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Sperm quality and selected biochemical parameters of seminal fluid in dogs with benign prostatic hyperplasia

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ABSTRACT

Benign prostatic hyperplasia (BPH) in dogs is most commonly associated with age and increasing concentrations of dihydrotesterone, a hormone that stimulates growth and secretion of the prostatic epithelial cells. During this process, the biochemical composition of prostatic secretion changes, which can affect the quality of semen and limit the ability of the sperm to contribute to fertilization. Therefore, the present study was conducted to examine possible correlation between BPH and biological quality of semen. The study was performed in 11 sexually mature dogs of various breeds. Animals were divided into two groups: healthy dogs (Group I; $n = 5$; mean age 4.32; SEM = 1.28) and dogs with BPH (Group II $n = 6$; mean age 6.16; SEM = 0.65). Semen and prostate secretions were collected and evaluated in this study. Standard semen examinations were conducted in the ejaculates collected; moreover, the extent of apoptosis and DNA defragmentation was determined. The selected biochemical parameters were determined in the prostate secretion. According to the examination results, there were no significant differences in standard semen parameters between the two groups of dogs. Nevertheless, morphological tests of semen in dogs with BPH demonstrated elevated percentages of primary defects in spermatozoa. A significant increase ($P = 0.01$) in DNA defragmentation of sperm was found in dogs with BPH. Moreover, changes in the biochemical composition of prostate secretion were demonstrated. In dogs with BPH, pH of prostate secretions was greater ($P = 0.03$), concentrations of cholesterol increased while concentrations of Zn and Cu decreased. The study findings reveal that BPH does not change semen quality in dogs.

1. Introduction

The seminal fluid is physiologically composed of secretions by various glands of the male reproductive system and its biochemical composition diversely affects many sperm functions. In dogs, the prostate is the only accessory sex gland. Its secretion constitutes 90% of the entire ejaculate volume and has several important functions (Hewitt, 2001). First of all, it affects the sperm transport while proteins and bio-elements contained in it influence sperm metabolism and motility (Arienti et al., 2004; Dacheux and Dacheux, 2001). Among the basic constituents of seminal fluid, phospholipid-binding proteins, metal ions and some bio-elements, such as potassium, zinc, sodium, magnesium, copper, calcium and iron, are essential (Branam et al., 1984). For example, zinc ions have bactericidal effects

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protecting against urinary infections and affects the stabilization of DNA contained in sperm heads (Bjorndahl and Kvist, 2011; Dacheux and Dacheux, 2001). Compared to other species of animals, the prostate secretion in dogs contains lesser amounts of sugars which constitute energy component and affect survivability and motility of sperm (Branam et al., 1984). Moreover, the prostatic alveoli contain large amounts of cholesterol, proteins and calcium that are transported to sperm through membrane fusion (Dube et al., 1987; Gobello et al., 2002). Additionally, several studies indicate that the prostatic secretion is important for fertilization. For example, pH changes and zinc deficiencies can decrease the rate of sperm capacitation and impair acrosomal reactions, which can result in lack of fertilization (Dacheux and Dacheux, 2001; Fontbone, 2011; Henkel et al., 1999; Meyers-Wallen, 1991; Neal Jr et al., 1993; Riffo et al., 1992).

One of the most common reproductive disorders in dogs is benign prostatic hyperplasia (BPH) (Berry et al., 1986; Blum et al., 1985; Johnston et al., 2000; Krawiec and Heflin, 1992). Benign hyperplasia of this gland is a natural consequence of ageing in dogs. According to the literature, the prostatic glandular tissue undergoes modification in 80% of dogs greater than 5 years of age, which leads to prostatic hyperplasia (Atalan et al., 1999; Berry et al., 1986; Barsanti et al., 1983; Krawiec and Heflin, 1992; Paclikova et al., 2006). The prostatic gland requires testosterone produced in the testicular tissue to develop and function properly. The enzyme, 5-alpha-reductase, converts testosterone to dihydrotestosterone, a hormone which interacts with prostate receptors causing its growth. As a dog ages, the number of receptors as well as testosterone secretion increase. The key role in BPH pathogenesis is also ascribed to the effect of estradiol and other mitogenic factors causing prostatic hyperplasia. Furthermore, chronic inflammations also predispose dogs to BPH (Branam et al., 1984).

Due to uncontrolled proliferation of cells, dogs develop prostatic hyperplasia and health-related complications with increasing age. BPH in dogs as well as in humans is likely to be one of the major causes of impaired fertility (Dacheux and Dacheux, 2001; Dohle et al., 2005; Fontbone, 2011; Leib et al., 1994). The significant changes in biochemical composition of the prostatic secretion can impair semen nutrition, which results in poor viability, motility and greater numbers of secondary morphological changes in sperm (Branam et al., 1984; Marconi et al., 2009; Sirivaidyapong et al., 2001).

The available information regarding the effects of hyperplastic prostate changes in dogs on semen quality are sparse. The purpose of the present study, therefore, was to evaluate the effects of prostatic hyperplastic changes in dogs on selected biochemical parameters of seminal fluid and sperm characteristics.

2. Materials and methods

2.1. Animals

The study was conducted in 11 healthy, sexually mature dogs of various breeds (one Labrador, one Pointer, two Golden Retrievers, three Dobermans, two Bernese Mountain, one Fox Terrier and one Mastiff) aged 3–7 years and weighing 16–80 kg, from private kennels. Dogs were fed individually with balanced diets, and water was offered ad libitum. Dogs were regularly used for reproduction.

2.2. Clinical examination

Each dog underwent a prostate examination, first per-rectum and subsequently by trans-abdominal ultrasonography using the frequency of 7.5 MHz (Aloka SSD500, Mitaka-Shi, Tokyo, Japan). Based on the measurements performed (length, width and depth of lesions in the glandular tissue), dogs were divided into two groups: Group I ($n = 5$; mean age 4.32 ± 1.28) – healthy dogs with normal prostates and Group II ($n = 6$; mean age 6.17 ± 0.65; $P = 0.25$) dogs with prostatic hyperplasia. Dog body weight in both groups was not statistically different $P = 0.74$; Table 2. Diagnosis was based on clinical and ultrasonography examination only and was not confirmed by histopathology.

2.3. Sperm and prostatic fluid collection

Two ejaculates were collected from each dog at 1-week intervals. Semen was collected using manual massage (Linde-Forsberg and Forsberg, 1993) into sterile silicon tubes using the following protocol: initially the first and second semen fraction and subsequently the third fraction (prostatic fluid secretion). Seminal fluid was separated by centrifugation at $700 \times g$ for 15 mintes and frozen at $-80\,^{\circ}C$ until used for investigation.

2.4. Sperm analysis

The initial study of ejaculates involved pH, volume, concentration, motility, viability (live/dead) and sperm morphological characteristics. Prostatic fluid pH was determined using a highly sensitive pHydrion test paper (pH value ranges from 6.0 to 8.0, Micro Essential Lab, USA) and compared with colours on a colour chart metre.

The sperm concentration was determined photometrically (SpermaCue – Minitüb Abfüll- und Laboretechnik GmgH, Tiefebach, Germany). The percentage of static, non-progressive motility, progressive motility and the percentage of slow, medium and rapid spermatozoa was determined using a sperm class analyser (SCA) (MICROPTIC S.L, Barcelona, Spain). The system is composed of a light microscope Olympus CX4 with a Basler A312 camera and a computer with suitable software (SCA® 2005). The 20 μl Leja slides were used for motility evaluation (Leja Amsterdam, The Netherlands); 5 μl of diluted semen (25×10^6 spz/ml) heated to $37\,^{\circ}C$ were used for each analysis. Analysis was conducted in 3 of 4 fields containing at least 300 sperm, each. Morphological examinations were performed using the Diff Quik® kit (Sigma–Aldrich, Vienna, Austria) described by Mota and Ramalho-Santos (2006). The percentages of morphologically normal spermatozoa and those with primary and secondary defects were determined. The percentage of live/dead spermatozoa was determined by staining the specimens with eosin and nigrosin, and evaluating 300 sperm. Subsequently, sperm