



## Canine testicular disorders and their influence on sperm morphology

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### Abstract

The objective of this study was to evaluate the influence of testicular disease on sperm morphology. The reproductive tracts of 33 dogs were evaluated clinically and with ultrasound, followed by orchiectomy and harvesting of fluid from the vas deferens to evaluate sperm morphology. A section from each testis was used to conduct histological analyses. Histological changes were noted in 71.2% of testes (47/66). Regardless of dog age, the most frequent pathology was testicular degeneration (80.8%; 38/47), whereas testicular tumors were observed only in adult and old dogs (25.9%; 7/27). Harvesting fluid from the vas deferens for sperm morphology assessment was effective in 87.9% of cases (58/66), and severe testicular degenerative processes induced an increase ( $P < 0.05$ ) in the percentage of abnormal sperm when compared with normal testes or those with moderate testicular degeneration (special attention given to detached heads). In conclusion, regardless of dog age, breed or origin, a severe testicular degeneration process led to a significant increase in detached heads. Furthermore, the collection of sperm from the vas deferens proved to be an alternative and reliable technique for future research.

**Keywords:** dog, sperm morphology, testicular disorder, Vas deferens.

### Introduction

The search for the correct etiology and treatment of reproductive diseases in dogs has received much attention in the past few years (Ström Holst *et al.*, 2000; Memon, 2007), and early diagnosis of canine testicular disorders may be useful before selecting a male dog for mating or artificial insemination (Domingos and Salomão, 2011). Many techniques have been used to estimate the reproductive potential of a male dog, to examine and diagnose disorders of the male reproductive tract, or to detect a decline in the reproductive function. Techniques have included classical evaluation of canine ejaculates (Peña Martínez, 2004), genital tract ultrasound (Santos *et al.*, 2004), fluorescent staining for assessment of specific sperm functions (Rijsselaere *et al.*, 2005), computer-assisted

sperm analyses (Root Kustritz, 2007), testicular fine needle aspiration cytology (Gouletsou *et al.*, 2010), testicular biopsy (Mascarenhas *et al.*, 2006), and serum or plasma hormone concentrations (Veronesi *et al.*, 2009).

The limited availability of some procedures mentioned above emphasizes the importance of studies that identify simple techniques that help veterinary practitioners achieve the correct diagnoses of testicular disease. Studies on morphological characteristics of dog semen are dated (Bartlett, 1962), and assessment of sperm morphology is based on subjective criteria (Peña Martínez, 2004), even though variations in fixation, staining technique, quality of the microscope and the observer's experience can influence evaluation (Rijsselaere *et al.*, 2005). However, the identification of sperm morphologic changes is relatively easy and can be used by veterinary practitioners to select supplementary diagnostic tools for a precise diagnosis.

Moreover, the collection of canine semen by digital manipulation results in sperm from both testes; allows evaluation of the suitability of semen for artificial insemination, preservation, or investigation of subfertility or infertility (Root Kustritz, 2007); and has resulted in several reports relating specific sperm defects to canine infertility (Plummer *et al.*, 1987; Kawakami *et al.*, 2005; Peña Martínez *et al.*, 2007). However, the semen collection technique (digital manipulation) makes it impossible to observe the real influence of unilateral testicular disease on sperm morphology. Thus, the objective of the present study was to evaluate the influence of canine testicular disease on the morphology of sperm harvested from the vas deferens.

### Materials and Methods

#### *Animals, physical examination and ultrasound examination*

Initially 52 dogs of different breeds, ages, and body conditions were examined. Dogs were classified as young (1-2 years), adult (3-5 years) or old ( $\geq 6$  years; Ortega-Pacheco *et al.*, 2006a). During clinical examination, particular attention was paid to seminological and ultrasonographic alterations of the testes or epididymides.

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Ultrasound evaluation was performed using a portable device (10 MHz linear-array transducer; CTS 900V, Siui, Hong Kong, China) with minimal compression to avoid organ-shape alterations, and the testes and epididymides were completely examined in both longitudinal and transverse axes (Paltiel *et al.*, 2002). Throughout the experimental period, the presumptive diagnosis from ultrasound scans was analyzed by the same operator and described according to Nyland and Mattoon (2005).

After clinical and ultrasound evaluation, 19 dogs were excluded from the experiment because they were diagnosed with a scrotal or epididymal disease, either alone or in association with testicular disorder. These exclusions were made to eliminate those dogs with potential sperm defects (Oettlé and Soley, 1988) that may not be associated with testicular disease.

#### *Orchiectomy, sperm morphology evaluation, and testicular histological analysis*

All 33 dogs were anesthetized and orchiectomized (Fossum, 2002), followed by dissection of the vas deferens at the furthest point from the epididymal tail. For sperm collection the vas deferens was mildly compressed using the thumb and index fingers, sliding the fingers onto the previously sectioned vas deferens area. Fluid drops were smeared onto microscope slides in duplicate, air dried, and stained with a rapid Romanowsky-type stain (Instant-Prov, NewProv Laboratory, Brazil). Sperm morphology was assessed by counting 200 sperm from each testis, whenever possible, under a microscope (Nikon, Tokyo, Japan) at 1000X magnification (Mies Filho, 1987).

Additionally, fragments (approximately 1 cm<sup>3</sup>) were removed from areas of diseased testes or non-specific areas of normal testes that had previously been macroscopically and ultrasonographically examined and diagnosed. Testicular sections were fixed in 10% buffered formalin solution, submitted to histological procedures, and five-micrometer sections were cut and stained with hematoxylin and eosin (Veronesi *et al.*, 2009). The findings were described according to McGavin and Zachary (2007). Testicular degeneration was classified as slight to moderate in the presence of reduced seminiferous tubular diameter, decreased numbers of germinal cells, and interstitial fibrosis, either alone or in association. Testicular degeneration was classified as severe when testes presented reduced seminiferous tubular diameter, a thickened basement membrane, Sertoli cell vacuolization, decreased numbers of germinal cells, and interstitial fibrosis.

#### *Statistical analyses*

The experimental unit for the influence of testicular disease on sperm morphology was each testis from the 33 dogs included in the experiment (n = 66). Differences in sperm morphology data between normal testes and those with histological changes were assessed using a one-way ANOVA, followed by Tukey's test, with SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). For all analyses P < 0.05 was considered significant.

#### **Results**

Among the 33 dogs included in the experiment, six were classified as young, 12 as adult, and 15 as old. There were histopathological alterations in 71.2% of testes (47/66). Slight to moderate testicular degeneration was present in 61.7% (29/47) of dogs with histopathological alterations, followed by severe testicular degeneration in 19.2% (9/47), interstitial cell tumours in 6.4% (3/47), and Sertoli cell tumours in 4.3% (2/47) of dogs with histopathological alterations. Seminoma, orchitis, testicular hypoplasia, and a mixed tumour (seminoma and Sertoli cell tumour) all had the same prevalence (2.1%; 1/47).

A greater prevalence of testicular disease was observed in adult (83.3%; 10/12) and old dogs (73.3%; 11/15) compared to young dogs (50.0%; 3/6). Neoplastic processes were histopathologically diagnosed only in adult and old dogs, with a frequency of 25.0% (3/12) and 26.7% (4/15), respectively. All dogs with a unilateral neoplastic process presented some degree of testicular degeneration on the contralateral testis. No dog in the current study had bilateral testicular tumors.

Harvesting fluid from the vas deferens for sperm morphology assessment was effective in 87.9% of cases (58/66). Of the eight azoospermic samples, two were from testes histopathologically diagnosed as having severe degeneration and one sample was from each of the following: Sertoli cell tumour, seminoma, interstitial cell tumour, mixed tumour, orchitis, and hypoplasia. Vas deferens fluid samples obtained from 19 testes classified as normal after histopathological examination contained  $80.7 \pm 5.7\%$  (mean  $\pm$  SD) morphologically normal sperm. This finding was similar (P > 0.05) to results observed for slight to moderate testicular degeneration ( $74.7 \pm 14.0\%$ ) and higher (P < 0.05) than that observed for severe testicular degeneration ( $54.6 \pm 12.8\%$ ). Only the prevalence of detached head abnormalities differed significantly between samples from normal testes and those with severe testicular degeneration (Table 1). In both testes with Leydig cell tumors that had sperm in the vas deferens fluid, the rate of morphologically normal sperm was  $47.0 \pm 4.2\%$ .

Table 1. Morphology of sperm (mean  $\pm$  SD) harvested from vas deferens fluid<sup>†</sup> of canine testes classified as normal or diagnosed with different levels of testicular degeneration after histological evaluation.

Sperm morphology (%)	Testicular histological diagnosis		
	Normal (n = 19)	Slight to moderate testicular degeneration (n = 29)	Severe testicular degeneration (n = 9)
Normal	80.7 $\pm$ 5.7 <sup>a</sup>	74.7 $\pm$ 14.0 <sup>ab</sup>	54.6 $\pm$ 12.8 <sup>b</sup>
Detached head	3.8 $\pm$ 2.0 <sup>a</sup>	7.2 $\pm$ 6.0 <sup>ab</sup>	15.0 $\pm$ 6.3 <sup>b</sup>
Pyriform head	0.0	0.2 $\pm$ 0.4	0.3 $\pm$ 0.6
Giant head	0.1 $\pm$ 0.2	0.2 $\pm$ 0.6	0.0
Narrow head	0.0	0.2 $\pm$ 0.6	0.2 $\pm$ 0.7
Ruptured head	0.0	0.1 $\pm$ 0.1	0.0
Micro-head	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2	0.0
Macro-head	0.0	0.1 $\pm$ 0.2	0.2 $\pm$ 0.4
Distal droplet	0.1 $\pm$ 0.5	0.1 $\pm$ 0.3	0.0
Abnormal midpiece	0.0	0.1 $\pm$ 0.2	0.0
Coiled tail	6.5 $\pm$ 2.6	5.6 $\pm$ 4.3	8.5 $\pm$ 9.7
Broken tail	1.6 $\pm$ 1.2	3.8 $\pm$ 4.2	5.8 $\pm$ 7.4
Bent tail	2.8 $\pm$ 2.6	2.4 $\pm$ 2.0	3.1 $\pm$ 3.9
Double tail	0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2

<sup>a,b</sup>Within a row, means without a common superscript differed ( $P < 0.05$ ). <sup>†</sup>Sperm harvested from the vas deferens fluid after orchiectomy.

### Discussion

The prevalence of testicular pathologies (71.2%) and testicular degeneration (57.6%) in the present study was higher than the 33.9 and 15.1%, respectively, reported previously (Ortega-Pacheco *et al.*, 2006a), even though both studies used the same histological analysis parameters (McGavin and Zachary, 2007). It is well known that testicular degeneration is progressive and more prominent with increasing age (Lowseth *et al.*, 1990), and is considered the most common cause of low fertility in domestic animal species (Domingos and Salomão, 2011). Most samples obtained in the present study were harvested from old dogs. Thus, differences in the average age of the dogs in the present study and that conducted by Ortega-Pacheco *et al.* (2006a) could explain the differences observed in the overall prevalence of testicular pathologies.

Neoplastic processes were diagnosed only in adult and old dogs, which is supported by findings in a previous report (Lowseth *et al.*, 1990). Data from the present study are also supported by a recent study with over 200 dogs, wherein authors reported that the mean age of dogs with testicular tumors was 10.7 years (Grieco *et al.*, 2008). In contrast, in our study, the frequency of dogs with testicular tumors (21.2%) was lower than the 48.4% previously reported (Peters *et al.*, 2000). This difference is likely attributable to the approximately 9.5% (9/95) of cryptorchid dogs studied by those authors, whereas in the present study the frequency was 3.0%. Cryptorchidism can increase the occurrence of Sertoli cell tumors and seminomas by 26- and 15-fold, respectively (Kim and Kim, 2005).

The presence of azoospermia in cases of testicular tumors, orchitis, hypoplasia, and testicular

degeneration has often been reported (Ortega-Pacheco *et al.*, 2006a; Memon, 2007; Goedken *et al.*, 2008; Domingos and Salomão, 2011; Fontbonne, 2011). In the present study testes that were diagnosed with severe testicular degeneration and had azoospermic vas deferens fluid, whereas their seminiferous tubules contained germ cells, possibly had an extra-testicular obstruction causing impairment of sperm release (Westlander *et al.*, 2001; Memon, 2007).

An association between occlusive lesions and testicular degeneration has been reported in dogs (Dahlbom *et al.*, 1997b; Souza *et al.*, 2004) and could result from a previous infection or inflammation of the seminal ducts (Romagnoli *et al.*, 2009). Furthermore, testicular degeneration may have been exacerbated by the presence of anti-sperm antibodies, given that anti-sperm antibody production may be related to impairment of sperm transit caused by congenital occlusion of the epididymal duct or sperm granuloma formation (Kawakami *et al.*, 2003).

Although the harvesting of vas deferens fluid cannot be adopted for assessment of sperm morphology in routine dog breeding soundness examinations, it is noteworthy that sperm samples from euthanized dogs are often recovered from the epididymal tail, resulting in a high percentage of sperm with distal cytoplasmic droplets even in sperm samples from normal testes (Ortega-Pacheco *et al.*, 2006a). Moreover, even though this sperm pathology has been considered of minor importance to dog fertility (Ortega-Pacheco *et al.*, 2006b), its occurrence can complicate the determination of the real influence of testicular disorders on sperm morphology.

When compared to normal testes, severe testicular degeneration significantly decreased the



percentage of normal sperm, which in turn indicated reduced reproductive performance due to a percentage of abnormal sperm greater than 40.0% (Dahlbom *et al.*, 1997a). The significant increase in the percentage of detached heads is in accordance with the findings of Ortega-Pacheco *et al.* (2006a), who reported that detached heads were one of the most frequent sperm abnormalities in canine testicular degeneration. Similarly, studies in others species, such as bovine and buffalo bulls (Blom, 1950; Garcia, 2009) and bucks (Refsal *et al.*, 1983) have shown the same pattern of sperm pathology in testicular degeneration.

Separation between the sperm head and tail can occur at the moment of spermiation (Chemes and Rawe, 2003), in the caput epididymis (Blom and Birch-Andersen, 1970), or increase gradually during epididymal transit (Kawakami *et al.*, 2005). Furthermore, sperm is easily decapitated during manipulation after semen collection (Kamal *et al.*, 1999). Despite the origin, abnormalities of the head-neck attachment display varying degrees of alteration in the relationship between centrioles and the sperm nucleus (Chemes and Rawe, 2003). Moreover, the connecting pieces of many sperm show ultrastructural defects that include degeneration or absence of the basal plate and abnormalities of the proximal centriole (Blom and Birch-Andersen, 1970; Kamal *et al.*, 1999; Toyama *et al.*, 2000).

In conclusion, the present investigation observed that regardless of dog age, breed, or origin, severe testicular degeneration significantly increased the percentage of detached heads. More research is needed to demonstrate if this finding could be used as a tool for diagnosis of canine testicular degeneration. Furthermore, the collection of sperm from the vas deferens proved to be an alternative and reliable technique for future research.

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