Sperm Granuloma and Sperm Agglutination in a Dog with Asthenozoospermia

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ABSTRACT. A mongrel dog, aged 2 years, was found to have only a small number of sperm, immobilization of all sperm, and many sperm agglutinations in its ejaculates, and scrotal palpation revealed a small nodule in the left cauda epididymis. Addition of the dog's seminal plasma or serum to the semen of 2 normal dogs caused immobilization and agglutination of their sperm. Histological examination showed that the nodule was a sperm granuloma. Many lymphocytes were seen in the stroma around the sperm granuloma. Anti-sperm antibodies are presumed to be present in the semen and serum of the asthenozoospermic dog.

KEY WORDS: asthenozoospermia, canine, sperm granuloma.

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It has been reported that anti-sperm antibodies were detected in the serum of 6% of infertile men [6]. Anti-sperm antibodies consist of sperm-immobilizing antibodies and sperm-agglutinating antibodies [1, 3, 8], and sperm granulomas in the testis or epididymis have been identified as one of the causes of the production of anti-sperm antibodies [3, 7]. There have been two reports of vasectomy [2] and congenital occlusion of the epididymal duct [4] causing a sperm granuloma in dogs. The other causes of sperm granuloma are severe stenosis or occlusion of the genital tract secondary to infectious orchitis and epididymitis [13] or injury of the testis and epididymis [9, 10]. We report the cause of a dog with poor semen quality in which a sperm granuloma was found in the epididymis, and we examined the relation between spermatogenic dysfunction and the sperm granuloma in the dog.

A mongrel dog (21 kg body weight), aged 2 years, and cared for at our university, was diagnosed with asthenozoospermia based on evalutions of the quality of semen collected by digital manipulation 3 times weekly, and scrotal palpation revealed a small, projecting, firm nodule in the left cauda epididymis. Semen samples were examined for total semen volume, total number of sperm, and percentages of actively motile sperm, viable sperm, and morphologically abnormal sperm by the methods described previously [11]. Peripheral vein blood samples were collected from the dog and 3 normal male beagle dogs, Dogs A, B, and C, 13–16 kg body weight and 2–4 years old, at the time of the semen collection. Peripheral vein plasma testosterone and estradiol-17β concentrations were measured by radioimmunoassay methods [12, 15].

The seminal plasma and serum collected from the mongrel dog and Dog C were used to perform the sperm immobilizing test and sperm agglutinating test. A 500 μl volume of seminal plasma obtained by centrifugating (700 g for 20 min) the semen and a 100 μl of serum inactivated at 56°C for 30 min were added to 500 μl of the semen of Dogs A and B, and the motility and agglutination of the sperm in the semen were examined after incubation for 15 min at 38°C in an atmosphere of 5% CO_2 in air. The left testis and epididymis of the asthenozoospermic dog were removed under halothane inhalation anesthesia, and the testis and the caput, corpus, and cauda epididymis, and the nodule were embedded in paraffin, sectioned at 3 μm, and stained with PAS-hematoxylin. Statistical significance was tested by the unpaired Student's t test. The data are summarized as mean values ± standard deviation (S.D.).

The total number of sperm collected from the mongrel dog with asthenozoospermia was below the mean value in normal dogs, and no motile sperm were observed (Table 1). Many head-to-head sperm agglutinations were seen in the semen (Fig. 1). The peripheral vein plasma testosterone concentration in the asthenozoospermic dog was lower than the mean value in the normal dogs (Table 2). There were no marked changes in the sperm motility of Dogs A and B after the addition of the seminal plasma and serum from Dog C (Table 3), but the addition of the seminal plasma and serum from the asthenozoospermic dog induced low sperm motility and head-to-head sperm agglutination.

No evidence of injury or inflammation of the scrotum or

Table 1. Semen quality of the asthenozoospermic (AS) dog[a)] and 3 normal beagle dogs[b)]

	Total volume of semen (ml)	Total number of sperm ($\times 10^6$)	%motile sperm	%viable sperm	%abnormal sperm
AS dog	15.8	107.8	0	57.2	15.0
Normal dogs	14.4 ± 1.2	511.4 ± 43.6	91.7 ± 1.5	95.8 ± 0.5	4.5 ± 0.5

a) Mean values of the quality of the semen, which was collected 3 times weekly.
b) Means ± S.D. of the mean semen quality of each normal dog.

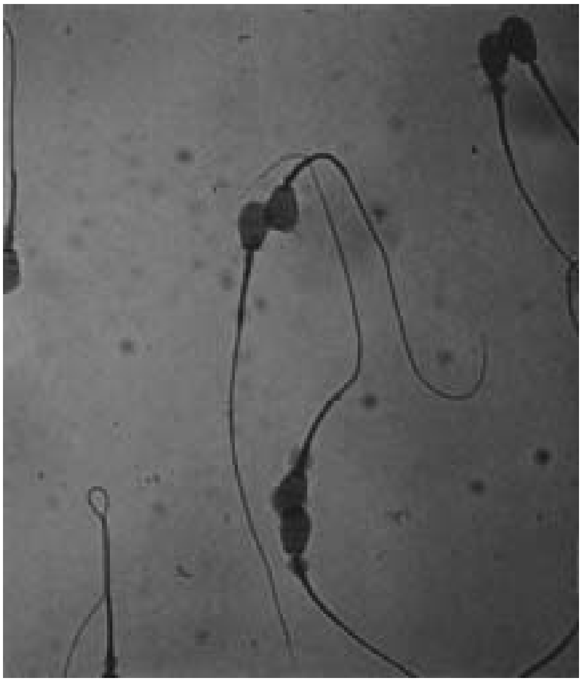

Fig. 1. Head-to-head agglutination of ejaculated sperm from the asthenozoospermic dog (Rose bengal stain, × 1,000).

Table 2. Peripheral vein plasma testosterone and estradiol-17β concentrations of the asthenozoospermic (AS) dog[a)] and 3 normal beagle dogs[b)]

	Estradiol-17β (pg/m*l*)	Testosterone (*n*g/m*l*)
AS dog	10.2	1.26
Normal dogs	11.4 ± 0.7	2.01 ± 1.12

a) Mean values of the concentrations in the plasma, which was collected 3 times weekly.
b) Means ± S.D. of the mean concentrations of each normal dog.

of the left testis and epididymis was detected in the asthenozoospermic dog. A gray, projecting nodule (8 mm in diameter) was observed in the cauda epididymis (Fig. 2). The numbers of germ cells and sperm in the seminiferous tubules of the left testis were low (Fig. 3). The lumen of the nodule was filled with a large number of sperm, and many sperm agglutinations were seen (Fig. 4). The lumen was surronded by thick connective tissue, and the structure of the epididymal ducts in the nodule had been completely lost. Many lymphocytes were seen in the stromal tissue around the nodule (Fig. 5). Although the sperm in the duct of the corpus epididymis were not agglutinated, head-to-head agglutinated sperm were observed in the cauda epididymal duct that passed through the nodule (Fig. 5).

Histological examination of the left cauda epididymis of the asthenozoospermic dog confirmed that the nodule was a sperm granuloma. It is assumed that the nodule was occurred due to collapse of the neighboring epididymal ducts. Cases of sperm granuloma of the epididymis have been reported in dogs [2, 4]. The presence of the head-to-head agglutinated sperm in the cauda epididymal duct that passed through the sperm granuloma in our dog proved that the epididymal duct near the sperm granuloma was not completely occluded and the sperm agglutinations had occurred in the sperm granuloma. The formation of a sperm granuloma has been described as inducing infiltration by immune response cells, lymphocytes and macrophages in the stromal tissue around the granuloma and the production of anti-sperm antibodies [3, 7, 14]. In humans, anti-sperm antibodies in serum cause head-to-head agglutination of ejaculated sperm [8] and infertility [6], and the anti-sperm antibodies in seminal plasma and serum have been found to be sperm-immobilizing antibodies and sperm-agglutinating antibodies [5]. In our dog many lymphocytes were also observed in the stromal tissue around the sperm granuloma, suggesting that an immune response to the sperm in the granuloma of the asthenozoospermic dog occurred, and that sperm-immobilizing antibodies and sperm-agglutinating antibodies were then produced and released into the seminal plasma and serum. The sperm immobilization and agglutination and low spermatogenic function in our dog appeared to have been caused by anti-sperm antibodies. No evidence of infection, inflammation or injury of the scrotum was found in the dog. The formation of the sperm granuloma in the

Table 3. Sperm motility and agglutination before and 15 min after the addition of 500 μl of seminal plasma and 100 μl of serum[a)] collected from the asthenozoospermic (AS) dog and a normal dog (Dog C) to 500 μl of the semen of 2 normal dogs (Dogs A and B)

Addition of seminal plasma or serum	Sperm in Dog A		Sperm in Dog B	
	%motile	Agglutination	%motile	Agglutination
Seminal plasma				
Before	95	(–)	90	(–)
After (AS dog)	30	(+++)	30	(+++)
(Dog C)	90	(–)	90	(–)
Serum				
Before	95	(–)	90	(–)
After (AS dog)	20	(++)	30	(++)
(Dog C)	80	(–)	85	(–)

a) The sera were inactivated at 56°C for 30 min.

Fig. 2. Left testis and epididymis of the asthenozoospermic dog. The small nodule (arrow) is seen in the cauda epididymis.

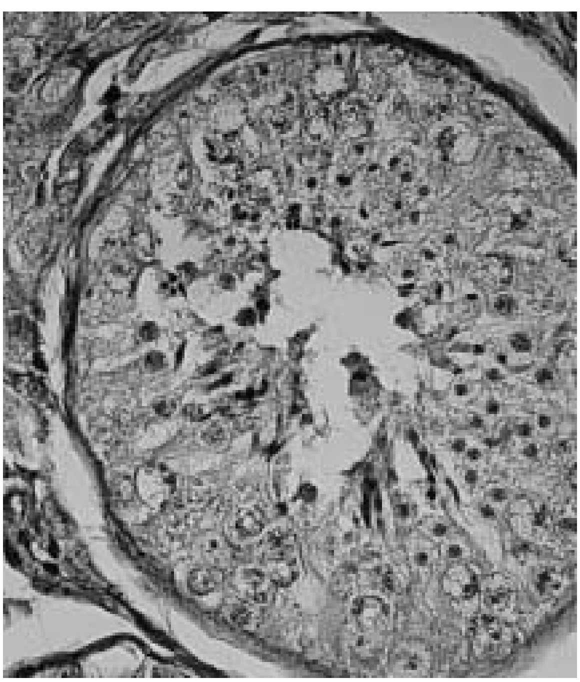

Fig. 3. Histological findings in the left testis of the asthenozoospermic dog (PAS-hematoxylin stain, × 400). Small numbers of germ cells and sperm are seen in the seminiferous tubule.

Fig. 4. Histological findings in the nodule in the left epididymis (PAS-hematoxylin stain, ×200). A large number of sperm are seen in the lumen and are surrounded by thick connective tissue (*), and the epithelium of the epididymal duct has been lost. Based on these findings, the nodule was diagnosed as a sperm granuloma.

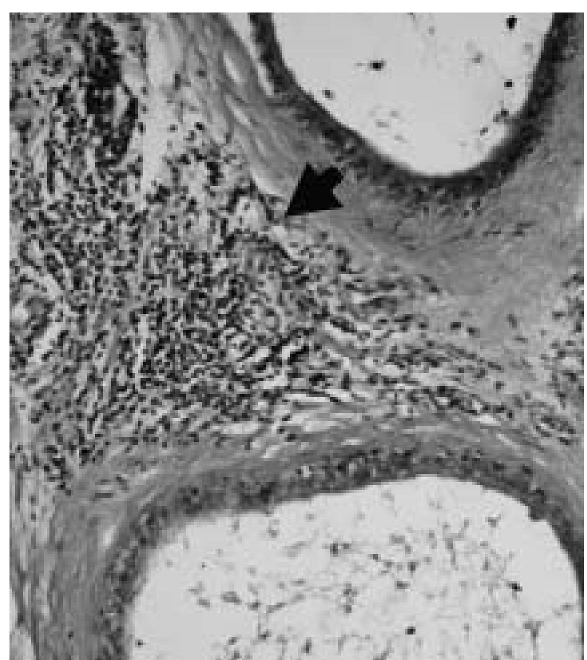

Fig. 5. Histological findings in the stromal tissue around the nodule (PAS-hematoxylin stain, × 200). Many lymphocytes (arrow) are observed in the stroma.

asthenozoospermic dog may have been caused by congenital severe stenosis of the cauda epididymal duct.

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