duration of treatment and prudently weigh the risks versus benefits. To increase the chances of fertility, at least some sperm cells must be present in the ejaculate. If
antisperm antibodies are present in serum and/or seminal fluid, washing the sperm before insemination may be beneficial. However, once the sperm are bound to the antibodies, they form irreversible complexes and the antibodies cannot be washed off. Another option is to perform intrauterine insemination close to the ovaries or more sophisticated methods such as intracytoplasmic sperm injection. In reality though, by the time dogs are presented for infertility due to autoimmune orchitis, the disease has usually progressed to aspermia and is at that point likely irreversible.

**Conclusion**

Autoimmune orchitis in dogs is a poorly understood disease. Future studies are needed to understand the pathophysiology and potentially inciting causes. As in other species, the role of antisperm antibodies in the development of autoimmune orchitis remains unclear. This is perhaps due to the fact that there are no reliable, standardized methods of assessing antisperm autoantibodies. Currently, diagnosis of autoimmune orchitis is best made by obtaining a testicular biopsy along with a detailed history, physical examination, and semen evaluation. Future studies will focus on less invasive diagnostics and elucidation of the pathophysiology, so that better targeted therapies may be developed.

**References**