Azoospermia in two Labrador Retrievers

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Azoospermia is described in two sibling Labrador Retriever dogs. Clinical investigations following failure to sire pups after normal matings revealed testicular hypoplasia and degeneration. Sperm were absent on repeated ejaculate examination in both dogs. Histopathological examination of testicular needle aspirate biopsy and whole testicle of the first dog displayed an absence of spermatids and spermatocytes. Seminiferous tubules containing Sertoli cells with or without primary spermatogonia were present in the second dog. Peritubular lymphocyte accumulation was also present in both dogs. The dogs had been conceived using frozen-thawed semen.

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ALP	Alkaline phosphatase
H&E	Haematoxylin and eosin
PAS	Periodic acid-Schiff

Two Labrador Retrievers forming part of the stud dog group used at Guide Dogs Association of Victoria were assessed for reproductive failure. This report describes the nature of the problem and the difficulties in determining the aetiology.

Case reports

The two affected dogs had been fed a standard pelletised food, received puppy and annual immunisation against canine parvovirus, distemper, hepatitis and kennel cough. Deworming had been performed at 3-month intervals with a combination tablet of pyrantel embonate, febantel and praziquantel. Monthly heartworm prevention with ivermectin and oral flea control with lufenuron had been used. Neither dog had suffered from any severe illness nor received any medication known to affect sperm production.

Dog 1

A 21-month-old Labrador Retriever was assessed for infertility after a single episode of failure to sire pups after a normal 'tied' mating. The bitch had conceived and whelped as a result of mating at its previous oestrus. On physical examination we palpated bilaterally descended, small, soft testicles and small epididymides. No prostate abnormalities were present on digital palpation per rectum. No other physical abnormalities were detected. There was no history of previous endocrinopathy or other disease. The dog exhibited normal libido with the ability to obtain and maintain normal erection following manual stimulation.

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An ejaculate was collected for analysis by masturbation using a silicone artificial vagina. Semen was collected into a sterile 14 mL test tube and centrifuged at 300 *g* for 10 min. The supernatant was removed. The remaining centrifugate was examined in a modified Nubauer haemocytometer counting chamber using light microscopy. No sperm were present in the ejaculate. A second ejaculate collected 1 week later also contained no sperm. Seminal ALP activity was 60,720 IU/L. The dog was sedated with medetomidine and percutaneous needle aspirate biopsy was obtained from each testis using an 18-gauge needle. Ultrasonography revealed no focal lesions of the epididymides, testes or prostate. Blood was collected into a lithium heparin tube and despatched on ice to Waite Veterinary Cytogenetics Services, Glen Osmond, South Australia for lymphocyte karyotyping.

Histopathological examination of the testicular biopsy revealed a single seminiferous tubule with Sertoli cells and an absence of germ cells. Morphologically normal interstitial cells were present. There were no inflammatory cells. A third ejaculate 3 months after the initial evaluation contained no sperm.

Judging from these results, the dog had a very poor prognosis for breeding. It was castrated. The vas deferens on both sides was ligated at the level of the external inguinal ring. Ten mL of blood was collected into a clot tube immediately before induction of anaesthesia. At the completion of the surgery the blood was centrifuged at 300 *g* for 15 min, 1 mL aliquots of serum were transferred to 1.8 mL cryovials and frozen at -80°C for later testosterone measurements. The testes and epididymides were cleaned of excess blood, dried, weighed and then placed in Bouin's fixative prior to histological examination (Figure 1). Sections were stained with H&E or PAS.

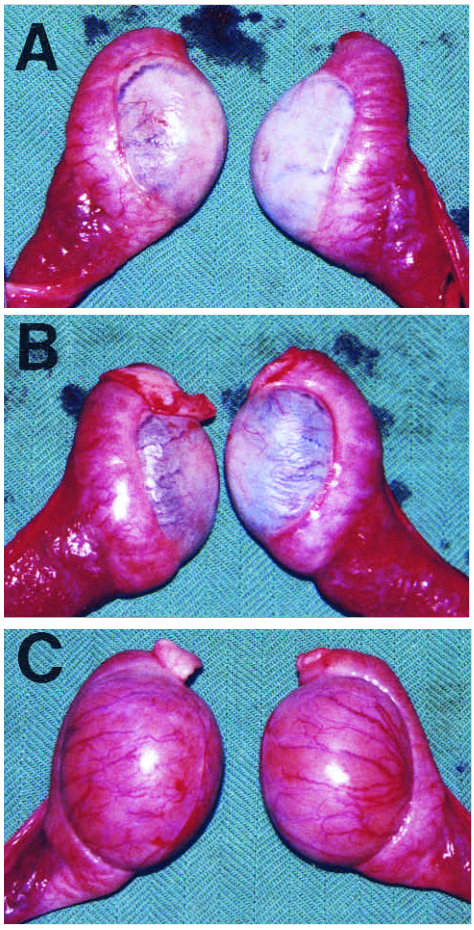

Figure 1. Testes immediately after castration. A is dog 1, B is dog 2 and C is unrelated Labrador.

Histopathological examination of the heads of the epididymides showed absence of sperm and mild vacuolation and degeneration of the epithelium compared to that of the control, 12-month-old unrelated Labrador. A systematic uniform random sampling was performed. This technique involves examining all tubules in 10, 100 µm apart, 5 µm thick sections of testes cut in the sagittal plane with 5 sections each side of the midline.[1] Tubular degeneration with Sertoli-cell-only tubules containing prominent cytoplasmic extensions and protein exudate comprised 90% of the tubules examined in the right testis and 94% in the left testis (Figure 2). Spermatogonia and spermatocytes were present in the remaining tubules with no evidence of maturation beyond the spermatocyte stage. The substance of the testes contained a spectrum of normal interstitium with morphologically normal interstitial cells to abnormal interstitium with small interstitial cells and peritubular lymphocyte accumulation around degenerating tubules (Figure 3).

Dog 2

Dog 2 was examined after failing to mate and then failure to sire pups after a normal mating at 21 months of age. On physical examination bilaterally descended, small, soft testicles and small epididymides were palpated. No other physical abnormalities were detected. The prostate was small with no abnormalities present on digital palpation per rectum. There was no history of previous endocrinopathy or other disease. The dog exhibited normal libido with the ability to obtain and maintain a normal erection after manual stimulation. An ejaculate was collected and centrifuged as described for dog 1. No sperm were found on examination. A second ejaculate 2 months later also revealed no sperm. Seminal ALP activity was 83 IU/L. Ultrasonography did not reveal any focal lesions of epididymides, testes or prostate. Blood collection for karyotyping, testicular biopsy and castration were performed in a similar fashion as with dog 1 (Figure 1).

Histopathological examination of the testicular biopsy was inconclusive due to very poor cell recovery. No germ cells or inflammatory cells were present. Histopathological examination of the heads of the epididymides showed similar vacuolar and degenerative changes as those in dog 1. No sperm were present in the epididymides. The testis showed tubular hypoplasia and degeneration. Tubules contained Sertoli cells only with prominent cytoplasmic extensions. The substance of the testes contained a spectrum of normal interstitium with morphologically normal interstitial cells to abnormal interstitium with hypotrophic Leydig cells. Intense peritubular lymphocytic infiltrates were present around degenerating tubules.

Discussion

Azoospermia refers to the complete absence of spermatozoa in the ejaculate.[2] In the reported cases azoospermia was diagnosed after examination of repeated ejaculates over a 3-month period. This is important because the sperm production cycle in the dog may take 55 to 70 days and sampling prior to re-establishment of sperm maturation after arrest may result in a false positive diagnosis of azoospermia. Centrifugation allows ejaculated sperm and other cells to be concentrated prior to examination. Bacterial, fungal or mycoplasma cultures were not performed because in all cases the ejaculates contained no cellular matter and clinical assessment did not suggest a cellular infectious aetiology. Seminal ALP activity of less than 5000 IU/L indicates an absence of fluid from the epididymides and may be caused by obstruction to the vas deferens, retrograde ejaculation and/or epididymal hypoplasia or aplasia.[3] No evidence of aplasia or obstruction of the vas deferens was evident at the time of castration in dog 2.

The testicular biopsy evaluations indicated germ-cell arrest. Correctly performed percutaneous needle aspirate biopsy has the advantage over excisional wedge biopsy that it can be performed when the animal is under sedation rather than under general anaesthesia. It is quicker, requires no suturing and is associated with little haemorrhage.[4,5] Recovery of inadequate cellular material may be due to technical failure during aspiration or to the inherent nature of the testicular tissue.[6] Nondiagnostic cell recovery has been recorded in 6 to 11% of azoospermic men and 25% of testicular tumours.[7-9] Excisional wedge biopsy provides better samples for histological assessment. Because of the potential complications from testicular biopsy (haemorrhage, infection, immune-mediated orchitis, sperm granuloma, scrotal swelling, transient and permanent decrease in sperm production) biopsy should only be performed after other less-invasive diagnostic techniques have been used.[2,6] Fixation in Bouin's fixative

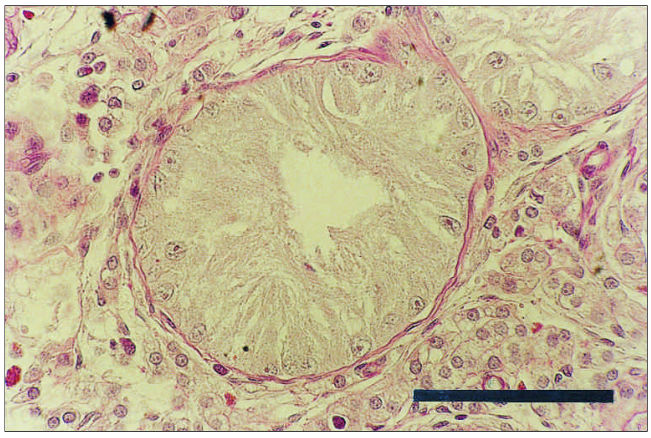

Figure 2. Section through testis of dog 1 showing Sertoli-cell-only tubule. PAS, bar = 100 µm.

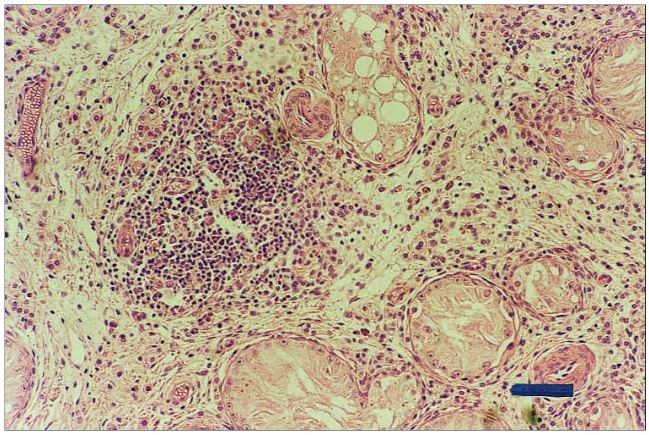

Figure 3. Section through testis of dog 1 showing lymphocyte accumulation around degenerating tubules. H&E, bar = 200 µm.

is considered preferable to that in buffered formalin. Formalin causes seminiferous tubule shrinkage and other artefacts that preclude meaningful interpretation.

Although the peritubular lymphocyte accumulation is usually consistent with a diagnosis of immune-mediated or lymphocytic orchitis, this is a morphological diagnosis only. It is difficult to interpret because of the previous testicular biopsy and possible disruption to the normally immunologically privileged tubules. In the two cases reported, lymphocyte accumulation was only present around tubules that had disrupted and degenerating basement membranes but not the degenerate tubules that had intact basement membranes.

Sertoli-cell-only tubules may be present either as a developmental anomaly or as an end-stage of germ cell destruction.[10,11] It is difficult to conclusively distinguish these two situations without examination of repeated biopsy specimens over time and/or use of additional diagnostic tools including electron microscopy, immunohistochemistry and chromosome analysis.

Serum testosterone concentrations, measured by radioimmunoassay at the time of castration, were 5.8 and 6.5 nmol/L for dogs 1 and 2, respectively. Poor correlation exists between testosterone concentration and semen variables.[12] Serum testosterone concentration may range from 1.7 to 34.7 nmol/L in normal dogs and from 0.3 to 6.9 nmol/L in those with cryptorchidism.[2] Dogs with normal libido and the ability to mate rarely have plasma testosterone concentrations below 1.4 nmol/L.[13] In the cases reported, it is possible that the total Leydig cell population was reduced. However, since testicular concentrations of testosterone may be 50 to 100 times greater than those in blood, plasma concentrations may not reflect alterations in testicular testosterone nor be a useful diagnostic marker for spermatogenesis.[12]

Chromosomal abnormalities such as XXY, XX sex reversal, XX/XY chimerism and mosaicism have been associated with azoospermia in non-Labrador Retriever dogs.[14-17] Developmental abnormalities in phenotypic sex such as hypospadias and cryptorchidism are usually present.[18] Cryptorchidism and testicular maldescent are recognised as the most common developmental causes of testicular hypoplasia and azoospermia. Lymphocyte metaphase spreads from both these cases displayed the normal dog 78 XY chromosome pattern. Specific Y chromosome deletions in men and autosomal deletions in mice have been associated with azoospermia but these deletions have not been investigated in the dog with normal karyotype.[19,20]

Table 1. Weight of testes and epididymides (g).

Variable	Dog 1[a]	Dog 2[a]	Control[a]
Right testis	6.04	5.73	12.83
Right epididymis	3.59	4.07	3.25
% testis : testis + epididymis	62.7	58.5	79.8
Left testis	6.89	6.94	13.49
Left epididymis	3.52	3.63	3.13
% testis : testis + epididymis	66.2	65.6	81.2

[a]Body weights at castration for dogs 1, 2 and control were 31, 32 and 28 kg, respectively.

The testicular weights were considerably smaller than that of the control dog. The ratios of testis to testis plus epididymal weight in both cases were outside the 99.99% confidence interval of measurements collected from known normal, fertile dogs (mean 81.25, SD 2.66, n = 22) of a range of breeds between the ages of 12 months and 7 years (unpublished). These comparisons in combination with the small testes weights confirm gross testicular hypoplasia/atrophy (Table 1). A mean ratio of testis to testis and epididymal mass of 81.5% has been reported previously.[21] Inbreeding may have detrimental effects on reproductive variables such as reduced ejaculate quality and testicular volume.[22] However, four-generation-pedigree review of the sire and dam showed no common ancestors.

The two dogs were from a litter of five pups conceived using frozen-thawed semen. The first female pup died 3 days after birth. No necropsy was conducted and the cause of death was undetermined. The second female pup died at 12 weeks of age. Necropsy and histopathological examination confirmed renal dysplasia with end-stage renal failure, secondary hyperparathyroidism with chondrodysplasia, myocarditis and megaoesophagus. No infectious aetiology was found. No urogenital abnormalities were found in the remaining bitch at the time of ovariohysterectomy at 8 months of age. To date no other significant reproductive abnormalities have been detected in half-siblings from either sire (8 females and 13 males from 3 litters) or dam (6 females and 9 males from 3 litters).

There are no previous reports of azoospermia in dogs conceived using frozen semen. The semen was imported from the USA. *Brucella canis* is recognised as a cause of acute and latent immune-mediated orchitis and epididymitis in dogs. It is possible for a bacteraemic bitch to deliver live and dead foetuses with the surviving pups being bacteraemic for several months.[23] *B canis* is currently exotic to Australia. The semen was collected from a certified *Brucella*-negative dog.

Familial azoospermia in Labrador Retrievers has been previously reported.[24,25] In the first report the offspring were diagnosed as infertile when they were between 2 and 3 years of age. Both dogs had a history of successful matings prior to developing azoospermia. The affected dogs were produced from the same dog-bitch mating but in separate litters. In contrast to the cases presented, up to 80% normal tubules with apparently normal sequence of spermatogenesis were present on histopathological assessment of the testes in the sire and one offspring. Identified lesions ranged from localised peritubular lymphocyte accumulation to degenerate tubules containing spermatogonia and/or only Sertoli cells. Similar to the cases presented, minimal changes occurred to the Leydig cells. The second report indicated that the two affected Labrador Retrievers had male relatives with reproductive dysfunction but provided no further specific information. A familial occurrence of interstitial, lymphocytic orchitis has also been documented in an inbred Beagle colony. Immune-mediated factors have been implicated because of the concurrent lymphocytic thyroiditis present in the affected dogs.[26]

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Diagnosis and therapy from page 566

Assessment

The diagnosis is feline herpesvirus corneal ulceration and conjunctivitis.[1] The superficial nature of the ulcer is typical of a feline herpesvirus lesion. Herpetic ulcers often take up fluorescein in a branching pattern (dendritic ulcer), but this is an unreliable feature and ulcers can assume any shape.

Treatment

Idoxuridine eye drops (Herplex D, Allergan) should be instilled five or six times daily after cleaning away discharges. Ulcers will resolve in most cases within a few days. Concomitant infection with calicivirus and/or *Chlamydia* should be considered and for this reason additional therapy with oral doxycycline may be advisable in most cases.

Comment

Feline herpesvirus is ubiquitous in cats. The virus is epitheliotrophic and replication takes place in the conjunctiva, cornea and mucosa of the respiratory tract.[2] Following exposure to the virus for the first time, infection typically involves the respiratory tract and conjunctivae, with sneezing and bilateral conjunctival hyperaemia and serous discharges. Rhinotracheitis and pneumonitis may occur. Conjunctivitis progresses with swelling of the mucous membrane. The discharge becomes mucopurulent. Superficial corneal ulceration may occur with uptake of fluorescein dye frequently in a dendritic pattern.

Recovered cats become latent carriers in 80% of cases.[3] Many of these will shed virus without signs, but some succumb to recurrent episodes of keratoconjunctivitis in one or both eyes. Spasm becomes a variable sign depending on corneal involvement. Discharges tend to be serous in these adult infections. Recurrent respiratory infections are far less common.

Diagnostic confirmation of herpesvirus conjunctivitis can be sought using conjunctival swabs tested for herpesvirus DNA by polymerase chain reaction methods. Other antiherpetic drugs (acyclovir, vidarabine) are not as effective for treatment as idoxuridine. Use of steroidal or nonsteroidal anti-inflammatory medications topically should be avoided with corneal ulcers.

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