Polish Journal of Veterinary Sciences Vol. 26, No. 4 (2023), 621–628

DOI 10.24425/pjvs.2023.148281

Original article

Nitric oxide production by spermatozoa and sperm characteristics in dogs with benign prostatic hyperplasia

A. Domoslawska-Wyderska[1], A. Orzołek[2], S. Zduńczyk[1], A. Rafalska[1]

[1] Department of Animal Reproduction with Clinic,
University of Warmia and Mazury, Oczapowskiego 14, 10-719 Olsztyn, Poland
[2] Department of Animal Biochemistry and Biotechnology,
University of Warmia and Mazury, Oczapowskiego 5, 10-719 Olsztyn, Poland

Abstract

The aim of this study was to evaluate the effect of benign prostatic hyperplasia (BPH) on nitric oxide (NO) production by spermatozoa and sperm parameters in dogs. The study was conducted on 40 intact dogs of various breeds. The dogs were assigned to two groups: BPH group (n=20) and non-affected group (n=20). The sperm concentration and motility parameters of spermatozoa were assessed using computer-assisted sperm analysis. For the assessment of sperm morphology monochromatic Diff-Quick stain was used. Plasma membrane integrity, mitochondrial membrane potential and the spermatozoa producing nitric oxide and with apoptotic-like changes were determined using fluorescent stain methods. The percentages of motile sperm, sperm with progressive motility and normal sperm were statistically significantly ($p<0.05$) lower in dogs with BPH than in non-affected dogs. The proportion of sperm in motility subcategory RAPID was statistically significantly ($p<0.05$) lower in dogs with BPH than in control dogs, whereas in the STATIC motility subcategory the proportion was significantly ($p<0.05$) higher in dogs with BPH. The percentage of spermatozoa producing NO was significantly ($p<0.05$) higher in dogs with BPH than in control dogs. In conclusion, the results of this study showed that BPH adversely affects semen quality, especially motility, in dogs. The decreased semen quality was associated with an increased proportion of spermatozoa generating NO. Further research is needed to clarify the mechanisms by which BPH affects semen quality.

Keywords: dog, benign prostatic hyperplasia, nitric oxide, semen

Correspondence to: A. Domosławska-Wyderska, e-mail: anna.domoslawska@uwm.edu.pl

Introduction

Benign prostatic hyperplasia (BPH) is the most common age-related prostatic disease in male, intact dogs, accounting for approximately 50% of all prostate disorders (Lévy et al. 2014, Polisca et al. 2016). Histologic evidence of BPH developed at an early age with a prevalence of 16% of the dogs by the age of 2 years. Fifty percent of the dogs had BPH, determined histopathologically by the age of 4.1-5.0 years. With time, almost all intact male dogs will develop BPH, with > 95% affected by 9 years of age (Berry et al. 1986).

BPH develops under the influence of androgen metabolite dihydrotestosterone (DHT) and the increased ratio of oestrogen to androgen (Carson et al. 2003, Smith et al. 2008). With age, oestrogen concentration increases and testosterone concentration decreases. This enhances the concentration of androgen receptors and increases the conversion of testosterone by 5α-reductase to DHT in the prostate. More recently, it has been thought that oxidative stress may play an important role in the development of BPH (Minciullo et al. 2015, Domosławska et al. 2022). Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the ability of the antioxidant system to detoxify these reactive products. Age-related hormonal changes may activate a chronic inflammatory response in the prostate (Tong and Zhou 2020). This causes the generation of ROS and results in oxidative stress. ROS may induce oxidative DNA damage and hyperplastic transformation of prostatic cells (Minciullo et al. 2015, Vital et al. 2016). In BPH there is an increase in prostate epithelial cell size (hypertrophy) and cell numbers (hyperplasia) and enlargement of the prostate. The hyperplastic gland has increased vascularity, which results in vascular leakage or haemorrhage into the gland. Enlargement increases intraglandular pressure and results in the accumulation of fluid within the excretory ducts and cyst formation (Smith 2008, Lévy et al. 2014, Cunto et al. 2022).

Initially, most affected animals do not develop clinical signs associated with BPH. The first symptom in stud dogs is often a decrease in libido and fertility. Sperm from dogs with BPH have increased DNA fragmentation, increased numbers of morphologic defects and altered sperm movement pattern (Aquino-Cortez et al. 2017, Flores et al. 2017, Angrimani et al. 2020, Niżański et al. 2022). With the progressive enlargement of the prostate, BPH can lead to sanguineous discharge from the urethra, dysuria, hematuria, hemospermia and tenesmus (Smith 2008, Lévy et al. 2014, Cunto et al. 2022).

The mechanisms by which BPH exerts its effects on fertility are still not completely elucidated. It is assumed that decreased semen quality in dogs with BPH may be related to hormonal disturbance (lower testosterone level) associated with ageing (Flores et al. 2017) and biochemical changes of the prostatic fluid (Krakowski et al. 2015, Aquino-Cortez et al. 2017). As BPH is associated with oxidative stress, the accumulation of ROS can induce sperm cell damage, resulting in decreased sperm motility, velocity and morphological integrity (Agarwal et al. 2014, Flores et al. 2017).

ROS represent a broad category of molecules including radical (hydroxyl ion, superoxide, peroxyl etc.) and non-radical (ozone, singlet oxygen, lipid peroxide, hydrogen peroxide) and oxygen derivatives. A subclass of ROS, reactive nitrogen species (nitric oxide [NO], peroxynitrite, nitroxide ion, etc.) are responsible for a subtype of oxidative stress termed nitrosative stress (Agarwal et al. 2014). Some studies in men showed that nitrosative stress may be involved in male infertility associated with BPH (Doshi et al. 2012). Mouse and human spermatozoa contain nitric oxide synthase (NOS) and can synthesize NO (Herrero et al. 1996). Higher NOS activity was found in sperm from infertile men compared to healthy controls (Fafula et al. 2018). Physiological levels of NO are necessary for sperm motility, morphology and viability (Luo et al. 2021). However, an excess of NO can adversely affect semen parameters (Rosselli et al. 1995, Balercia et al. 2004).

NO production in the spermatozoa of dogs with BPH has not yet been studied. There are also very few data on functional sperm parameters such as mitochondrial membrane potential, plasma membrane integrity and apoptic-like changes in dogs with BPH. Thus, the aim of this study was to evaluate the effect of BPH on sperm NO production, motility, morphological and functional parameters of spermatozoa in dogs.

Materials and Methods

Animals and study design

The study was conducted on 40 intact dogs of various breeds. The males were presented at the Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine in Olsztyn, either because of sanguineous discharge from the urethra or for the evaluation of semen quality. The dogs were assigned to two groups: BPH group (n=20) and non-affected group (n=20). The diagnosis of BPH was based on history, clinical symptoms such as sanguineous discharge from the urethra, dysuria, tenesmus and enlargement of the prostate on rectal palpation and ultrasound examination (Mindray Bio-Medical 2 with a 7.5-MHz convex transducer). Ultrasound diagnostic criteria for BPH

were enlargement of the prostate and heterogeneity of the prostatic parenchyma with the presence of small hypoechogenic cysts. Reference values for the normal size of the prostate depending on age and weight were taken from Ruel et al. (1998).

The control animals showed no clinical signs and the prostate was not enlarged. The age of the dogs ranged from 5 to 8 years and averaged 7.3±1.3 years in the BPH group and 6.7±1.1 years in the control group. The animals in both groups were of similar weight. The dogs were fed with commercial premium dry diets. Semen was collected from all the animals and analyzed macroscopically, microscopically and by computer-assisted semen analysis (CASA) and functional tests. The samples were analyzed individually, immediately after semen collection. The study was conducted according to good veterinary practice.

Semen collection

Semen was collected by manual manipulation as described by Linde-Forsberg (1991) in the presence of a teaser bitch in heat. Three fractions of the ejaculate were collected separately into prewarmed (36-38°C) sterile glass tubes.

Semen evaluation

The sperm concentration and motility parameters of spermatozoa in the sperm-rich fraction were assessed by CASA (Hamilton Thorne Sperm Analyzer, version IVOS 12.3), according to a previously described protocol (Domosławska et al. 2013). For the assessment of sperm morphology monochromatic Diff-Quick stain was used. Two hundred spermatozoa were evaluated per slide (Olympus BX50, 400x magnification).

NO production by spermatozoa, plasma membrane integrity (PMI), mitochondrial membrane potential (MMP) and apoptotic-like changes in spermatozoa were also determined. Before analyses, sperm samples were extended to a final concentration of 30 x 10^6 spermatozoa/ml with HEPES-buffered saline solution (130 mM of NaCl, 4 mM of KCl, 14 mM of fructose, 10 mM of HEPES, 1 mM of CaCl, 0.5 mM of MgCl, 0.1% BSA). The sperm concentration was estimated by means of a light microscope and a Bürker counting chamber (Equimed-Medical Instruments, Kraków, Poland). The samples were analyzed individually, immediately after semen collection.

Plasma membrane integrity (PMI)

The fluorescent dyes SYBR-14 and propidium iodide (PI) (Live/Dead Sperm Viability Kit; Molecular Probes, Eugene, USA) were used to assess the percentage of membrane-intact spermatozoa. The staining was performed according to the method given by Garner and Johnson (1995), with modifications proposed by Fraser et al. (2022). Extended sperm samples were incubated with SYBR-14 solution (1 mM of SYBR-14 in DMSO) for 10 min, and then with PI solution (2.4 µM of PI in Tyrode salt solution) for another 10 min at 37°C. An aliquot (10 µl) of each sample was placed on a sterile microscopic slide, covered with a coverslip and examined at 600x magnification under an epifluorescence microscope (Olympus CH 30 RF-200). A minimum of 200 cells were assessed in ten random fields of view using the appropriate filters. Spermatozoa demonstrating green fluorescence were classified as viable with an undamaged plasma membrane, whereas those showing red fluorescence were classified as non-viable with a damaged membrane. The results were expressed as the percentage of sperm cells with an intact membrane. The samples were analyzed individually, immediately after semen collection.

Determination of NO production

Cell-permeable fluorescent dye – DAF-2DA (4,5-diaminofluorescein-2-diacetate; Sigma-Aldrich, St. Louis, USA) – was used to determine the percentage of spermatozoa that generated nitric oxide (NO). Sperm staining was carried out according to the method proposed by Lampiao et al. (2006). Diluted sperm samples were incubated with DAF-2DA solution (20 µM DAF-2DA in PBS) for 120 min at 37°C, in the dark. A minimum of 200 cells per slide were examined in each aliquot under an epifluorescence microscope (Olympus CH 30 RF-200) using the appropriate filters. Spermatozoa demonstrating blue-green fluorescence in any segment (head, mid-piece or tail) were considered as NO-producing cells. The results were expressed as the percentage of sperm cells that were capable of producing nitric oxide. The samples were analyzed individually, immediately after semen collection.

Mitochondrial membrane potential (MMP)

The fluorescent dyes JC-1 (Molecular Probes, Eugene, USA) and PI were used to estimate the mitochondrial membrane potential (MMP) of sperm. The protocol was based on the method described by Thomas et al. (1998). Sperm suspensions were incubated at 37°C with JC-1 solution for 10 min, and then with PI solution (2.4 µM PI in Tyrode salt solution) for another 10 min. Approximately 200 cells per slide were examined in each aliquot under an epifluorescence microscope (Olympus CH 30 RF-200) as described above. Viable spermatozoa with high mitochondrial potential exhibited orange/red fluorescence in the

Table 1. Sperm quality parameters (mean±SD) in dogs with benign prostatic hyperplasia (BPH) and non-affected (control) dogs.

Parameter	Unit	BPH n=20	Control n=20
Volume of the sperm-rich fraction	ml	2.50±0.79	2.53±0.75
Concentration	x 10^6/ml	123.87±87.68	162.01±98.35
TSC	x 10^6	295.25±247.60	405.82±284.24
MOT	%	64.15±17.51[a]	71.15±12.74[b]
PMOT	%	41.25±19.64[a]	55.05±14.31[b]
VAP	µm/s	121.36±38.94	127.14±32.37
VSL	µm/s	111.29±39,93	115.73±32.50
VCL	µm/s	162.46±54.33	185.51±41.66
ALH	µm	6.54±1.36	6.89±1.25
BCF	Hz	33.10±4.19	32.93±3.42
STR	%	88.45±9.56	86.70±8.77
LIN	%	65.10±11.51	65.10±7.97
RAPID	%	46.35±19.80[a]	58.60±16.52[b]
MEDIUM	%	17.70±11.39	17.05±11.08
SLOW	%	13.25±8.20	3.80±1.96
STATIC	%	22.75±12.77[a]	14.35±7.28[b]
NORMAL	%	62.33±9.73[a]	79.50±8.04 [b]

TSC – Total Sperm Count, MOT – motile spermatozoa, PMOT – progressive motility, VAP – velocity average pathway, VSL – velocity straight line, VCL – velocity curvilinear, ALH – amplitude lateral head, BCF – beat cross frequency, STR – straightness, LIN – linearity, motility subcategories: RAPID, MEDIUM, SLOW, STATIC
a, b – values with different superscripts are statistically different between the groups ($p<0.05$)

mid-piece region. Sperm exhibiting green fluorescence were considered as nonviable spermatozoa with low MMP. The results were expressed as the percentage of spermatozoa with high mitochondrial potential. The samples were analyzed individually, immediately after semen collection.

Apoptotic-like changes (vitality of spermatozoa)

Apoptotic-like changes in sperm were assessed using YO-PRO-1/PI fluorochromes (Vybrant Apoptosis Assay Kit #4; Molecular Probes, Eugene, USA) according to the method of Trzcińska and Bryła (2015). Sperm suspensions were incubated initially with YO-PRO-1 (100 µM) for 5 min and then with PI (2 µM) solution for 10 min at 37°C. Approximately 200 cells per slide were examined in each aliquot under an epifluorescence microscope (Olympus CH 30 RF-200) as described above. Sperm cells with apoptotic-like changes showed green fluorescence in the head region, while dead sperm showed red fluorescence. The results were presented as the percentage of spermatozoa presenting apoptotic-like changes (green fluorescence), dead (red fluorescence) and live (no fluorescence). The samples were analyzed individually, immediately after semen collection.

Statistical analysis

The results were presented as mean and standard deviation and compared between both groups using Student's *t*-test or the Mann-Whitney test according to the distribution of variables (GraphPAD PRISM, Version 4.00, GraphPad Software, San Diego, CA, USA). The level of significance was set at $p<0.05$.

Results

The mean values of semen quality parameters (volume of the sperm-rich fraction, sperm concentration, total sperm count, motility parameters and percentage of normal sperm) of dogs with BPH and in non-affected dogs are shown in Table 1.

The volume of the sperm-rich fraction was similar in dogs with BPH and in non-affected dogs ($p>0.05$). Sperm concentration and total sperm count were numerically lower in dogs with BPH compared to controls, but the differences were not statistically significant ($p>0.05$). The mean percentages of motile sperm and sperm with a progressive motility were statistically significantly ($p<0.05$) lower in dogs with BPH than in non-affected dogs (64.15±17.51% vs 71.15±12.74% and 41.25±19.64% vs 55.05±14.31%, respectively). The proportion of spermatozoa in motility subcategory

Table 2. Percentage (mean±SD) of spermatozoa producing nitric oxide (NO), spermatozoa with intact membranes, spermatozoa with high mitochondrial membrane potential, apoptotic, necrotic and viable spermatozoa in dogs with benign prostatic hyperplasia (BPH) and non-affected (control) dogs.

Variables	BPH n=20	Control n=20
NO production %	36.46±20.92[a]	25.48±9.98[b]
PMI %	76.54±14.73	82.03±6.42
MMP%	79.28±20.92	84.34±9.64
apoptotic spermatozoa %	11.32±5.98	9.89±8.92
dead spermatozoa %	29.97±14.81	25.21±7.34
live spermatozoa %	59.70±17.09	63.54±15.04

NO nitric oxide, PMI plasma membrane integrity, MMP mitochondrial membrane potential
a, b – values with different superscripts are statistically different between the groups ($p<0.05$)

RAPID was statistically significantly ($p<0.05$) lower in BPH dogs than in control dogs (46.35±19.80% vs 58.60±16.52%), whereas in motility subcategory STATIC the proportion was significantly higher (22.75±12.77% vs 14.35±7.28%). The percentage of normal spermatozoa was statistically significantly ($p<0.05$) lower in dogs with BPH than in non-affected dogs (62.33±9.73 % vs 79.50±8.04%). There were no statistically significant ($p>0.05$) differences between both groups in other motility parameters (VAP, VSL, VCL, ALH, BCF, LIN, SLOW, MEDIUM).

The percentage of NO-producing spermatozoa, spermatozoa with intact membranes, spermatozoa with high membrane potential, apoptotic, necrotic and viable spermatozoa are shown in Table 2.

The percentage of spermatozoa producing NO was significantly ($p<0.05$) higher in BPH dogs than in control dogs (36.46±20.92% vs 25.48±9.98%). The percentage of spermatozoa with intact membranes, spermatozoa with high mitochondrial membrane potential and live spermatozoa tended to be lower in dogs with BPH compared to non-affected dogs, but the differences were not statistically significant ($p>0.05$). The percentage of spermatozoa with apoptic-like changes and dead spermatozoa was numerically higher in dogs with BPH than in control dogs, but the differences were not statistically significant ($p>0.05$).

Discussion

The dogs with BPH showed decreased semen quality. In general, there were large individual differences in semen quality parameters. However, the mean percentage of motile sperm and sperm with a progressive motility, and the proportion of sperm in motility subcategory RAPID was significantly lower in dogs with BPH than in non-affected dogs, whereas the proportion of sperm in motility subcategory STATIC was significantly higher. The percentage of normal sperm was significantly lower in BPH dogs than in controls. Similarly, low semen quality in dogs with BPH was found also in other studies. Krakowski et al. (2015) found no significant changes in standard semen parameters, but a significant increase in DNA defragmentation of sperm and elevated percentages of primary defects in spermatozoa in dogs with BPH compared to healthy dogs. Flores et al. (2017) reported that BPH decreases sperm DNA integrity and mitochondrial activity, and also alters sperm movement pattern. In the study of Aquino-Cortez et al. (2017) dogs with BPH presented lower sperm motility, vigour, concentration and percentage of sperm with a functional membrane than control dogs. The percentage of sperm with primary morphological changes was greater in BPH dogs. Angrimani et al. (2020) found that BPH dogs had a lower sperm count, a higher percentage of minor sperm defects, and altered sperm kinetics. More recently, Niżański et al. (2022) observed a lower proportion of spermatozoa with progressive motility and in the RAPID motility subcategory, as well as a higher proportion of sperm defects in dogs with BPH compared to healthy dogs.

In this study, NO production in the spermatozoa of dogs with PBH was determined for the first time. The percentage of spermatozoa producing NO was significantly higher in BPH dogs than in control dogs. It is therefore possible that the reduced sperm motility parameters in dogs with BPH are related to increased production of NO. Rosselli et al. (1995) showed that treatment of human spermatozoa with NO donors decreased forward progressive sperm motility and straight line velocity, and also increased the percentage of immotile spermatozoa in a concentration-dependent manner. Balercia et al. (2004) reported a significant negative correlation between NO concentration in human semen and the percentage of total sperm motility.

MMP is the parameter that reflects mitochondrial function and is an indicator of mitochondrial energy status. In humans, a correlation has been found bet-

ween impaired mitochondrial function, demonstrated by a reduction in MMP, and reduced sperm motility and reproductive ability (Paoli et al. 2011). High levels of reactive nitrogen species, such as peroxynitrate (a nitrogen oxide-derived oxidant), resulted in a decrease in the MMP of human spermatozoa (Uribe et al., 2015). In our study, MMP tended to be lower in BPH dogs than in control dogs. There have been only a few studies on MMP in canine spermatozoa. Volpe et al. (2009) reported that the presence of a high MMP was more strongly correlated to sperm viability than to sperm motility. Strzeżek et al. (2015) observed seasonal variation in the percentage of spermatozoa with high MMP and motility parameters. Flores et al. (2017) observed decreased mitochondrial activity in BPH dogs compared to controls.

Analysis of PMI, responsible for the preservation of cellular homeostasis, allows the evaluation of sperm viability and the prediction of potential sperm survival inside the female reproductive tract (Lampiao et al. 2014). High levels of NO cause adverse effects on the sperm plasma membrane and stimulate apoptosis (Doshi et al. 2012). There was a negative correlation between NO generation in boar spermatozoa preserved in short-term extender and plasma membrane integrity (Orzołek et al. 2018). In our study, there was only a tendency to a lower proportion of spermatozoa with intact membranes in dogs with BPH compared to control dogs. Similarly, Flores et al. (2017) found no significant difference in PMI between dogs with BPH and healthy dogs.

It has been shown that high concentrations of NO are associated with an increase in sperm cell apoptosis rate in men (Lampiao et al. 2014). In this study the percentage of spermatozoa with apoptic-like changes and dead spermatozoa was numerically higher in dogs with BPH than in control dogs but was not statistically different. This is consistent with the results of Krakowski et al. (2015), who found no differences in the percentage of viable sperm between BPH dogs and controls.

The results of our study indicate a trend towards nitrosative stress, a subtype of oxidative stress, characterized by overproduction of NO in the spermatozoa of dogs with BPH. This suggests the potential importance of NO in reducing sperm quality in BPH dogs. So far there have been only a few studies on oxidative stress in dogs with BPH. Dearakhshandeh et al. (2019) reported a decrease in antioxidant enzyme level activities in the blood serum of dogs induced for BPH by testosterone and estradiol. Angrimani et al. (2020) found no significant differences in the oxidative profile of prostatic fluid between BPH dogs and non-affected dogs. Our earlier study showed significantly lower serum total antioxidant activity in dogs with BPH compared to non-affected dogs (2022). In addition to oxidative stress, haematospermia, hormonal imbalance and biochemical changes in the prostatic fluid are discussed as possible causes of reduced semen quality in dogs with BPH.

Haematospermia is a common clinical symptom observed in BPH dogs. The effect of the presence of erythrocytes in the semen on fertility of dogs is controversial. Dogs with some blood in their ejaculates may be fertile (Fontbonne et al. 2011). On the other hand, iron contained in haemoglobin can contribute to generation of ROS (Agarwal et al. 2014). Rijsselaere et al. (2004) showed that blood additions of up to 10% exerted no negative effects on the functional characteristics of chilled canine spermatozoa, but had negative effects on cryopreserved spermatozoa due to the high amount of haemoglobin originating from the red blood cell haemolysis observed after freezing and thawing.

Lowered sperm quality in dogs with BPH may be the result of hormonal imbalance. BPH is associated with an increased ratio of estrogen to androgen. BPH dogs had significantly lower testosterone levels than healthy dogs (Cochran et al. 1981, Angrimani et al. 2020, Niżański et al. 2022). Testosterone plays an essential role in maintaining spermatogenesis (Ramaswamy and Weinbauer 2015). Decreased testosterone concentration in dogs with BPH may affect sperm quality.

The prostate is the sole accessory gland in dogs. The prostatic fluid has a major role in the production of seminal plasma during ejaculation. It constitutes approximately 90% of seminal fluid volume and provides transport and sperm support (Ferré-Dolcet et al. 2022). Prostate diseases can cause significant changes in the seminal plasma, resulting in reduced semen quality. However, there are only few studies on the biochemistry of the prostatic fluid in dogs with BPH. Krakowski et al. (2015) observed that in dogs with BPH, the pH of prostatic secretions was higher and cholesterol concentrations increased, while zinc (Zn) and copper concentrations decreased. Ferré-Dolcet et al. (2022) reported low Zn concentrations in the the prostatic fluid when BPH was diagnosed in dogs. Zn concentration in the prostatic fluid has been positively correlated with an improvement in semen quality during treatment with osaterone acetate. Concentrations of glucose, cholesterol and triglycerides in prostate fluid were higher in dogs with BPH than in healthy dogs, suggesting that these could be used as biomarkers of prostate enlargement in dogs with BPH (Aquino-Cortez et al. 2017).

We are aware that our study has a limitation. BPH was diagnosed only on the basis of ultrasound examination and clinical symptoms. Prostatic biopsy for cyto-

logy or histology is considered the gold standard for the diagnosis of BPH in the living animal (Smith 2008). The study was conducted on a clinical population of client-owned dogs and the dog owners did not agree to the biopsy.

Conclusions

In conclusion, the study showed that BPH adversely affects semen quality, especially motility parameters, in dogs. The decreased semen quality was associated with an increased proportion of spermatozoa generating NO. This suggests that increased NO production may play a causal role in the impairment of semen quality in dogs with BPH. Further research is needed to clarify the mechanisms by which BPH affects semen quality.

References

Agarwal A, Virk G, Ong C, du Plessis SS **(2014)** Effect of oxidative stress on male reproduction. World J Mens Health 32: 1-17.

Angrimani DS, Brito MM, Rui BR, Nichi M, Vannucchi CI **(2020)** Reproductive and endocrinological effects of Benign Prostatic Hyperplasia and finasteride therapy in dogs. Sci Rep 10: 14834.

Aquino-Cortez A, Pinheiro BQ, Lima DB, Silva HV, Mota-Filho AC, Martins JAM Rodriguez-Villamil P, Moura AA, Silva LD **(2017)** Proteomic characterization of canine seminal plasma. Theriogenology 95: 178-186.

Balercia G, Moretti S, Vignini A, Magagnini M, Mantero F, Boscaro M, Ricciardo-Lamonica G, Mazzanti L **(2004)** Role of nitric oxide concentrations on human sperm motility. J Androl 25: 245-249.

Berry SJ, Strandberg JD, Saunders WJ, Coffey DS **(1986)** Development of canine benign prostatic hyperplasia with age. Prostate 9: 363-73.

Carson C 3rd, Rittmaster R **(2003)** The role of dihydrotestosterone in benign prostatic hyperplasia. Urology 61 (4 Suppl 1): 2-7.

Cochran RC, Ewing LL, Niswender GD **(1981)** Serum levels of follicle stimulating hormone, luteinizing hormone, prolactin, testosterone, 5 alpha-dihydrotestosterone, 5 alpha-androstane-3 alpha, 17 beta-diol, 5 alpha-androstane-3 beta, 17 beta-diol, and 17 beta-estradiol from male beagles with spontaneous or induced benign prostatic hyperplasia. Invest Urol 19: 142-147.

Cunto M, Ballotta G, Zambelli D **(2022)** Benign prostatic hyperplasia in the dog. Anim Reprod Sci 247: 107096.

Dearakhshandeh N, Mogheiseh A, Nazifi S, Ahrari Khafi MS, Abbaszadeh Hasiri M, Golchin-Rad K **(2019)** Changes in the oxidative stress factors and inflammatory proteins following the treatment of BPH induced dogs with an anti-proliferative agent called tadalafil. J Vet Pharmacol Ther 42: 665–672.

Domosławska A, Zduńczyk S, Kankofer M, Bielecka A **(2022)** Oxidative stress biomarkers in dogs with benign prostatic hyperplasia. Ir Vet J 75: 21.

Domosławska A, Zduńczyk S, Niżański W, Janowski T **(2013)** Assessment of semen quality in infertile dogs using computer-assisted sperm analysis by the Hamilton-Thorne Semen Analyser. Bull Vet Inst Pulawy 57: 429-432.

Doshi SB, Khullar K, Sharma RK, Agarwal A **(2012)** Role of reactive nitrogen species in male infertility. Reprod Biol Endocrinol 10: 109.

Fafula RV, Iefremova UP, Onufrovych OK, Maksymyuk HV, Besedina AS, Nakonechnyi IA, Vorobets DZ, Vorobets ZD **(2018)** Alterations in Arginase-NO-synthase System of Spermatozoa in Human Subjects with Different Fertility Potential. J Med Biochem 37: 134-140.

Ferré-Dolcet L, Frigotto L, Contiero B, Bedin S, Romagnoli S **(2022)** Prostatic fluid composition and semen quality in dogs with benign prostatic hyperplasia undergoing treatment with osaterone acetate. Reprod Domest Anim 57: 72-79.

Flores RB, Angrimani D, Rui BR, Brito MM, Abreu RA, Vannucchi CI **(2017)** The influence of benign prostatic hyperplasia on sperm morphological features and sperm DNA integrity in dogs. Repro Domest Anim 52 (Suppl 2): 310-315.

Fontbonne A **(2011)** Infertility in male dogs: recent advances. Rev Bras Reprod Anim 35: 266-273.

Fraser L, Lecewicz M, Strzeżek J **(2002)** Fluorometric assessments of viability and mitochondrial status of boar spermatozoa following liquid storage. Pol J Vet Sci 5: 85-92.

Garner DL, Johnson LA **(1995)** Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. Biol Reprod 53: 276–284.

Herrero MB, Pérez Martínez S, Viggiano JM, Polak JM, de Gimeno MF **(1996)** Localization by indirect immunofluorescence of nitric oxide synthase in mouse and human spermatozoa. Reprod Fertil Dev 8:931-934.

Krakowski L, Wąchocka A, Brodzki P, Wrona Z, Piech T, Wawron W, Chałabis-Mazurek A **(2015)** Sperm quality and selected biochemical parameters of seminal fluid in dogs with benign prostatic hyperplasia. Anim Reprod Sci 160: 20-125.

Lampiao F, Huussen J, du Plessis SS **(2014)** Effects of nitric oxide exposure on human sperm function and apoptosis markers. Open Reprod Sci J 6: 17-20.

Lampiao F, Strijdom H, du Plessis SS **(2006)** Direct nitric oxide measurement in human spermatozoa: flow cytometric analysis using the fluorescent probe, diaminofluorescein. Int J Androl 29:564-567. DOI: 10.1111/j.1365-2605.2006.00695.x.

Lévy X, Niżański W, von Heimendahl A, Mimouni P **(2014)** Diagnosis of common prostatic conditions in dogs: an update. Reprod Domest Anim 49 (Suppl 2): 50-57.

Linde-Forsberg C **(1991)** Achieving canine pregnancy by using frozen or chilled extended semen. Vet Clin North Am Small Anim Pract 21: 467-485.

Luo Y, Zhu Y, Basang W, Wang X, Li C, Zhou X **(2021)** Roles of Nitric Oxide in the Regulation of Reproduction: A Review. Front Endocrinol (Lausanne) 12: 52410.

Minciullo PL, Inferrera A, Navarra M, Calapai G, Magno C, Gangemi S **(2015)** Oxidative stress in benign prostatic hyperplasia: a systematic review. Urol Int 94: 249-254.

Niżański W, Eberhardt M, Ochota M, Fontaine C, Levy X, Pasikowska J **(2022)** A Comparative Study of the Effects of Osaterone Acetate and Deslorelin Acetate on Sperm

Kinematics and Morpho-Functional Parameters in Dogs. Animals (Basel) 12: 1548.

Orzołek A, Zasiadczyk Ł, Wysocki P, Kordan W, Krysztofiak P **(2018)** Relation between nitric oxide (NO) level in semen and certain properties of boar spermatozoa stored at 17°C. Pol J Vet Sci 21: 423-426.

Paoli D, Gallo M, Rizzo F, Baldi E, Francavilla S, Lenzi A, Lombardo F, Gandini L **(2011)** Mitochondrial membrane potential profile and its correlation with increasing sperm motility. Fertil Steril 95: 2315-2319.

Polisca A, Troisi A, Fontaine E, Menchetti L, Fontbonne A **(2016)** A retrospective study of canine prostatic diseases from 2002 to 2009 at the Alfort Veterinary College in France. Theriogenology 85: 835-840.

Ramaswamy S, Weinbauer GF **(2015)** Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. Spermatogenesis 4: e996025.

Rijsselaere T, Van Soom A, Maes D, Verberckmoes S, de Kruif A **(2004)** Effect of blood admixture on in vitro survival of chilled and frozen-thawed canine spermatozoa. Theriogenology 61: 1589-1602.

Rosselli M, Dubey RK, Imthurn B, Macas E, Keller PJ **(1995)** Effects of nitric oxide on human spermatozoa: evidence that nitric oxide decreases sperm motility and induces sperm toxicity. Hum Reprod 10: 1786-1790.

Ruel Y, Barthez PY, Mailles A, Begon D **(1998)** Ultrasonographic evaluation of the prostate in healthy intact dogs. Vet Radiol Ultrasound 39: 212-6.

Smith J **(2008)** Canine prostatic disease: a review of anatomy, pathology, diagnosis, and treatment. Theriogenology 70: 375-383.

Strzeżek R, Szemplińska K, Filipowicz K, Kordan W **(2015)** Semen characteristics and selected biochemical markers of canine seminal plasma in various seasons of the year. Pol J Vet Sci 18: 13-18.

Thomas CA, Garner DL, DeJarnette JM, Marshall CE **(1998)** Effect of cryopreservation of bovine sperm organelle function and viability as determined by flow cytometry. Biol Reprod 58: 786-793.

Tong Y, Zhou RY **(2020)** Review of the roles and interaction of androgen and inflammation in benign prostatic hyperplasia. Mediators Inflamm 2020: 7958316.

Trzcińska M, Bryła M **(2015)** Apoptotic-like changes of boar spermatozoa in freezing media supplemented with different antioxidants. Pol J Vet Sci 18: 473-480.

Uribe P, Boguen R, Treulen F, Sánchez R, Villegas JV **(2015)** Peroxynitrite-mediated nitrosative stress decreases motility and mitochondrial membrane potential in human spermatozoa. Mol Hum Reprod 21: 237-243.

Vital P, Castro P, Ittmann M **(2016)** Oxidative stress promotes benign prostatic hyperplasia. Prostate 76: 58-67.

Volpe S, Leoci R, Aiudi G, Lacalandra GM **(2009)** Relationship between motility and mitochondrial functional status in canine spermatozoa. Reprod Domest Anim 44 (Suppl 2): 275-278.