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Original article

COMPUTER-ASSISTED SPERM ANALYSIS AND COMPARATIVE DIAGNOSTIC IMAGING OF BENIGN PROSTATIC HYPERPLASIA IN DOGS BY ULTRASOUND, X-RAY AND COMPUTED TOMOGRAPHY

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Summary

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Early diagnosis of benign prostatic hyperplasia (BPH) in dogs using imaging methods has become increasingly prevalent in recent years. That allows accurate differentiation of BPH from other diseases, as well as timely prophylaxis and treatment. The aim of the research was to make a comparative analysis of BPH diagnosis by ultrasound, X-ray and computed tomography (CT) and computer-assisted sperm analysis (CASA) of ejaculates from sexually mature male dogs. Two hundred and fifty patients of different breeds, 80 of whom with previous clinical history and BPH-related complaints at 3 to 7 years of age, were followed. The results of echographic studies of dogs with clinical signs of BPH showed structural prostate changes. Focal inflammation, small or larger intra- or paraprostatic cysts (7%), neoplasia (3%), acute or chronic prostatitis (15%) and prostate abscesses (3%) were detected. The remaining 72% of patients demonstrated evidence of hypo -to hyper heterogenic parenchyma with moderate heterogeneity, which are characteristic of BPH. X-ray findings about prostate volume showed that 85% of the patients studied had an increase in prostate volume, enlarged soft tissue shadow in the caudal abdomen or cranially to the pelvis but caudally to the bladder. These data were found out in 13% more patients than those with ultrasound data for BHP. In this study, computed tomography demonstrated structural changes and precise prostate size in all 10 patients with suspected BPH. The CASA results showed that the percentage of static sperm was significantly higher in dogs with BPH than in healthy dogs. At the same time, there was a decline in the percentage of cells with progressive movement at a rapid and medium rate of movement in patients with BPH compared to those data in healthy dogs. These changes could have a negative effect on the fertilising potential of the sperm. The obtained results suggested that the application of CT and CASA for early diagnosis of BPH in dog breeders from rare and valuable breeds is advisable and useful for timely prevention and treatment of the disease.

Key words: benign prostatic hyperplasia, CASA, dogs, imaging

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a disease that develops in older male dogs, a natural result of the aging and action of some hormones on the prostate gland. Testosterone secreted by the testes is converted by 5-α reductase to dihydrotestosterone (DHT) in prostate epithelial cells. DHT regulates prostate gland growth by interacting with DHT-receptors in the prostate gland. With increasing age, the number of receptors for DPH grows, as does the percentage of testosterone that becomes DHT. Testosterone levels are known to decline with age, while estrogen levels remain the same. This altered ratio of androgen to estrogen influences the number of DHT receptors and their sensitivity to DHT. Recent publications indicate that prolactin may also play a role in this process (Wolf *et al.* 2012; Lai *et al.*, 2013). The result of hormonal changes is the development of BPH, which involves increase in the size of the prostate epithelial cells (hypertrophy) and their number (hyperplasia: the predominant mechanism in dogs), often accompanied by multiple cysts or abscesses involved in the prostatic cavities.

Cysts occur in about 15% of dogs 7 years of age or older. Bacterial contamination is often found in 42% of cases of prostate cysts (Levy *et al.,* 2014). According to previous studies, BPH accounts for over 50% of cases of prostate disease in dogs, <20% are caused by infections, about 7% by tumours, and <2% by squamous metaplasia (Krawiec & Hefl, 1992; Foster, 2012). When comparing BPH in humans and dogs, it can be argued that endocrine factors are similar, but hyperplasia in humans involves periurethral stromal tissues more than glandular tissues, which is a specific pathomorphologic difference when compared with signs of BPH in dogs. These are factors that can explain the various clinical signs and responses to human pharmacological agents in the dog. Despite these differences, the dog has served as an excellent model for human disease research, and this research has greatly helped to increase our knowledge of the disease and its potential treatment.

Dogs with BPH are usually not clinically ill, but early detection of BPH is especially important because this disease is a major cause of infertility (Fontbonne, 2007; Wilson, 2011). Therefore, early diagnosis and treatment of BPH reduces the likelihood of future pathological conditions. Primary clinical examination is normal, except for the enlarged prostate gland at rectal palpation, but the examination is sometimes painful, subjective and not very accurate (Levy & Mimouni, 2009). Urinalysis results may be normal or show red blood cells and/or enlargement of squamous epithelial cells. Haematuria may occur mainly by the end of urination (Johnston *et al*., 2001; Maurey-Guenec, 2007). Haematospermia without changes in sperm quality is the most common sign in the early stages of BPH and prostatitis. In the later stages of BPH, sperm motility and changes in their morphology are especially commonly observed by increasing tail abnormalities (Johnston *et al.* 2001; Maurey-Guenec, 2007; Walker & Challacombe, 2013). The microbiological analysis of urine is negative or may exhibit up to <10,000 colonies/mL (established by catheterisation), unless the dog has secondary prostatitis.

Imaging by radiography may show prostatomegaly and other abnormalities. However, in some cases, the use of X-ray analysis alone is not reliable enough to

investigate and diagnose BPH, as there is a tendency to overestimate the size of the gland (Atalan *et al.,* 1999). Ultrasound diagnostics can show a rather homogeneous texture of the prostate gland, which is normal to slightly hyperechogenic. There may be small cysts filled with fluid. The imaging method depicts the size of the gland as well as the homogeneity of the parenchyma (Gunzel Apel *et al.,* 2001). Ultrasound can sometimes diagnose early stages of clinical BPH. The range of normal prostate sizes published by various authors is rather broad, and some of them did not take into account the breed of dog or the probe position (Atalan *et al.,* 1999; Levy & Mimouni, 2013). The use of other technologies such as magnetic resonance imaging and computed tomography (CT) are excellent tools, but they are quite expensive. These methods allow for accurate determination of prostate volume and visualisation of structures and lymph nodes around the gland (Jia *et al.,* 2005).

The purpose of the present studies was to perform a comparative analysis of the diagnosis of BPH by means of ultrasound, radiography, computed tomography and CASA of ejaculates from sexually mature male dogs with regard to the timely diagnosis and prevention of the disease in dogs.

MATERIALS AND METHODS

In this study, 250 patients with suspected BPH from different dog breeds, aged 3 to 7 years, were followed up. Dogs are privately owned and were included in studies with informed consent from owners. The studies were carried out in accordance with the rules of operation of the Central Veterinary Clinic, Sofia, as well as all veterinary norms and the law for the protection of animals, in terms of protection of life, health and well-being of patients. Clinical studies for BPH included: history, rectal palpation to evaluate gland position and symmetry, urine analysis and preparation of dogs for further analysis.

Imaging studies

All 250 patients were submitted to ultrasound imaging of the prostate using ultrasound systems MyLab 70 (Esaote) and Mindray DC 7 and convex 10 MHz transducer.

All patients were subjected to radiographic examination without sedation in lateral recumbency. Reference digital X-ray is used for the analysis, with TOSHIBA ROTANOTE^{TM} E7865X X-ray tube. Exposure data were as followed: 80 kVp, 320 mA, 8.00 mAs and 0.025 s.

Computed tomography was made on 10 patients with the Siemens SOMATOM Scope. Patients were positioned in the sternal position after i.m. sedation with 0.2 mg/kg butorphanol (Butomidor) and 5–20 μg/kg medetomidine. The thickness of the sections was 1.5 mm.

Semen collection and analysis

Ejaculates were obtained manually from healthy dogs (n=24) and dogs with suspected BPH (n=28). The performed spermatological analysis included determination of ejaculate volume, pH, concentration, progression and rate of movement of sperm. Semen was analyzed by the CASA System Sperm Class Analyzer® (Microptic®, Spain), with software analytical module Motility and concentration. The CASA parameters assessed were: sperm concentration ($\times 10^6$/mL); motility and progressive movement of spermatozoa (%) (static,

progressive, non-progressive); sperm velocity (%) (fast, medium and slow velocity); head area (μm^2); kinematic parameters (total and in individual sperm populations – static, slow, medium and fast).

Cytological studies

In some patients with suspected BPH, a cytomorphological analysis of prostate gland samples taken by thin 22 G needle aspiration biopsy was performed under ultrasound control after i.m. sedation with 0.2 mg/kg butorphanol (Butomidor) and 5–20 µg/kg medetomidine. Samples were stained with Diff Quick.

Statistical analysis

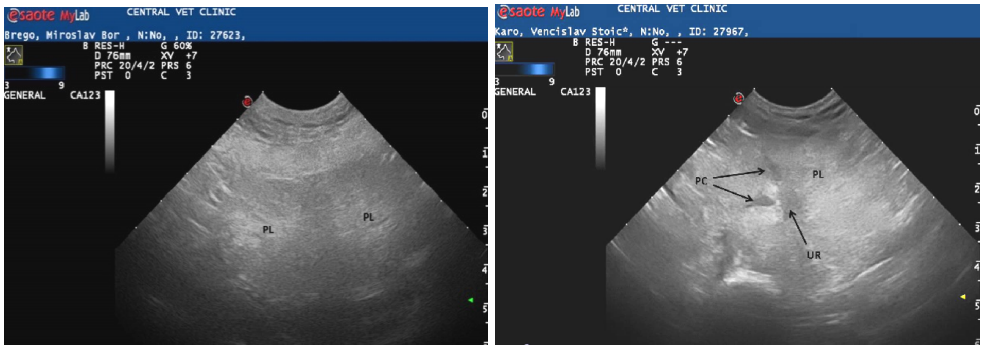
All results are presented as mean ± standard deviation. Statistical significance was determined by Student's *t*-test. Statistical analyses were performed on MS Excel and through the CASA software product.

RESULTS

On the basis of initial clinical examinations and history taken from all patients, half of the dogs showed signs of acute prostatitis, and dysorexia or diarrhoea was reported in some patients. Dogs showing problems with the genitourinary system were the least. Haematuria, polakisuria, stranguria and pyuria were reported. Rectal palpation of the prostate was indicative but did not provide definitive data. In the current study on 250 dogs 68% were healthy and 32% had enlarged prostates. Of these, there were intraprostatic or paraprostatic cysts (7%), neoplasia (3%), acute or chronic prostatitis (15%), prostate abscesses (3%), and BPH (72%).

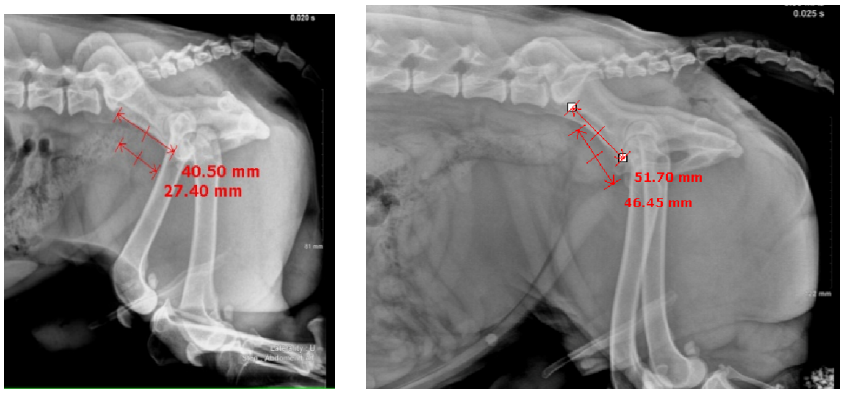
The ultrasonographic appearance of the prostate gland provides information on the shape, dimensions, lobular structure, and echotexture of the gland parenchyma. In the present studies, prostate was found to be symmetrical in healthy dogs, with a homogeneous structure without subcapsular oedema, with a freely visible urethra. In patients with BPH, the prostate was found to be symmetrical but with a moderately heterogeneous structure, often with visible subcapsular oedema and, in more advanced cases, urethral pressure was registered. In some cases, due to increased pressure in the prostate gland, increased Doppler signal and cystic structures were observed. (Fig. 1).

The results of the radiography about



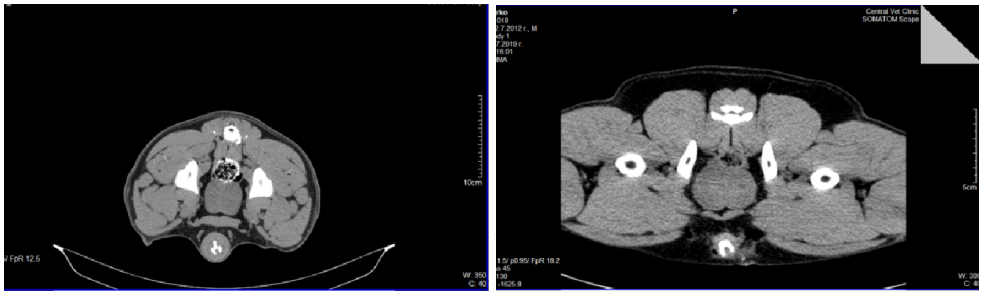
A B

Fig. 1. Ultrasonogram of prostate gland. A) healthy dogs with homogeneous echodensity pattern; B) dogs with benign prostatic hyperplasia. PL – prostatic lobe; PC – prostate cyst, UR – urethra.



A B

Fig. 2. Lateral radiography of a healthy dog (A) and a dog with benign prostatic hyperplasia (BPH). The ratio of prostate diameter to the distance from the cranial edge of the sacrum to the cranial edge of the pecten pubis, in healthy dogs is up to 0.7, while in patients with BPH it is >0.7. Very often, patients with BPH patients have inadequate bladder emptying.



A B

Fig. 3. Computed tomography of a healthy dog (A) and a dog with benign prostatic hyperplasia (B). In healthy subjects, the prostate lies freely in front of the pelvic cavity without displacing or compressing the vertebral column dorsally, while in BHP, the prostate almost fills the pelvic cavity by compressing or displacing the vertebral column in dorsal direction. In both cases (A and B), the prostate has a homogeneous structure.

the volume of the prostate showed that in 85% of the studied patients the prostate volume was increased, as well as soft tissue shadow in the caudal abdomen or cranially to the pelvis, but caudally to the bladder. These data were present in 13% more patients with suspected BPH than in diagnoses obtained from the ultrasound examination (Fig. 2). For more accurate differentiation and more convincing BPH diagnosis, computed tomography (applied to 10 patients) was used for the first time in Bulgaria. In all 10 patients BPH was demonstrated by accurate and well visualised data (healthy dogs and dogs with BHP) on structural changes and accurate prostate volume determination (Fig. 3).

A comparative diagnosis of normal prostate, prostatitis and neoplasia in dogs was made by histological analysis of samples (Fig. 4).

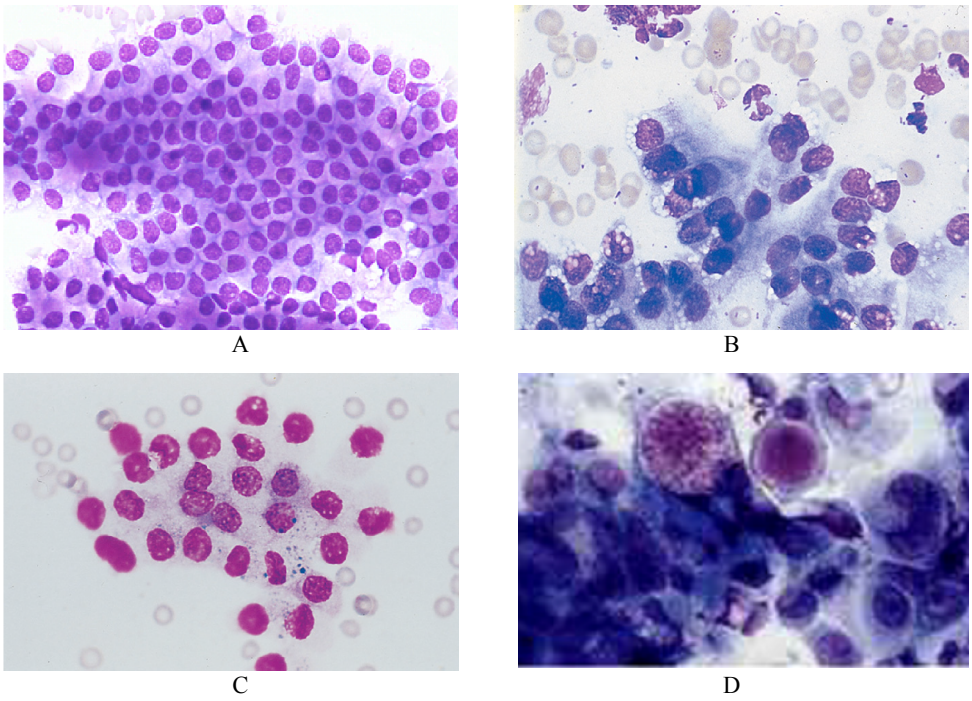


Fig. 4. Cytological smears of canine prostatic gland. A) Normal prostate gland revealing simple columnar epithelium. The tissue stroma is composed by collagen fibres and fibroblasts; B) Benign prostatic hyperplasia – glandular hyperplasia, larger prostate epithelial cell layer and stromal cell space; C) Prostatitis – atrophy of the glandular epithelium, arranged in two or more layers and inflammatory mononuclear infiltration; D) Neoplasia – low-grade canine prostate cancer composed by small glandular proliferation with tubules showing more than two layers with visible nucleoli. Diff Quick staining, ×100.

It was of interest to determine whether BPH affected the reproductive potential of the patients. The results of the spermatological analysis showed that ejaculatory volume in dogs with BPH was significantly increased (5.13±1.99), compared with healthy dogs (3.66 ± 2.15) (P=0.03), regardless of breed. Analysis of changes in semen pH showed that was more alkaline in dogs with BPH (7.20±0.25) compared to healthy dogs (pH 6.77±0.33). A substantial decrease in sperm concentration (× 10^6/mL) was also found in dogs with BPH (75.25±55.14) compared with healthy dogs (139.66±61.41) (P≤0.01). A negative correlation between ejaculatory volume and sperm concentration was recorded in healthy dogs (n=24; r=–0.677), whereas in dogs with BPH this correlation was weak (n=28; r = –0.289) (Fig. 5).

A more specific test for the analysis of changes in the semen of dogs with a proven BPH was performed by computer-assisted sperm analysis. The data allowed obtaining additional information on the fertility potential of sperm. The data from the comparative CASA analysis of motility, progression and rate of movement of sperm showed specific and statistically

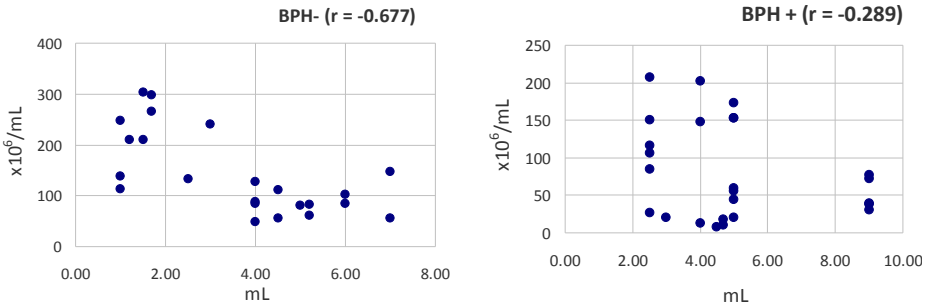


Fig. 5. Correlation between ejaculatory volume and sperm concentration in healthy dogs (n=24; r = –0.677) (left) and in dogs with BPH (n = 28; r = -0.289) (right).

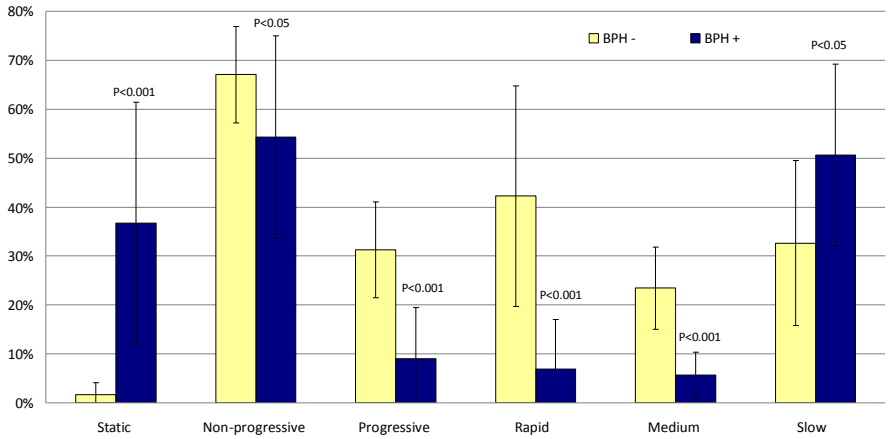


Fig. 6. CASA analysis on sperm motility, progression and velocity in healthy dogs (BHP–; n=24) and dogs with benign prostatic hyperplasia (BHP+; n=27). Data are presented as mean ± SD.

significant differences between healthy dogs and those with BPH (Fig. 6). The percentage of static sperm was significantly higher in dogs with BPH (40.22±19.66) compared to healthy dogs (3.22±1.15) (P<0.001). A smaller percentage of cells with progressive movement (9.05±5.37), rapid (7.55±8.15) and moderate (5.92±3.33) velocity were observed in patients with BPH compared to those in healthy subjects (32.33±6.97, 44.35±22.46, 25.15±6.66, respectively) (P<0.001).

DISCUSSION

In the present study we showed that prostate disorders can vary in severity and manifestation. There are at least six diseases affecting the prostate other than BPH, such as: squamous metaplasia, cystic hyperplasia, paraprostatic cysts, bacterial infection, abscess and prostate cancer. Ultrasonography is an excellent diagnostic tool in evaluating the prostate gland. In the present study this technique

was very useful for visualising the internal architecture, external texture, as well as cystic structures within the prostate. The prostate should have a homogeneous pattern. We established that in cases of inflammation, hyperplasia or neoplasia, the homogeneous pattern of the prostate image was lost, and focal to multifocal areas of hyperechoic and/or hypoechoic tissue became apparent. Calcification (hyperechoic area underlined by a cone shadow) may be observed in prostatic tumours or chronic prostatitis. In contrast, anechoic or hypoechoic areas are present in cases of prostatic cysts or prostatic abscessation (Lévy & Mimouni, 2013). At the same time in practice, it is not always possible to make a precise differentiation of this disease from some other diseases of the reproductive system (Wolf *et al.,* 2012). It is well known that BPH disease often has no clinical signs or is accompanied by nonspecific or common manifestations (Fontbonne, 2007; Wilson, 2011). Therefore, veterinarians may find it difficult to make an accurate diagnosis and, in many cases, make a general diagnosis of prostate syndrome. Moreover, various disorders of the prostate gland may be simultaneously present, for example BPH and prostatitis and cysts or abscesses, or abscess and prostate cancer, etc. (Wallace, 2001). In order to obtain a correct diagnosis, it is necessary to perform physical examination of the patient, but ultrasound and/or radiographic examination are also advisable. Some authors also recommend that bacteriological, cytology and blood tests are performed (Levy *et al.*, 2014). For more accurate diagnosis of BPH, comparative cytomorphological analyses were performed in the present studies. The data obtained allowed us to differentiate cases of normal prostate, prostatitis and neoplasia in dogs. In some clinics, up-to-date diagnostic tests, such as canine prostate specific arginine esterase test, are used to seek appropriate medical treatment (Christensen, 2018). Other procedures may sometimes be required to confirm the diagnosis or permanently rule out infection or the presence of a tumour. Transrectal digital palpation of the prostate should always be included in the examination, although it has relatively low sensitivity, specificity and is painful. Imaging, including ultrasound and radiographic examinations, is of particular importance and allows visualisation of the prostate gland, the urethra, the bladder, and surrounding lymph nodes. Prostate volume and structure data are obtained. However, this diagnosis is not always definitive (Atalan *et al.,* 1999; Levy & Mimouni, 2013). The current data from the X-ray examination confirm these claims. This necessitates a more accurate examination of dogs using computed tomography. On the other hand, an emergency diagnosis is often required, for example in cases of suspected prostate cancer, therefore the need from appropriate markers or new and specific approaches (Levy & Mimouni, 2013; Levy *et al.,* 2014). The present studies provide, through CASA analysis, new data on the role of BPH on the reproductive status of dogs. DPH disease affects the basic parameters of sperm, which probably affects their fertilising ability of spermatozoa. The percentage of static sperm has been shown to be significantly higher in dogs with BPH than in healthy dogs. At the same time, there is a decline in the percentage of cells with progressive movement at a rapid and medium rate of movement in patients with BPH compared to those data in healthy subjects. These

changes have a negative effect on the fertilising potential of the sperm.

In this study, computed tomography was applied in Bulgaria for the first time for BPH diagnostics, which gave 100% success in the diagnosis of BPH in comparison with ultrasound or X-ray. Computed tomography data along with specific CASA results about the reproductive potential of patients with BPH would not only allow timely treatment to be undertaken, but would also provide complete information for breeders about the potential for future use of dogs for breeding.

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