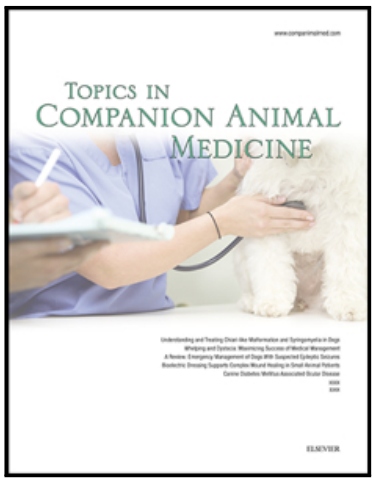

Advances in prostatic diagnostics in dogs: The role of Canine Prostatic Specific Esterase (CPSE) in the early diagnosis of prostatic disorders

Salvatore Alonge , Monica Melandri , Giulio Aiudi , Giovanni Michele Lacalandra

PII: S1938-9736(18)30087-4
DOI: https://doi.org/10.1053/j.tcam.2018.09.002
Reference: TCAM 329

To appear in: *Topics in Companion Animal Medicine*

Received date: 20 May 2018
Revised date: 2 September 2018
Accepted date: 4 September 2018

Please cite this article as: Salvatore Alonge , Monica Melandri , Giulio Aiudi , Giovanni Michele Lacalandra , Advances in prostatic diagnostics in dogs: The role of Canine Prostatic Specific Esterase (CPSE) in the early diagnosis of prostatic disorders, *Topics in Companion Animal Medicine* (2018), doi: https://doi.org/10.1053/j.tcam.2018.09.002

ADVANCES IN PROSTATIC DIAGNOSTICS IN DOGS:

THE ROLE OF CANINE PROSTATIC SPECIFIC ESTERASE (CPSE) IN THE EARLY DIAGNOSIS OF PROSTATIC DISORDERS

Role of CPSE in a preventive screening programme for prostatic health in dogs.

Salvatore ALONGE DVM, PhD[1,2]; Monica MELANDRI DVM[1];

Giulio AIUDI DVM, PhD[2]; Giovanni Michele LACALANDRA DVM, Professor[2]

[1]Società Veterinaria "Il Melograno" Srl, Sesto Calende, Varese, Italy;

[2]Dipartimento di Medicina Veterinaria (DiMeV), Section of Surgery and Obstetrics, University of Bari Aldo Moro, Italy.

e-mail: drsalvatorealonge@gmail.com; monica.melandri@email.it;

giulioguido.aiudi@uniba.it; giovannimichele.lacalandra@uniba.it

Corresponding Author:

Salvatore Alonge

Dipartimento di Medicina Veterinaria (DiMeV)

Sezione di Chirurgia e Ostetricia

University of Bari Aldo Moro, Italy.

Phone +39 392 8058524

e-mail: drsalvatorealonge@gmail.com

Author contributions

All authors contributed to design and draft the paper. All authors have approved the final version.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Highlights

- CPSE is a suitable biomarker to include in a preventive screening for prostate health program in dog.
- CPSE is the major androgen-dependent secretory product of canine prostatic gland.
- Canine CPSE was widely compared to human PSA.
- Higher CPSE was reported in dogs suffering from several prostatic diseases.
- CPSE is a very promising non-invasive tool to early diagnose prostatic diseases in dogs.

ABSTRACT

In the last years, following the increased canine life expectancy and the rising attention pet-owners devote to their animals, several authors have carried on investigations concerning new techniques to early identify canine prostatic disorders that might affect the dog's quality of life. Prostatic disorders often have an asymptomatic onset and their early diagnosis is difficult: hence, they are usually identified at an advanced stage, only. Traditionally, the diagnosis of prostatic disorders is based on non-invasive tools, such as transrectal and abdominal palpation, seminal or prostatic fluid

evaluation, urinalysis and imaging. On the other hand, a definite diagnosis of prostatic abnormalities could be achieved through prostatic parenchyma FNA or biopsy. However, these investigations are performed rarely because of their invasiveness. Thus, several authors investigated canine serum biomarkers in order to achieve an earlier diagnostic timing and to apply therapeutic strategies for better outcomes. The Canine Prostatic Specific Esterase (CPSE) has been identified as a suitable biomarker to be included in a prostate health screening programme, following the model of Prostatic Specific Antigen (PSA) in human medicine. A higher CPSE in dogs suffering from several prostatic diseases, such as benign prostatic hyperplasia (BPH), bacterial prostatitis or prostatic carcinoma, was reported in literature. Thanks to the potential usefulness in clinical practice, further studies should investigate the potential role of CPSE in monitoring the medical treatment success in the male reproductive system. Moreover, the spreading availability of serum biomarkers, easily carried out on blood samples in clinical practice, could assure a more accurate evaluation of the actual prevalence of prostatic disorders. The CPSE is actually recognized as a promising diagnostic tool for the detection of prostatic disorders in a "prostate health screening programme", in order to properly select those patients requiring further more accurate and expensive diagnostic investigations.

Running head: Role of CPSE in a preventive screening programme for prostatic health in dogs.

Keywords: BPH, CPSE, dog, prostatic disease, ultrasonography.

Introduction

Recently, several authors have carried on research activities concerning new techniques to early identify canine prostatic disorders that might also affect life quality. Such improvements have been allowed from the increased attention pet-owners devote to their animals, the increased canine life expectancy, the improved veterinary care and the most recent diagnostic possibilities [1-4].

The most important diseases of the gland include benign prostatic hyperplasia (BPH), prostatic cyst, prostatitis and prostatic neoplasia [5]. Canine prostatic disorders remain subclinical at their onset, thus it is difficult to diagnose them in early stages and they are mostly recognized at an advanced stage, only [2,6]. In clinical practice, the delayed diagnosis usually follows late clinical signs reported in combination with an enlarged gland though dogs might have an enlarged prostate in absence of symptoms [7].

When defining the stage of the disease at diagnosis, the dog's lifestyle plays an important role: in pet-dogs, prostatic disorders are often unnoticed at their beginning because they are asymptomatic or show only very mild clinical signs, such as the detection of blood drops in urine [6,8]. In stud dogs, these disorders are diagnosed more frequently as they are presented for poor fertility [9] or they undergo deeper male breeding soundness examinations after decreased libido or detection of blood in the prostatic fluid of the ejaculate [6,8].

In human medicine, diagnostic tools for the investigation of prostatic disorders recorded a great improvement, mainly through the serum dosage of the prostate specific antigen (PSA). As a result, the incidence of diagnosed prostatic diseases increased, thanks to the possibility of identifying subclinical asymptomatic cases [4]. The PSA is a proteolytic glycoprotein present in normal prostatic tissue in men while it increases with BPH, prostatitis and malignant neoplasia [10,11,12,13,14,15].

For the above mentioned reasons, several researchers began to look for canine serum biomarkers similar to those routinely used in human andrology, in order to achieve a similar diagnostic timing and to apply earlier therapeutic strategies for better outcomes [16,17,18,19]. A more accurate

evaluation of the prevalence of prostatic disorders could be achieved with a prostate health screening programme, easily performed on blood samples thanks to the availability of serum biomarkers [1]. The three most important markers in the canine male genital tract are represented by alkaline phosphatase, carnitine and canine prostatic specific esterase (CPSE) [20]. Alkaline phosphatase and carnitine are linked to epididymis and ductal network disorders [20]. Serum prostatic acid phosphatase and PSA are successfully used to diagnose prostatic carcinoma in men, while these markers are still controversial in dogs because acid phosphatase resulted nonspecific and PSA does not seem to increase in canine prostatic neoplasia [21,22], the latter being excluded from canine clinical practice [23]. Recently, some literature studies reported a higher CPSE in dogs suffering from several prostatic diseases: benign prostatic hyperplasia (BPH), bacterial prostatitis or prostatic carcinoma [3,18,24,25,26]. Since then, serum arginine esterase has routinely been used in the diagnosis of prostatic hyperplasia [19,27], though its role in different prostatic alterations has not yet been completely understood [25,28].

The objective of the present review was to give a complete clinical description of the diagnostic value of CPSE, describing in detail several aspects of the composition and of the biological role of this enzyme, in order to highlight the diagnostic usefulness of this marker and its possible perspectives for future research and clinical applications.

Discovering Canine Prostatic Specific Esterase

The seminal fluid of all mammalian species contains various proteinases, thus the presence of proteolytic activity in canine seminal plasma was expected [29]. In humans, PSA, seminal plasma acidic protease, neutral protease and plasminogen activator have deeply been investigated [30,31,32]. In dogs, instead, a markedly different arginine esterase activity was first described in 1956, when it was identified in great levels in canine prostatic fluids [33]. Since the '80s, Canine Prostatic Specific Esterase, i.e. the major androgen-dependent secretory product of the canine prostatic gland, was suggested as an useful diagnostic biomarker to identify dogs suffering from

prostatic diseases [16,22]. Arginine esterase is one of the most abundant proteins in the canine seminal plasma [34], accounting for more than 90% of seminal proteins in dogs [16,28], identified in similar quantities in all ejaculate fractions [35]. The CPSE is mainly present in the apical region of the canine prostatic secretory epithelial cells [36]. In polyacrylamide gel electrophoresis, CPSE has a molecular weight of 29 kDa. Under denaturant conditions, in the presence of mercaptoethanol and SDS gel, it undergoes the rupture of disulfide bridges linking its two constituting protein chains, originating two protein bands of 15 kDa and 14 kDa, respectively [16]. Different isoforms were identified by column chromatofocusing and two-dimensional electrophoresis, basing on their isoelectric points [37,38]. Their heterogeneity is probably due to a different glycoside content [36]. The CPSE was also associated to the class of kallikreins [39] as its aminoacid sequence has a 64% homology with swine pancreatic kallikrein and its molecular weight is similar to salivary glands kallikrein, though, contrary to them, it is not able to hydrolyse canine seminal plasma kininogen [40,41].

The CPSE was compared to PSA: their enzymatic activity turned out not to be completely superimposable because CPSE is a trypsin-like enzyme and PSA has also a chymotrypsin-like effect [42]. Though being two clearly distinct molecules [42], they have similar molecular weight (29 kDa for CPSE and 34kDa for PSA); they are both enzymes of the serine-proteases class and have a 58% homology in the 30^{th} -NH2 terminal portion of the aminoacid sequence [42]. Recently, a strong taxonomic and genomic correlation between PSA and CPSE was found, making the dog a very suitable model for further studies and comprehension of human prostatic disorders [43]. In men, the function of PSA is to hydrolyse the clot formed by seminal substances just after their emission; canine ejaculate does not form any clot, thus the CPSE activity turns out to be not clear though its role in the melting of the female genital tract mucus can be assumed [44].

Seminal plasma proteins can exert diverse effects on sperm functions [45,46]; among them, CPSE is a multifunctional protein thanks to its zinc-binding properties [38]. The enzyme was first detected in the post-acrosomal region and in the sperm tail of ejaculated spermatozoa, but it was not found in

147 epididymis spermatozoa [35]. The CPSE plays a relevant role in the binding of phosphorylcholine-
148 binding proteins to choline phospholipids of the sperm plasma membrane; at ejaculation, this
149 process induces a cholesterol efflux, an essential step for the capacitation process [47]. Thus, CPSE
150 can be counted among sperm-binding proteins and is possibly implicated in sperm fertilization-
151 related events [38].
152 The CPSE may also affect the tail of spermatozoa where it was identified by immunofluorescence
153 [40]. Further studies are needed to clarify the specific role of CPSE in the egg-sperm fertilization
154 process [48].
155 Along with prostatic pathologies, when the architecture of the gland is widely damaged,
156 independently from its grounding cause [49], CPSE is released by the prostate gland also in blood,
157 where it can be dosed on serum samples [25]. At the very beginning, CPSE was dosed by a
158 radioimmunoassay determination [50], while today new techniques are available in ELISA
159 immunoassay kits (Speed CPSE™, Virbac BVT, France), which are fast, easy and ready to use
160 afield in everyday clinical settings. Thus, CPSE has been identified as a suitable biomarker to be
161 included in a prostate health screening programme, following the model of PSA in human medicine
162 [1,25].
163
164 **Using Canine Prostatic Specific Esterase in canine clinics**
165 Traditionally, the diagnosis of prostatic disorders is based on non-invasive tools, such as the
166 transrectal and abdominal palpation, the seminal or prostatic fluid evaluation, urinalysis and
167 imaging. On the other hand, a definite diagnosis of prostatic disorders could be reached by prostatic
168 parenchyma FNA or biopsy, but they are rarely performed due to their invasiveness [23]. Moreover,
169 to identify any co-existence of BPH and prostatitis, a bacterial exam complete with antibiogram and
170 a cytological exam should be performed either on prostatic fluids or on fine-needle aspiration of the
171 prostatic parenchyma or on urine [51], even though a surely more invasive prostatic tissue culture
172 could represent a more accurate technique for the diagnosis of a prostatic infection [52,53].

Obviously, the clinical evaluation of a prostatic disease must include complete haemato-biochemical exams, with special attention to CPSE. In addition to the ultrasonographic exam, CPSE, marker for prostatic secretion, can widely be used to explore the canine prostate, though its exact role in the different prostatic disorders has not yet been completely understood [25,50]. Higher serum CPSE concentrations were reported in dogs affected by BPH, bacterial prostatitis and prostatic carcinoma than in normal dogs, but they did not differ significantly among specific pathologies [22]. Coexistence of prostatitis or neoplasia together with BPH may justify some overlapping in serum CPSE [22]. A different study reported a higher CPSE in BPH dogs than in dogs affected by bacterial prostatitis or prostatic carcinoma [54]. The CPSE was also dosed in urine samples: in healthy dogs, it ranged from 20 to 300 µg/ml, while it was possible to distinguish patients with acute (1000-2000 µg/ml) or necrotizing (5-10 µg/ml) prostatitis [24].

An ultrasonographic exam of the prostate is strongly recommended when a dog is presented for poor fertility [9] as well as when a presumptive diagnosis of BPH is based on the detection of blood in the prostatic fluid of the ejaculate or in case of a presumptive diagnosis of chronic prostatitis based on signs of infertility or decreased libido [6,8]. A recent retrospective work, involving 1003 intact male dogs, assessed that over 40% of the expected life of the dog, calculated according to its breed, an ultrasonographic screening programme would be advisable as it is highly probable that abnormal prostatic findings are detected with the sonographic exam, irrespectively of clinical evidence [2]. The ultrasonographic examination should evaluate an altered aspect of echotexture, abnormal borders as well as the presence of single or multiple cysts [55]. Several authors evaluated the echographic volume of the prostate as a possible indicator for BPH. On the other hand, the increased prostatic volume is not enough to surely diagnose BPH, because it is present also in case of prostatitis and neoplasia [5]. However, a clear threshold measure to identify the normal volume of the canine prostate does not exist. A tool that has been developed to make objective prostatic volume considerations is represented by the ratio between the actual volume of the prostate (V=H*L*W*0.523) [56] and the estimated normal one (V=0.33*BW+3.28) [57]. Recent studies

correlated the echographic findings of the prostate, focusing the attention on the actual/normal estimated volume ratio (V-ratio), and the serum CPSE, either symptomatic or asymptomatic. In 2017, the diagnostic potential of CPSE was exploited in a prospective research enrolling seventy-nine intact male dogs. The authors of the study assessed that in symptomatic patients, a V-ratio ≥ 2.5 and a serum CPSE ≥ 90 mg/ml have to be expected [19]. In 2018, the role of CPSE in a wider concept of preventive screening for canine prostatic health was defined, establishing the CPSE threshold to early identify asymptomatic dogs already affected by ultrasonographically detectable signs of prostatic alterations that could require further clinical investigations [1]. Among the nineteen patients enrolled in this prospective study – though they were all asymptomatic - 60% showed ultrasonographic prostatic alterations. The concentration of CPSE was significantly associated with the presence of ultrasonographic prostatic abnormal findings. By a ROC curve (AUC=0.974, SE 95.6%, SP 89.2%), the cut-off CPSE threshold of 52.3 ng/ml was endorsed to early identify those asymptomatic dogs already having sonographic alterations and thus requiring further evaluations, before becoming clinically ill. In the study, normal dogs had a V ratio < 1.5, while dogs requiring further evaluations resulted to have a V ratio ≥ 1.5 [1].

Finally, it was recently proved that ejaculation induces a spread in the prostatic vascularization [58,59]. Thus the increased blood flow turns a greater CPSE amount away from the gland, resulting in higher serum CPSE, as recently reported by the authors in the 2018 European Veterinary Society for Small Animal Reproduction (EVSSAR) Congress [60]. The higher basal CPSE is, the higher is the peak, they both being more pronounced in dogs with prostatic alterations. Twenty-four hours after ejaculation, serum CPSE achieves basal values again. However, the relationship between basal and post-ejaculation CPSE was the same in healthy dogs, in animals suspected for prostatic disorders as well as in dogs diagnosed with glandular diseases. These physiological effects of ejaculation should be taken into account whenever CPSE dosage is planned to preventively select patients needing further prostatic evaluations. A 24 hours' proper sexual rest is recommended before the examination to exploit this diagnostic tool at best [60].

Canine Prostatic Specific Esterase perspectives

The CPSE prostatic screening suggested for clinical practice aims at identifying subclinical under-diagnosed prostatic diseases, affecting male fertility, life quality and canine breeding performances. A non-invasive screening of the prostatic health would be advisable as part of the routine preventive medicine programme in dogs. The early detection of the most common prostatic disorders would allow the clinician to suggest specific follow-up and to recommend efficient therapeutic protocols [2]. Thus, because of the potential usefulness of this tool in clinical practice, further studies should investigate the suitable role of CPSE in monitoring the medical treatment success in the male reproductive system. The secretion of CPSE is regulated by androgen hormones, mainly represented by testosterone: thus, it could be inhibited by surgical or medical castration or by anti-androgen treatments [16,51,61,62,63]. On the contrary, CPSE synthesis could be promoted by an exogenous androgen administration following surgical castration [61,62]. Finally, it has recently been demonstrated that in dogs with subclinical BPH, serum CPSE dosages significantly decrease under osaterone treatment [27]. The debate on the management and therapy of canine prostatic abnormalities is still in progress [3,5,64,65,66], but the current availability of medical treatments prompts the development of a screening programme, aiming at avoiding potentially more invasive options, i.e. surgical orchiectomy, to prevent the adverse effects of surgical castration [67] and/or to preserve the reproductive career of a stud.

Conclusions

Evidently, though having a different biological activity (CPSE: trypsin-like; PSA: chymotrypsin-like), the canine CPSE and the human PSA follow the same hormonal regulation [41,42]. This is the reason why CPSE is recognised as a promising diagnostic tool for the detection of prostatic disorders in a “prostate health screening programme”, as it is the case for PSA in human medicine [25]. In Table 1, clinical applications of blood serum CPSE are summarised. In clinical practice, CPSE can be used as a diagnostic tool for

prostatic disorders to recognise affected dogs, with a cut-off of CPSE > 90 ng/ml, in the presence of clinical symptoms and a V-ratio ≥ 2.5 [19]. Moreover, CPSE has to be considered in planning preventive screening for prostatic disorders to preventively select dogs that would require further evaluations, with a CPSE threshold of 50 ng/ml, in absence of symptoms but already showing ultrasonographic alterations and a V-ratio ≥ 1.5 [1]. Finally, the serum CPSE would become a suitable biomarker to monitor the medical treatment of prostatic disorders thanks to its non-invasiveness, cheapness, rapidity and practicality.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. Laura Colombi, certified translator for English language, for the linguistic support.

CONFLICT OF INTEREST

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper content.

FORMATTING OF FUNDING SOURCES

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1] Alonge S, Melandri M, Leoci R, Lacalandra GM, Aiudi G. Canine prostate specific esterase (CPSE) as an useful biomarker in preventive screening programme of canine prostate: CPSE threshold value assessment and its correlation with ultrasonographic prostatic abnormalities in asymptomatic dogs. Reprod Dom Anim 2018;53:359–64.

[2] Mantziaras G, Alonge S, Faustini M, Luvoni GC. Assessment of the age for a preventive ultrasonographic examination of the prostate in the dog. Theriogenology 2017;100:114–9.

277 [3] Levy X, Nizanski W, von Heimendahl A, Mimouni P. Diagnosis of common prostatic conditions
278 in dogs: an update. Reprod Dom Anim 2014;49(2):50-7.

279 [4] Mukaratirwa S, Chitura T. Canine subclinical prostatic disease: histological prevalence and
280 validity of digital rectal examination as a screening test. J. South. Afr. Vet. Assoc. 2007;78:66-8.

281 [5] Johnston SD, Root-Kustritz MV, Olson PN. Disorders of the canine prostate , in: Johnston SD,
282 Root-Kustritz MV, Olson PN (Eds.), Canine and feline theriogenology. Saunders Co, Philadelphia,
283 USA; 2001, pp. 337-55.

284 [6] Johnston SD, Kamolpatana K, Root-Kustritz MV, Johnston GR. Prostatic disorders in the dog.
285 Anim Reprod Sci 2000;60-61:405-15.

286 [7] Russo M, Vignoli M, England GC. B-mode and contrast-enhanced ultrasonographic findings in
287 canine prostatic disorders. Reprod Dom Anim 2012;47(6):238-42.

288 [8] Lopate C. The problem stud dog. Vet Clin North Am Small Anim Pract 2012;42:469-88.

289 [9] Polisca A, Troisi A, Fontaine E, Menchetti L, Fontbonne A. A retrospective study of canine
290 prostatic diseases from 2002 to 2009 at the Alfort Veterinary College in France. Theriogenology
291 2016;85:835-40.

292 [10] Pontes J. Biological markers in prostatic cancer. J Urol 1983;1301:1037-47.

293 [11] Stamey T, Yang N, Hay A, Prostatic-specific antigen as a serum marker for adenocarcinoma of
294 the prostate. New Eng J Med 1987;317:909-16.

295 [12] Lokant MT, Naz RK. Presence of PSA auto-antibodies in men with prostate abnormalities
296 (prostate cancer/benign prostatic hyperplasia/prostatitis). Andrologia 2015;47:328-32.

297 [13] Capitanio U, Pfister D, Emberton M. Repeat prostate biopsy: rationale, indications, and
298 strategies. Eur Urol Foc 2015;1(2):127-36.

[14] Vasanwala FF, Wong MYC, Ho HSS, Foo T. Benign prostatic hyperplasia and male lower urinary symptoms: A guide for family physicians. Asian J Urol 2017;4:181-4.

[15] Fu S, Zhang X, Niu Y, Wang RT. Prostate Specific Antigen, Mean Platelet Volume, and Platelet Distribution Wdth in combination to discriminate prostate cancer from benign prostate hyperplasia. Asian Pac J Cancer Prev 2018;19(3):699-702.

[16] Chapdelaine P, Dube JY, Frenette G, Tremblay RR. Identification of arginine esterase as the major androgen-dependent protein secreted by dog prostate and preliminary molecular characterization in seminal plasma. J Androl 1984;5:206–10.

[17] Levy X. Mimouni P. Hyperplasie benigne de la prostate: actualites. Le Point Veterinaire 2009;293:39–43.

[18] Levy X, Fontbonne A, Derre G, Claret E, Audhuy S, Morlet J, Performance Evaluation of the New Blood test Odelis CPSE in the Diagnosis of Benign Prostatic Hyperplasia (BPH) in Dogs. Proceeding of the 6th EVSSAR meeting; 2009, p, 46.

[19] Holst BS, Holmroos E, Frilling L, Hanas S, Langborg LM, Franko MA, et al. The association between the serum concentration of canine prostate specific esterase (CPSE) and the size of the canine prostate. Theriogenology 2017;93:33-9.

[20] Johnston SD. Performing a complete canine semen evaluation in a small animal hospital. Vet Clin North Am Small Anim 1991;21:545-51.

[21] Weaver AD. Fifteen cases of prostatic carcinoma in the dog. Vet Rec 1981;100:1-75.

[22] Bell FW, Klausner JS, Hayden DW, Lund EM, Liebenstein BB, Feeney DA. Evaluation of serum and seminal plasma markers in the diagnosis of canine prostatic disorders. J Vet Int Med 1995;9:149-53.

[23] Kutzler MA, Yeager A. Prostatic disease, in: Ettinger, S.J., Feldman, E.C., Core, E. (ed.), Textbook of Veterinary Internal Medicine. Elsevier Saunders, St. Louis, USA; 2005.

[24] Teinflat M, Miller I, Loupal G, Thalhammer JG, Gemeiner M. Quantitative determination of canine prostatic-specific protein and its clinical relevance. Tierarzt Prax Aus Kleim 2000;28:127-31.

[25] Gobello C, Castex G, Corrada Y. Serum and seminal markers in the diagnosis of disorders of the genital tract of the dog: a mini-review. Theriogenology 2002;57:1285-91.

[26] Wolf K, Kayacelebi H, Urhausen C, Piechotta M, Mischke R, Kramer S. Testicular steroids, prolactin, relaxin and prostate gland markers in peripheral blood and seminal plasma of normal dogs and dogs with prostatic hyperplasia. Reprod Dom Anim 2012;47(6):243-6.

[27] Sagols E, Navarro C, Follow up of CPSE concentrations after Osaterone treatment in dogs. Proceeding of the 10th EVSSAR meeting; 2013, p.169.

[28] Frenette G, Dube JY, Tremblay RR. Origin of alkalin phosphatase of canine seminal plasma. Arch Androl 1986;16:235-41.

[29] Zaneveld LJD, Polakoski KL, Schumacher GFB. Proteases and biological control: The proteolytic enzyme systems of mammalian genital tract secretions and spermatozoa (eds.). Cold Spring Harbor conferences on cell proliferation 1975;2:682-706.

[30] Syner F.N, Moghissi KS. Purification nd properties of human seminal proteinase. Biochem J 1972;126:1135-40.

[31] Ruenwongsa P, Chulavatnatol M. Acidic protease from human seminal plasma. Purification and some properties of active enzyme and proenzyme. J Biol Chem 1975;250:7574-8.

[32] Propping D, Zaneveld LJD, Tauber PF, Schumacher GF. Purification of plasminogen activators from human seminal plasma. Biochem J 1978;171:435-44.

[33] Gotterer G, Banks J, Williams-Ashman HG, Hydrolysis of arginine esters by male accessory sexual tissues. Proceedings of the Society for Experimental Biology and Medicine, 1956;92:58-61.

345 [34] Acquino-Cortez A, Pinheiro BQ, Silva H, Lima D, Silva T, Souza MB, et al. Serum
346 testosterone, sperm quality, cytological, physicochemical and biochemical characteristics of the
347 prostatic fraction of dogs with prostatomegaly. Reprod Dom Anim 2017;52(6):998-1003.

348 [35] Isaacs W, Coffey D. The predominant protein of canine seminal plasma is an enzyme. J Biol
349 Chem 1984;259:11520-6.

350 [36] Isaacs W, Shaper J. Isolation and characterization of the major androgen-dependent
351 glycoprotein of canine prostatic fluid. J Biol Chem 1983;258:660-5.

352 [37] Dube JY, Chapdelaine P, Tremblay RR. Effect of testicular hormones on synthesis of soluble
353 proteins by dog prostate slices. Canad J Biochem Cell Biol 1983;61:756-63.

354 [38] Mogielnicka-Brzozowska M, Kowalska N, Fraser L, Kordan W. Proteomic Characterization of
355 Zinc-Binding Proteins of Canine Seminal Plasma. Reprod Dom Anim 2015;50(6):1017-21.

356 [39] Fieldler F, Fink E, Tschesche H, Fritz H, Porcine glandular kallikreins, in: Colowick, S.P.,
357 Kaplan, N.O. (Ed.), Methods in enzymology. Academic Press, New York; 1980, pp. 493-532.

358 [40] Frenette G, Dube J, Marcotre JR. Arginine esterase from isolated dog prostate secretory
359 granules is fully active enzymatically. Can J Physiol Pharmacol 1985;63:1603-7.

360 [41] Clements JA. The glandular family of enzymes: tissues-specific expression and hormonal
361 regulation. Endocr Rev 1989;10:39-419.

362 [42] Dube JY, Lazure C, Tremblay, RR. Dog prostate arginine esterase is related to human prostatic-
363 specific antigen. Clin Invest Med 1986;9:51-4;

364 [43] Olsson AY, Lilja H, Lundwall A. Taxon-specifin evolution of glandular kallikrein genes and
365 identification of a progenitor of prostate-specific antigen. Genomics 2004;84:147-56.

366 [44] Dubé JY, Frenette G, Chapdelaine P. Biochemical characteristics of the proteins secreted by
367 dog prostate. Exp Biol 1985;43:149-59.

368 [45] Schäfer-Somi S, Palme N. Seminal Plasma Characteristics and Expression of ATP-binding
369 Cassette Transporter A1 (ABCA1) in Canine Spermatozoa from Ejaculates with Good and Bad
370 Freezability. Reprod Dom Anim 2016;51(2):232-9.

371 [46] Zelli R, Orlandi R, Verstegen J, Troisi A, Elad Ngonput A, Menchetti L, et al. Addition of
372 different concentrations of prostasome-like vesicles at neutral or slightly alkaline pH decreases
373 canine sperm motility. Andrology 2016;5(1):160-8.

374 [47] Thérien I, Bousquet D, Manjunath P. Effect of seminal phospholipid-binding proteins and
375 follicular fluid on bovine sperm capacitation. Biol Reprod 2001;65(1):41-51.

376 [48] Mogielnicka-Brzozowska M, Słowińska M, Fraser L, Wysocki P, Strzeżek R, Wasilewska K, et
377 al. Identification and characterization of non-phosphorylcholine-binding and phosphorylcholine-
378 binding proteins of canine seminal plasma. Ann Anim Sci 2017;17:771–86.

379 [49] Oesterling JE, Chan DW, Epstein JI. Prostate specific antigen in the preoperative and
380 postoperative evaluation of localized prostatic cancer treated with radical prostatectomy. J Urol
381 1988;139:766-72.

382 [50] Frenette G, Dube JY, Lacoste D, Tremblay RR. Radioimmunoassay in blood plasma of arginine
383 esterase: the major secretory product of dog prostate. Prostate 1987;10:145:52.

384 [51] Barsanti JA, Finco DR. Medical management of canine prostatic hyperplasia , in: Bonagura
385 JD, Twedt DC (ed.), Current veterinary therapy. Saunders, Philadelphia, USA; 1995.

386 [52] Ling GV, Nyland TG, Kennedy PC, Hager DA, Johnson DL. Comparison of two sample
387 collection methods for quantitative bacteriologic culture of canine prostatic fluid. J Am Vet Med
388 Assoc 1990;196:1479–82.

389 [53] Kustritz MVR. Collection of tissue and culture samples from the canine reproductive tract.
390 Theriogenology 2006;66:567–74.

391 [54] Klaunsner JS, Johnston SD, Bell FW. Canine prostatic disorders, in: Bonagura JD, Twedt DC
392 (ed.), Current veterinary therapy. Saunders Co, Philadelphia, USA; 1995.

393 [55] Davidson AP, Baker TW. Reproductive Ultrasound of the dog and tom. Top Comp Anim Med
394 2009;24:64-70.

395 [56] Ruel Y, Barthez PY, Mailles A, Begon D. Ultrasonographic evaluation of the prostate in
396 healthy intact dogs. Vet Radiol Ultrasound 1998;39:212-6.

397 [57] Sannamwong N, Saengklub N, Sriphuttathachot P, Formula derived prostate volume
398 determination of normal healthy intact dogs in comparison to dogs with clinical BPH Proceedings
399 of the 7th international symposium on canine and feline reproduction; 2012.

400 [58] Alonge S, Melandri M, Fanciullo L, Lacalandra GM, Aiudi G. Prostate vascular flow: The
401 effect of the ejaculation on the power doppler ultrasonographic examination. Reprod Dom Anim
402 2018;53:110-5.

403 [59] Alonge S, Melandri M, Leoci R, Lacalandra GM, Aiudi G. Ejaculation effect on blood
404 testosterone and prostatic PW Doppler ultrasound in dogs. 22nd European Society for Domestic
405 Animal Reproduction (ESDAR) Annual Conference, Cordoba, Spain, September 27 – 29, 2018,
406 *accepted*.

407 [60] Alonge S, Melandri M, Leoci R, Lacalandra GM, Caira M, Aiudi G. Ejaculation effect on
408 CPSE serum concentration in dogs. Proceedings 21st European Veterinary Society Small Animal
409 Reproduction (EVSSAR) Congress, Venice, Italy, 22-23 June, 2018, *accepted.*

410 [61] Frenette G, Dube JY, Marcotre JR, Tremblay RR. Effects of castration and steroid synthesis on
411 the activity of some hydrolitic enzymes in the dog prostate. Prostate 1983;4:206-10.

412 [62] Isaacs W, Sharper JH. Immunological localization and quantification of the androgen
413 dependent secretory protease of canine prostate. Endocrinology 1985;117:1512-20.

414 [63] Juniewicz PE, Barbolt TA, Egy MA, Frenette G, Dube JY, Tremblay RR. Effects of androgen
415 and antiandrogen treatment on canine prostatic arginine esterase. Prostate 1990;17:101-11.

416 [64] Krawiec DR. Urologic disorders of the geriatric dog. Vet Clin North Am Small Anim Pract
417 1989;19(1):75-85.

418 [65] Tsutsui T, Hori T, Shimizu M, Tatsuzawa C, Kawakami E. Effect of osaterone acetate
419 administration on prostatic regression rate, peripheral blood hormone levels and semen quality in
420 dogs with benign prostatic hypertrophy. J Vet Med Sci 2001;63(4):453-6.

421 [66] Gonzalez G, Guendulain.C, Maffrand C, Gobello C. Comparison of the effect of the aromatase
422 inhibitor, anastrazole, to the antioestrogen, tamoxifen citrate, on canine prostate and semen. Reprod
423 Dom Anim 2009;44(2):316-9.

424 [67] McKenzie B. Review: Evaluating the benefits and risks of neutering dogs and cats. CAB
425 Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources,
426 2010;5:No.045.

427

Table 1. Clinical meaning of CPSE blood serum concentration [1,19] and recommendations on complementary exams.

Blood Serum Concentration	**Clinical meaning**	**Volume ratio expected**	**Complementary exams**
CPSE ≤ 50ng/ml	Prostatic disorders unlikely	V ratio < 1.5	-
CPSE 50 – 90ng/ml	Prostatic disorders possible	1.5 ≤ V ratio < 2.5	Suggested
CPSE ≥ 90 ng/ml	Prostatic disorders likely	V ratio ≥ 2.5	Required

Volume ratio expected (V ratio) [1,19]: actual/normal expected prostatic volume [57].

Complementary exams: seminal or prostatic fluid evaluation, urinalysis and imaging, prostatic parenchima FNA or biopsy, bacterial exam complete with antibiogram [23,51-53].