Severe Tail Defects in the Spermatozoa Ejaculated by an English Bulldog

Ada ROTA1)*, Elisabetta MANUALI2), Simona CAIRE3) and Simonetta APPINO4)

1)Department of Animal Pathology, University of Turin, 2)Laboratory of Histopathology and Electron Microscopy, Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche, Perugia, 3)Practitioner and 4)University of Sassari Italy

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ABSTRACT. This paper describes the case of a 2-year-old English Bulldog with severe teratozoospermia that consisted mainly of “Dag-like” defects, which is strong coiling of the tail. Although libido, semen volume and sperm concentration were normal, sperm motility was 5.0%, and 93.3% of spermatozoa exhibited morphological abnormalities affecting the tail. Transmission electron microscopic examination of the spermatozoa revealed strong folding, coiling and fracture of sperm midpieces and tails, axonemal defects and the presence of swollen and unevenly distributed mitochondria. Taking into account the dog’s history and examinations and the constantly high percentage of abnormal spermatozoa over time, the defect was considered to be genetic in origin.

KEY WORDS: canine, sperm, tail defect.

A variety of morphological defects that affect the acrosome, head, midpiece and tail have been sporadically identified in dog spermatozoa [3, 6, 8–10]. The “Dag defect” is a tail defect that was first identified in a Jersey bull named “Dag” [1], and it consists of a strong coiling of the tail. The “Dag” or “Dag-like” defects have also been found in dogs, sometimes affecting a low proportion of spermatozoa in association with other sperm abnormalities [8, 9] and sometimes representing the major sperm defect [6]. Structural changes occur in the epididymis and result in multiple fractures of the axonemal fibers and disruption of the mitochondrial sheath [7]. This paper describes the case of a Bulldog with severe teratozoospermia, mainly consisting of “Dag-like” defects.

The dog, aged 2, was referred to the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine of the University of Turin because of very low sperm motility, as assessed in four successive ejaculates collected at monthly intervals. The dog’s history showed an hCG treatment at 3 months of age for a partially undescended testis; the dog had never suffered from any injury or inflammation of the genital organs and his libido was normal. He was seronegative for brucellosis, and only Streptococcus canis was isolated from semen culture.

Physical examination, palpation and ultrasound examination of the genital system did not reveal any abnormalities. A blood sample was collected for plasma testosterone concentration determination (Chemiluminescence; Immulite, Medical Systems S.p.A, Genoa, Italy). The method had been previously validated for canine serum; data not shown), and a semen sample was collected by digital manipulation without an estrous bitch. Immediately after collection, the percentage of motile sperm was estimated under light microscopy on a warm stage at 100 × magnification. Viable spermatozoa were assessed by eosin-nigrosin staining, and sperm morphology was examined after Diff-Quick staining by observing 200 cells under oil immersion (1,000 × magnification). The sperm concentration was determined by hemocytometer count. Seminal pH was also measured.

Semen aliquots were prepared for transmission electron microscopy (TEM) by adding 2.5% glutaraldheyde (TAAB, England) in 0.1 M phosphate buffer (v/v), pH 7.4, for 10 min at 4°C and centrifugation at 3,000 rpm for 5 min. The supernatant was then removed. The pellet was resuspended in 1% glutaraldheyde in 0.1 M phosphate buffer for 45 min at 4°C and centrifuged as before. The pellet was then rinsed three times, post-fixed in 1% osmium tetroxide (OsO4; Next Chimica, Germiston, South Africa) in 0.1 M phosphate buffer (v/v) for 1 hr at 4°C, dehydrated in a graded series of ethanol, incubated in propylene oxide (TAAB, Aldermaston, England) for 5 min at room temperature and embedded in Epon 812. Resin blocks were solidified at 60°C for 48 hr. Semithin sections of sperm cells were stained with 1% toluidine blue (w/v; pH 3.5). Silver color ultrathin sections were collected onto copper grids coated with a Formvar-carbon layer and double stained with uranyl acetate-lead citrate. Samples were examined and photographed at 80 kV on a CM12 STEM electron microscope (Philips, Eindhoven, The Netherlands).

The testosterone concentration was 3.8 ng/ml. Although the semen volume (6 ml) and sperm count (816 × 10⁶ spermatozoa) were normal, semen motility was very low (5.0%) and sperm viability was 59.2%. Under light microscope, the vast majority of spermatozoa (93.3%) exhibited morphological abnormalities affecting their tails. The defective spermatozoa had coiled or folded tails (93.8%), and 6.2% of the tails were detached (Fig. 1). The degree of coiling was variable: ‘Dag-like’ defects affected 58.4% spermatozoa, while other tails were either folded or exhibited a lower degree of coiling that was localized only at their apical part (35.4%). The ultrastructural characteristics of the defective sperm are shown in Fig. 2 and Fig. 3. The most important abnor-
malities observed were related to the flagellar compartment, and these consisted of strong folding, coiling and fracture of the sperm midpiece; coiled tails were encapsulated in a thin membrane that trapped several membranous structures, the mitochondria were swollen and unevenly distributed, and axonemal defects (numerical aberrations of the microtubules and dislocation of axial fibers) were also observed. Previous works reporting the Dag defect in dog spermatozoa describe coiling of the sperm tail within an extended, but intact, cell membrane [8, 9] along with derangement of fibers and mitochondrial vesiculation [9].

Sperm tail defects have been correlated with low testosterone secretion from the testis affecting epididymal functionality in the dog [5] and with severe infection/inflammation of the genital tract [9]. Both conditions can be excluded in our case because the circulating testosterone concentration was high and the dog’s history did not show any pathologies affecting the genital area.

Unfortunately, no fertility data was available for his siblings. The “Dag defect” has a heritable basis in some cattle breeds, and when a bull is homozygous for this recessive gene, 60–100% of sperm cells are defective [7]. In the literature, sporadic reports describe Dag-like defects in individuals of other species, such as a subfertile stallion [4], a subfertile boar [2] and an infertile goat [7], although a genetic basis was not established in these cases. Some sperm defects are considered to be genetic in origin when they appear in the semen “at a fairly constant rate and in a very high proportion of the sperm cells without any indication of environmental influence” [2]. In consideration of the percentage of spermatozoa affected and of the permanence of the abnormality over time, the defect that we observed could be considered genetic.

REFERENCES

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Fig. 1. Diff-Quick stained smear showing a high proportion of defective spermatozoa and various degrees of tail coiling. Bar=10 µm.

Fig. 2. Longitudinal section of a spermatozoon displaying a coiled tail surrounded by a membrane and swollen mitochondria. TEM. Bar = 1 µm.

Fig. 3. Cross section of an abnormal sperm tail showing numeric aberrations of the axonemal microtubules. TEM. Bar = 1 µm.