

Age-related ultrasonography, cytology, and microbiologic exam of canine prostate

[*Ultrassonografia, citologia e exame microbiológico da próstata de cães em diferentes idades*]

C.R.F. Gadelha¹, W.R.R. Vicente¹, A.P.C. Ribeiro¹, M.F. Apparício¹, G.J. Covizzi¹, L.D.S. Machado²

¹Faculdade de Ciências Agrárias e Veterinárias - UNESP
Via de Acesso Professor Paulo Donato Castellane s/n
14884-900 – Jaboticabal, SP

²Faculdade de Veterinária - UECE – Fortaleza, CE

ABSTRACT

The physiological parameters that could be reference for trustful diagnosis and prognosis of prostate disorders in dogs were obtained. Thirty six intact male dogs without clinical signs of neither prostatic nor reproductive disorders were allocated according the age in three groups. These animals were submitted to semen manual collection for microbiological exams; transabdominal ultrasonography to evaluate dimensions, ecogenicity, and texture of prostatic parenchyma and aspirative puncture with fine needle for cytological and microbiological analyses. Ultrasonography revealed that the predominant prostatic shape was round with regular surface. Dimensions varied according to age, being small in young animals and large in old ones. There was a positive correlation between prostatic dimensions and body weight. Microbiological exams detected microorganisms on seminal plasma from 11 dogs and prostate tissue aspirated from 10 animals, although they were healthy. Cytology did not reveal any inflammatory, proliferative, or neoplastic alteration in young and middle age dogs, but in three older dogs signs of hyperplasia/hypertrophy was found. It was observed positive correlation between age and cellular area but a negative correlation was observed between nucleus:cytoplasm ratio and craniocaudal dimension.

Keywords: dog, cytology, microbiology, prostate, ultrasonography

RESUMO

Obtiveram-se parâmetros fisiológicos que pudessem ser utilizados como referência para diagnóstico e prognóstico confiáveis de doença prostática em cães. Trinta e seis cães, sem sinais clínicos de doença prostática ou distúrbios reprodutivos, foram distribuídos em três grupos de acordo com a idade. Os animais foram submetidos à colheita manual de sêmen para exames microbiológicos, à ultrassonografia transabdominal, para avaliar as dimensões, a ecogenicidade e a ecotextura prostática, e à punção aspirativa com agulha fina, para análise citológica e microbiológica. A ultrassonografia revelou que a forma predominante da próstata foi globosa, com superfície de contorno regular. As dimensões variaram de acordo com a idade, sendo pequena em animais jovens e grande nos animais idosos. Houve correlação positiva entre as dimensões prostáticas e o peso corporal. Os exames microbiológicos detectaram microrganismos no plasma seminal de 11 cães e no tecido prostático aspirado de 10 animais, embora eles fossem saudáveis. A citologia não revelou nenhuma alteração inflamatória, proliferativa ou neoplásica nos cães jovens e de meia idade, mas, em três cães idosos foram encontrados sinais de hiperplasia/hipertrofia. Foi observada correlação positiva entre a idade e a área celular e correlação negativa entre a relação núcleo:citoplasma e a dimensão craniocaudal.

Palavras-chave: cão, citologia, microbiologia, próstata, ultrassonografia

INTRODUCTION

Prostate gland is present in all mammals, but it is more important in men and dogs due to frequency of diseases. The canine prostate gland is one of the best experimental model to study prostate, because dog is the only domestic animal that spontaneously develops benign prostatic hyperplasia (BPH) and adenocarcinoma, like men (Lowseth et al., 1990; Waters and Bostiwick, 1997a; Waters et al., 1997). In puberty, prostate gland is pelvic and stays there until adult age. In older dogs, the position can be more cranial due to a progressive increase of the gland. The dimensions can be correlated with body size and weight (Feeney et al., 1987; Ruel et al., 1998).

Disorders of prostate are common in old and intact dogs, specially BPH, bacterial prostatitis, cysts, abscess, squamous metaplasia, and neoplasia (Barsanti and Finco, 1992; Krawiec and Heflin, 1992; Krawiec, 1994). These alterations can occur simultaneously and clinical signs are similar difficulting definitive diagnosis. The most frequent disorder in dogs is BPH. It is correlated to age and has an endocrine cause. Prostate infection usually results from ascending bacteria from urethra, but blood contamination or spread infection from bladder and semen are possible (Ling et al., 1990; Johnston et al., 2000). Old dogs are more predisposed to infection because the decreasing of prostatic antibacterial factor after four years old. Prostate neoplasia incidence in dogs is low, but it is aggressive and highly invasive, leading to metastasis. The late diagnosis can be responsible for poor prognosis associated to prostate cancer (Waters and Bostiwick, 1997b).

The prostate evaluation must be firstly made by the history, clinical signs, and rectal palpation, but ultrasonography is an important diagnosis method that allows to characterize size, contour, parenchyma, and fluid structures in the gland (Cartee and Rowles, 1983; Di Santis et al., 2001; Sampaio and Araújo, 2002). Another important exam is the cytology by fine needle aspirative puncture to identify alterations in number, kinds, and morphology of cells and their elements, allowing evaluate inflammatory and proliferative process (Rogers et al., 1986; Kay et al., 1989; Guedes et al., 2000; Ferian et al., 2006). Prostatic fluid, obtained by spontaneous ejaculation and prostate tissue, is important to evaluate infection signs

The objective of this study was to evaluate the prostate of healthy dogs at different ages by ultrasonography, cytology, and microbiologic exams in order to obtain physiologic parameters that could help to establish definitive and trustful diagnosis and prognosis of prostate disease.

MATERIAL AND METHODS

Thirty-six adult, intact, mixed breed, healthy male dogs from castration program of the Veterinary Hospital, Faculdade de Ciências Agrárias e Veterinárias at the Universidade Estadual Paulista, Campus de Jaboticabal were studied after being submitted to clinical examination. The dogs were allocated in three groups (G), with 12 animals in each, according to the age: GI – dogs from one to three-year-old, GII – dogs from four to six-year-old, and GIII – dogs older than seven-year-old.

The semen was collected as previously described (Seager and Platz, 1977) in a sterile funnel and collector tube. Only the third semen fraction was collected and send to bacterial culture. To perform the ultrasonography and fine needle aspirative puncture, the dogs were anesthetized with levomepromazine (1mg/kg IV) as pre-medication and epidural anesthesia was performed with lidocaine 2% (3mg/kg) associated with bupivacaine 0.75% (1mg/kg) in a total volume of 0.3mL/kg of body weight and not exceeding 5mL/animal.

The ultrasonography was made with setorial 5.0 and 7.5 MHz probe on pre-pubic region, trying to evidence the craniocaudal and ventrodorsal dimensions and characterize gland ecogenicity plus parenchyma structure. The aspirative puncture was associated to ultrasonography after antiseptic proceedings. The probe was positioned close to the penis and a sterile fine needle (Spinal 22Gx3.5) was inserted by transabdominal via. After achieving the correct area of prostatic tissue, 6-8mL suction were applied by a syringe for three or four times. The aspirated material was expelled onto a glass microscope slide and a smear technique was used to spread the material. After that, the slides were submitted to Rosenfeld stain (Romanowsky type stain) and the cytological examination was made on a light optic microscope with 100x (oil immersion) objective as well as morphometric evaluation. The images were evaluated by computational

program (Image Pro plus 3.0) after they had been caught by a light optic microscope (Olympus Bx50) connected to a digital camera (CCD Iris Sony). It was verified the cellular area, the nuclear area, and the nucleus:cytoplasm ratio in morphometric evaluation. Another portion of the aspirated material stayed at needle to proceed the bacterial culture. Any bacterial growth in the microbiological exam would indicate a positive sample.

The statistical analyses were carried out using Pearson correlation method, variance analysis, and Tukey test to the parametric variables (body weight, prostatic dimensions, cellular area, nuclear area, and nucleus:cytoplasm ratio). To non-parametric variables (prostate localization and size by rectal palpation, gland ecogenicity, and bacterial growth), the frequency distribution was used and the Fisher test was applied.

RESULTS

By rectal palpation, it could be firstly evaluated localization, size, consistence, symmetry, mobility, and prostate sensibility to most of the dogs. Three older dogs presented abdominal prostate localization and rectal palpation became difficult. No one presented any surface alteration at physical examination. Prostate localization and size varied according to the age. The pelvic

localization of the prostate (66%) and a little gland (83%) were mainly observed in younger dogs, while half of dogs from GIII presented abdominal localization of the prostate and a big gland. These data were confirmed by the ultrasonographic exam.

The prostate shape, size, contour, and integrity were efficiently determined by ultrasonography. The canine prostate was predominantly round with a regular surface and presented homogenous parenchyma texture, characterized by hyperechoic areas within lower ecogenicity areas (transonic regions) in all animals of GI (Fig. 1) and GII, but little anechoic sites were observed in only one dog of GIII, suggesting micro cysts (Fig. 2).

Craniocaudal and ventrodorsal dimensions were recorded and indicated that GII and GIII animals had a similar prostate size, but it was statistically higher than that from GI dogs (Table 1). There was a positive correlation between prostate dimensions and animal age, suggesting that the prostate size increase with the age. There was also a positive correlation between body weight and prostate dimensions. However, there was not statistical difference among body weight of dogs from the three distinct groups, suggesting that animal body weight did not interfere in prostate dimensions in this study.

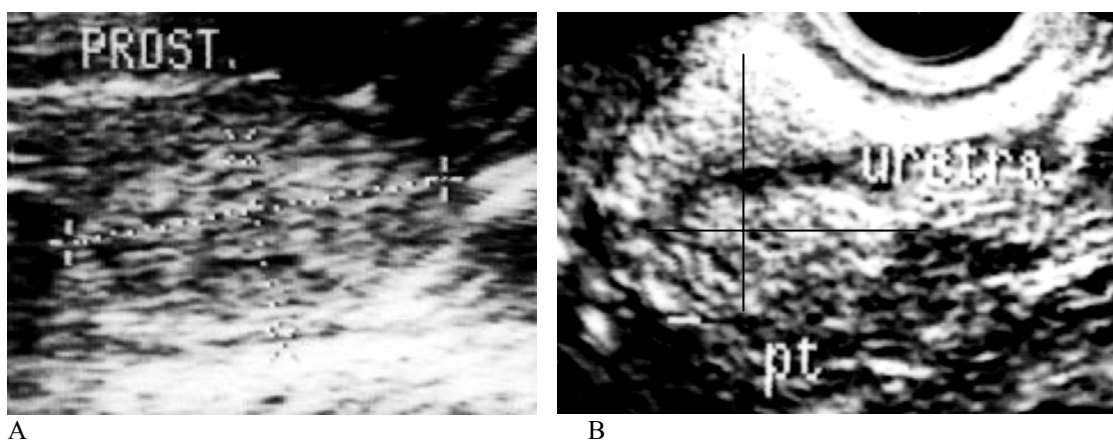


Figure 1. Real time ultrasonographic image of canine prostate, transabdominal via, sagittal plane, in two to three-year-old dogs. Images captured by setorial probe, 5 MHz (B) and 7.5 MHz (A) of frequency. Observe elliptic (A) and globe shape (B), regular surface, and homogenous ecotexture (A and B). The urethra is visible in B.

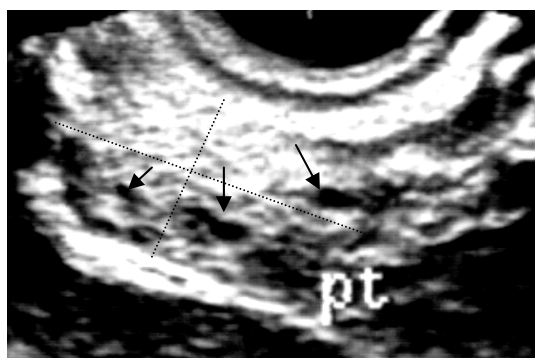


Figure 2. Real time ultrasonographic image from canine prostate (pt), sagittal plane of an older seven-year dog. Image captured by setorial probe, 5 MHz of frequency. Observe micro cysts in prostatic parenchyma (narrowes).

Table 1. Averages and standard deviations of ventrodorsal (VD) and craniocaudal (CC) dimensions of prostate (cm) according the age group

Group	Prostate dimensions	
	VD (cm)	CC (cm)
I	2.16±0.8853b	2.25±0.8876b
II	3.00±0.7780a	3.22±1.0186a
III	3.00±0.5477a	3.84±0.8969a

Group I: dogs were one to three-year-old; group II: dogs were four to six-year-old; group III: dogs were seven-year old or more. Average followed by different letters in columns indicate statistical difference by Tukey test (P<0.05).

At cytological exam, it was not observed proliferative, inflammatory, and neoplastic alteration in dogs from GI (one to three-year-old) and GII (four to six-year-old). Few hemacias, neutrophils, and rare lymphocytes and eosinophils were found, but many prostate epithelial cells, characterized by cuboidal shape, granular and basophilic cytoplasm; and peripheric, round, and big nucleus, presenting reticular chromatin pattern were observed. In slides from GIII dogs, epithelial cells blocks

Table 2. Averages and standard deviations of cellular area, nuclear area (µm), and nucleus:cytoplasm ratio (%) from morphometric evaluation according to the age group

Groups	Cellular área (µm)	Nuclear area (µm)	Nucleus:cytoplasm ratio (%)
GI	286.56±66.53	79.27±9.56	28.40±3.99
GII	329.09±40.12	87.14±4.39	26.85±3.72
GIII	327.17±54.21	82.89±11.84	25.51±2.58

Averages were not different by Tukey test (P≤0.05).

large than those from GI and GII were observed, but with the same characteristics, except for three samples presented signs of benign prostatic hyperplasia (BPH) like higher cellular blocks, augmented nucleus:cytoplasm ratio, and more basophilic cytoplasm, sometimes with vacuolization (Fig. 3). Two of these dogs presented prostate dimensions that could indicate BPH, but no one had clinical signs.

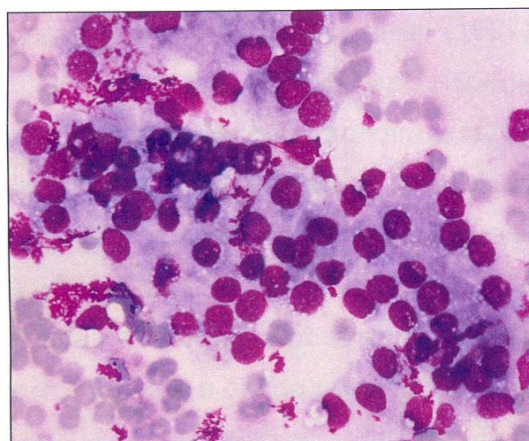


Figure 3. Photomicrography of prostatic cells obtained by fine needle aspirative puncture (Rosenfeld stain, x 100). Observe normal prostatic cells of an eight-year-old dog with round nucleus and granular cytoplasm.

The morphometric evaluation allows measure the cellular area, nuclear area, and nucleus:cytoplasm ratio (Table 2). There was not statistical difference among groups to these parameters, but it was a observed positive correlation between age and cellular area and between cellular area and nuclear area. It was found a negative correlation between craniocaudal dimension and nucleus:cytoplasm ratio as well as cellular area and nucleus:cytoplasm ratio for all groups of animals.

Microorganisms were present in seminal plasma of 11 animals, two (18%) from GI, three (27%) from GII, and six (55%) from GIII. In spite of GIII had more than half part of positive culture, there was not statistical difference among the groups ($P < 0.05$). Ten samples from aspirated material were positive to bacterial culture, three (30%) from GI, four (40%) from GII, and three (30%) from GIII dogs. There was not statistical difference among the groups either ($P < 0.05$). Five dogs presented microorganisms in both cultures.

The microorganisms isolated from seminal plasma were *Escherichia coli* (54.5%), *Staphylococcus aureus* spp. (27.3%), *Klebsiella* spp. (27.3%) and *Proteus* spp. (9%). The same agents were identified in aspirated samples but in different proportions: *Staphylococcus aureus* spp. (40%), *Proteus* spp. (30%), *Escherichia coli* (20%) and *Klebsiella* spp. (10%). No one animal presented clinical signs of infection neither the cytological exam indicated that.

DISCUSSION

Palpation per rectum allowed obtaining initial information about prostate condition and it was a valuable method to verify some kind of gland alterations. Localization and size indicate that, as the dogs became older, the prostate increased and moved from pelvic to abdominal cavity location.

The ultrasonographic exam was essential to characterize the morphology of canine prostate and detect probable cavity lesions. The round form was previous related (Cartee and Rowles, 1983; Cooney et al., 1992; Bussadori, 1993). Prostate homogeneity texture was reported too (Bussadori, 1993; Matoon and Nyland, 1995), but it is still a controversy. Muzzi (1999) verified an increased diffuse parenchyma ecogenicity in altered prostate; but, in this study, it was not observed any alteration in ecogenic pattern even in cases of increased prostate or micro cysts.

Prostates dimensions were nearly those previously related (Ruel et al., 1998; Di Santis et al., 2001) in dogs at same ages. According to this study, the prostate growth is higher until six years old and after that, the growth is lower, since gland alterations did not occur.

In this study, it was verified that it was easier to

obtain cells from older dogs prostates than from younger ones. It is possible that in older dogs, intrinsic gland alterations became prostate cells more free than those from intact or healthy glands (Zinkl, 1999). The cellular characteristics observed in this study are in agreement with previous reports (Thrall et al., 1985; Muzzi, 1999).

The morphometric evaluation allows obtaining more data about prostate cells dimensions and nucleus:cytoplasm ratio in different canine ages which facilitate subsequent studies. The negative correlation found between craniocaudal dimension and nucleus:cytoplasm ratio indicate that when cell grows this ratio decreases. However, alterations were observed in prostate: cytological signs of BPH and an increase in nucleus:cytoplasm ratio in this study. It suggests that prostate growth is a physiological event in dogs life without cells alterations. But, when BPH occurs, the growth is associated with cells alterations. Although any statistical difference among groups to cellular and nucleus area had been observed, an increase of these dimensions according to age was verified, especially until six-year-old. After that period, the dimensions were almost equal among the dogs. This agrees with what was related before, that the prostate growth is physiologically higher until six years old in dogs.

The major part of positive bacterial cultures of seminal plasma were from older dogs (GIII) and these data have clinical importance, because they can indicate that natural defenses against bacteria were decreased, making them more susceptible to infections (Arantes and Ferreira, 2002). It is possible that ascending dissemination of bacteria from urethra to prostate had occurred, because it was verified the presence of bacteria in prostate tissue. However, any dog present neither clinical signs of prostate infection nor infection signs by cytological exams. This suggests that pathogenic bacteria can be present in prostate of healthy dogs without causing infection. The positive animals stayed under clinical observation for six months and were kept healthy.

The microorganisms isolated from seminal plasma and prostate tissue are implicated in prostate infection and can be found in urethra and prepuce (Siqueira et al., 2008). Only bacterial culture from seminal plasma was not a

good indicator to prostate bacteriological evaluation, but associated with culture and cytology of aspirated prostate tissue became more trustful to diagnose the infection.

The association of various parameters from canine prostate evaluation, performed in this study, allowed knowing better the gland condition and this can propitiate more efficient treatment and prognosis of prostate disease.

REFERENCES

- ARANTES, L.V.; FERREIRA, A.M.R. Avaliação histopatológica das principais afecções prostáticas dos cães. *Rev. Bras. Med. Vet.*, v.24, p.171-175, 2002.
- BARSANTI, J.A.; FINCO, D.R. Moléstias prostáticas do cão. In: ETTINGER, S.J. *Tratado de medicina interna veterinária: moléstias do cão e gato*. 3.ed. São Paulo: Manole, 1992. p.1941-1943.
- BUSSADORI, C. Ecografia della prostata nel cane. *Paxis Vet.*, v.14, p.16-18, 1993.
- CARTEE, R.E.; ROWLES, T. Transabdominal sonographic evaluation of the canine prostate. *Vet. Radiol.*, v.24, p.156-164, 1983.
- COONEY, J.C.; CARTEE, R.E.; GRAY, B.W. et al. Ultrasonography of the canine prostate with histologic correlation. *Theriogenology*, v.38, p.877-895, 1992.
- DI SANTIS, G.W.; AMORIM, R.L.; BANDARRA, E.P. Aspectos clínicos e morfológicos das alterações prostáticas em cães – revisão. *Rev. Educ. Cont. CRMV-SP*, v.4, p.46-52, 2001.
- FEENEY, D.A.; JOHNSTON, G.R.; KLAUSNER, J.S. et al. Canine prostatic disease – comparison of radiographic appearance with morphologic and microbiologic findings: 30 cases (1981-1985). *J. Am. Vet. Med. Assoc.*, v.190, p.1018-1026, 1987.
- FERIAN, P.E.; SILVA, E.F.; GUEDES, R.C. et al. Diagnóstico citológico de neoplasia pulmonar por meio do lavado broncoalveolar em uma cadela: relato de caso. *Arq. Bras. Med. Vet. Zootec.*, v.58, p.776-780, 2006.
- GUEDES, R.M.C.; ZICA, K.G.B.; COELHO-GUEDES, M.I.M. et al. Acurácia do exame citológico no diagnóstico de processos inflamatórios e proliferativos dos animais domésticos. *Arq. Bras. Med. Vet. Zootec.*, v.52, p.437-439, 2000.
- JOHNSTON, S.D.; KAMOLPATANA, K.; ROOT-KUSTRITZ, M.V. et al. Prostatic disorders in the dog. *Anim. Reprod. Sci.*, v.60, p.405-415, 2000.
- KAY, N.D.; LING, G.V.; NYLAND, T.G. et al. Cytological diagnosis of canine prostatic disease using a urethral brush technique. *J. Am. Anim. Hosp. Assoc.*, v.25, p.517-526, 1989.
- KRAWIEC, D.R. Canine prostate disease. *J. Am. Vet. Med. Assoc.*, v.204, p.1561-1564, 1994.
- KRAWIEC, D.R.; HEFLIN, D. Study of prostatic disease in dogs: 117 cases (1981). *J. Vet. Med. Assoc.*, v.200, p.1119-1122, 1992.
- LING, G.V.; NYLAND, T.G.; KENNEDY, P.C. et al. Comparison of two sample collection methods for quantitative bacteriologic culture of canine prostatic fluid. *J. Am. Vet. Med. Assoc.*, v.196, p.1479-1482, 1990.
- LOWSETH, L.A.; GERLACH, R.F.; GILLETTN, A. et al. Age related changes in the prostate and testes of the beagle dogs. *Vet. Pathol.*, v.27, p.347-353, 1990.
- MATTON, J.S.; NYLAND, T.G. Ultrasonography of the genital system. In: NYLAND, T.G.; MATOON, J.S. (Ed.). *Veterinary Diagnostic Ultrasound*. Philadelphia: WB Saunders, 1995. p.141-163.
- MUZZI, L.A. Ultrassonografia e citologia das afecções prostáticas em cães. *Arq. Bras. Med. Vet. Zootec.*, v.51, p.9-16, 1999.
- ROGERS, K.S.; WANTSCHKE, L.; LEES, G.E. Diagnostic evaluation of the canine prostate. *Cont. Educ. Comp. Small Anim.*, v.8, p.799-811, 1986.
- RUEL, Y.; BARTHEZ, P.Y.; MAILLES, A. et al. Ultrasonographic evaluation of the prostate in healthy intact dogs. *Vet. Radiol. Ultrasound*, v.39, p.212-216, 1998.
- SAMPAIO, K.M.O.R.; ARAUJO, R.B. Ultrassonografia de características lineares e estimativas do volume de rins de cães. *Arq. Bras. Med. Vet. Zootec.*, v.54, p.248-252, 2002.
- SEAGER, S.W.J.; PLATZ, C.C. Collection and evaluation of the canine semen. *Vet. Clin. N.*

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Am.: Small Anim. Pract., v.7, p.765-773, 1977.

SIQUEIRA, A.K.; RIBEIRO, M.G.; SALERNO, T. et al. Perfil de sensibilidade e multirresistência em linhagens de *Escherichia coli* isoladas de infecção do trato urinário, de piometra e de fezes de cães. *Arq. Bras. Med. Vet. Zootec.*, v.60, p.1263-1266, 2008.

THRALL, M.A.; OLSON, P.N.; FREEMAYER, F.G. Cytology diagnosis of canine prostatic disease. *J. Am. Anim. Hosp. Assoc.*, v.12, p.95-102, 1985.

WATERS, D.J.; BOSTIWICK, D.G. Prostatic intraepithelial neoplasia occurs spontaneously in the canine prostate. *J. Urol.*, v.157, p.713-716, 1997a.

WATERS, D.J.; BOSTIWICK, D.G. The canine prostate is a spontaneous model of intraepithelial neoplasia and prostate cancer progression. *Anticancer Res.*, v.17, p.1467-1470, 1997b.

WATERS, D.J.; HAYDEN, D.W.; BELL, F.W. et al. Prostatic intraepithelial neoplasia in dogs with spontaneous cancer. *Prostate*, v.30, p.92-97, 1997.

ZINKL, J.G. Cytology of the male reproductive tract. In: *Diagnostic cytology and hematology of the dog and cat*. 2.ed. St. Louis: Mosby, 1999. p.230-234.