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Diagnostic Value of Fine Needle Aspiration Cytology in Testicular Disorders of Red Deer (*Cervus elaphus*): A Case Report

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ABSTRACT: We used fine needle aspiration cytology (FNAC) to diagnose Sertoli cell-only pattern and hypospermatogenesis in an Iberian red deer (*Cervus elaphus hispanicus*). Cytologic diagnosis was confirmed by histology and epididymal sperm analysis. We conclude that FNAC can be an important diagnostic tool in testicular diseases of wildlife.

Assessment of spermatogenesis plays a key role in the evaluation of male fertility. Although several methods have been described, fine needle aspiration cytology (FNAC) is currently the simplest and least-invasive technique for obtaining testicular samples (e.g., Gouletsou et al. 2012). Developed by Posner and Cohn (1904) as a diagnostic tool in human infertility, FNAC has been applied in veterinary medicine only recently (e.g., Romagnoli et al. 2009).

Several studies report testicular disorders in cervids including white-tailed deer (*Odocoileus virginianus*; Taylor et al. 1964), black-tailed deer (*Odocoileus hemionus*; Tiller et al. 1997; *Odocoileus hemionus columbianus*; DeMartini and Connolly 1975; *Odocoileus hemionus sitkensis*; Bubenik et al. 2001), red deer (*Cervus elaphus*; Carrasco et al. 1997), and pampas deer (*Ozotoceros bezoarticus*, Ungerfeld 2013). We describe the diagnosis of Sertoli cell-only pattern and hypospermatogenesis by FNAC in an Iberian red deer (*Cervus elaphus hispanicus*).

An adult Iberian red deer (age>4.5 yr, body mass>130 kg) was culled in February (nonbreeding season) 2011 in Spain in accordance with Spanish harvest regulations. Testes, within the scrotal sac, were transported to the laboratory at 20–25 C and processed 4–8 hr postmortem. The testes were descended into the scrotal sac and, together with epididymides, exhibited normal consistency. Macroscopic analysis revealed no abnormality except for marked testicular asymmetry. Testicular mass was 48.10 g and 34.26 g in the right and left testes, respectively. Mean (±SD) testicular masses in stags culled at the same date and location were similar between the left and right testes (44.92±6.85 g and 42.09±7.05 g, respectively; $P=0.221$, paired t-test, $t=1.396$, df=5, $n=6$).

Cytologic and histologic analyses were performed as described by Pintus et al. (2014). Briefly, a 20-G needle, connected to a 5-mL syringe, was inserted into the testis avoiding the epididymis. After gentle in-out movements, the content was flushed onto a glass slide and smeared. Smears were air-dried and stained with Hemacolor® (Merck, Darmstadt, Germany). Percentages of spermatogenic cells and cytologic indices were determined on at least 200 spermatogenic and Sertoli cells as follows (Pintus et al. 2014): the Sertoli cell index (SEI) was the number of Sertoli cells per 100 spermatogenic cells as an index of spermatogenic activity; spermatic index (SI) was the number of spermatozoa per 100 spermatogenic cells as an index of spermiogenic activity; spermatozoa-Sertoli index (SSEI) was the number of spermatozoa per Sertoli cell; Sptd ab:sptc I was the number of round spermatids per primary spermatocyte as an index of meiotic activity; and number of

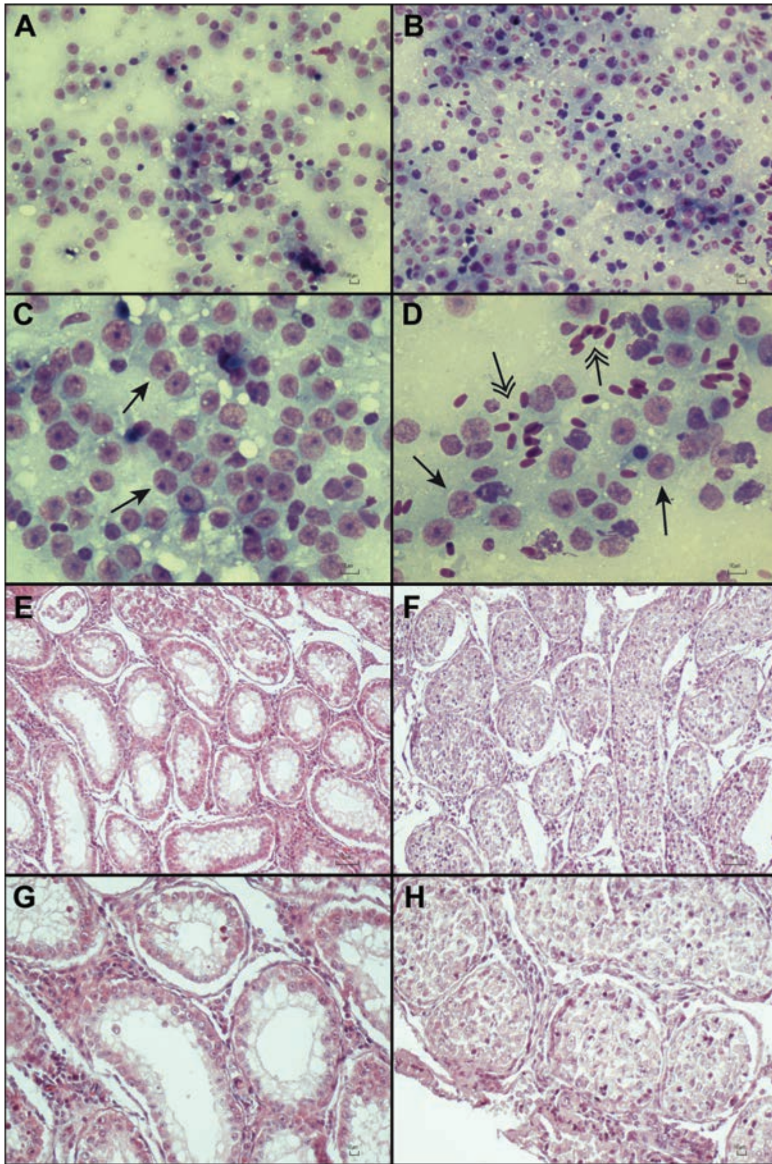

FIGURE 1. Testicular cytology and histology from Iberian red deer (*Cervus elaphus hispanicus*) affected by spermatogenic disorders. Aspermatogenesis and Sertoli cell-only pattern were found in the left testis (left column); hypospermatogenesis was found in the right testis (right column). (A) Cytologic feature of aspermatogenesis. Note the low cellularity represented by the Sertoli cell-only pattern. Bar = 10 μm. (B) Cytologic feature of hypospermatogenesis showing moderate cellularity represented both by germ cells and Sertoli cells. Bar = 10 μm. (C) Sertoli cell-only pattern at higher magnification. Sertoli cells (arrows) usually appear as naked nuclei with finely granular chromatin and a single, clearly visible nucleolus. Bar = 10 μm. (D) Germ cells (double arrows) and Sertoli cells (single arrows) at higher magnification. Bar = 10 μm. (E) Histologic feature of Sertoli cell-only tubules (H&E). Interstitial tissue appeared normal and no signs of inflammatory infiltrate or fibrosis were detected. Bar = 50 μm. (F) Histologic feature of hypospermatogenesis (H&E). Bar = 50 μm. (G) Histologic feature of Sertoli cell-only at higher magnification (H&E). Bar = 10 μm. (H) Histologic feature of hypospermatogenesis at higher magnification (H&E). Bar = 10 μm.

TABLE 1. Sperm parameters of an Iberian red deer (*Cervus elaphus hispanicus*) affected by testicular disorders and control values from Iberian red deer ($n=6$) with normal spermatogenesis culled on the same date in Spain.

Parameter[a]	Iberian red deer with hypospermatogenesis	Iberian red deer with normal spermatogenesis[b]	
		Mean±SD	Range
Sperm concentration ($\times 10^6$/mL)[c]	656	1,872±923	766–3,144
Total sperm number ($\times 10^6$)	261	1,052±673	292–2,101
Viability (%)[d]	83.05	55.80±8.55	44.51–67.27
Total motility (%)[e]	99.62	78.50±23.92	34.82–99.92
Progressive motility (%)	12.73	10.76±5.04	1.02–14.79
VAP (μm/sec)	86.11	46.25±25.57	9.88–81.32
VSL (μm/sec)	47.83	25.07±12.78	5.77–43.20
VCL (μm/sec)	138.30	63.34±32.53	19.80–113.04
LIN (%)	34.15	37.06±4.75	28.62–41.37
STR (%)	54.99	56.40±3.02	52.30–60.48
WOB (%)	61.16	65.61±8.85	50.10–74.65
ALH (μm)	5.60	2.48±0.98	1.29–4.15
BCF (Hz)	6.71	4.97±1.58	2.39–7.13

[a] VAP = average path velocity; VSL = straight linear velocity; VCL = curvilinear velocity; LIN = linearity; STR = straightness; WOB = wobble (VAP/VCL); ALH = lateral head displacement; BCF = beat cross frequency.

[b] Values refer to the content of both epididymides.

[c] Sperm concentration was evaluated using a Makler chamber.

[d] Sperm viability was evaluated by flow cytometry (propidium iodide/YO-PRO®-1, Invitrogen, Barcelona, Spain).

[e] Sperm motility was assessed by Sperm Class Analyzer (SCA, Microptic S.L., Barcelona, Spain).

germ cells per Sertoli cell (GC:SC) was assessed as an index of Sertoli cell workload. Histologic samples were analyzed according to the Johnsen (1970) score.

Findings for testicular cytology and histology are illustrated in Figure 1. Analysis of the left testis revealed low cellularity represented by a Sertoli cell-only pattern (SEI: 100; Fig. 1A). Sertoli cells exhibited normal morphology (Fig. 1C). Data were confirmed by histology showing that Sertoli cells were the only cells per tubular cross-section (Johnsen score: 2.02; Fig. 1E, G). No sperm sample was recovered from the epididymal cauda.

Smears from the right testis showed both spermatogenic and Sertoli cells (Fig. 1B, D). Percentages of spermatogenic cells were 3.27% spermatogonia, 20.91% primary spermatocytes, 15.03% round spermatids, and 57.52% elongated spermatids. Secondary spermatocytes were not observed due to their short lifespan (Leme and Papa 2010). The SI and SSEI were much lower (3.27 and 0.09, respectively) than normal values (mean±SD, SI: 16.13±5.49; SSEI: 1.67±0.79; $n=6$). Conversely, SEI was higher than normal values (35.29 vs. 11.74±5.90) whereas Sptd ab:sptc I and GC:SC ratios were low (0.72 vs. 2.21±0.48 and 2.83 vs. 6.94±3.37, respectively). Although histologic sections were not optimal due to the delay of testes fixation, tubules showed no lumen, with few spermatozoa or only spermatids (Fig. 1F, H). Both cytologic and histologic data from the right testis were indicative of hypospermatogenesis. However, the Johnsen score was similar to the normal value of the same period (7.78 vs. 7.69±0.26; $n=4$). Sperm samples were recovered from the epididymal cauda. Although sperm concentration of the abnormal testis was low, sperm viability and motility were within the range or even higher than normal values (Table 1).

Possible etiologies of hypogonadism include an inflammatory process, vascular obstruction, heat stress, toxicants, nutritional deficiency, poisonous plants, and congenital aplasia, among others (Carrasco et al. 1997; Tiller et al. 1997). We discarded

an infectious or inflammatory process due to the lack of inflammatory infiltrate or connective tissue replacement. Poisonous plants may induce testicular hypogonadism in deer with lesions that are usually bilateral and affect several individuals within the same population (Taylor et al. 1964). Differentiation of testicular atrophy from congenital aplasia is extremely difficult in the absence of data regarding antler history (Carrasco et al. 1997), as in our case. Ischemia determines testicular atrophy with Sertoli cell-only pattern (Young et al. 1988) and affects spermatogenesis even in the contralateral testis (Sukhotnik et al. 2005). Thus, hypogonadism might be a consequence of a traumatic or vascular obstruction which is also affecting the contralateral testis. Although sperm quality was within or above the normal values, sperm production was low as a consequence of the reduced spermatogenic efficiency. Low efficiency of spermatogenesis together with high Sertoli cells:germ cells ratio characterize testicular degeneration (Papa and Leme 2002). However, a major limitation of this case report is the lack of follicle stimulating hormone, testosterone, and inhibin B blood levels in order to confirm our diagnosis. In conclusion, FNAC is an important diagnostic tool in testicular diseases of wildlife.

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