THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Agricultural Biotechnology)

DEGREE

Agricultural Biotechnology | Interdisciplinary Graduate Program

FIELD | **PROGRAM**

TITLE: Effect of Deslorelin and Finasteride on Benign Prostatic Hypertrophy Treatment in Dogs: A Clinical Trial

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THESIS

EFFECT OF DESLORELIN AND FINASTERIDE ON BENIGN PROSTATIC HYPERTROPHY TREATMENT IN DOGS: A CLINICAL TRIAL

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A Thesis Submitted in Partial Fulfilment of
the Requirements for the Degree of
Master of Science (Agriculture Biotechnology)
Graduate School, Kasetsart University
2012

Chunsumon Limmanont 2012: Effect of Deslorelin and Finasteride on Benign Prostatic Hypertrophy Treatment in Dogs: A Clinical Trial. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Kaitkanoke Sirinarumitr, Ph.D. 103 pages.

The study was aimed to proof, a GnRH agonist, the new alternative medical treatment in dog with benign prostatic hypertrophy (BPH). The study design was a clinical trial on the effects and adverse effects of deslorelin (a GnRH agonist) compared to finasteride (5 alpha reductase blocker) treatments and the disease recurrent time after both treatments cessations on natural BPH in dogs. Eight BPH dogs were assigned and be implanted a single dose 4.7 mg deslorelin, and another eight BPH dogs were received finasteride orally, once a day for 16 wk. Each dog was evaluated for clinical sings, skin reaction (only implanted dogs), prostatic volume, testicular volume, semen quality, semen bacterial culture, seminal cytology, blood profile, serum testosterone (T) and dyhydrotestosterone (DHT)concentrations. Adverse effects were continued to follow up at 8 and 16 wk after both treatments cessations. The clinical signs were resolved, and prostatic size and volume were decreased to normal approximately 4 wk after treatment of both medications. Deslorelin is effect on follicular stimulating hormone and luteinizing hormone down regulation, following with decrease in androgens production and secretion, including T and DHT, consequencing with spermatogenesis suppression, prostate gland shrinkage, decrease in testicular volume, and leading to anejaculation. Finasteride is effect on hypertrophic prostatic cells by type II – 5 alpha reductase inhibitor causing decrease in DHT and consquencing with prostate gland shrinkage. Finasteride does not effect on T, testicular volume, spermatogenesis, so it does not effect on semen quality. The adverse effects of both treatments were not found in the study. Anejaculation phenomenon effected on dogs at least for 40 wk after deslorelin implantation. Prostatitis should be cautioned with finasteride treatment dogs because the inflammation may be complicated during treatment and/or after treatment cessation. Conclusion, both medications are effective on BPH treatment. The disease recurrent should be monitored after treatment cessations because both medications are temporary.

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Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to sincerely acknowledge many persons who supported this research. The research could not been successfully finished without them.

Special acknowledgement to Assoc. Prof. Dr. Kaitkanoke Sirinarumitr, my thesis advisor who is my inspirations. The numerous of her kindness always be taken for advising, supporting and promoting to me. She is also my second mother and my lovely sister.

For this research successful, there are many persons who always supported me, including my lecturers, colleagues, and academic officers both at the Center of Agricultural Biotechnology, Kasetsart University, Kamphansean campus and Kasetsart University Veterinary Teaching Hospital (KUVTH), Bangkean. Mrs. Pimon Verasit, a special technician at Reproductive Clinic, is my lovely technical trainer. Unfortunately, she was passed away during my research working. Dr. Janjira Phavaphutanon is my kindly thesis co-advisor. Twenty two dogs, my subjects in the clinical trial, were attended by their enthusiastic owners. Place and some facilities were provided by the Department of Companian Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, KUVTH and Kasetsart University Veterinary Diagnosis Centre, Bangkhaen campus. Deslorelin, hormone implantations, was kindly supported by Peptech Animal Health, Australia. Statistical analysis was cordially consulted Dr. Chaithep Poolkhet, lecturer at the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Kasetsart University.

The last from my heart, no more words is adequate to thank my family, including my parents, my elder brother, my elder sister, and their families with my lovely nephew and nieces. All of them are my soul to do everything.

Chunsumon Limmanont

July 13, 2012

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LIST OF ABBREVIATIONS

ALKP	=	Alkaline phosphatase
ALT	=	Alanine transaminase
BMP4	=	Bone morphogenetic protein 4
BPH	=	Benign prostatic hypertrophy
BUN	=	Blood urea nitrogen
CBC	=	Complete blood count
CFU	=	Colony forming unit
CT	=	Computed tomography
EIA / ELISA	=	Enzyme immunoassays/ Enzyme-linked immunosorbent assay
DHT	=	Dihydrotestosterone
FGF10	=	Fibroblast growth factor 10
FSH	=	Follicle stimulating hormone
GnRH	=	Gonadotrophin releasing hormone
LH	=	Luteinizing hormone
MRI	=	Magnetic resonance imaging
PO	=	Per os (to take orally, by mouth)
SD	=	Standard deviation
Shh	=	Sonic hedgehog
T	=	Testosterone
TAUS	=	Transabdominal ultrasonography
TGF-ß	=	Transforming growth factor beta
TRUS	=	Transrectal ultrasonography

EFFECT OF DESLORELIN AND FINASTERIDE ON BENIGN PROSTATIC HYPERTROPHY TREATMENT IN DOGS: A CLINICAL TRIAL

INTRODUCTION

Benign Prostatic Hypertrophy (BPH) is a common and spontaneous prostatic disorder in an intact old dog. The higher risk of BPH will be found in dog over 5 years old. There are many factors for BPH initiation such as dihydrotestosterone, testosterone, estrogen, and growth factors. However, dihydrotestosterone (DHT) which is converted from testosterone by 5-alpha reductase is considered a main cause of prostate gland enlargement. The recommended permanent treatment for dogs with BPH is castration. However, medical treatment should be considered in breeding valuable stud dogs or dogs with high risk of anesthesia.

Finasteride, a 5-alpha reductase blocker, is accepted and used widely for BPH and hair loss treatment in men. Finasteride, type II 5-alpha reductase inhibitor, has been used for treating dog with BPH. However, finasteride is an expensive drug and only temporary decrease in prostatic size, and dog needs to be administered daily. Drug should be cautious in pregnant woman, especially while she carries male fetus. She may be contaminated and absorbed with medicine during administer finasteride to dogs with BPH. Type II 5-alpha reductase inhibitor may cause abnormalities of the external genitalia of a male fetus in these pregnant women.

Deslorelin, GnRH agonist, affects at gonadotropin-producing cells of anterior pituitary gland. Long term effect of deslorelin on anterior pituitary gland causing a down regulation and followed with reversibly blocks production and releasing of the follicle stimulating hormone (FSH) and luteinizing hormone (LH). Deslorelin is used treating in fertility control, behavioral related sex hormone control, urinary incontinence and estrus control in dogs. Although there are amount of deslorelin experiments in dog in the area of fertility control, estrus control and urinary incontinence, there is no report a treatment on clinical dog with BPH. Deslorelin is determined to treat BPH in dog due to the action of testosterone suppression.

At the present time, most old intact male dogs were referred to Reproduction Clinic, at Kasetsart University Veterinary Teaching Hospital, Bangkok with problems of prostatic disorders, including BPH. Treatment protocols for dogs with BPH are either castration or finasteride treatment. Castration is a permanent BPH treatment; however finasteride is used for BPH temporary treatment. Dog with BPH has to be received finasteride daily for at least 1-4 months. Finasteride is an expensive medicine, so client has to spend a lot of money for the medical treatment protocol, and also need to administer drug daily to the dog. Finasteride is also cautious for pregnant women. Deslorelin, a GnRH agonist, is more convenient for BPH treatment protocol. One deslorelin implantation is lasted for 6-12 months, however, the side effects have not been reported. Deslorelin is considered may be an alternative medical treatment for dogs with BPH.

OBJECTIVES

1. Overall objective - To study in a clinical trial on the effects of deslorelin compared to finasteride on natural benign prostatic hypertrophy treatment in dogs.

2. Specific objectives

2.1 To study the effects of deslorelin compared to finasteride on clinical signs, prostatic volume, testicular volume, semen quality, serum testosterone and dihydrotestosterone concentrations on dogs with BPH.

2.2 To study the adverse effects of desolerin and finasteride treatment on dogs with BPH.

2.3 To study the reversible of the disease after treatment cessation of deslorelin and finasteride on dogs with BPH.

LITERATURE REVIEW

1. Prostate gland

Prostate gland is only one accessory sex gland in male dog. The other accessory sex glands, such as seminal vesicle and bulbourethral gland (cowper's) are not developed in dog. The main function of prostate gland is prostatic fluid secretion which is the majority component of semen in dog. Prostatic fluid nourishes sperm cells and creates large amount volume and helps much easier for sperm movement in female reproductive tract (Samuelson, 2007). There are some antibacterial properties in prostatic fluid, and they help to decrease the chances of infection from semen contamination in the female genital tract (Finco, 1980).

1.1 Embryonic development In embryonic period, prostate develops from the sinus urogenitalis which is controlled by androgens, growth factors and epithelio-mesenchymal interactions. The prostate parenchyma is a complex of prostatic buds (Figure 1) (Poul *et al*, 2010). On molecular and gene expression, transcription factors, Hoxa-13 and Hoxd-13, are the keys of gene expression found in the mouse. Dihydrotestosterone (DHT), sex hormone which is converted from Testosterone (T), acts through receptors in the mesenchymal cells, and induce fibroblast growth factor 10 (FGF10) and transforming growth factor beta (TGF-ß) to regulate the expression of sonic hedgehog (Shh) in the urogenital sinus epitheliums (Poul *et al*, 2010). Sonic hedgehog protein sends the cell-signalling to prostatic epithelium bud to evaginate from urogenital sinus to surrounding mesenchymal cells. Some of surrounding mesenchymal cells differentiates to smooth muscle cells. Moreover, prostatic bud is controlled by inhibition of bone morphogenetic protein 4 (BMP4) (Poul *et al*., 2010).

1.2 Prostate anatomy In male puppy at birth to 2 months old, the prostate is located in an abdominal cavity, after that, the prostate is moved into pelvic cavity, located at neck of urinary bladder, and directly below the rectum. Prostate is a bi-lobular structure, covered with thick smooth muscle fiber capsule, separated from prominent fibrous medial septum into right and left lobes. Dorsal longitudinal ridge of mucosa and the urethral crest cause prostate in cross section

like V-shape (Johnston *et al*., 2001). The pair of ductus defferents is opened to craniolateral of each prostate lobe (Figure 2) (Smith, 2008). Colliculus seminalis, the opening of prostatic duct, is located in a part of prostatic urethra (Figure 3) (Johnston *et al*., 2001). Prostate glands is also divided by histology into body type and disseminate type. In dog, human and some species, their prostates are body types which are located in a compact mass around the urethra, well defined by connective tissue and histologically differentiable into secretory areas and ducts. Other species have a disseminate type which the glandular tissue is embedded and diffused in the urethra mucosa. The cuboidal to columnar epithelium provides the lining for tubuloaveolar gland for prostatic secretion. There are numerous prostatic ducts intra prostate, so prostate is not collapsed when it is empty from secretion (Figure 1). Prostate is supplied by prostatic arteries, which arise from the pudendal artery. Arterial branches enter to dorsal or dorsolateral of gland surface, and penetrate to capsule and glandular tissue. Vein circulations are the prostatic and urethral veins. Lymph vessels empty into iliac lymph nodes (Smith, 2008). Pelvic nerve, parasympathetic, is related prostatic plexus and it induces prostatic fluid secretion. Hypogastric nerve, sympathetic, stimulates smooth muscle contraction for prostatic fluid secretion into prostatic ducts and urethra (Johnston *et al*., 2001)

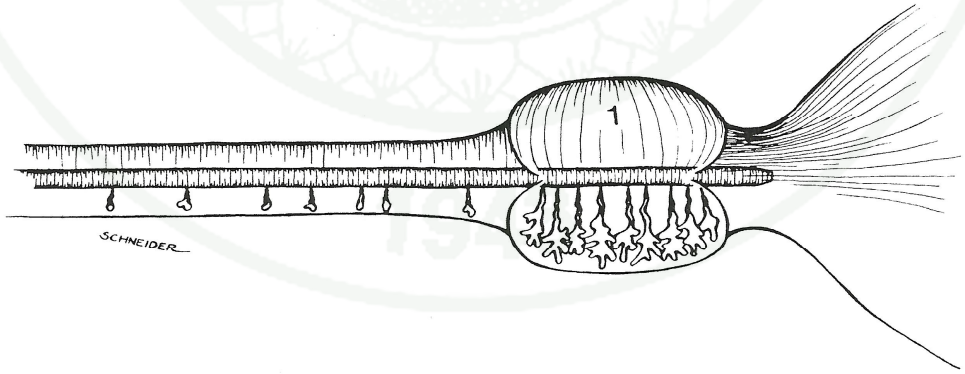

Figure 1 Dorsal view of schematic urethra, prostate and urinary bladder. There are the numerous prostatic ducts intra prostate.

Source: Adams (2004).

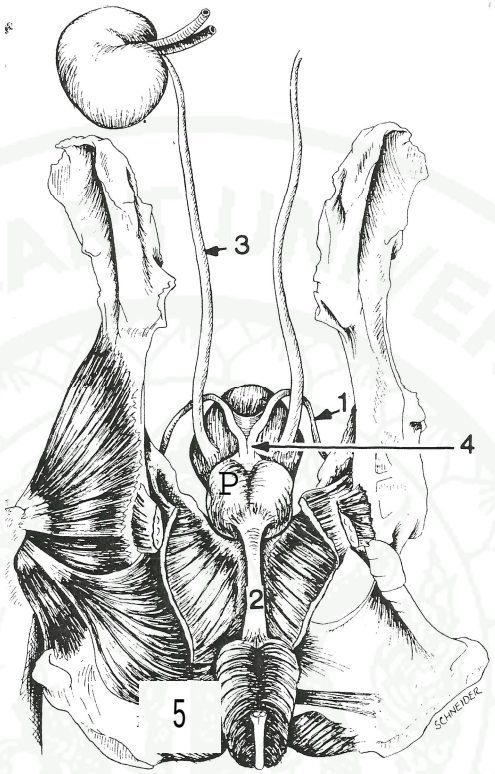

Figure 2 Dorsocaudal view of urogenital organ in male dog; ductus deference (1), pelvic urethra (2), ureter (3), ampulla of the ductus deferens (4), prostate (P).

Source : Adams (2004)

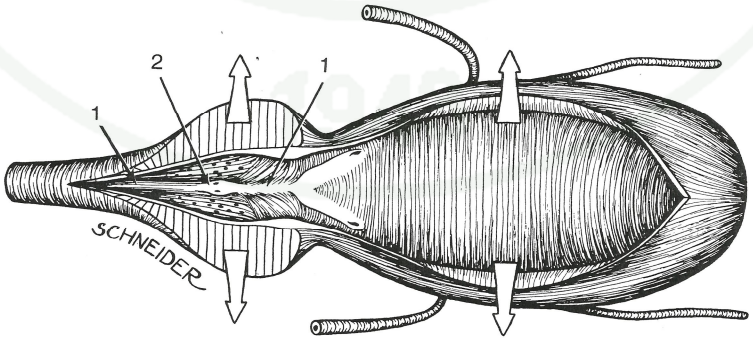

Figure 3 The portion of the ventral wall of the urethra and urinary bladder removed; urethral crest (1), colliculus seminalis (2).

Source : Adams (2004)

1.3 Prostatic regulation Androgen, a male hormone including T, is responsible for male sex characteristics and function. This is a prerequisite for normal development, physiological control, normal metabolic and secretory in the male. The prostate, an accessory sex gland, is also an androgen- dependent organ; DHT mainly regulates the prostate's development and function. DHT is converted from T by 5-alpha reductase type II intra canine prostate gland (Figure 4). Therefore, persistent androgen secretion over the life of dog causes gradual enlargement of the prostrate because of the proliferation of glandular and stromal components, leading to increase in size (hypertrophy) and number (hyperplasia) of prostatic epithelium cells. Castration will cause significant atrophy of the prostate gland because of lacking in androgen production. (Johnston *et al*., 2001)

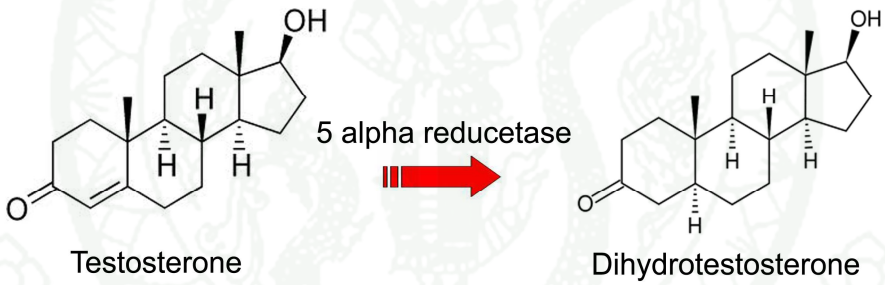

Figure 4 Chemical structure of T (left) and DHT (right) that is converted by 5 alpha reducetase (type II).

Source: Adapted from Sirinarumitr *et al*. (2001)

2. Benign prostatic hypertrophy (BPH)

Benign prostatic hypertrophy is urogenital diseases which only find in dog and human. It is a common and spontaneous prostatic disorder in an old intact male dog. BPH will cause the difficult urination and defecation in dogs due to an enlargement of prostate gland. Normal prostate weight was less than 15 g in mature intact male dog (Breg, 1958). The prostatic volume of dog with BPH was averaged 2-6.5 times compared with normal dog in the same body weight. Clinically, prostate volume can be calculated by ultrasonography (Ruel *et al*., 1998). Some sources described BPH in glandular hyperplasia and complex or cystic hyperplasia. Glandular

hyperplasia type is mostly found in younger intact male dog with age of 5-6 years old, however, 70 % of complex or cystic hyperplasia is found in older intact male dogs with age of 8- 9 years old (Robert, 1998). More than 80% of sexually intact male dog over 5 years have the BPH clinical signs, either blood on gross or microscopic finding in semen (Sirinarumitr *et al.,* 2001). Breed predisposition of BPH has not been reported (Memon, 2007).

2.1 Pathophysiology The intact male dogs have regularly androgen secretion, causing prostate cell growth continuous stimulated. Whenever the ratio of prostatic cell growth and cell death is imbalanced, such as prostatic cell growth is over than prostatic cell death, the prostate will develop prostatic hyperplasia. However, there are many factors involved in BPH development, but DHT is well accepted as a key hormone for prostate gland enlargement. DHT stands for 5- alpha DHT which is converted from circulating T by specific enzyme, 5- alpha reductase type II in canine prostatic cells. Most of DHT is synthesized in stromal cells. DHT acts in an autocrine action on the prostatic stromal cells, and paracrine action on prostatic glandular epithelial cells. The mitogenesis of both stromal and epithelial cells are processed when DHT binds to nuclear androgen receptors, and sends the signaling for growth factors transcription. Besides DHT, the other sex hormones such as intraprostatic estrogen, some growth factors and intracellular receptors, are all influence to BPH development (Lee *et al*. , 1997, Johnston *et al*., 2001 and Sirinarumitr *et al.,* 2001).

On the other hand, the development of BPH in human has been proved that it is involved in aging and the present of functional testicles for over centuries (White, 1893 and Rand, 1895). A few decades, etiological factors of BPH are proposed in possible role of intrinsic and extrinsic factors in prostatic cell growth. Intrinsic factors are the signalling interaction between stromal and epithelium compartments within the prostate. Growth factors, TGF-ß, keratinocyte growth factor, and their receptor proteins are signalled to cells in a paracrine fashion. Some of mechanisms possibly coupled with stromal cell autocrine and/or systemic factors. Extrinsic factors are testicular function, nonandrogenic testicular factors, other systemic environment and genetic background (Table 1). Most of extrinsic factors are mediated through intrinsic factors by modified the relationship between stromal and epithelium of prostatic cells (Lee *et al*., 1997).

Table 1 Etiological factors in man with BPH.

Intrinsic factors	Extrinsic factors
• Stromal- epithelial interaction • Stromal effect on epithelia • Epithelial effect on stromal	• **Testicular factors**: androgen, estrogen, nonandrogenic testicular factors. • **Other somatic factors**: endocrine organ, neurotransmitters, immunology. • **Environment factors**: dietary factors, microorganisms, genetic predisposition

Source: Adapted from Lee *et al*. (1997)

2.2 Diagnosis of BPH The diagnoses of BPH are based on signalment, history, clinical signs, physical examination, complete blood count (CBC), radiography, ultrasonography, and examination of prostatic fluid (Feldman and Nelson, 2004). Computed tomography (CT) and magnetic resonance imaging (MRI) are unlikely to use for BPH diagnosis in clinical practice.

2.2.1 Clinical signs and physical examination Dogs with BPH show sign of constipation or ribbon shape of feces because the rectum is compressed by the enlargement of the prostate. Some dogs have tenemus associated with defecation (Smith, 2008). Perineal hernia may be occurred in some dogs with BPH and chronic constipation. Sanguineous prostatic fluid or blood contaminated in urine (hematuria) or semen (hemospermia) is also found in dogs with BPH.

Digital rectal palpation, transrectal palpation by using gloved and lubricated finger, will find a symmetric, painless and enlargement of prostate gland. Dog with BPH and prostatic cyst may be found asymmetrical gland. Sometimes in large dog or dog with severe BPH, another hand press from ventral abdominal to remark and stabilizes the prostate (Figure 5) (Memon, 2007).

Figure 5 The drawing picture of digital rectal palpation demonstration. One hand under a glove with lubricant gel, using the index finger passes to survey the prostate including size, texture, painful, symmetrical of gland, and the another hand should press from ventral abdomen for prostate stabilized.

Source : Barsanti (1995)

2.2.2 Radiography and ultrasonography Abdominal radiography reveals prostatomegaly from various prostatic disorders. Retrograde urethrocystography may be normal or reveal narrowing of prostatic urethra. Radiographic finding at lateral position of normal canine prostate gland should not larger than 50% of width of pelvic inlet on ventrodorsal view (VD). Prostatomegaly is remarked when prostatic dimensions exceed 70% of the distance between the sacral promontory and pubic brim (Figure 6 A-B) (Feeney *et al.,* 1987 and Atalan *et al.,* 1999).

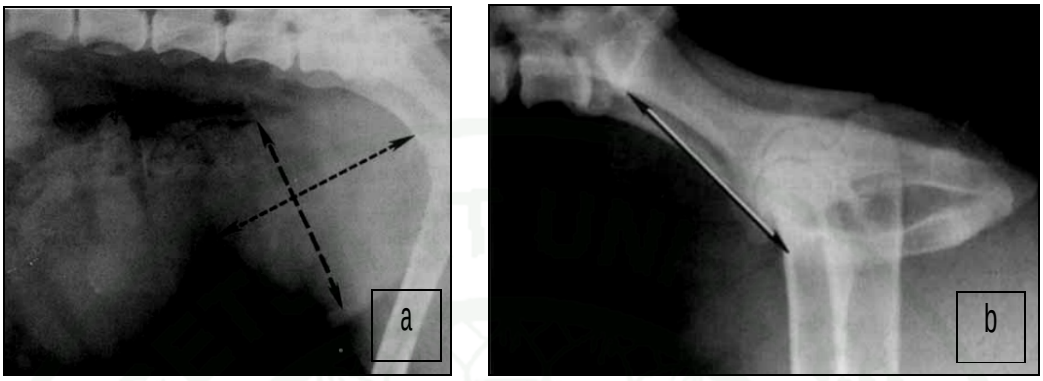

Figure 6 The lateral caudal abdominal radiograph of an old intact male dog. Prostate gland was measured in craniocaudal (length) and ventrodorsal (depth), and radio plaque of cystic calculi was also presented in urinary bladder (a). The distance was measured between the sacral promontory and pubic brim (arrow) (b). Prostatomegaly was remarked because of prostatic dimensions exceed 70% of the distance between the sacral promontory and pubic brim.

Source : Alalan *et al*. (1999)

Ultrasonographic finding of canine normal prostate is homogeneous, medium echogenicity and symmetry of prostate bi-lobular (Peninck, 2008). Ultrasonography is a useful technique and still widely used for prostatic-disorder diagnoses. The importance details to investigate prostatic problems are prostatic parenchyma, prostatic texture, prostatic size and prostatic volume. The ultrasonographic finding of prostatic gland varies with age, neuter status, type and quality of the ultrasound machine, and machine setting (Nyland and Mattoon, 2002). Transrectal ultrasonography (TRUS) and transabdominal ultrasonography (TAUS) are ultrasonographic techniques in prostate gland. TRUS is usually used in human for finding prostatic details. In animal practice, TRUS technique is preferred to use in large animals rather than in small animals because this technique is inconvenience, pain, and it is necessary to sedate or anesthetize in small animals. TAUS has still been a currently imaging examination in small animal though some artifacts may be found (Figure 7-9) (Nyland and Mattoon, 2002; Penninck and d' Anjou (2008).

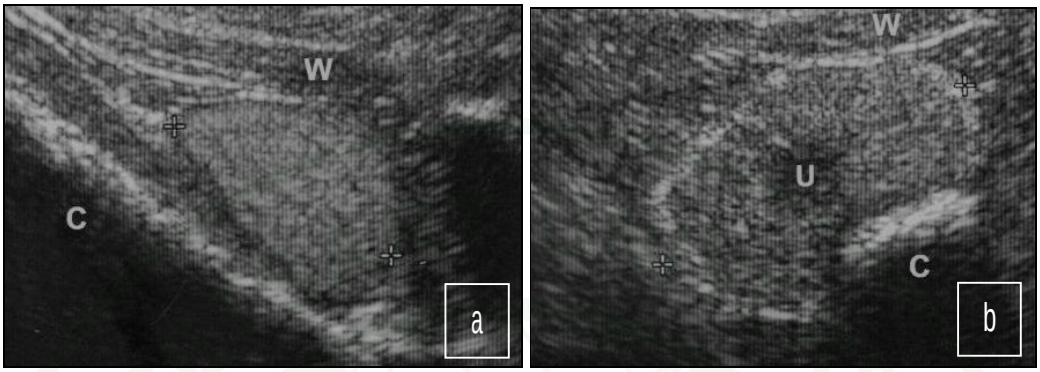

Figure 7 Normal prostate in a 1-year-old-intact Boston Terrier by TAUS in saggital view (a) and transverse view (b). Prostate presents in a homogeneous of oval shape on saggital view. Abdominal wall (W) stands dorsal part of the prostate. The irregular hyperechogenicity at ventral is affected from artifact of feces in the colon (C). Urinary bladder is not presented in this section. Prostate presents in the symmetrical and homogeneous of bilobular gland on transverse view. Prostate is in oblique because of the angulation of the probe. The central of prostate parenchyma is hypoechogenicity because of prostatic urethra and urethalis muscle (U).

Source: Penninck and d' Anjou (2008)

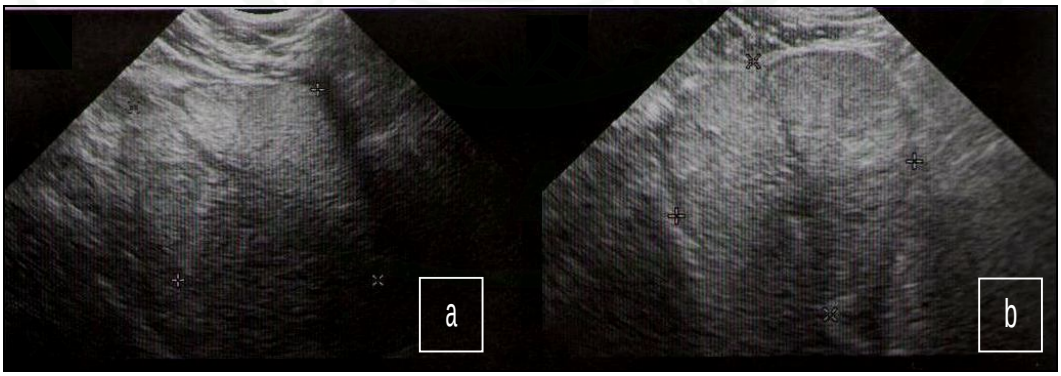

Figure 8 Nine-year-old German Shorthair Pointer with BPH was TAUS in saggital view (a) and transverse view (b). The prostatic parenchyma was mostly hyperechoic and slightly inhomogeneous. Left and right lobes were symmetrical on transverse view. Prostate size was 5.8 × 4.6 × 4.0 cm.

Source: Penninck and d' Anjou (2008)

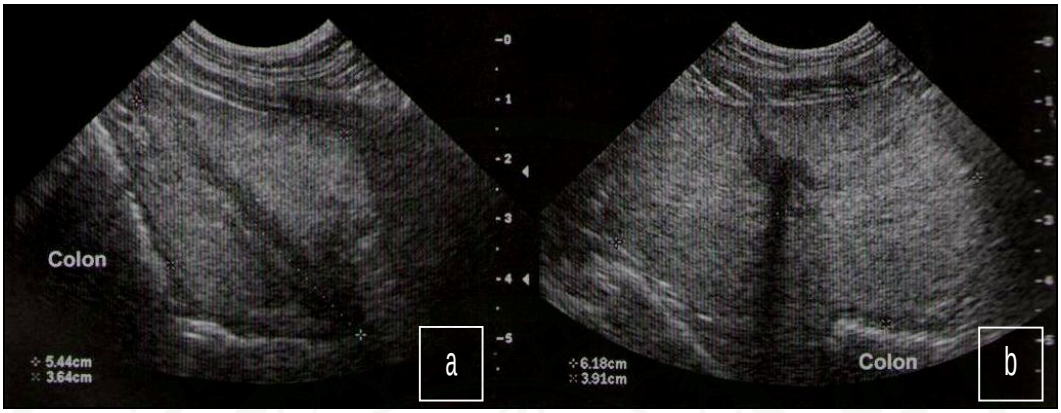

Figure 9 Six-year-old Rottweiler with BPH saggital (a) and transverse views (b) of prostate was increase in size. Homogeneous echogenicity, an edge shadow remarked on transverse view was caused by prostatic urethra.

Source: Penninck and d' Anjou (2008)

Ultrasonography provides not only the prostate parenchyma, but also in prostatic size which is useful to determine prostatic volume for prostomegaly investigation. There are many reports about prostatic volume measurement. In a study of prostatic volume estimation by TRUS, data was collected from 20 intact dogs. Prostatic volume is calculated by using the formula: gravimetric prostatic volume (cm^3, mL) = {0.642 × prostatic length (L) × height (H) × width (W)} + 1.84 (Suzuki *et al*., 1998). One study in 12 intact mature dogs, prostatic volume estimation by TAUS is calculated by use the following formula: volume (mL) = {1/2.6 × prostatic length (L) × width (W) × depth (D)} + 1.8. Normal prostate volume of dog under 20 kilograms should not be larger than 10 mL (Sirinarumitr *et al*., 2001 and Kamolpatana, *et al*., 1999). In another study of TAUS prostatic measurement in 43 dogs, prostatic volume = {0.625 × prostatic length (L) × width (W) × depth (D)}-0.216 (Alatan *et al*., 1999). Nevertheless, TAUS is occasionally used for guidance when prostatic biopsy or fine needle aspiration is performed (Smith, 2008).

2.2.3 Prostatic fluid evaluation Prostate fluid from dog with BPH, collected by ejaculation or prostatic massage, is clear or hemorrhagic. The ejaculation is completely collected in three fractions by manual stimulation, and prostatic fluid is the last fraction which is the most composition of semen (Sirinarumitr *et al*., 2001). Prostatic fluid evaluation should be submitted for bacterial culture and cytology. Lack of ejaculation in dog with low libido, pain, stress or other complications, prostatic massage will be considered. Some dogs may need to be sedated in order to perform prostatic-massage prostatic fluid collection. The urinary bladder is empty with steriled urethral catheter draining, and then the bladder is washed with saline several times. The residual saline is collected for a pre-massage sample. A steriled urethral catheter is caudally moved to prostatic urethra by digital rectum guidance. Prostate is massaged transrectal, whenever 10 mL of saline is slowly followed. Then the prostatic fluid is aspirated, and collected for a post-massage sample. Pre massage sample should be submitted for quantitative bacterial culture and cytology to compare with post- massage sample (Smith, 2008). Quantitative of bacterial culture is recommended due to the sample is occasionally contaminated with normal flora from urogenital tract. Pathogenic bacterial infection is accepted when it is over than 10,000 CFU/mL (Sirinarumitr *et al*., 2001). Inflammatory cells and neoplastic cells should not be found in semen cytology.

2.3 BPH treatment The recommended permanent treatment for dogs with BPH is surgical treatment by castration. However, some Dogs with BPH with high risk of anesthesia during castration procedure, stud dog, and dog with BPH and prostatitis with abscess should be considered in medical treatment.

2.3.1 Surgical treatment Castration is a surgical technique for removal testicles (orchiectomy). It is the best recommend for prevention of BPH initiation in young dog, and the method also accepted as the effective permanent treatment in dog with BPH. After castration, here is no longer androgen; T and DHT; available for prostate gland enlargement. Prostate is permanently atrophy within 3 to 12 wk after castration. (Fossum *et al*., 2002)

2.3.2 Medical treatment There are many medicine reported for treatment in dog with BPH, such as finasteride (Proscar™) (Sirinarumitr *et al*., 2001), diethylstilbestrol, medroxyprogesterone, megestrol acetate, flutamide, progesterone (Johnston *et al*., 2000), atamestane (Etreby, 2003), and FK143 (Hirosumi *et al*., 1995). Some of these medications have been reported causing some adverse effects during medical treatment and now they are not routinely used for BPH treatment in dog. Adverse effects of diethylstilbestrol in dogs are anemia, thrombocytopenia, pancytopenia, infertile, and squamous metaplasia of the prostate gland resulting in ductal obstruction and cystic formation (Fossum *et al*., 2002). Medroxyprogesterone acetate, a synthetic progestin, had been reported for BPH treatment in dogs. The hormone may cause permanent local alopecia, atopy and depigmentation at subcutaneous injection area. Adverse effects of megestrol acetate are lethargy, changes in behavior or hair color (Sirinarumitr *et al*., 2001).

a. Finasteride is a 4-azasteroid synthetic, type-II 5-alpha reductase blocker. It is also known as finasteridum, MK-0906 and MK-906. Finasteride is used to treat human BPH and hair loss for a long time. There are 2 dosage of human label products; 1 mg (Propecia™) and 5 mg (Proscar™) (Donald and Phar, 2008). Five mg/day, continuous 6-12 months is recommended for BPH treatment in man. One mg/day, 6 months is used for improving hair growth in man, however, the problem recurrence is about 12 months after drug withdrawal. Some of patients may have low libido or infertile during finasteride administration (Glina *et al*., 2004). Finastride at 1 mg/day did not affect on spermatogenesis on young healthy men in a clinical trial (Overstreet *et al*., 1999). However, the effects of long term finasteride treatment on the human fertility is not completed defined.

Since (1995) there were studies of finasteride for BPH treatment in dogs. Finasteride at dosages of 0.1-0.5 mg/kg, PO, every 24 hours for 16 wk did not affect on serum T. Clinical signs, percentage of prostatic diameter, prostatic volume and serum DHT were decreased, but libido and semen quality were not changed (Sirinarumitr *et al*., 2001). After treatment, the prostate was shrinkage due to apoptosis of prostatic cells, so there was no inflammation process in the prostate, and the prostate still had normal function (Sirinarumitr *et al*.,

2002). At the present time, finasteride is usually used for BPH treatment in dogs. However, it is a temporary treatment in order to decrease in prostatic size, dogs with BPH need to be on medication everyday for a period of time, and the total cost of finasteride treatment is higher than castration. After medical treatment, the dogs with BPH need to be rescheduled every 6 months to monitor the clinical signs and prostatic size. Another disadvantage of finasteride treatment in Dogs with BPH is that the medicine should be handled with cautious in pregnant client since finasteride may be caused the abnormality of external genitalia organ in male fetus who may be accidentally received 5 alpha reductase blocker form maternal drug contamination when she administered medicine to dog with BPH. There is not vet label product of finasteride (Donald and Phar, 2008), so the dog is also used in human product of 5 mg finasteride (Proscar™) for BPH treatment. However, finasteride is not approved to be used out of label for dogs in USA.

b. Deslorelin, a GnRH agonist, has been used in livestock industry for ovulation induction in nonlactating dairy cows and mares (Bartolome *et al*., 2004). In this century, GnRH agonist was introduced to be used in small animal reproduction. The mechanism of GnRH agonist in small animal reproduction are composed of 2 steps: the first step is agonist properties on the pituitary gland. The anterior pituitary gland is positively stimulated by GnRH agonist, and then their productions; follicle stimulating hormone (FSH) and luteinizing hormone (LH); were secreted into blood circulation, and consequently elevation the concentration of sex steroid hormones. The second step is desensitization of pituitary gland, after the constant administration of GnRH agonist, and then FSH and LH are decreased (Richler *et al.,* 2003; Trigg *et al*. 2006; Fontaine and Fontbonne, 2011). As two steps of the mechanisms, GnRH agonists are used to both stimulation and sterilization effects.

Deslorelin has been used in fertility control, estrus control (Trigg *et al*. 2006), behavioral related sex hormone control and urinary incontinence treatment in dogs (Richler *et al.,* 2003). In female dogs, six of 4 month-old intact dogs, which were implanted with deslorelin at the dosage of 4.7 mg/dog, had no signs of heat, and serum progesterone was less than 2 ng/mL. In contrast, six of seven month-old dogs were induced and showed sign of estrus cycle after deslorelin implantation. In male dogs, fifty six intact male dogs which were implanted

with deslorelin at the dosage of 4.7 mg/dog, range 0.11-1.32 mg/kg, had decrease in libido and testicular functions within 180 days. Serum T was suppressed to 0 ng/mL within 180 days during implantation period. Deslorelin at the dosage of 9.4 mg/dog was effective in reducing T to lower than 1 ng/mL for 400 days (Trigg *et al*., 2006). The effect of deslorelin implantation on dogs with normal prostate size. Deslorelin at the dosage of 0.5-1 mg/kg resulted in decreasing prostatic volume starting from 6 wk of treatment and remaining for 42 wk and returning to pre-treatment volume at 48 wk of treatment (Ponglowhapan *et al*., 2002). In conclusion, deslorelin implantation had long term effect on reproduction in dog. No adverse effect was reported in these studies. Nevertheless, GnRH agonist effectively inhibited the pituitary gonad axis, leading to suppression of fertility in both sexes, and was safe for contraceptive control.

3. The other prostatic disorders

Basically, most prostatic disorder found in intact male dog is BPH. Nonetheless, the abnormalities affecting canine prostate is not only BPH, but also prostatitis, prostatic cysts and prostatic neoplasia (Smith, 2008). Paraprostatic cyst is rarely found, and the pathogenic of disease is unclear. One theory instated that paraprostatic cyst might be a remnant of the uterus masculinus or prostatic origin (neck of urinary bladder) (Robert, 1998). However, there is often found histological evidence of one more disease processing in the same individual prostatic tissue, so some prostatic disorders may be complicated together (Feldman, E.C. and Nelson, 2004).

3.1 Prostatitis Prostatitis, an inflammation of prostate gland, is usually caused by bacterial infection. It is also the important prostatic disorder commonly seen in veterinary practices. Bacteria isolated from prostatitis dogs is generally similar to urinary tract infection. The predisposing factors of prostatic infection include urinary tract infection, altered urine flow, altered prostatic secretions, disruption of normal parenchyma architecture such as prostatic cystic hyperplasia, and BPH. Prostatitis can be either an acute or chronic forms. Concurrently, prostatitis can be developed to prostatic abscess. Prostatitis treatment is either medical or surgical treatments. Though castration is the recommend treatment for most of prostatic disorders, an appropriate antibiotic treatment is necessary for prostatitis to clear the infection before castration.

In case of large abscess forming, castration is not recommended without other surgical protocols for abscess drainage. The recommendation antibiotic for bacterial prostatitis treatment is based on bacterial culture, and course duration treatment should be lasted for 3-4 wk (Fossum *et al*., 2002 and Robert, 1998).

3.2 Prostatic neoplasia The primary prostatic neoplasm is considered in malignancy tumor including adenocarcinoma, transitional cell carcinoma, squamous cell carcinoma and lymphoma. Clinical sign of prostatic neoplasia is unlike BPH, it is based on prostatomegaly with neoplastic condition including metastasis. Prostatic palpation is irregular of prostatic surface, and asymmetrical mass can be found in some dogs (Robert, 1998). Hetero- hyperechogenicity is remarked in ultrasonography of prostate neoplasia. Malignant cells may be detected from prostatic fluid analysis. The prognosis of prostatic neoplasia is poor because it is usually found at the metastasis stage. There are some evidences that prostatic neoplasia was found more in castrated than intact male dog.

Practically, an intact male dog should be examed for prostate disorders by digital rectal palpation when dog is immunized a booster annual vaccination (Robert, 1998). In clusion, prostatic diseases are differences in cause, clinical signs, prostate textures, ultrasonographic echogenicity, and treatment protocol (Feldman and Nelson, 2004).

4. GnRH in canine reproduction

4.1 Gonadotrophin releasing hormone (GnRH) GnRH is, a decapeptide hormone, a key hormone in mammal reproduction. GnRH is primary synthesis in certain neurons of hypothalamus, and it is secreted pulse–like manner into the hypothalamo-hypophyseal portal system before carried to the pituitary gland. Anterior pituitary responds to GnRH stimulation by secreting FSH and LH into blood stream. FSH and LH have mainly cell targets in the gonad, and consequently, sex steroids hormones are stimulated and released. Testis is a male gonad, and mainly secreting T. Ovary is a female gonad, and mainly secreting estradiol and progesterone (Figure 10) (Fontaine and Fontbonne, 2011).

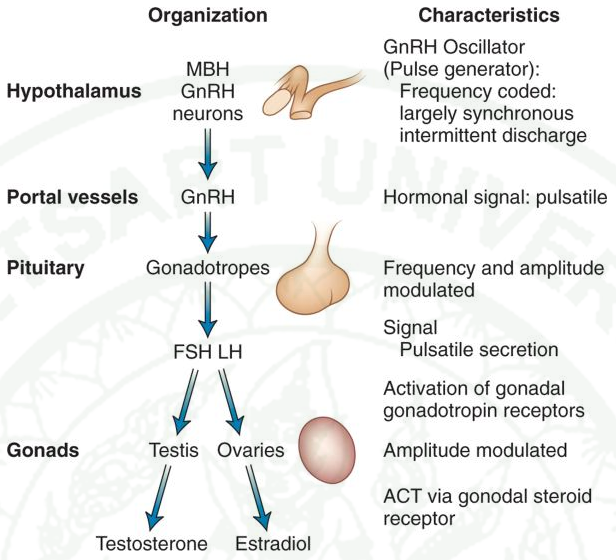

Figure 10 Organization and characteristics of the hypothalamic-pituitary gonadotroph-gonadal system.

Source : Adapted from Grumbach and Kaplan (1990)

4.2 GnRH analog As a GnRH mechanism, the key of reproduction hormone, the analogue compound in agonist and antagonist were developed to be used in a clinical treatment.

4.2.1 Agonist An agonist is a chemical that binds to a cell receptor, and triggers a response by that cell. Agonists often relate the action of a naturally occurring substance. GnRH agonist forms have been developed. Firstly, GnRH agonist was intravenous or subcutaneous injection, but they had a short half-life. For longer action, GnRH agonists were developed with sustain-release form like depot injection (leuprolide acetate, 7.5 mg, and lasted for 1 month, Abbott laboratories) and a biocompatible implant (deslorelin 4.7 mg, and lasted for 6 months, and 9.4 mg, and lasted for 12 months, Peptech Virbac Group). Long action of GnRH agonist compounds had 2 steps of mechanisms. The first step, GnRH agonist acts like natural GnRH, agonist acts to positive increase in GnRH, FSH, LH, sex hormones in blood stream. The second step, continuous stimulation of GnRH agonist leads to pituitary desensitization, so FSH, LH, sex hormone are decreased (Figure 11-a). The second step acts as sterilization. Therefore,

GnRH agonist effects on both stimulating and sterilizing in male and female animal reproduction. GnRH agonist is used for either estrous induction, but also contraception (Annie, 2008 and Fontaine and Fontbonne, 2011).

4.2.2 Antagonist antagonist is a class of compounds that are similar in natural structure, but they have an antagonistic effect. GnRH antagonist has a similar structure compared to natural GnRH structure and it binds to a GnRH receptor as a competitive binding. So, GnRH antagonist is a GnRH receptor blocker in orders to decrease or inhibit GnRH action (Figure 11-b) (Annie, 2008).

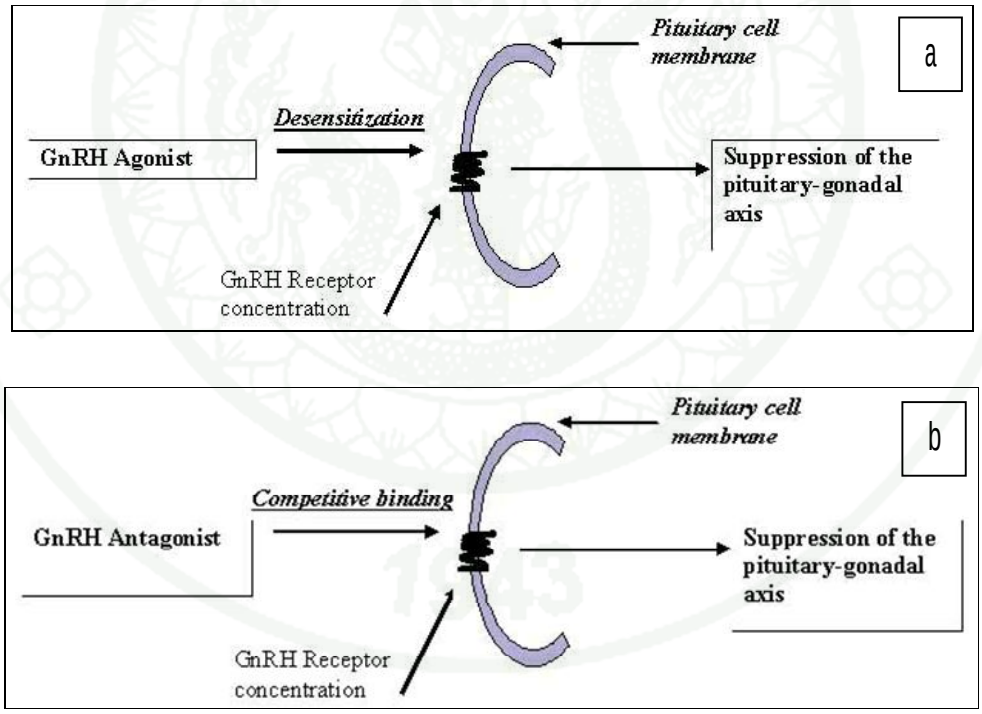

Figure 11 GnRH agonist pituitary action concentrations, receptor desensitization (a). GnRH antagonist pituitary action concentrations, receptor competition (b).

Source: Frontiers (2002).

MATERIALS AND METHODS

The experiment was conducted at Reproductive Clinic, Radiography and Imaging Unit, Kasetsart University Veterinary Teaching Hospital, and Laboratory of Blood Analysis, Kasetsart University Veterinary Diagnosis Centre, Bangkok.

1. Dog selection

Twenty two owned intact male dogs which were diagnosed natural BPH were recruited in the study. The clinical signs were including constipation, blood contaminated in urine or semen and/or urinary incontinence. All dogs were apparently in normal for complete blood count (CBC), creatinine, blood urea nitrogen (BUN), alanine transaminase (ALT) and alkaline phosphatase (ALPK), and were negative in canine brucella antibody test (FASTest™) (Appendix Figure 11). The prostatic volume was over 10 mL by ultrasonography measurement (Sirinarumitr *et al*., 2001) or found squamous metaplasia cells from semen cytology. The quantitative aerobic bacterial culture yields are not over than 10,000 colony forming units (CFU)/mL, and there were no inflammatory cell and neoplastic cell in semen cytology.

2. Experimental design

The experiment was conducted in a clinical trial. All dog owners signed consent forms before enrolling to the study (Appendix A). Dogs with BPH were assigned to each treatment groups either implanted with a single dose 4.7 mg of deslorelin (Suprelorin™, Peptech Animal Health), subcutaneous injection between base of scapular area (Appendix Figure 9, 12), and lasted for 24 wk or received finasteride (Proscar™, 5 mg/tablet, Merck) (Appendix Figure 10) 0.1-0.5 mg/kg, orally, once a day for 16 wk (Sirinarumitr *et al*., 2001). The treatment selection was depending on dog owner convenience. Each dog with BPH was evaluated for clinical signs, skin reaction (only implanted dog), prostatic volume, testicular volume, semen quality, semen bacterial culture, semen cytology, CBC, creatinine, BUN, ALT and ALPK and serum T at 0, 4, 8, 16, and 24 wk of deslorelin treatment period, and at 0, 4, 8 and 16 wk of finasteride treatment

period. Four dogs from each treatment were random to test DHT at 0, 4 and 8 wk of treatment period. Adverse effects were continued to follow up at 8 and 16 wk after both treatment cessations. The data was resulted in two parts. Part I was the effect on both treatments compared to before treatment. Part II was the adverse effect and the disease recurrence after both treatments cessation.

2.1 Clinical signs The clinical signs, constipation, blood in semen, were monitored. Constipation was observed by dog owner at home, and was checked by digital rectal palpation at the following weeks. Blood in semen was remarked in gross and microscope. The other clinical signs were monitored individual case at the following weeks compare before treatment. Deslorelin implanted dogs were inspected for skin reaction at an implantation site including allergy, hair loss, and hair change colour.

2.2 Prostatic volume Ultrasonography; HONDA™ Electronics HS-2000VET or GE™ LOGIQP6 was used to measure prostatic size. Dog was positioned in dorsal recumbency. After clipping hair, ultrasound transducer was located at ventrocaudal part of abdomen (Appendix Figure 13). Prostate gland was measured and recorded in centimeter unit. L, W and D were the greatest craniocaudal length, transverse width, and dorsoventral depth, respectively (Figure 14). Prostate volume was calculated in following formula; (1/2.6 × L × W × D) + 1.8 (Sirinarumitr *et al.*, 2001). Ultrasonographic character of the prostatic parenchyma was recorded. An example of prostate volume calculation following the formula was in the Appendix C.

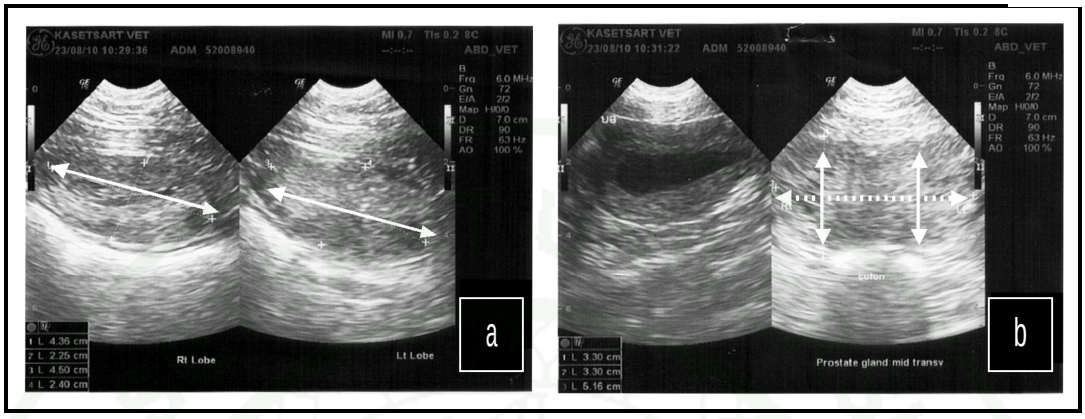

Figure 12 Prostatic size measurement by TAUS in saggital view (a) and transverse view (b). Saggital plain from right and left lobes of prostate gland is measured for length (L) measurement. Transverse plain of prostate gland is measured for width (W) (dot arrow) and depth (D) measurements for right and left lobes.

Source: KUVTH (2012)

2.3 Testicular volume Testicular size was measured by vernier calliper in centimeter for lenght (L) and width (W) of each testis. The distence between the proximal and distal poles of each testis was taken as L, and the distance between testis median and lateral surface was taken as W. The average of L and W from both testis were calculated for testicular volume (mL) following by this formula; $0.52 (LW^2)$. (Annie, 2008, Harriet *et al*., 2002)

2.4 Semen quality

2.4.1 Semen collection Semen was manually collected by using female pheromone and an artificial vagina. The prepuce was caudally slide to behind bulbus glandis. The finger of the collector constricted and stimulated for dog erection and ejaculation. Semen was collected. The first two fractions of semen; pre-sperm and sperm rich were collected in a 15 mL centrifugation tube attached to the artificial vagina. The last fraction, prostatic fluid, was separated collection in a new tube. (Appendix Figure 15)

2.4.2 Semen evaluation Semen quality was evaluated of sperm motility, sperm morphology, and percentage of dead and alive sperm, total semen volume, semen concentration, and total sperm number/ejaculation. The semen evaluation form was shown in Appendix Figure 2-3.

a. Sperm motility Suddenly, sperm rich fraction was ejaculation, a drop of semen was dropped on a warm glass slide. Sperm motility was examined at 400× of microscope, and manually evaluated in percent of progressive motile.

b. Sperm morphology and percentage of dead and alive sperm. One drop of semen was smeared on a warm glass slide, and allowed the slide to be dried, then slide was stained by Diff Quik, and evaluated for percentage of normal and abnormal sperm morphology. Another drop of semen and an eosin- nigrosin stain were mixed and smeared on glass slide and counted for percentage of dead and alive sperm. (Appendix Figure 16)

c. Total semen volume, semen concentration and total sperm number/ejaculation Total semen fluid volume was a total volume of three fractions. The last fraction had been collected until dog was loss of erection. Counting sperm in an aliquot of semen diluted 1:100 and using a hematocytometer was determined for semen concentration (Sirinarumitr *et al.*, 2001). Total sperm number/ejaculation was calculated by semen concentration multiplied the total semen volume.

2.5 Semen bacterial culture and semen cytology

Semen aerobic bacterial culture and semen cytology were submitted to Kasetsart University Veterinary Diagnosis Centre, Bangkhaen campus, Bangkok (Appendix Figure 4-5).

2.6 Haematology and serum biochemical analysis

Blood samples collected from each dog for CBC in EDTA tube, and creatinine, BUN, ALT, ALKP in heparin tube. All samples were submitted to Laboratory of Blood Analysis, Kasetsart University Veterinary Diagnosis Centre, Bangkhaen campus, Bangkok.

2.7 Measurement of serum T and serum DHT concentration

Three times of 3 mL blood samples were collected from cephalic or saphenous vein at approximately in 20 minute intervals. Sample from each dog was collected at the same time of the day. Serum was separated within 20 minutes after blood collection by centrifuge machine, and was stored at – 80 ºC. The serum from each collection was pooled for serum T and serum DHT concentration measurement. Serum T was assayed by chemiluminescence method (Immulite™) (Appendix Figure 17-19) at Laboratory of Blood Analysis, Kasetsart University Veterinary Diagnosis Centre, Bangkok. Serum DHT was submitted to enzyme immune assay (EIA) method at Bangkok R.I.A. LAB Co., LTD.

2.8 Statistical analysis

Clinical signs were described throughout treatment period and after treatment cessation. Repeated ANOVA measurements, Bonferroni Multiple- Comparison Test was used to compare the differences in prostatic volume, testicular volume, total semen fluid volume and total sperm per ejaculation in both groups during treatment period, and compare at the end of treatment time to 8 and 16 wk after treatment cessation. The significant difference was considered at a concentration of $P < 0.05$. The statistical program was run by NCSS 2007 (Hintze, 2006).

RESULTS

Twenty two natural dogs with BPH were recruited in the study. Ten dogs were recruited in deslorelin implantation group, but only 8 dogs were completed through the end of the study period. They were Rottweiler (1), Golden Retriever (1), Bull Terrier (1), Beagle (1) and crossbreed (4). Twelve dogs were recruited in finasteride treatment group, but only 8 dogs were completed through the end of the study period. The dogs were American Pit Bull Terrier (1), and crossbreed (7). The data of age and body weight from deslorelin and finasteride treatment dogs were shown in Table 2. There were not differences of age and body weight between treatment groups before treatment.

Table 2.1 Age and body weight from deslorelin.

Deslorelin dogs	Age (yr)	Weight (kg)
1.	8.43	29.50
2.	5.94	33.10
3.	6.44	17.00
4.	3.65	19.10
5.	3.65	21.10
6.	3.11	19.40
7.	4.10	16.70
8.	2.78	16.50
Mean ± SD	6.48 ± 2.57	21.78 ± 8.58

Table 2.2 Age and body weight from finasteride.

Finasteride dogs	Age (yr)	Weight (kg)
1.	6.44	38.00
2.	3.16	27.00
3.	8.72	20.23
4.	4.32	13.40
5.	8.68	-
6.	8.68	16.60
7.	8.68	14.60
8.	3.16	22.60
Mean ± SD	4.76 ± 1.97	21.55 ± 6.29

The results were reported in two parts. Part I was the effect of deslorelin and finasteride treatments on the treatment periods in dogs with BPH. Part II was the adverse effects of deslorelin and finasteride treatments on dogs with BPH and the disease recurrence after treatment cessation.

Part 1: The effect of deslorelin and finasteride treatments on the treatment periods in dogs with BPH.

The results were consisted of clinical signs, prostatic volume, testicular volume, semen quality, semen bacterial culture, semen cytology, CBC, creatinine, BUN, ALT, ALKP and serum T concentration at 0, 4, 8, 16 and 24 wk of deslorelin treatment period, and at 0, 4, 8 and 16 wk of finasteride treatment period. Four dogs from each treatment were randomly to measure for serum DHT concentration at 0, 4 and 8 wk of treatment period.

1.1 Clinical signs In deslorelin implantation group, blood in semen, which was only a clinical sign related to BPH (4/8), was observed. The sign was resolved within 4 wk (2/4) and 8 wk (2/4) of treatment period, respectively. In finasteride treatment group, clinical signs related to BPH included constipation (1/8) and blood contaminated in semen (3/8) (Figure 13). All clinical signs were resolved within 4 wk of treatment, except there was one dog found blood in the semen throughout 16 wk of treatment period.

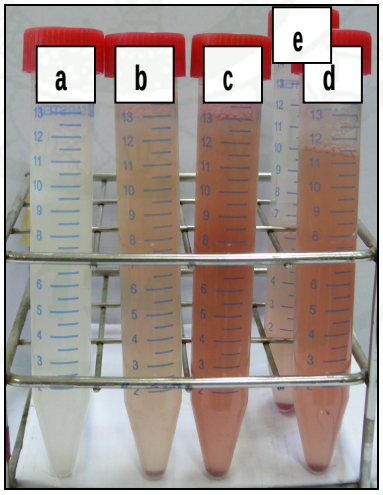

Figure 13 Semen collected from dogs with benign prostatic hypertrophy: (a) Sperm-rich fraction (opaque color); (b-e) Prostatic fluid contaminated with blood.

1.2 Prostatic volume Prostatic volumes from deslorelin and finasteride treatment groups were shown in Table 3.1-3.2.

Table 3.1 Prostatic volumes from deslorelin treatment dogs at 0, 4, 8, 16 and 24 wk of treatment period.

Deslorelin	Weeks of deslorelin treatment period				
dogs	0	4	8	16	24
1	36.00	51.75	31.47	8.40	12.59
2	36.06	17.58	9.57	5.05	6.70
3	16.60	12.95	8.08	4.23	4.15
4*	10.29	4.45	4.20	3.63	3.42
5*	7.60	5.66	3.40	2.48	2.45
6*	9.40	7.08	4.50	2.90	2.64
7	11.70	7.99	3.50	3.18	4.51
8	27.20	27.25	21.74	7.74	6.73
Mean ±SD	19.36 ± 11.97	16.84 ± 16.01	10.81 ± 10.33	4.70 ± 2.23	5.40 ± 3.33

* Dogs had prostatic volumes under 10 mL at the beginning time. They were recruited in the study because BPH was diagnosed with blood and squamous cells in their semen cytology.

Table 3.2 Prostatic volume from finasteride treatment dogs at 0, 4, 8 and 16 wk of treatment period.

Finasteride dog	Weeks of finasteride treatment period			
	0	4	8	16
1	22.48	-	19.83	16.00
2	18.05	12.40	16.40	8.84
3*	9.10	5.31	5.00	4.14
4	14.40	7.86	6.80	7.40
5	11.90	4.57	7.30	6.07
6*	9.20	6.16	5.00	5.15
7*	7.80	5.46	3.90	4.50
8*	8.20	5.16	3.40	5.68
Mean ±SD	12.64±5.31	6.70±2.72	8.45±6.17	7.22±3.87

* Dogs had prostatic volumes under 10 mL at the beginning time. They were recruited in the study because BPH was diagnosed with blood and squamous cells in their semen cytology.

Before treatment, mean ± SD of prostatic volumes from deslorelin and finasteride treatment groups were 19.36 ± 11.97 and 12.64 ± 5.31 mL, respectively. Mean of prostatic volumes and standard deviation (SD) during both treatments period were shown in Figure 14.

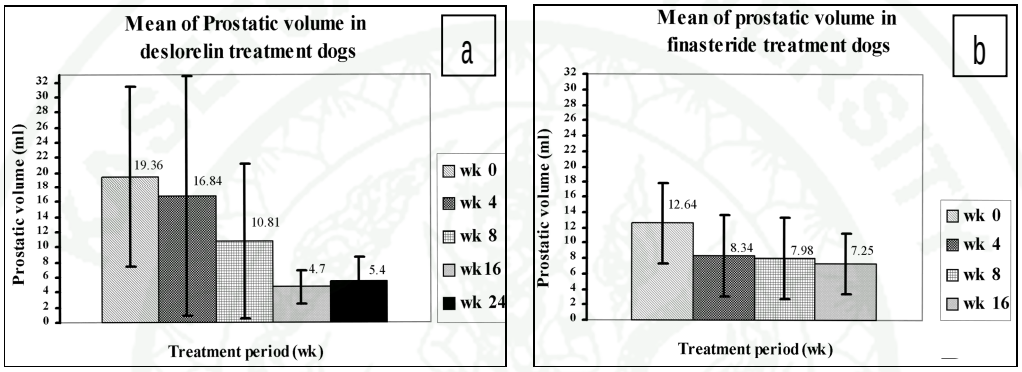

Figure 14 Mean and standard deviation (SD) of prostatic volumes in dogs with BPH. Deslorelin treatment group at 0, 4, 8, 16 and 24 wk of treatment (a). Finasteride treatment group at 0, 4, 8 and 16 wk of treatment (b).

In deslorelin treatment dogs, there was significant decrease in prostatic volume at 16, and 24 wk (P =0.0206) compared to before treatment (Figure 14-a). In finasteride treatment dogs, there was no significant difference in decreases in prostatic volume during the 16 wk of treatment period (P =0.1497). However, after 4 wk of treatment, mean of prostatic volume was under 10 mL which is the normal size of prostate gland (Figure 14-b). There was no significant difference in prostatic volume between deslorelin and finasteride treatment groups during 16 wk of treatment period (P =0.5455). Both medical treatments were effect on decreasing prostatic volume during their treatment periods.

Percentage changes of prostatic volume during deslorelin (at 4, 8, 16 and 24 wk) and finasteride (at 4, 8 and 16 wk) treatment groups compared to before treatment were shown in Figure 15. In deslorelin treatment dogs, prostatic volume was decreased in 13.02% at 4 wk, 50.77% at 8 wk of treatment, and maximum decrease in 75.72% at 16 wk of treatment period

compared to before treatment. In finasteride treatment dogs, prostatic volume was decreased in 34.02% at 4 wk of treatment, and maximum decrease in 55.88% at 8 wk of treatment period compared to before treatment. There was no significant difference in percentage change in prostatic volume between deslorelin and finasteride treatment groups during 16 wk of treatment period (P <0.005).

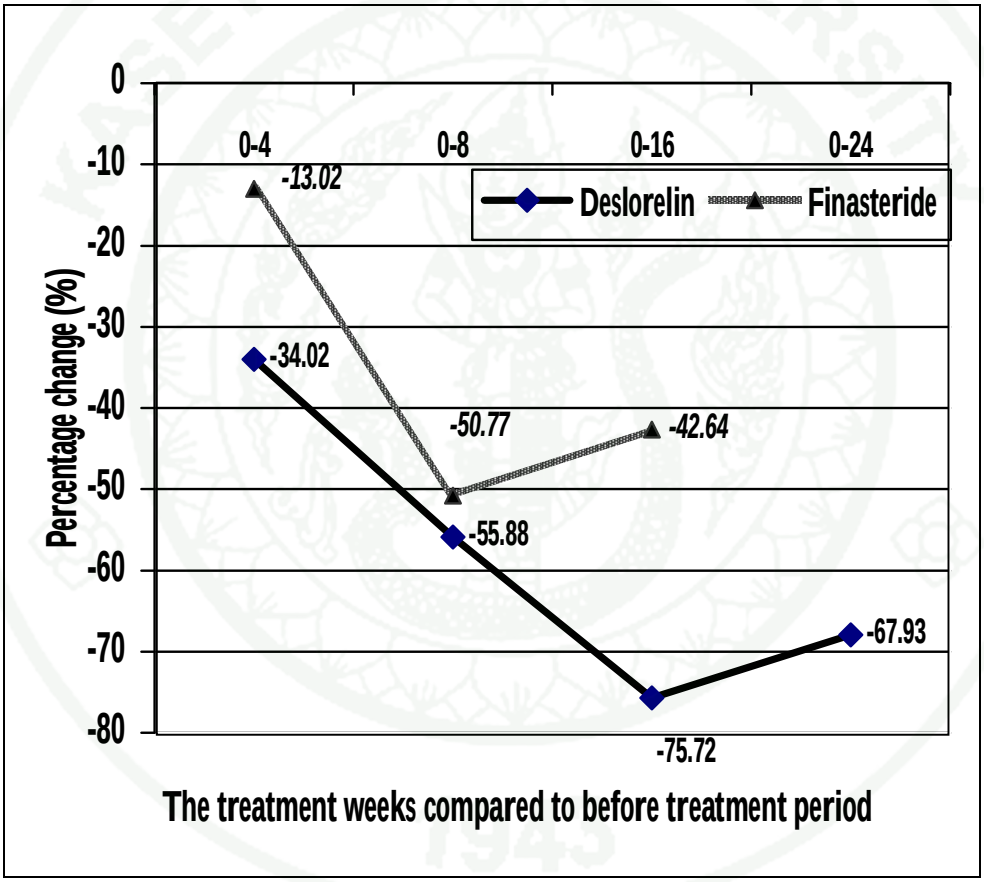

Figure 15 Percentage changes of prostatic volumes during deslorelin and finasteride treatment groups compared to before treatment.

1.3 Testicular volume Testicular volumes of deslorelin and finasteride treatments during treatment period were shown in Table 4.1-4.2.

Table 4.1 Testicular volumes (mL) from deslorelin dogs at 0, 4, 8, 16 and 24 wk of treatment period.

Deslorelin dogs	Weeks of deslorelin treatment period				
	0	4	8	16	24
1	15.35	17.16	13.33	10.53	9.63
2	19.27	12.65	12.52	4.68	6.13
3	14.89	9.62	4.87	4.33	4.37
4	7.70	2.90	2.42	2.32	1.96
5	6.88	6.15	3.35	2.10	2.51
6	5.22	4.31	4.48	2.04	2.94
7	6.20	6.76	2.43	2.20	2.47
8	6.72	5.33	6.05	3.11	2.48
Mean± SD	10.28±5.36	8.11±4.78	6.18±4.34	3.91±2.87	4.06±2.63

There was no significant difference in testicular volumes during the 24 wk of deslorelin treatment period (P =0.6471).

Table 4.2 Testicular volumes (mL) from finasteride dogs at 0, 4, 8 and 16 wk of treatment period.

Finasteride dogs	Weeks of finasteride treatment period			
	0	4	8	16
1	12.88	-	10.55	14.95
2	15.60	23.08	21.75	12.73
3	8.60	8.53	10.32	8.20
4	8.11	9.50	9.18	8.6
5	6.95	8.11	7.23	6.92
6	10.46	10.40	8.74	9.36
7	7.98	7.71	7.61	8.11
8	6.76	6.74	6.40	4.39
Mean ±SD	9.67±3.13	10.58±5.64	10.22±4.88	9.16±3.30

There was no significant difference in testicular volume during the 16 wk of finasteride treatment period (P =0.9923).

1.4 Semen quality Semen quality was consisted of percentage of progressive motility, percentage of normal morphology, percentage of alive sperm, total semen volume (mL), and total sperm number (million)/ejaculation.

In deslorelin treatment dogs, the semen quality was shown in Tables 5.1. There were significant decreases in percentage of progressive motility, in percentage of normal morphology, in percentage of alive sperm (P <0.0001), in semen volume (P =0.0157) and in mean of total sperm number per ejaculation (P =0.1400) at 16, and 24 wk of treatment period compared to before treatment. Anejaculation was completely found in all deslorelin treatment dogs at 16 and 24 wk of treatment periods. Dogs still had normal libido.

Table 5.1 Results of semen evaluations from dogs with BPH in 24 wk of deslorelin treatment.

Weeks of deslorelin treatment	0	4	8	16	24
Percentage of progressive motility	73.57±24 .28 [a]	67.14±23.07 [a]	70.00±26.46 [a]	0 [b]	0 [b]
Percentage of normal morphology	74.35±13.51 [a]	68.60±10.25 [a]	59.23±11.52 [a]	0 [b]	0 [b]
Percentage of alive sperm (%)	93.64±11.40 [a]	86.40±15.30 [a]	94.00±6.10 [a]	0 [b]	0 [b]
Semen volume (mL)	8.23±5.62 [a]	6.58±12.26 [a]	0.44±0.66 [a]	0 [b]	0 [b]
Total sperm number (10^6)/ ejaculation	230.63±263.85 [a]	162.70±161.73 [a]	74.23±128.62 [a]	0 [b]	0 [b]

Data were shown as mean ± SD. Values with different letter superscripts (a, b) within the same column were significantly different (P <0.05).

In finasteride treatment dogs, the semen quality was shown in Tables 5.2. There were no significant differences in percentage of progressive motility (P =0.5162), in percentage of normal morphology (P =0.9312) and in percentage of alive sperm during the treatment period (P =0.3530). There was significant difference decrease in semen volume at 4, 8, and 16 wk compared to before treatment (P =0.0006). However, there were no significant differences in mean of total sperm number per ejaculation (P =0.5334).

Table 5.2 Results of semen evaluations from dogs with BPH during 16 wk of finasteride treatment.

Weeks of finasteride treatment	0	4	8	16
Percentage of progressive motility	62.86 ± 14.10	77.14 ± 21.38	78.13 ± 25.00	81.25 ± 8.35
Percentage of normal morphology	74.36 ± 21.11	79.00 ± 18.96	75.08 ± 22.58	75.69 ± 16.00
Percentage of alive sperm (%)	95.30 ± 6.58	95.20 ± 8.14	98.80 ± 1.39	93.69 ± 5.02
Semen volume (mL)	12.03 ± 5.56 [a]	4.26 ± 3.03 [b]	3.76 ± 2.71 [b]	5.76 ± 3.06 [b]
Total sperm number (10^6)/ ejaculation	229.09 ± 255.78	327.40 ± 246.03	288.89 ± 185.60	410.74 ± 274.24

Data was shown as mean ± SD. Values with different letter superscripts (a, b) within the same column were significantly different (P <0.05).

1.5 Semen culture and cytology The results of semen bacterial culture from both treatment were shown in Table 6. In deslorelin treatment dogs, one dog was found 221,000 CFU/mL *Klebsiella* spp. from semen bacterial culture at 8 wk of treatment. Anejaculation was affected in most dogs at 4 and 8 wk of treatment period and affected in all dogs within 16 wk, so there was no sample submitted for semen bacterial culture or cytology test. In finasteride treatment dogs, a few bacterial colonies were growth in some samples, and they were less than 10,000 CFU/mL at 0 wk (1/7), 4 wk (2/6), 8 wk (2/7) and 16 wk (3/7), respectively. The result of pathogenic bacterial growth was considered when bacteria was over 10,000 CFU/mL.

Table 6 Results of bacterial growth in semen culture from deslorelin and finasteride treatment dogs.

Weeks of treatment	0	4	8	16
Deslorelin	0/8	0/3*	1/1**	-
Finasteride	0/7	0/6	0/7	0/7

Bacterial growth was considered when bacteria were cultured and got over 10,000 CFU/mL.

* At 4 wk of desorelin treatment, seven dogs were success in semen collection, and one dog had anejaculation. However, only 3 samples were submitted for bacterial culture.

** At 8 wk of desorelin treatment, only one dog was successful in semen collection and positive in semen bacterial culture. The other 7 dogs had anejaculation.

Cytology of semen from both groups were contained no inflammatory and neoplasia cells for the whole period of treatment, except one deslorelin treatment dog which was found 221,000 CFU/mL *Klebsiella* spp. and was also found inflammatory cell on semen cytology at 8 wk of treatment. Some photographies of semen cytology under microscope were shown in Figures 16 and 17.

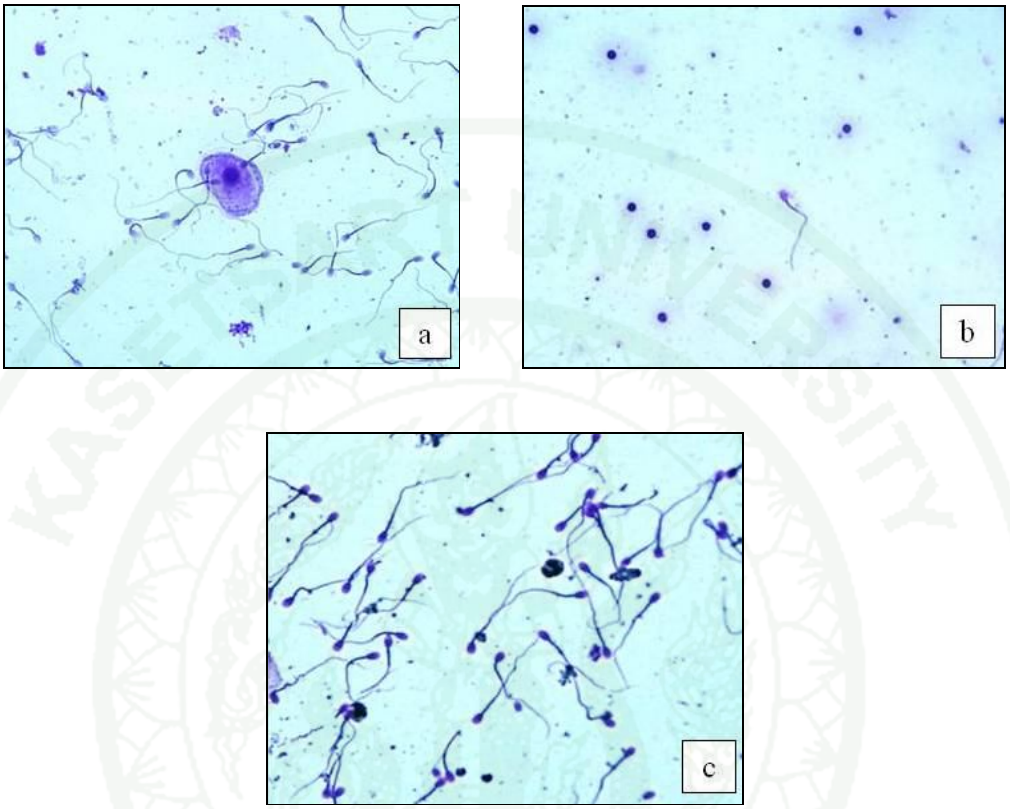

Figure 16 The semen cytology from one deslorelin treatment dog at 0, 4, 8 wk of treatment was shown on Figure 16a-c, respectively. This dog had 221,000 CFU/mL *Klebsiella* spp, and there were some inflammatory cells on the semen cytology at 8 wk of treatment (Figure 16c). There was no neoplastic cell found on the ejaculation during the treatment period. Semen cytology was stained with Diff Quik. The magnification was 200×.

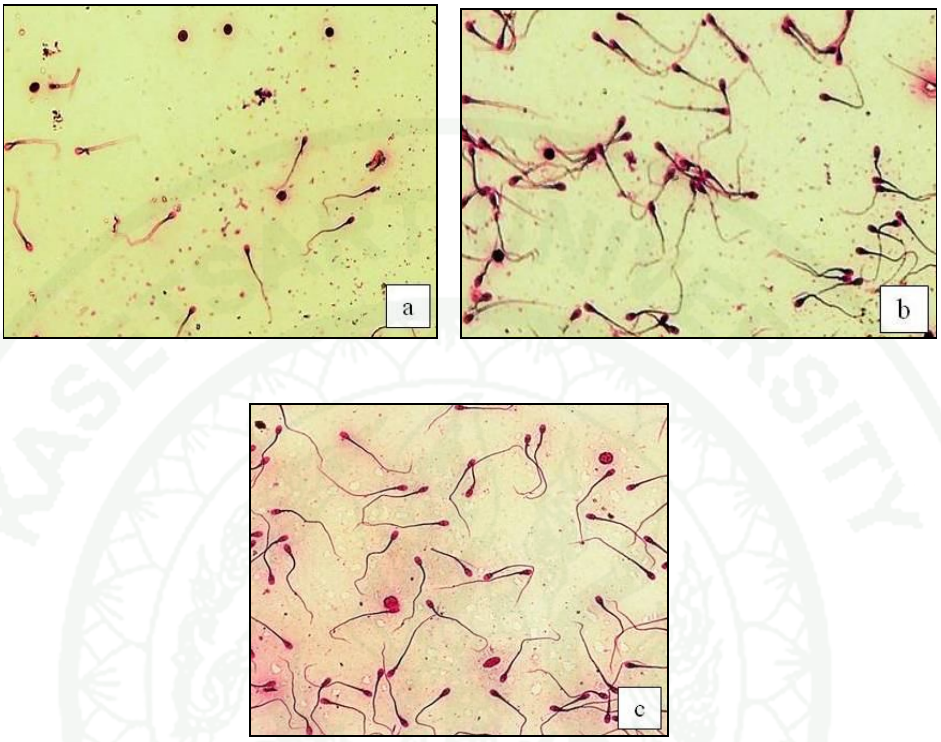

Figure 17 The semen cytology from one finasteride treatment dog at 0, 4, 8 wk of treatment was shown on Figure 17a-c, respectively. There were no inflammatory cell and neoplastic cell on the following wk of treatment. Semen cytology was stained with Diff Quik. The magnification was 200×.

1.6 Haematology and serum biochemical analysis There were no significant differences in both treatment groups throughout their treatment period in CBC and blood chemistry profile for creatinine, BUN, ALT, ALKP.

1.7 Serum T and serum DHT concentrations In deslorelin treatment dogs, serum T and serum DHT concentrations were shown in Table 7.1. Mean ± SD of serum T and DHT concentrations were shown in Figure 18.

Table 7.1 Serum T concentrations (ng/mL) from deslorelin treatment dogs at 0, 4, 8, 16, 24 wk of treatment period, and serum DHT concentrations (pg/mL) from 4 dogs at 0, 4, 8 wk.

Dog No.	Serum T and DHT concentrations at the weeks of deslorelin treatment period							
	0		4		8		16	24
	T	DHT	T	DHT	T	DHT	T	T
1.	5.33	3,953	0.49	1,475	0	353	0	0
2.	0.21	289	0.55	461	0.62	588	0	0
3.	4.04	3,163	0	1,646	0	302	0	0
4.	2.02	-	1.20	-	0	-	0	0
5.	2.38	-	0	-	0	-	0	0
6.	5.32	-	0.36	-	0.22	-	0	0
7.	5.68	-	0.23	-	0	-	0	0
8.	11.13	5,779	0	374	0	199	0	0
Means ±SD	4.51±3.29	3,296±2,284.5	0.35±0.41	989±664.5	0.10±0.22	360.5±164.7	0	0

The serum hormone assay validations were shown in Appendix Table 2-3.

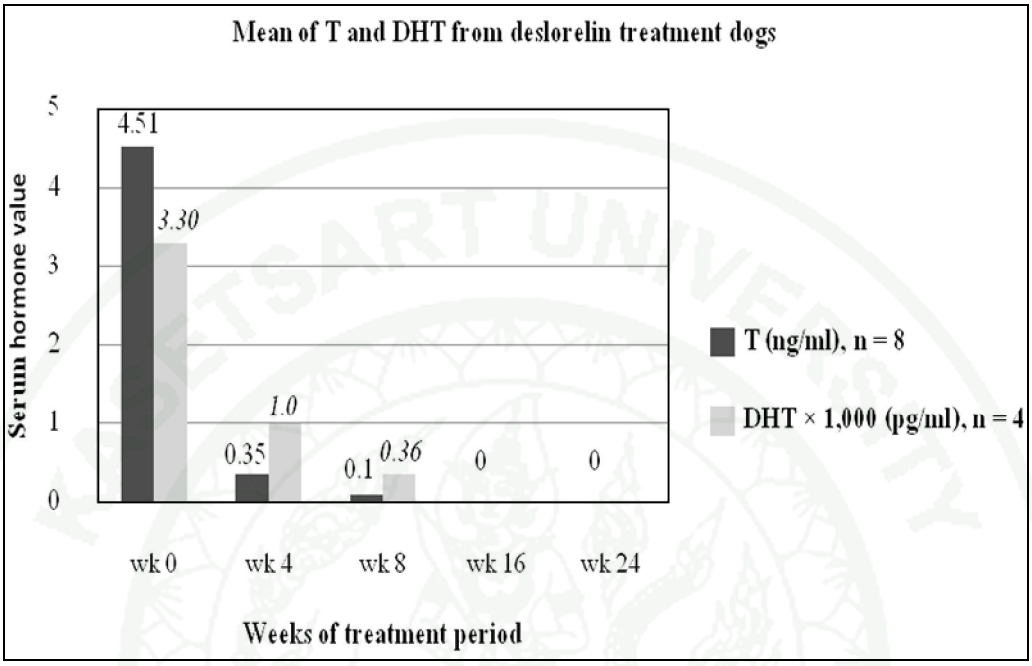

Figure 18 Means of serum T and serum DHT concentrations from deslorelin concentration treatment dogs.

Serum T concentration was trended to decrease at 4 and 8 wk of treatment period, and low to undetectable at 16 and 24 wk of treatment period. Serum DHT concentration was trended to decrease at 4 and 8 wk of treatment period, but it was not significant difference (P >0.05).

In finasteride treatment dogs, serum T and DHT concentrations were shown in Table 7.2. Mean ± SD of serum T and DHT concentrations were shown in Figure 19.

Table 7.2 Serum T concentrations (ng/mL) from finasteride treatment dogs at 0, 4, 8, and 16 wk of treatment period, and serum DHT concentration (pg/mL) from 4 dogs at 0, 4, 8 wk.

Dog No.	Weeks of finasteride treatment period						
	0		4		8		16
	T	DHT	T	DHT	T	DHT	T
1.	2.86	-	-	-	3.96	-	2.96
2.	1.43	1,029	0.776	610	1.56	794	1.06
3.	3.36	-	4.87	-	5.87	-	4.64
4.	1.74	-	1.14	-	3.33	-	6.17
5.	13.96	5,448	0.918	529	4.98	1,570	2.52
6.	5.6	-	3.5	-	6.11	-	1.2
7.	6.64	1,528	5.15	853	8.29	1,900	1.78
8.	7.81	6,497	0.72	2,271	2.28	1,671	4.26
Means ±SD	5.43±4.15	3,625.5±2,751.3	2.44±2.00	1,063±817.2	4.56±2.21	1,483±480.11	3.07±1.81

The serum hormone assay validations were shown in Appendix Table 2-3.

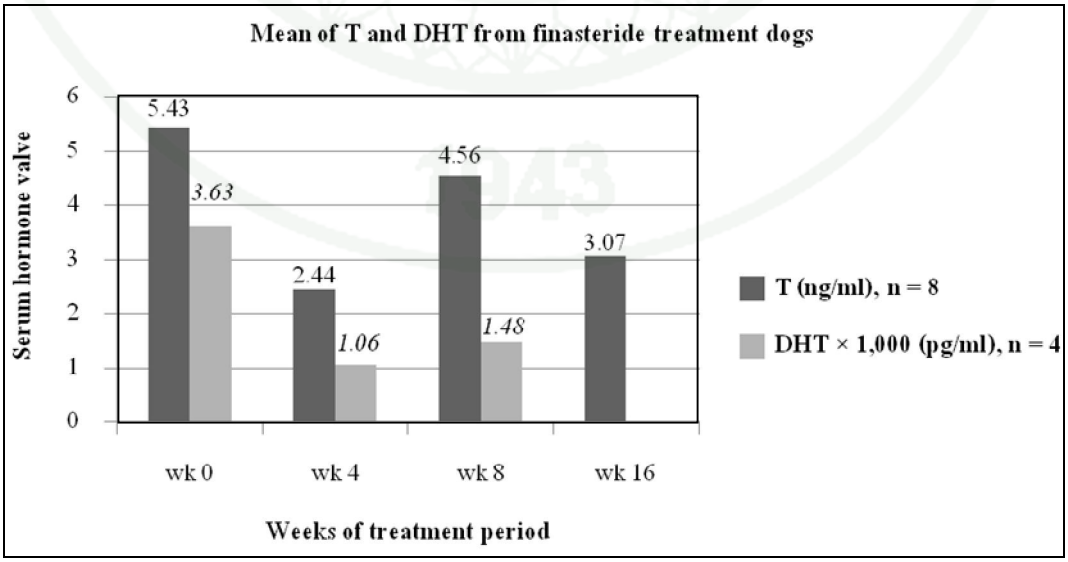

Figure 19 **M**eans of serum Tand serum DHT concentration from finasteride treatment dogs.

Serum DHT concentration was decreased at 4 and 8 wk of treatment period compared to before treatment but it was not significant difference. Serum DHT concentration was decreased following serum T concentration, however, they were in normal range.

Part 2: The adverse effects of deslorelin and finasteride treatments on dogs with natural BPH and the disease recurrence after treatment cessation

The results were consisted of clinical signs, skin reaction (only implanted dog), prostatic volume, testicular volume, semen quality, semen bacterial culture, semen cytology, CBC, creatinine, BUN, ALT, ALKPand serum T at 0, 8 and 16 wk after both treatment cessations. The last week of both treatments period; at 24 wk of deslorelin treatment and at the 16 wk of finasteride treatment; were counted in the cessation time and considered the 0 wk after treatment cessation.

2.1 Clinical signs After deslorelin treatment cessation, clinical signs related to BPH such as conspitation, difficult to urinate, blood contaminated in urine or semen were not complained from the dogs' owners. All dogs still had anejacution phenomenon at least 16 wk after treatment cessation (40 wk after deslorelin implanted). There was no adverse effect on skin reaction such as aloplacia, allergy, changed in hair color at the implantation site and at whole body coat in all dogs during 24 wk of treatment period and 16 wk after treatment cessation. After finasteride treatment cessation, clinical signs related to BPH were not complained from the dogs' owners, except blood contaminated in semen which was detected from semen fluid by gross and/or by microscopy in some dogs at 0 wk (1/8) (Figure 20), 8 wk (5/8) and 16 wk (6/7) after treatment cessation.

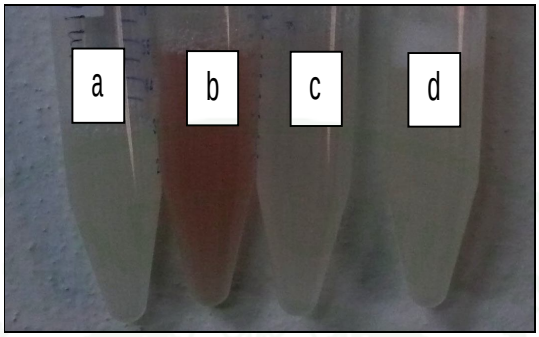

Figure 20 Semen fluid samples were collected from finasteride treatment dogs at the cessation time (at 16 wk of treatment time). Blood in semen was shown in one dog (b) compared to another three normal semen fluid samples (a, c, d) at 0 wk after treatment cessation.

2.2 Prostate volume In deslorelin treatment dogs, prostatic volumes were shown in Table 8.1. Mean ± SD of prostatic volumes were 5.40 ± 3.12, 5.30 ± 2.62 and 5.20 ± 2.94 mL at 0, 8 and 16 wk after treatment cessation, respectively (Figure 21). There was no significant difference in prostatic volume at 0, 8 and 16 wk after treatment cessation period (P =0.9549).

Table 8.1 Prostatic volumes from deslorelin treatment dogs at 0, 8 and 16 wk after treatment cessation period.

Deslorelin dogs	Weeks after deslorelin treatment cessation			
	0*	8	16	24**
1.	12.59	9.20	11.15	-
2.	6.70	8.45	-	-
3.	4.15	4.35	3.32	4.60
4.	3.42	4.17	4.11	3.55
5.	2.45	2.77	2.80	-
6.	2.64	2.34	4.78	-
7.	4.51	3.09	2.30	-
8.	6.73	8.03	6.10	-
Mean ±SD	5.40±3.12	5.30±2.62	5.2±2.94	-

* Deslorelin treatment cessation time was at the 24 wk of deslorelin treatment.

** Dogs No. 3, 4 were continued follow up to 24 wk after treatment cessation.

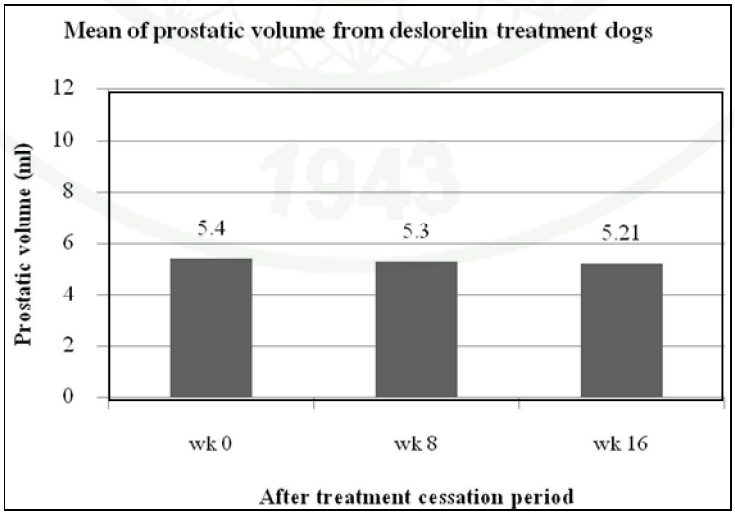

Figure 21 Means of prostatic volumes from deslorelin treatment dogs at 0, 8 and 16 wk after treatment cessation period.

In finasteride treatment dogs, prostatic volumes were shown in Table 8.2. Mean ± SD of prostatic volumes were 7.23 ± 3.93, 11.72 ± 8.69, 11.63 ± 10.06 mL at 0, 8 and 16 wk after treatment cessation, respectively (Figure 22).

Table 8.2 Prostatic volume from finasteride treatment dogs at 0, 8 and 16 wk after treatment cessation period.

Finasteride dogs	Weeks after finasteride treatment cessation			
	0*	8	16	24**
1.	16.00	16.20	30.36	-
2.	8.84	19.17	24.90	30.84
3.	4.14	5.40	7.42	9.28
4.	7.40	9.24	8.31	8.10
5.	6.07	8.70	5.10	9.30
6.	5.15	8.10	6.64	5.64
7.	4.50	6.10	6.40	3.30
8.	5.68	6.70	3.92	6.12
Mean ±SD	7.23±3.93	11.72±8.69	11.63±10.06	10.37±9.28

* Finasteride treatment cessation time was at the 16 wk of finasteride treatment.

** Seven dogs were continued follow up at 24 wk after treatment cessation.

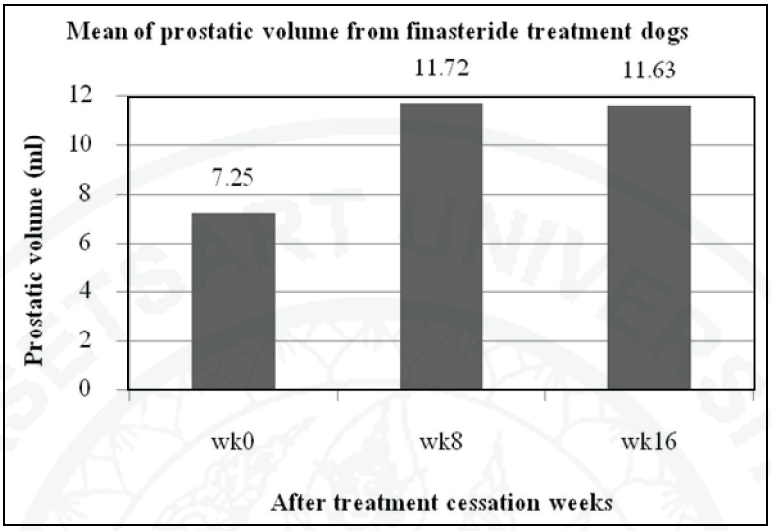

Figure 22 Means of prostatic volumes from finasteride treatment dogs at 0, 8 and 16 wk after treatment cessation period.

There was significant difference in prostate volume between subject and time (*P* =0.0439). There were two dogs (No.1 and No.2) which were large breed dogs and had a magnitude increase in prostatic volume at 8 and 16 wk after treatment cessation. When their data were excluded, the other six dogs were not significant in prostate volume after treatment cessation in prostate after treatment cessation (*P* =0.0565).

2.3 Testicular volume In deslorelin implantation group, testicular volumes after treatment cessation were shown in Table 9.1. The means ± SD of testicular volume were 4.06 ± 2.63, 3.62 ± 2.70 and 3.81 ± 2.72 mL at 0, 8 and 16 wk after treatments cessation, respectively. There was no significant difference in testicular volumes during the 16 wk after deslorelin treatment cessation (*P* =0.9863).

Table 9.1 Testicular volumes (mL) from deslorelin treatment dogs at 0, 8 and 16 wk after treatment cessation.

Deslorelin dogs	Weeks after deslorelin treatment cessation		
	0*	8	16
1.	9.63	9.44	9.44
2.	6.13	5.96	-
3.	4.37	3.03	4.66
4.	1.96	1.95	1.65
5.	2.51	2.01	3.21
6.	2.94	2.2	3.72
7.	2.47	2.23	2.47
8.	2.48	2.17	1.53
Mean ±SD	4.06±2.63	3.62±2.70	3.81±2.72

* At the 24 wk of deslorelin treatment was deslorelin treatment cessation starting time (at the 0 wk).

In finasteride treatment group, testicular volume after treatment cessation was shown in Table 9.2. The means ± SD of testicular volumes were 9.16 ± 3.30, 9.62 ± 4.06 and 10.61 ± 3.57 mL at 0, 8 and 16 wk, respectively after treatment cessation. There were no significant difference in testicular volume during 16 wk after finasteride treatment cessation (P =0.9111).

Table 9.2 Testicular volume (mL) from finasteride treatment dogs at 0, 8 and 16 wk after treatment cessation.

Finasteride dogs	Weeks after finasteride treatment cessation		
	0*	8	16
1.	14.95	17.55	11.01
2.	12.73	14.79	16.7
3.	8.20	10.80	8.22
4.	8.60	10.57	7.02
5.	6.92	5.49	8.41
6.	9.36	10.55	11.20
7.	8.11	9.12	9.45
8.	4.39	5.97	4.68
Mean ±SD	9.16±3.3	10.61±4.06	9.59±3.57

* At the 16 wk of finasteride treatment was finasteride treatment cessation starting time (at the 0 wk).

2.4 Semen quality In deslorelin treatment dogs, semen quality could not be evaluated because anejaculation was continuous found in all deslorelin treatment dogs at 0, 8 and 16 wk after treatment cessation, though all dog had normal libido and erection. In finasteride treatment dogs, the results of semen quality after treatment cessation periods were shown in Table 10.

Table 10 Results of semen quality from finasteride treatment dogs at 0, 8 and 16 wk after treatment cessation.

Weeks of deslorelin treatment	0	8	16
Percentage of progressive motility	74.00 ± 23.11	69.75 ± 34.46	72.44 ± 34.53
Percentage of normal morphology	69.06 ± 24.89	70.48 ± 23.80	70.33 ± 34.26
Percentage of alive sperm (%)	84.72 ± 26.15	82.39 ± 26.16	79.11 ± 36.72
Semen fluid volume (mL)	6.90 ± 4.45 [a]	15.21 ± 7.55 [b]	11.64 ± 9.91[ab]
Total sperm number (10^6)/ ejaculation	366.88 ± 288.31	335.93 ± 308.90	359.60 ± 341.61

Data was shown as mean ± SD. Values with different letter superscripts (a, b) within the same column were significantly different (P <0.05).

There were no significant difference in average of percentage of progressive motility (P =0.6375), percentage of normal morphology (P =0.9950), percentage of alive sperm (P =0.7518) at 0, 8 and 16 wk after treatment cessation period. There was significant difference increase in semen volume at 8 wk after treatment cessation compared to the 0 wk (P =0.0075), however, there was no significant differences in mean of total sperm number per ejaculation at 0, 8 and 16 wk after treatment cessation period (P =0.9683).

2.5 Semen bacterial culture and cytology In deslorelin treatment dogs, semen culture and cytology were not submitted because all dogs had anejaculation throughout 16 wk after treatment cessation period. In finasteride treatment dogs, bacterial culture and cytology were remarked in two dogs. One dog had 4.75×10^4 CFU/mL *Escherichia coli* with cytologic diagnosis in mild to moderate BPH, and 1.28×10^8 CFU/mL *Streptococcus* spp., and *Klebsiella* spp. with cytologic diagnosis in mild purulent prostatitis with cocci at 8 and 16 wk after treatment

cessation, respectively. The another one had 2.3× 10^5 CFU/mL *Pseudomonas aeruginosa*, and *Streptococcus* spp. at 16 wk after treatment cessation. This dog also had blood in the semen throughout treatment period, and during 16 wk after treatment cessation.

2.6 Haematology and serum biochemical analysis There were no significant differences in both treatment groups at 8 and 16 wk after treatment cessation period compared to the 0 wk of cessation treatment in CBC and blood chemistry profile for creatinine, BUN, ALT, and ALKP.

2.7 Serum T concentrations In deslorelin treatment dogs, one dog absented at 16 wk after treatment cessation. Serum T concentration still had been undetectable (<0.2 ng/mL) from all serum samples at 0 week (8/8), 8 weeks (8/8) and 16 weeks (7/7) after treatment cessation. In finasteride treatment dogs, serum T concentration from finasteride treatment group at the treatment cessation period was shown in Table 11. Serum T concentration was not significant difference; however, they were in normal range.

Table 11 Serum T concentration (ng/mL) from finasteride treatment dogs at 0, 8 and 16 wk after treatment cessation.

Finasteride dogs	Weeks after finasteride treatment cessation		
	0*	8	16
1.	2.96	3.76	2.82
2.	1.06	1.80	2.69
3.	4.64	7.98	3.54
4.	6.17	16.52	3.77
5.	2.52	15.39	1.39
6.	1.20	14.32	1.66
7.	1.78	5.95	3.76
8.	4.26	12.23	1.25
Mean ± SD	3.07±1.81	9.74±5.62	2.61±1.06

* Finasteride treatment cessation time was at the 16 wk of finasteride treatment.

DISCUSSION

1. Dog selection

The experimental dogs were variable in breed, age, body weight between treatments because the study was designed in a clinical trial, and also the limited of dog numbers which were recruited in the study period. The small numbers and variability of experimental animals would affect in statistic analysis. Dogs with spontaneous BPH without any complication such as prostatitis, prostatitis with cyst or abscess were rarely attended. Most of dogs with BPH were referred to KUVTH when BPH was developed with complication of prostatitis. In a retrospective study of canine prostatitis in 2007-2008 at KUVTH, Bangkok, Thailand reported that 36 dogs with prostatitis concurrent with BPH had the average age of 8 ± 3.3 years old. The prostatic volume was 19.4 ± 14.9 mL (Limmanont and Sirinarumitr, 2009). This report supported that most of the old intact male dogs with prostatitis might be have a BPH as a predisposing cause and followed with bacterial infection.

2. The experimental design

Deslorelin implants are available in two formulas. One formula is 4.7 mg for 6 months duration, the other formula is 9.4 mg for 12 months duration (Ponglowhapan, 2011). A single 4.7 mg deslorelin implant was used in the study because it was reasonable to follow up and completely collected the experimental data in 6 months period. Superolin™ (deslorelin) is prescript for implantation between bases of scapulars (scruff). Some studies used deslorelin implanted at umbilical area in which the implant material was possible to be removed. In this study, deslorelin was implanted subcutaneously at the scuff area because the experiment was designed to observe the long action of GnRH agonist. In worse case, if there were the serious skin reaction or some adverse effects, deslorelin implant would be considered to be removed. In this study, all dogs had no adverse effect on skin reaction and on other systems at least 40 wk after deslorelin implantation. This result was similar to result of the previous study (Trigg *et al.*, 2006).

They reported that a single 4.7 mg deslorelin was implanted in the interscapular region in 56 mature gonad-intact dogs of mixed breed. There were no local and systemic inflammatory reactions (Trigg *et al.,* 2006). However, there was no report about the adverse action of deslorelin at the difference of implant locations. Deslorelin for dog with BPH treatment whom was not concerned in lack of libido and semen quality at least 40 wk after implantation and the implantation site suggested in scruff area. In contrast, the dog which is treated for BPH only, and the owner concerned his libido and breeding status. Deslorelin should be implanted at umbilical area which is easier to remove the implant material. To prevent the long term action of GnRH, the implanted hormone could be removed from the implantation site. Although deslorelin is coming to be interested in small animal reproduction at this century, deslorelin is not available in some countries, including in Asian countries. All deslorelin implants in the study were directly contacted and kindly provided from Peptech Animal Health, Australia. At the present time, due to there is no distributor in Thailand, vet practitioners have to buy the hormone directly from the company.

Finasteride at the dosage of 0.10, 0.25 and 0.50 mg/kg, once a day, 7 days were significantly decreased on serum DHT concentration, but there was no effect on serum T concentration, and the experiment conducted in 3 sexually intact male dogs (Kamonpatana *et al.,* 1998). Serum DHT and serum T concentrations did not differ by finasteride dosage administration before and 7 days during treatment period (Kamonpatana *et al.,* 1998). Finasteride 0.10-0.5 mg/kg, orally, once a day for 16 wk was reported to reduced prostatic volume, resolved clinical signs, reduced DHT concentration and had no effect on semen quality, fertility, or libido in a group of 9 dogs with natural BPH (Sirinarumitr *et al*., 2001). Nonetheless, Rhodes (1996) and Memon (2007) reported finasteride might be used at high dosage of 0.5 - 5 mg/kg. In this study, the average of finasteride dosage was 0.22-0.32 mg/kg (0.27 ± 0.05). One dog who received finasteride 0.15 mg/kg still had blood in semen throughout the study time. Therefore, finasteride 0.1 mg/kg was inadequate completely clear clinical signs of BPH in some dogs though this dosage was effect to decrease serum DHT concentration and prostatic volume.

3. Clinical signs

Classically, a clinical sign of BPH is blood in semen due to the prostate gland is enlarged and increased blood vessels intra prostate gland. When the dog ejaculates especially at the last fraction of semen, the prostate is contracted. This is easily to cause bleed, cause blood contaminated in semen. However, this sign should also be ruled out from other diseases such as coagulopathy, blood parasite infection, or other prostatic disorders (Memon, 2007).

In the study, one dog that was 5.9 year-old Pitt bull terrier dog, 33.1 kg, still had blood in semen throughout 16 wk of finasteride treatment. He had not only blood in semen, but also had oligospermia before treatment and azoospermia after 16 wk after treatment cessation. There were no bacteria or inflammatory cell found in his semen. No evidence of other disorders, including coagulopathy, blood parasite infection and other prostatic disorders were diagnosed in this dog. He was received finasteride at the dosage of 0.15 mg/kg. Blood in semen may be resolved if the dosage of finasteride was increased to 0.5 - 5 mg/kg (Rhodes, 1996; Memon, 2007). However, the prostatic volume was decreased to normal size, and blood in semen was less effect on semen quality (Johnston *et al*., 2001). Finasteride high dosage was less considered because finasteride is an expensive drug. The treatment cost will be expanded if the higher dosage is prescribed. Due to the drug administering to the dog was owner responsibility; continuous drug receiving in this dog is concerned. On the other hand, finasteride is a temporary treatment, BPH will be recurrent when dog is discontinues or not in regularly finasteride administering.

4. Prostatic volume

There are many reports about prostatic volume measurements. Prostatic size is normally measured by ultrasonography, however, it is related to age, body weight and previous diseases (Nyland and Mattoon, 2002). Some investigations find the relationship between age and body weight in prostatic size and volume. One investigation was study to estimate prostatic volume in 100 clinically normal, intact, sexually mature dogs in order to find the relationship between age (A) and body weight (BW). The prostate was measured using ultrasonography to measure

prostatic length (L), width (W), height on saggittal image (HS), height on transverse image (HT) and the prostatic volume (V) was estimated by the following formula: L = (0.055 × BW) + (0.143 × A) + 3.31, W = (0.047 × BW) + (0.089 × A) + 3.45, HS = (0.046 × BW) + 0-083 × A) + 2.68, HT = (0.044 × BW) + (0.083 × A) + 2.25 and V = (0.867 × BW) + (1.885 × A) + 15.88 (Ruel *et al.*, 1997). Another study estimated prostatic volume in 157 healthy mature male dogs using the following formula: Prostatic volume (cm^3) = 8.48 + (0.238 × BW [kg]) or 9.97 + (0-871 × age [yrs]) (Atalan *et al.*, 1999). Although there are many prostatic volume references, there are some limits of each formula to access a correct prostate volume in dogs with various sizes and ages. Nevertheless prostatic volume is only a parameter to diagnose prostatic enlargement and prostatic disorders, the prostatic diseases are also diagnosed by other parameters (Feldman and Nelson, 2004).

In this study, prostatic volume was one of parameters to diagnose, monitor and compare the results of the study in each treatment period. Age and body weight were not used to calculate prostatic volume because this way was suitable in screening intact male dogs into BPH, but it is not appropriated in monitoring the treatment effect. Prostatic volume was calculated by TAUS following only one reference throughout the study (Sirinarumitr *et al*, 2001). Dog should be fasted and enema before TAUS because some contents in gastrointestinal tract and feces in rectum would interfere prostate images (Penninck and d' Anjou, 2008). However in this study, all dogs were not prepared to empty gastrointestinal tract and feces, and prostate images were detected without any interfering.

In deslorelin treatment group, prostatic volume was significant decrease in 16 and 24 wk of treatment period. In finasteride treatment group, the average of prostatic volume was decreased under 10 mL, from 12.64 ± 5.31 to 8.3 ± 5.24 mL in 8 wk, which was a maximum decrease in percentage change of prostatic volume compared with the beginning time (-55.88%) in Figure 10-a and 11. One finasteride treatment dog, 8.4 year-old Labrador retriever dog, 29.5 kg, was castrated due to other reasons after he was finished from the clinical trial. One month post castration, the prostatic volume was 15.4 mL which was comparable to his 16 mL of prostatic volume at 8 wk of finasteride treatment. This may be supported that finasteride mostly effected on

magnified decrease in prostatic volume at 8 wk of treatment period. Sirinarumitr *et al.* (2001) reported in the effect of finasteride on the size of prostate gland and semen quality in dogs with BPH that prostatic volume significant decrease in percentage change (mean ± SD) after 8 wk; 41.0 % ± 23.8 and 16 wk; 43.0 % ± 29.0 of treatment compared to before treatment of finasteride at 0.1-0.5 mg/kg for 16 wk (Sirinarumitr *et al.*, 2001 and Donald and Phar, 2008). This is possible that finasteride treatment time could be adjusted less than 16 wk by the improvements of signs and other parameters during treatment period.

5. Testicular volume

Testicular volume is measured to assess testicular function because 70 -80 % of testes mass are seminiferous tubules in which spermatogenesis is processing (Setchell and Brooks, 1988). There are a number of measurement methods to access testicular volume, including calipers, orchidometry and ultrasonography. In this study, testicular volume was measured by caliper following LW^2 (0.52) (Annie, 2008). Testicular volumes of both treatments were not difference during treatment periods compared to the beginning time. However, mean of testicular volume from deslorelin treatment dogs was more difference from the beginning time, especially after 16 wk of treatment period, and dogs also had anjecaculation. Percentage change of mean testicular volume and percentage change of mean total sperm per ejaculation were shown in Figure 23 for presenting the relationship between testicular volume and semen concentration.

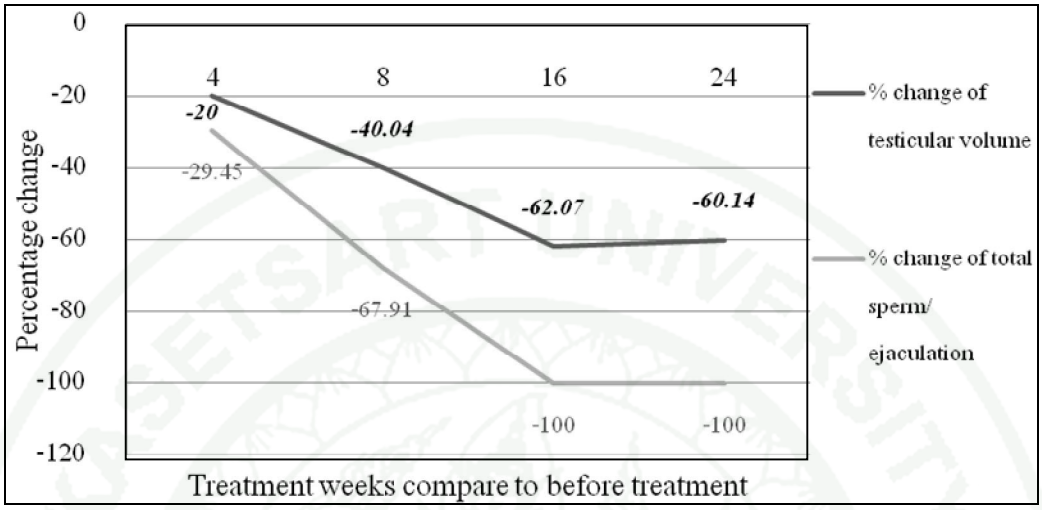

Figure 23 Percentage change of mean testicular volume and percentage change of total sperm per ejaculation from deslorelin treatment dogs at 4, 8, 16 and 24 wk compared to the beginning time.

Though there was no significant difference between testicular volumes during the treatment periods (P =0.6471), there were a relation between percentage change of mean testicular volume, sperm per ejaculation and anejaculation (Figure 23). At 16 wk of deslorelin treatment period, percentage change of mean testicular volume was decreased more than 60 % compared to the beginning time, and dogs had anejaculation and undetectable of serum T concentration (Table 4.1). Therefore, when testicular volume was decreased more than 60%, testicular function, in which sertoli cells and leydig cells were also affected and consequence with decrease in spermatogenesis and T production.

6. Semen quality

In deslorelin treatment dogs, deslorelin caused poor semen, so semen quality could not be evaluated due to anejaculation phenomenon which it was occurred within 16 wk of treatment period, and also continuous in all deslorelin treatment dogs for at least 16 wk after treatment cessation. Long action of GnRH agonist caused pituitary desensitization, subsequently, FSH, LH, T and T metabolite agents were completely depressed (Fontaine and Fontbonne, 2011). The dog

lacked of semen and prostatic fluid, following from the temporary shutdown of spermatogenesis and atrophy of prostate gland. Anejaculation was an unable to ejaculate while the dog still had normal erection. It is possible to classify in pretesticular azoospermia from hypoganadotropic effect. One deslorelin treatment dog, a 13.4 kg of 4.3 year-old Beagle, had monitored for 60 wk after a single 4.7 mg deslorelin implantation (equaled to the dosage of 0.35 mg/kg). The dog had a lack of libido and anejaculation on manual semen collection while he still had anal contraction in coital period and anejaculation was lasted for 56 wk after hormone implantation. Excellent libido and oligospermia were detected at 60 wk after hormone implantation. The suppression time of T concentration, spermatogenesis and prostatic fluid may be various depending on deslorelin dosage and dog's body weight. Trigg *et al*. (2006) reported that time of recovery from GnRH agonist was variable in which small dogs (<10 kg), in general took a longer time than in medium (10-25 kg) or large dogs (>25 kg). However, the action was at least 180 day on serum T suppression after 4.7 mg implantation (Trigg *et al*., 2006).

In finasteride treatment dogs, all results in semen evaluation did not change except only decrease in semen volume. The smaller size of prostate affects to decrease prostatic fluid due to the majority of semen in dog secreted from prostate gland (Johnston *et al*., 2001). Finasteride did not affect on other parameters in semen quality during treatment period and after treatment cessation. Actually less semen volume has less effect on fertility (Johnston *et al*., 2001). One finasteride treatment dog, a 33.1 kg, 5.9 year-old Pitt bull terrier dog, had azoospermia at 8 and 16 wk after treatment cessation. He had an excellent libido, and complete ejaculation due to semen ALKP was in normal range (above 5,000 U/L) (Memon, 2007 and Johnston *et al*., 2001). He had no signs of other abnormalities. His azoospermia was still unknown cause.

7. Semen culture and cytology

In deslorelin treatment group, there was one dog in deslorelin treatment group that found 2.2×10^5 CFU/ mL *Klebsiella* spp. at 8 wk of treatment, the dog was on antimicrobrial drug based on drug sensitivity test for 3 wk. Before using deslorelin treatment in dogs with BPH and coincident with prostatitis, the dog should be cleared from bacterial prostatitis before hormone

was implanted. After implantation, sex hormone will be suppressed, the prostate gland will be shrinkage, and therefore the antimicrobial drugs will be hardly distributed through prostate gland. This was similar in castrated dog that was hardly treatment for prostatitis (Johnston *et al*., 2001). In the further study, anejaculation dog should collect semen or prostatic fluid by prostatic massage in order to perform bacterial culture and cytology tests. This technique may provide more details in the anejaculation dogs with deslorelin implanted.

In finasteride treatment group, two dogs were found bacterial infection from semen culture after treatment cessation. One dog was found 4.75×10^4 CFU/mL *E.coli*. (Beta- hemolysis), and 1.28×10^6 CFU/mL *Klebsiella* spp. at 8 and 16 wk after treatment cessation, respectively. Another dog was found 2.3×10^6 CFU/mL *Pseudomonas aeruginosa*, and *Streptococcous pyogenes* at 16 wk after treatment cessation. Antimicrobrial drugs based on drug sensitivity test were subscribed in both dogs until semen bacterial culture was negative. The BPH recurrent time was approximately 8 wk after finasteride cessation, and prostatitis occasionally might be developed in this period.

BPH is predisposing to have an infection due to the enlargement and more fluid secretion in the gland. Dog with BPH is often found a complication with prostatitis from bacterial infection, especially aerobic bacteria which may be related to urinary tract normal flora. Semen bacterial culture and CFU/mL should be done every visit when the disease is followed up for the monitoring successful treatment. Prostatitis is diagnosis when semen pathogen is over than 10^4 CFU/mL (Sirinarumitr *et al.,* 2001). The most possible pathogens found are gram negative bacilli which are Enterobacteriaceae and *Pseudomonas aeruginosa*. Other gram positive bacilli are Enterococci, *Staphylococcus aureus*, and Beta-haemolytic streptococci. An appropriate antimicrobial drug is selected based on the results of drug sensitivity test and be subscribed to the dog for 3-8 wk (Feldman and Nelson, 2004).

8. Measurement of serum T and serum DHT concentratins

Serum T concentrations from eight deslorelin treatment dogs during 40 wk of study time were shown in Figure 24. The treatment period was 24 wk, and the dogs were followed up for 16 wk after treatment cessation.

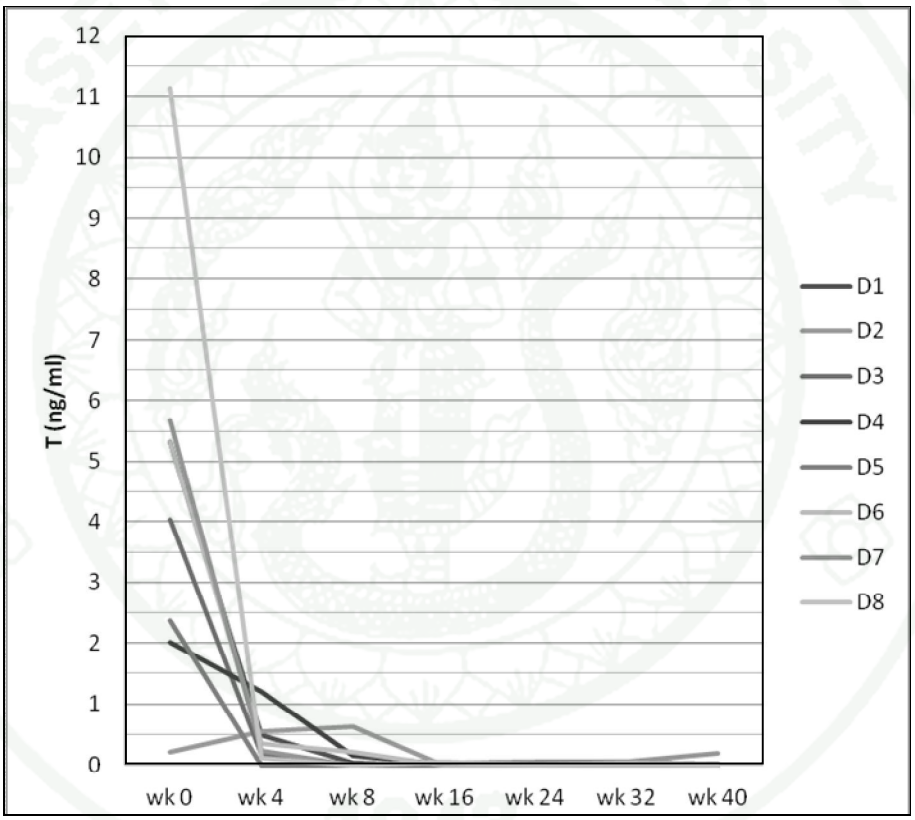

Figure 24 Serum T concentrations from eight deslorelin treatment dogs during 40 wk of study time; 24 wk of treatment period and followed with 16 wk after treatment cessation. This graph was represented from Table 7.1.

Basal T concentration is usually between 0.5-1.5 ng/mL (1.7-5.2 mmol/l), rising to a peak of 3.5-6.0 ng/mL (12.1 -20.8 mmol/l) (Keenam, 1998), or 1-10 ng/mL (Kamolpatana *et al*., 1998 and Kamolpatana, 1998). Seven dogs had serum T concentrations in normal range at the beginning time (wk 0), and then serum T concentrations tended to decrease until T concentration

was undetectable at the 16 wk after deslorelin implantation. Except there was one dog that serum T concentration was under the normal range (0.21 ng/mL) at the beginning time. He had a normal libido and semen quality. The underline cause of his low T concentration was unknown. Overall, the results were reasonable from a long action of GnRH agonist that shut down steroidogenesis (Fontaine and Fontbonne, 2011). The serum T concentration was designed to collect in next 4 wk after implantation. This period was too long to see the stimulation effect of short GnRH agonist action. If T concentration was collected everyday for a week after deslorelin implantation, we may see T concentration was increased from steroidogenesis stimulation following suppression effect.

Serum T concentrations from eight of finasteride treatment dogs during 32 wk of study time were shown in Figure 25. Treatment period was 16 wk, and the dogs were followed up for 16 wk after treatment cessation.

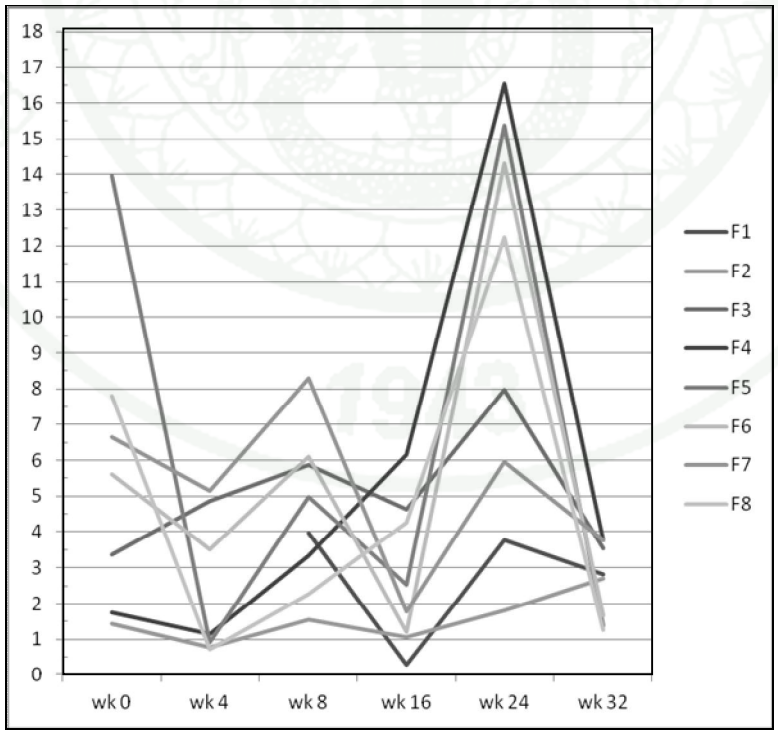

Figure 25 Serum T concentration from eight of finasteride treatment dogs during 32 wk of study time. This graph was represented from Table 7.2.

Serum T concentrations from all finasteride treatment dogs were in normal range thoughout 32 wk of study time. Finasteride did not effect on T concentration (Kamonpatana *et al*., 1998). Four weeks after treatment cessation (24 wk of the study), peak of T concentration (> 12 ng/mL) were detected in four dogs (Table 7.2 and Figure 25). All dogs were stayed in the same house with same owner. This phenomenon may be effected from the estrous bitch that was in the next cage. T, libido and semen quality were stimulated by pheromone (Keenam, 1998), and this effect may cause to increase in T concentration in these dogs.

Serum DHT concentrations from deslorelin and finasteride treatment dogs at 0, 4, 8 wk of treatment were represented in Figure 26 a-b.

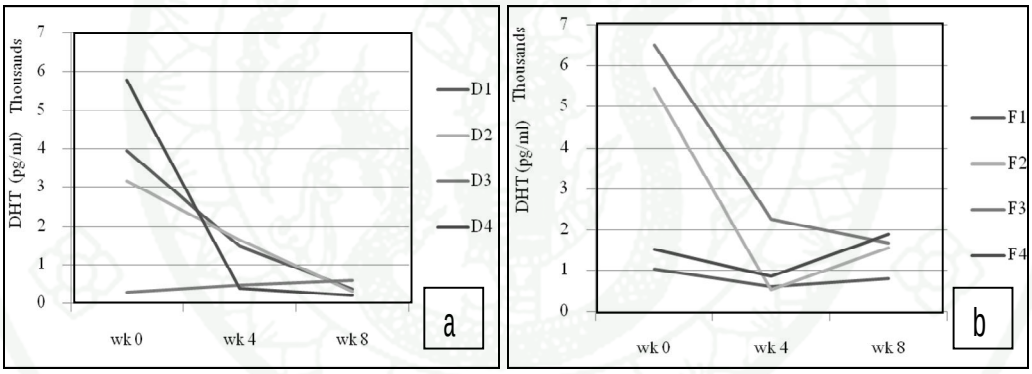

Figure 26 Serum DHT concentration from deslorelin treatment dogs (a) and finasteride treatment dogs (b) at 0, 4, 8 wk of treatment.

Both medications effected on decrease in serum DHT concentrations. The maximum decrease in percentage of DHT concentration were found at 8 wk of deslorelin and 4 wk of finasteride. There was no report available on the effect of deslorelin on DHTconcentration in dogs, however the results from this study were reasonable based on long action of GnRH agonist. Serum DHT concentration from finasteride treatment dogs was reported that finasteride at 0.10, 0.25, 0.5 mg/kg for 7 days were associated with a mean decrease in concentration of DHT of 55% (range, 155±32.3 pg/mL to 70±15 pg/mL) in normal mature intact male dogs (Kamonpatana *et al*., 1998). Deslorelin effected in DHT suppression more than finasteride at 4 wk of treatment.

This also related to effect of desolerin on decrease in T concentrations. Inclusion, desolerin was effected on decrease in both T and DHT concentrations. However, finasteride effected only on DHT but it was no effect on T concentrations.

There was not many reports available on normal DHT concentration in dog. There were 2 reportes available. One study reported that the normal range of serum DHT concentration was 155±32.3 pg/mL (Kamonpatana *et al.*, 1998). Another study reported the normal ranges of DHT concentration (mean±SEM) of 2 groups of dogs with BPH were 299±71 pg/mL (range = 35-649, n=9), and 429±111.3 pg/mL (range = 145-690, n=4), respectively (Kamolpatana, 1998). Most data available on DHT concentration were human DHT concentration. The National University Hospital (S) Pte Ltd, Singapore where was the laboratory that dog with BPH serums were sent to run DHTconcentration and it provided only normal ranges of human DHT concentrations as shown in Table 12. The normal DHT concentration in male is much higher than in previous reports in dogs.

Table 12 References range of human serum DHT concentration.

	Reference range (pg/ mL)
Male	250-990
Premenopausal female	24-368
Postmenopausal female	10-181

Source : Nationl University Hospital (S) Pte Ltd, Singapore 119074.

There were many factors effected on DHT results that can cause difficult interpretation, including small sample numbers, and or hormone measurement technique (Simoni, 2004). DHT measurement were randomed 4 samples from each treatment because of the expensive cost of DHT measurement. There was no laboratory available to measure DHT in Thailand, so serum samples were sent to Nationl University Hospital (S) Pte Ltd, Singapore. Serum DHT was measured by enzyme-linked immunosorbent assay (EIA). Immunoassays based on non-radioatively lables tracers, EIA, was the most popular alternative to radioactive method (RIA), but it was less accurate than radioimmonuassay (Simoni, 2004). The purified hormone extraction was also remarked on the high value results (Simoni, 2004). Hormone validiation data including sensitivity, specificity, and other valiation methods of this assay was not provided from the company, which may be very useful to interpeat between human DHT and canine DHT concentrations (Keenam, 1998).

CONCLUSION

Both deslorelin and finasteride were effective to treat BPH in dogs. The clinical signs were solved, and prostatic size and volume were decrease to normal approximately 4 weeks after treatment of both medications. Deslorelin was effect on FSH and LH down regulation, following with decrease in androgens production and secretion including T and DHT, consequencing with spermatogenesis suppression, prostate gland shrinkage, and leading to anejaculation. Finasteride was effect on hypertrophic prostatic cells by type II – 5 alpha reductase inhibitor causing decrease in DHT and consquencing with prostate gland shrinkage. Finasteride did not effect on T, and it also did not effect on semen quality.

The adverse effect of both treatments were not found on CBC, blood chemistry profiles, including skin reaction in deslorelin treatment dogs. Anejaculation phenomenal effected on dog at least 40 weeks after deslorelin implantation. Prostatitis should be cautioned on dog with finasteride treatment because the inflammation may complicate during treatment and/or after treatment cessation. The next appointments for monitoring the disease recurrent are recommended at least 16 and 8 weeks after treatment cessation for deslorelin and finasteride treatment, respectively.

As the differences in drug mechanisms and their effects, veterinarians should consider on treatment selection. Deslorelin is more suitable for dogs with anesthetic risk, and no longer concern in breeding. Finasteride is the suitable drug for breeding stud dogs. Disease recurrent should be monitored after both treatment cessations because both medications are temporary BPH treatment in dogs.

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APPENDIXES

Appendix A

Consent form in a clinical trial

การเปรียบเทียบผลข้างเคียงของ Deslorelin และ Finasteride ต่อการรักษาโรคต่อมลูกหมากโตในสุนัข

Side Effects of Deslorelin and Finesteride on Benign Prostatic Hypertrophy Treatment in Dogs.

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โทรสาร 02-579-7541

ความสำคัญ และที่มาของปัญหาที่ทำการวิจัย

โรคต่อมลูกหมากโต (Benign Prostatic Hypertrophy, BPH) เป็นภาวะที่เกิดขึ้นตามธรรมชาติ พบได้ในมนุษย์และสุนัขเท่านั้น สำหรับสุนัขจะพบในเพศผู้ที่ยังไม่ได้ทำหมัน โดยพบว่า 80% ของสุนัขที่ยังไม่ได้ทำหมันที่มีอายุ 5 ปีขึ้นไปจะเป็นโรคนี้ อาการที่พบในสุนัข ได้แก่ ท้องผูก มีเลือดปนในน้ำปัสสาวะและน้ำอสุจิ และโรคนี้อาจเป็นสาเหตุโน้มนำทำให้เกิดการอักเสบของต่อมลูกหมากหรือกระเพาะปัสสาวะได้

สาเหตุของโรคยังไม่สามารถอธิบายลักษณะทางพยาธิกำเนิดได้แน่ชัด อย่างไรก็ตามเป็นที่ยอมรับว่า Dihydrotestosterone (DHT) เป็นฮอร์โมนสำคัญที่ทำให้มีการขยายขนาดของต่อมลูกหมาก โดย DHT ถูกสังเคราะห์จากฮอร์โมนเพศ testosterone ภายในต่อมลูกหมาก โดยอาศัยเอ็นไซม์ 5 alpha reductase นอกจากนี้ยังมีปัจจัยอื่น ๆ ร่วมด้วย เช่น ฮอร์โมน testosterone estrogen และ growth factor อื่นๆ

การรักษาโรคต่อมลูกหมากโตในสุนัขแบ่งออกได้เป็น 2 วิธี คือ การทำหมันหรือการรักษาทางยา การทำหมันโดยการนำอัณฑะออกในสุนัขที่เป็นโรคนี้เป็นการรักษาที่ถาวร แต่ในสุนัขบางตัวอาจมีข้อจำกัดที่ไม่สามารถทำหมันได้ เช่น สุนัขที่มีอายุมาก สุนัขที่มีความเสี่ยงในการวางยาสลบ เช่น สุนัขที่เป็นโรคหัวใจ โรคตับ และโรคไตวาย สุนัขที่เจ้าของไม่ต้องการให้เข้ารับการผ่าตัดทำหมัน สุนัขพ่อพันธุ์ที่เจ้าของยังอยากได้ลูกสุนัข หรือสุนัขที่มีภาวะต่อมลูกหมากโตร่วมกับต่อมลูกหมากอักเสบแบบมีถุงหนองที่จำเป็นต้องได้รับการรักษาภาวะต่อมลูกหมากอักเสบด้วยยาปฏิชีวนะในระยะแรกให้ดีขึ้นก่อนที่จะผ่าตัดทำหมัน การรักษาทางยาจึงเป็นอีกทางเลือกหนึ่งสำหรับสุนัขกลุ่มดังกล่าว โดยยาที่นิยมใช้รักษามากที่สุด ได้แก่ Finesteride ยากลุ่มนี้จะลดระดับ DHT ทำให้ต่อมลูกหมากลดขนาดลง

ปัจจุบันโรงพยาบาลสัตว์มหาวิทยาลัยเกษตรศาสตร์มีสุนัขเพศผู้ที่มีปัญหาต่อมลูกหมากและมาทำการรักษาที่คลินิกระบบสืบพันธุ์เป็นจำนวนมากโดยพบสูงถึงประมาณ 70 % ของโรคทางระบบสืบพันธุ์ในตัวผู้ โดยเฉพาะโรคต่อมลูกหมากโตร่วมกับต่อมลูกหมากอักเสบจากการติดเชื้อแบคทีเรีย โดยหลักการรักษาโรคต่อมลูกหมากโตนั้น พบว่ามักเกิดร่วมกับต่อมลูกหมากอักเสบ ในกรณีนี้จะต้องทำการรักษาการอักเสบของต่อมลูกหมากก่อนด้วยยาปฏิชีวนะ จากนั้นจึงทำการรักษาต่อมลูกหมากโตโดยการทำหมัน หรือในสุนัขที่ไม่พร้อมทำการวางยาเพื่อผ่าตัด สุนัขที่เจ้าของไม่ต้องการให้ทำหมัน สุนัขที่ยังต้องการใช้เป็นพ่อพันธุ์ จะได้รับการรักษาทางยาด้วย finesteride (Proscar®) ในขนาด 0.1-0.5 มก/กก เป็นระยะเวลาต่อเนื่องประมาณ 1-4 เดือน อย่างไรก็ตามการใช้ยาตัวนี้อาจพบผลข้างเคียงจากการใช้ยาได้ โดยเฉพาะในกรณีที่ผู้ป้อนยาสุนัขเป็นหญิงตั้งครรภ์ เมื่อสัมผัสยาแล้วตัวยาอาจสามารถซึมผ่านทางผิวหนังเข้าสู่ร่างกาย และอาจมีผลต่อทารกเพศชายในครรภ์ได้ ดังนั้นในหญิงตั้งครรภ์จะแนะนำให้ใส่ถุงมือขณะที่ป้อนยาให้สุนัข นอกจากนี้ยังพบปัญหาอื่นๆในระหว่างการรักษา เช่น การใช้ยาอย่างต่อเนื่องนาน 1 ถึง 4 เดือน เจ้าของสัตว์ต้องดูแลและป้อนยาสัตว์ทุกวันตลอดช่วงการรักษา ยามีราคาสูงมาก ด้วยเหตุผลเหล่านี้จึงมีการริเริ่มในการศึกษายากลุ่มอื่น เช่น กลุ่ม GnRH agonist ชื่อ deslorelin โดยการใช้ยานี้จะเป็นการฝังตัวยาในรูปแบบแคปซูลขนาดเล็กเข้าชั้นใต้ผิวหนัง การใช้ deslorelin อาจเป็นทางเลือกใหม่ในการรักษาโรคต่อมลูกหมากโตในสุนัขที่สะดวกขึ้น ลดค่าใช้จ่ายในการรักษาทางยาที่ต่อเนื่อง เนื่องจากในการฝังครั้งหนึ่งตัวยาจะถูกปล่อยออกมาช้า ๆ โดยมีระยะเวลาออกฤทธิ์นาน 6 หรือ 12 เดือน โดยตัวยามีผลลดการสร้างฮอร์โมน testosterone และทำให้ต่อมลูกหมากมีการลดขนาดลง

ในปัจจุบันยังไม่มีการศึกษาถึงผลข้างเคียงจากการใช้ deslorelin และผลของการใช้ยากลุ่มนี้โดยการฝังใต้ผิวหนัง จึงควรมีการศึกษาถึงผลข้างเคียงของยาและผลของการใช้ยากลุ่มนี้โดยการฝังใต้ผิวหนัง เช่น การเปลี่ยนแปลงของค่าเคมีโลหิต ปฏิกิริยาการแพ้ของผิวหนังหรือการเปลี่ยนแปลงสี ลักษณะขนหรือผิวหนังบริเวณที่มีการฝังตัวยา และขนาดอัณฑะรวมถึงอาการอื่น ๆ ที่อาจแสดงขึ้น รวมทั้งยังไม่มีการศึกษาระยะเวลาของต่อมลูกหมากที่โตขึ้นหลังจากครบระยะเวลาที่ออกฤทธิ์ของฮอร์โมน ส่วนการใช้

ยาในกลุ่ม finasteride เองก็ยังไม่มีการศึกษาระยะเวลาของต่อมลูกหมากที่โตขึ้นหลังจากหยุดใช้ยา ดังนั้น ในการศึกษานี้กลุ่มทดลองที่ได้รับการรักษาด้วย finasteride หรือ deslorelin จะมีการติดตามผลการรักษาหลังหยุดใช้ยานาน 4 เดือน โดยการตรวจและเก็บข้อมูลของค่าโลหิตวิทยา ค่าเคมีโลหิต ระดับฮอร์โมน testosterone ขนาดของอัณฑะทั้งสองข้าง คุณภาพน้ำเชื้อ และปริมาตรต่อมลูกหมากจากการวัดขนาดต่อมลูกหมากโดยการทำ อัลตร้าซาวด์

วัตถุประสงค์ของโครงการวิจัย

1. เพื่อศึกษาผลข้างเคียงของ deslorelin (Suprelorin®) ต่อค่าโลหิตวิทยา เคมีโลหิต คุณภาพน้ำเชื้อ ปฏิกิริยา การแพ้ของผิวหนังหรือการเปลี่ยนแปลงลักษณะ สี ขนหรือผิวหนังบริเวณที่มีการฝังตัวยา รวมถึงอาการอื่น ๆ ที่อาจแสดงขึ้น ในการรักษาโรคต่อมลูกหมากโตในสุนัข
2. เพื่อเปรียบเทียบผลและผลข้างเคียงของ deslorelin (Suprelorin®) และ finasteride (Proscar®) ในการรักษาโรคต่อมลูกหมากโตในสุนัข
3. เพื่อศึกษาระยะเวลาการกลับมาเพิ่มขนาดของต่อมลูกหมากหลังจากหยุดยา finasteride (Proscar®) และ deslorelin (Suprelorin®) ในการรักษาโรคต่อมลูกหมากโตในสุนัข

วิธีการดำเนินการวิจัย และสถานที่ทำการทดลอง/เก็บข้อมูล

1. <u>สุนัขทดลอง</u>

สุนัขเพศผู้ที่มีอายุตั้งแต่ 5 ปีขึ้นไปและยังไม่ทำหมันจำนวนทั้งหมด 20 ตัว ที่มารับการรักษาที่โรงพยาบาลสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ โดยสุนัขได้รับการตรวจและยืนยันว่าเป็นโรคต่อมลูกหมากโต สุนัขโดยทั่วไปมีสภาพร่างกายอยู่ในเกณฑ์ปกติ สุนัขแสดงอาการทางคลินิกของโรคต่อมลูกหมากโต เช่น ท้องผูก มีลักษณะของเลือดหยดที่ปลายอวัยวะเพศ มีเลือดปนออกมาพร้อมปัสสาวะหรือน้ำอสุจิ มีอาการปัสสาวะลำบากหรือ กะปริกะปรอย สุนัขทุกตัวจะต้องตรวจค่าโลหิตวิทยา ค่าการทำงานของตับและไต การทำอัลตร้าซาวด์ได้ผลคำนวณปริมาตรต่อมลูกหมากพบว่ามีปริมาตรมากกว่าปกติ การเก็บน้ำเชื้อเพื่อตรวจคุณภาพน้ำเชื้อและผลการเพาะเชื้อแบคทีเรียที่ได้ปริมาณเชื้อไม่เกินค่ามาตรฐาน และไม่พบเซลล์อักเสบจากการตรวจเซลล์วินิจฉัย และสุนัขทุกตัวได้รับอนุญาตจากเจ้าของในการเข้ารับการศึกษานี้

2. <u>แผนการทดลอง</u>

สุนัขจะถูกสุ่มเลือกในการได้รับยา finasteride หรือ deslorelin โดยแต่ละกลุ่มใช้สุนัข 10 ตัว สุนัขกลุ่มที่ใช้ finasteride (Proscar®, Merck) จะได้รับยาในขนาด 0.1-0.5 มก./กก. (1 เม็ด/50 กก) กินวันละ 1 ครั้ง ทุกวันติดต่อกัน 16 สัปดาห์ ส่วนสุนัขอีกกลุ่มใช้ deslorelin ขนาด 4.7 มก. (Suprelorin® 4.7 มก., Peptech Animal Health) โดยฝังตัวยาเข้าชั้นใต้ผิวหนังเพียงครั้งเดียว บริเวณกึ่งกลางของสะบักในวันแรกของการทดลอง

3. **<u>ทำการตรวจและเก็บข้อมูล</u>**

สุนัขทุกตัว (ทั้งกลุ่มที่ใช้ finasteride และ deslorelin)ได้รับการตรวจค่าเคมีโลหิตในส่วนค่าการทำงานของตับและไต การวัดระดับฮอร์โมน testosterone ในเลือด** วัดขนาดอัณฑะทั้งสองข้าง การตรวจคุณภาพน้ำเชื้อ และวัดขนาดปริมาตรต่อมลูกหมากโดยคำนวณจากผลการทำอัลตร้าซาวด์ ในสัปดาห์ที่ 0, 4, 8, 16, 24, 32 และ 40 สัปดาห์ของการทดลอง และค่าโลหิตวิทยาในสัปดาห์ที่ 0, 16, 24 และ 40 สุนัขกลุ่มที่ฝังฮอร์โมนบันทึกปฏิกิริยาการแพ้ของผิวหนังหรือการเปลี่ยนแปลงสี ลักษณะขนหรือผิวหนังบริเวณที่มีการฝังตัวยา รวมถึงอาการอื่น ๆ ที่อาจแสดงขึ้นในสัปดาห์ที่ 0, 4, 8, 16, 24, 32 และ 40

<u>หมายเหตุ</u> ** การวัดปริมาณ testosterone ในซีรั่มทุกครั้งที่เก็บเลือดของสุนัขควรเก็บในช่วงเวลาเดียวกันของแต่ละตัว เก็บเลือดครั้งละ 3-5 มล. ทั้งหมด 3 ครั้ง โดยแต่ละครั้งเก็บห่างกัน 20-30 นาที

สถานที่ทำการทดลอง/เก็บข้อมูล

1. ภาควิชาเวชศาสตร์คลินิกสัตว์เลี้ยงคณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน
2. คลินิกเฉพาะทางระบบสืบพันธุ์ โรงพยาบาลสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน
3. หน่วยรังสีวินิจฉัย โรงพยาบาลสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน
4. ศูนย์ชันสูตรโรคสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน

ผลสำเร็จและความคุ้มค่าของการวิจัยที่คาดว่าจะได้รับ

ศึกษาผลการรักษาและผลข้างเคียงของการใช้ตัวยา deslorelin ในรูปแบบแคบซูลขนาดเล็กฝังเข้าชั้นใต้ผิวหนังในการรักษาโรคต่อมลูกหมากโตในสุนัข ซึ่งอาจเป็นทางเลือกใหม่ในการรักษาที่สะดวกขึ้น ลดค่าใช้จ่ายในการรักษาทางยาที่ต่อเนื่อง โดยให้ผลการรักษาที่น่าพอใจโดยที่ยังไม่มีการรายงานถึงผลข้างเคียงทั้งต่อสุนัขและผู้ใช้ยา นอกจากนั้นแล้วยังรายงานถึงระยะเวลาการกลับมาเพิ่มขนาดของต่อมลูกหมากหลังจากหยุดยา finasteride (Proscar®) และ deslorelin (Suprelorin®) ในการรักษาโรคต่อมลูกหมากโตในสุนัข

โรงพยาบาลสัตว์มหาวิทยาลัยเกษตรศาสตร์ บางเขน

หน่วยระบบสืบพันธุ์

ใบอนุญาตให้สัตว์เข้ารับการรักษาในโครงการ

"การศึกษาผลของ Deslorelin และ Finasteride ต่อการรักษาโรคต่อมลูกหมากโตในสุนัข

วันที่..... HN☐☐-☐☐☐☐☐☐

ข้าพเจ้า นาย/ น.ส./นาง.....สกุล.....

เจ้าของสุนัขชื่อ**.....พันธุ์.....อายุ.....

ติดต่อโดยโทรศัพท์..... มือถือ**.....

E-mail address.....ที่อยู่.....

ข้าพเจ้ามีความยินดีที่จะนำสุนัขเข้าร่วมโครงการศึกษาครั้งนี้ โดยที่

1. ข้าพเจ้าจะนำสุนัขเข้ารับการตรวจตามกำหนดที่นัดหมายทุกครั้ง
2. สุนัขอยู่ในความดูแลและเลี้ยงดูของข้าพเจ้าซึ่งเป็นเจ้าของสุนัข
3. สุนัขที่เข้าร่วมโครงการจะได้รับยา ในวันที่เริ่มโครงการ .....(ว/ด/ป)

O Finasteride 1 เม็ดต่อน้ำหนักไม่เกิน 50 กิโลกรัม ให้สุนัขกินวันละครั้ง ติดต่อกันนาน 16 สัปดาห์

O Desloreline 1 เข็มต่อตัว โดยสัตวแพทย์จะฝังยาไว้ที่ชั้นใต้ผิวหนังบริเวณกึ่งกลางของสะบักในวันแรกที่เริ่มการวิจัย

4. สุนัขจะได้รับยา การตรวจเลือด การตรวจวิเคราะห์คุณภาพน้ำเชื้อ การอัลตร้าซาวด์ช่องท้อง โดยที่ไม่เสีย ค่าใช้จ่ายตลอดระยะเวลาที่เข้าร่วมโครงการ
5. คณะวิจัยขอสงวนสิทธิ์ที่จะยกเลิกการเข้าร่วมโครงการได้ หากเจ้าของสุนัขไม่ได้ปฏิบัติตามระเบียบของโครงการวิจัยข้างต้น

ข้าพเจ้ายินยอมให้สุนัขของข้าพเจ้าเข้ารับการรักษาในโครงการการศึกษาผลของ Deslorelin และ Finasteride ต่อการรักษาโรคต่อมลูกหมากโตในสุนัข โดยที่ได้รับทราบรายละเอียดของโครงการเป็นอย่างดีแล้ว และข้าพเจ้าตระหนักดีว่าสัตวแพทย์และสถาบันแห่งนี้ไม่อาจรับผิดชอบต่ออุบัติเหตุใดๆ ที่อาจเกิดขึ้น เนื่องจากสัตวแพทย์ได้ให้ความดูแลและระมัดระวังอย่างดีที่สุดแล้ว

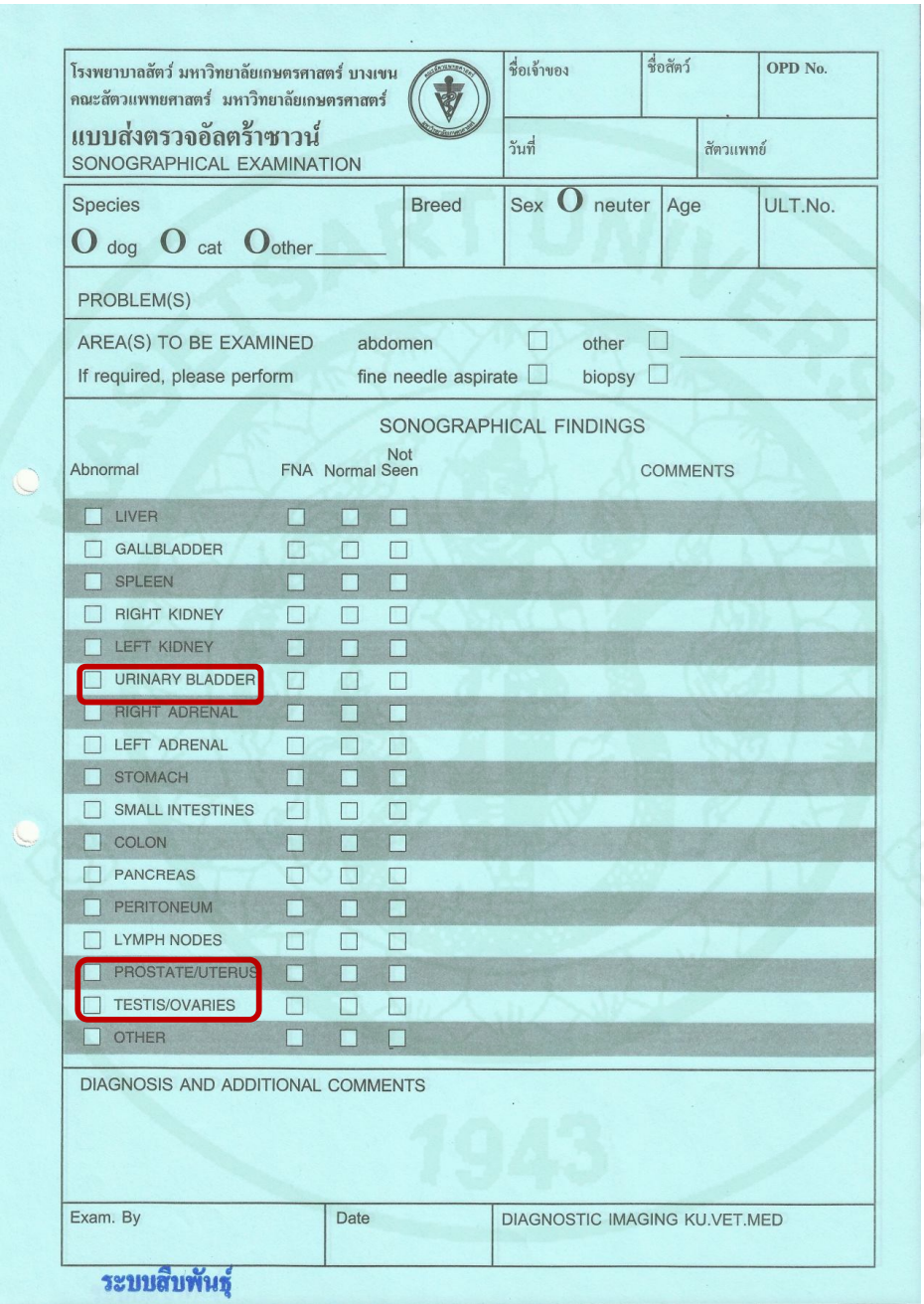
ลงชื่อ .....
(.....)
เจ้าของสุนัข

ลงชื่อ .....
(.....)
หัวหน้าคณะวิจัย

ลงชื่อ .....
(.....)
พยาน

Appendix B

Experiment forms in a clinical trial



โรงพยาบาลสัตว์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์
แบบส่งตรวจอัลตร้าซาวน์
SONOGRAPHICAL EXAMINATION

ชื่อเจ้าของ | ชื่อสัตว์ | OPD No.
วันที่ | สัตวแพทย์

Species	Breed	Sex	Age	ULT.No.
O dog O cat O other_____		O neuter		

PROBLEM(S)

AREA(S) TO BE EXAMINED abdomen ☐ other ☐
If required, please perform fine needle aspirate ☐ biopsy ☐

SONOGRAPHICAL FINDINGS

Abnormal	FNA	Normal	Not Seen	COMMENTS
☐ LIVER	☐	☐	☐	
☐ GALLBLADDER	☐	☐	☐	
☐ SPLEEN	☐	☐	☐	
☐ RIGHT KIDNEY	☐	☐	☐	
☐ LEFT KIDNEY	☐	☐	☐	
☐ URINARY BLADDER	☐	☐	☐	
☐ RIGHT ADRENAL	☐	☐	☐	
☐ LEFT ADRENAL	☐	☐	☐	
☐ STOMACH	☐	☐	☐	
☐ SMALL INTESTINES	☐	☐	☐	
☐ COLON	☐	☐	☐	
☐ PANCREAS	☐	☐	☐	
☐ PERITONEUM	☐	☐	☐	
☐ LYMPH NODES	☐	☐	☐	
☐ PROSTATE/UTERUS	☐	☐	☐	
☐ TESTIS/OVARIES	☐	☐	☐	
☐ OTHER	☐	☐	☐	

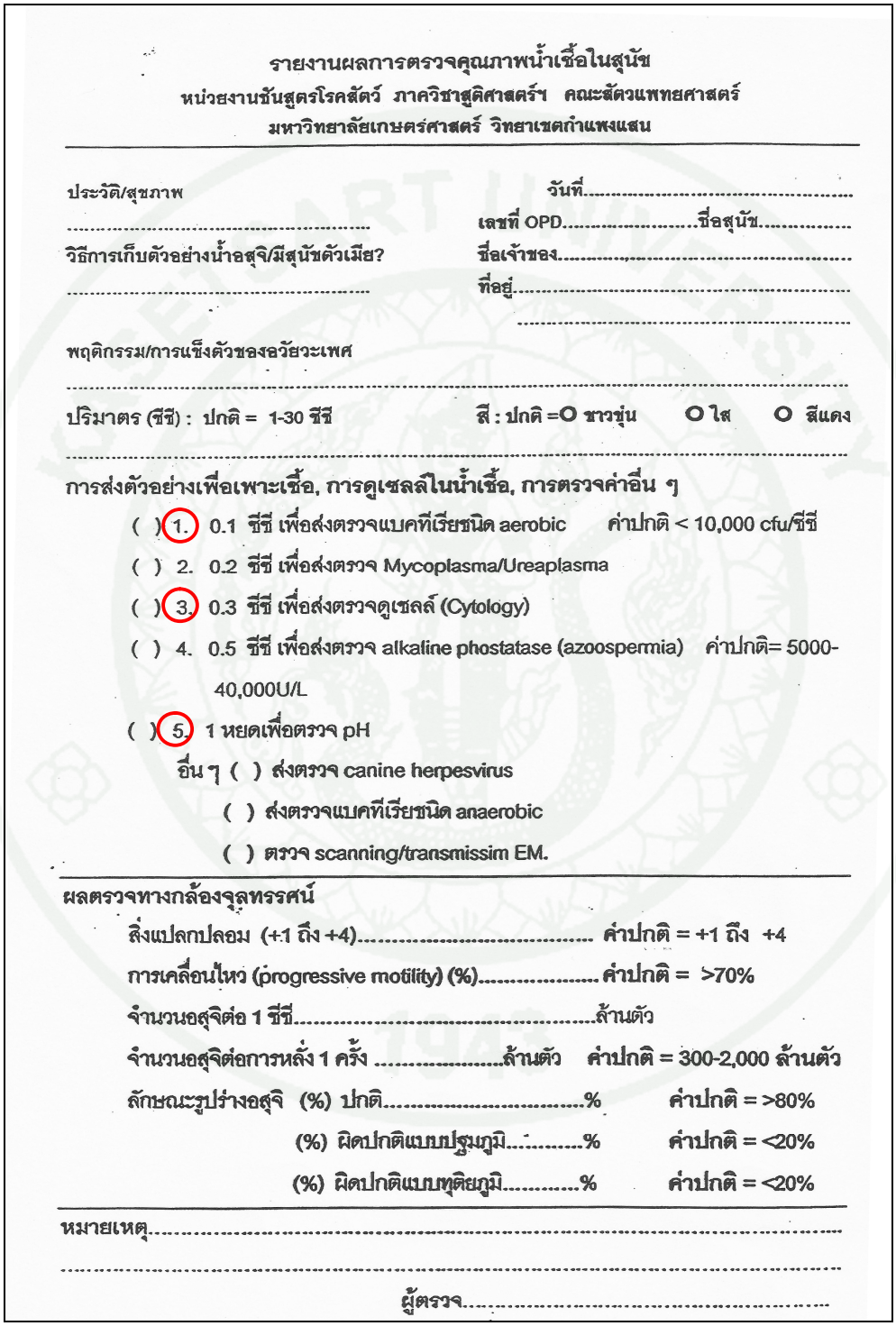
DIAGNOSIS AND ADDITIONAL COMMENTS

Exam. By | Date | DIAGNOSTIC IMAGING KU.VET.MED

ระบบสืบพันธุ์

Appendix Figure 1 Ultrasonography examination form of KUVTH. All dogs with BPH in the study were examined in ultrasonography of prostatic and testicular at Radiographic and Imaging Unit.

Source: KUVTH (2012)



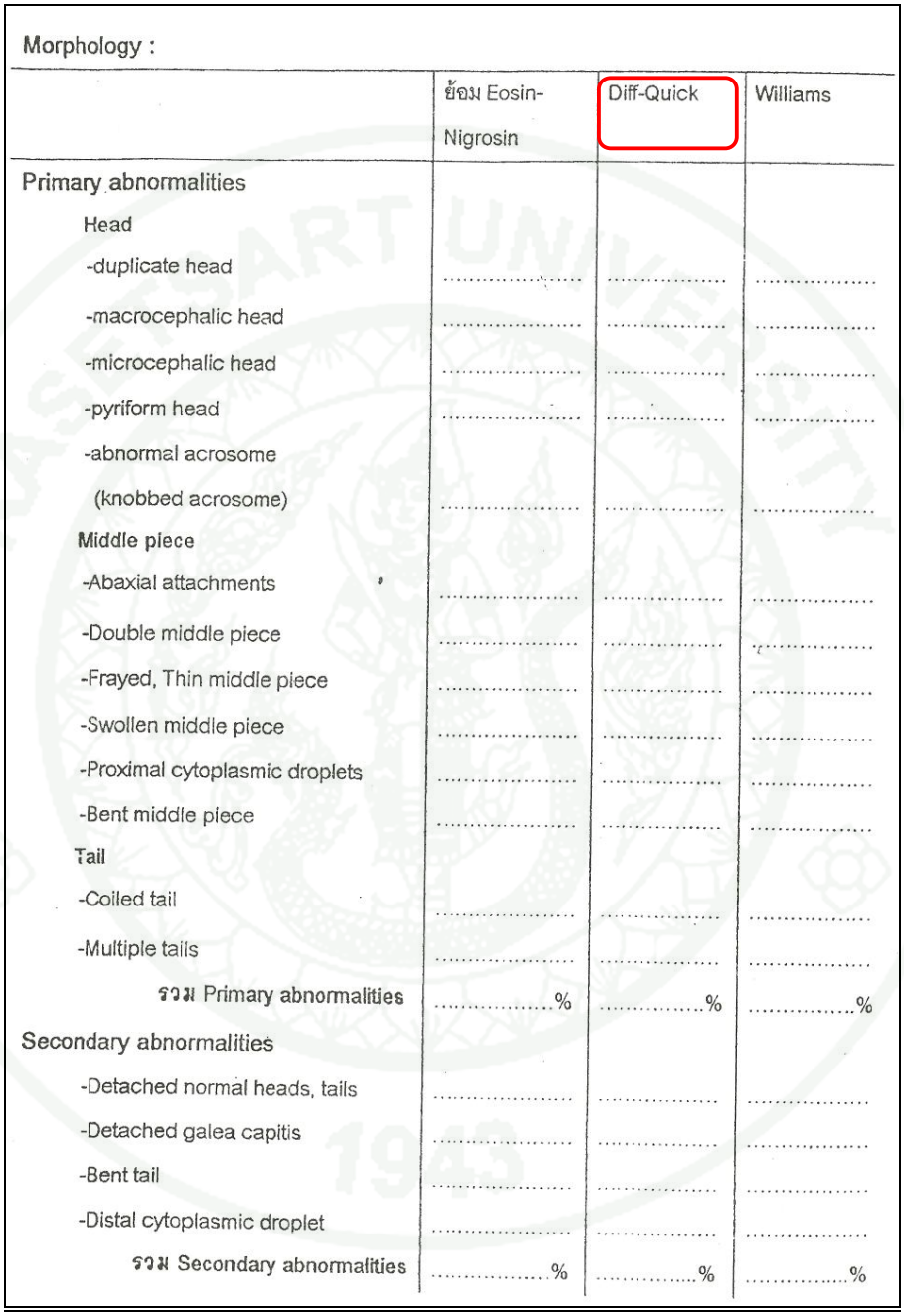
รายงานผลการตรวจคุณภาพน้ำเชื้อในสุนัข
หน่วยงานชันสูตรโรคสัตว์ ภาควิชาสูติศาสตร์ฯ คณะสัตวแพทยศาสตร์
มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน

ประวัติ/สุขภาพ วันที่.....
..... เลขที่ OPD.....ชื่อสุนัข.....
วิธีการเก็บตัวอย่างน้ำอสุจิ/มีสุนัขตัวเมีย? ชื่อเจ้าของ.....
..... ที่อยู่.....
.....
พฤติกรรม/การแข็งตัวของอวัยวะเพศ
.....
ปริมาตร (ซีซี) : ปกติ = 1-30 ซีซี สี : ปกติ =O ขาวขุ่น O ใส O สีแดง
.....
การส่งตัวอย่างเพื่อเพาะเชื้อ, การดูเซลล์ในน้ำเชื้อ, การตรวจค่าอื่น ๆ
( ) 1. 0.1 ซีซี เพื่อส่งตรวจแบคทีเรียชนิด aerobic ค่าปกติ < 10,000 cfu/ซีซี
( ) 2. 0.2 ซีซี เพื่อส่งตรวจ Mycoplasma/Ureaplasma
( ) 3. 0.3 ซีซี เพื่อส่งตรวจดูเซลล์ (Cytology)
( ) 4. 0.5 ซีซี เพื่อส่งตรวจ alkaline phostatase (azoospermia) ค่าปกติ= 5000-40,000U/L
( ) 5. 1 หยดเพื่อตรวจ pH
อื่น ๆ ( ) ส่งตรวจ canine herpesvirus
( ) ส่งตรวจแบคทีเรียชนิด anaerobic
( ) ตรวจ scanning/transmissim EM.

ผลตรวจทางกล้องจุลทรรศน์
สิ่งแปลกปลอม (+1 ถึง +4)..... ค่าปกติ = +1 ถึง +4
การเคลื่อนไหว (progressive motility) (%)..... ค่าปกติ = >70%
จำนวนอสุจิต่อ 1 ซีซี.....ล้านตัว
จำนวนอสุจิต่อการหลั่ง 1 ครั้ง .....ล้านตัว ค่าปกติ = 300-2,000 ล้านตัว
ลักษณะรูปร่างอสุจิ (%) ปกติ.....% ค่าปกติ = >80%
(%) ผิดปกติแบบปฐมภูมิ.....% ค่าปกติ = <20%
(%) ผิดปกติแบบทุติยภูมิ.....% ค่าปกติ = <20%

หมายเหตุ.....
.....
ผู้ตรวจ.....

Appendix Figure 2 Semen evaluation report form
Source: Reproductive Clinic, KUVTH (2012)

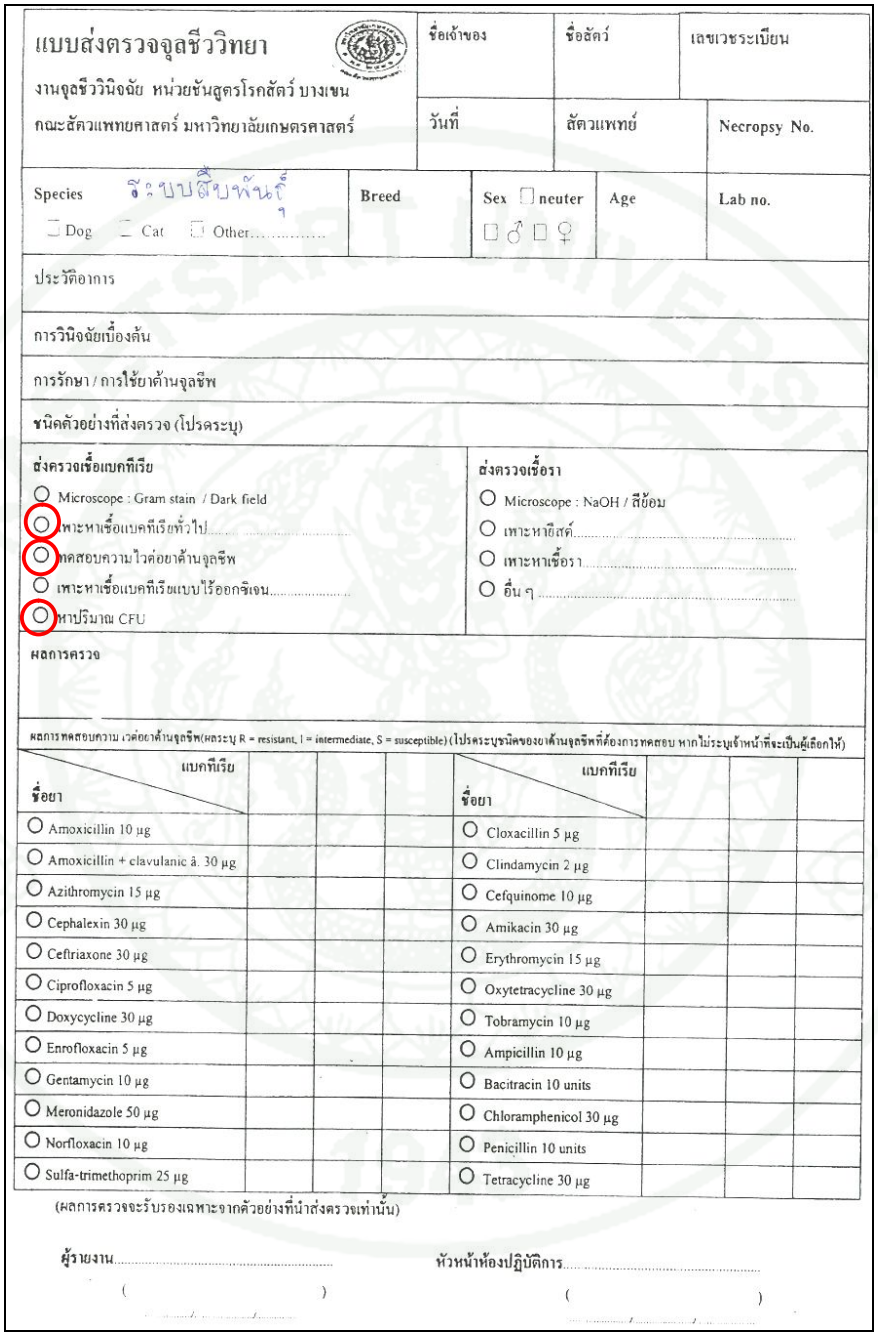


Morphology :

	ย้อม Eosin-Nigrosin	Diff-Quick	Williams
Primary abnormalities			
Head			
-duplicate head	.....	.....	.....
-macrocephalic head	.....	.....	.....
-microcephalic head	.....	.....	.....
-pyriform head	.....	.....	.....
-abnormal acrosome (knobbed acrosome)	.....	.....	.....
Middle piece			
-Abaxial attachments	.....	.....	.....
-Double middle piece	.....	.....	.....
-Frayed, Thin middle piece	.....	.....	.....
-Swollen middle piece	.....	.....	.....
-Proximal cytoplasmic droplets	.....	.....	.....
-Bent middle piece	.....	.....	.....
Tail			
-Coiled tail	.....	.....	.....
-Multiple tails	.....	.....	.....
รวม Primary abnormalities	.....%	.....%	.....%
Secondary abnormalities			
-Detached normal heads, tails	.....	.....	.....
-Detached galea capitis	.....	.....	.....
-Bent tail	.....	.....	.....
-Distal cytoplasmic droplet	.....	.....	.....
รวม Secondary abnormalities	.....%	.....%	.....%

Appendix Figure 3 Sperm morphology examination form

Source: Reproductive Clinic, KUVTH (2012)



แบบส่งตรวจจุลชีววิทยา

งานจุลชีววินิจฉัย หน่วยชันสูตรโรคสัตว์ บางเขน
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์

ชื่อเจ้าของ | ชื่อสัตว์ | เลขเวชระเบียน
วันที่ | สัตวแพทย์ | Necropsy No.

Species ระบบสืบพันธุ์ ☐ Dog ☐ Cat ☐ Other..... | Breed | Sex ☐ neuter ☐♂ ☐♀ | Age | Lab no.

ประวัติอาการ

การวินิจฉัยเบื้องต้น

การรักษา / การใช้ยาต้านจุลชีพ

ชนิดตัวอย่างที่ส่งตรวจ (โปรดระบุ)

ส่งตรวจเชื้อแบคทีเรีย
- ○ Microscope : Gram stain / Dark field
- ○ เพาะหาเชื้อแบคทีเรียทั่วไป.....
- ○ ทดสอบความไวต่อยาต้านจุลชีพ
- ○ เพาะหาเชื้อแบคทีเรียแบบไร้ออกซิเจน.....
- ○ หาปริมาณ CFU

ส่งตรวจเชื้อรา
- ○ Microscope : NaOH / สีย้อม
- ○ เพาะหายีสต์.....
- ○ เพาะหาเชื้อรา.....
- ○ อื่น ๆ .....

ผลการตรวจ

ผลการทดสอบความไวต่อยาต้านจุลชีพ(ผลระบุ R = resistant, I = intermediate, S = susceptible) (โปรดระบุชนิดของยาต้านจุลชีพที่ต้องการทดสอบ หากไม่ระบุเจ้าหน้าที่จะเป็นผู้เลือกให้)

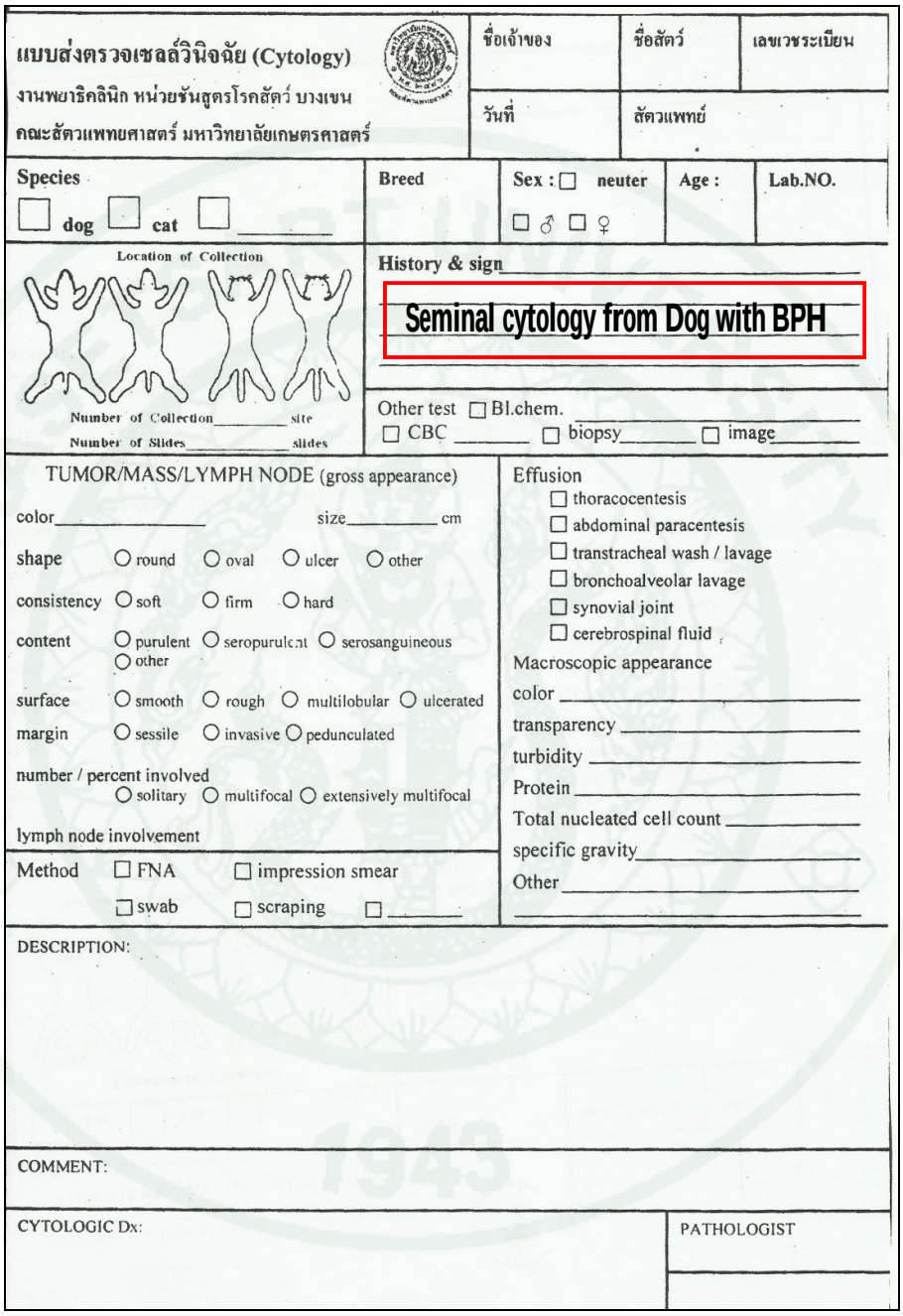
ชื่อยา \ แบคทีเรีย				ชื่อยา \ แบคทีเรีย			
○ Amoxicillin 10 µg				○ Cloxacillin 5 µg			
○ Amoxicillin + clavulanic a. 30 µg				○ Clindamycin 2 µg			
○ Azithromycin 15 µg				○ Cefquinome 10 µg			
○ Cephalexin 30 µg				○ Amikacin 30 µg			
○ Ceftriaxone 30 µg				○ Erythromycin 15 µg			
○ Ciprofloxacin 5 µg				○ Oxytetracycline 30 µg			
○ Doxycycline 30 µg				○ Tobramycin 10 µg			
○ Enrofloxacin 5 µg				○ Ampicillin 10 µg			
○ Gentamycin 10 µg				○ Bacitracin 10 units			
○ Meronidazole 50 µg				○ Chloramphenicol 30 µg			
○ Norfloxacin 10 µg				○ Penicillin 10 units			
○ Sulfa-trimethoprim 25 µg				○ Tetracycline 30 µg			

(ผลการตรวจจะรับรองเฉพาะจากตัวอย่างที่นำส่งตรวจเท่านั้น)

ผู้รายงาน..... ( ) ...../...../.....

หัวหน้าห้องปฏิบัติการ..... ( ) ...../...../.....

Appendix Figure 4 Bacterial culture submitted form
Source: KUVTH (2012)



แบบส่งตรวจเซลล์วินิจฉัย (Cytology)
งานพยาธิคลินิก หน่วยชันสูตรโรคสัตว์ บางเขน
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์

ชื่อเจ้าของ | ชื่อสัตว์ | เลขเวชระเบียน
วันที่ | สัตวแพทย์

Species ☐ dog ☐ cat ☐ _____ | Breed | Sex : ☐ neuter ☐ ♂ ☐ ♀ | Age : | Lab.NO.

Location of Collection

Number of Collection_____ site
Number of Slides_____ slides

History & sign

Seminal cytology from Dog with BPH

Other test ☐ Bl.chem. _____
☐ CBC _____ ☐ biopsy_____ ☐ image_____

TUMOR/MASS/LYMPH NODE (gross appearance)

color_____ size_____ cm

shape ○ round ○ oval ○ ulcer ○ other

consistency ○ soft ○ firm ○ hard

content ○ purulent ○ seropurulent ○ serosanguineous ○ other

surface ○ smooth ○ rough ○ multilobular ○ ulcerated

margin ○ sessile ○ invasive ○ pedunculated

number / percent involved ○ solitary ○ multifocal ○ extensively multifocal

lymph node involvement

Method ☐ FNA ☐ impression smear ☐ swab ☐ scraping ☐ _____

Effusion
☐ thoracocentesis
☐ abdominal paracentesis
☐ transtracheal wash / lavage
☐ bronchoalveolar lavage
☐ synovial joint
☐ cerebrospinal fluid

Macroscopic appearance
color _____
transparency _____
turbidity _____
Protein _____
Total nucleated cell count _____
specific gravity_____
Other _____

DESCRIPTION:

COMMENT:

CYTOLOGIC Dx: | PATHOLOGIST

Appendix Figure 5 Cytology form
Source: KUVTH (2012)

โรงพยาบาลสัตว์มหาวิทยาลัยเกษตรศาสตร์ หน่วยระบบสืบพันธุ์	Date:	HN:
	Time:	Animal name:
Rectal palpation	size…….. shape…….. surface…… pain……	

Testis size	**Right**	**Left**
Utrasonography		
Prostate border		
Prostate parenchyma		
Prostate volume (ml)	L , W , D Vol.=	
Ejaculation		
O AV with female dog		O AV with female pheromone
Libido :		
Erection:		

Semen quality						
	vol.	**normal**	**colour**	**normal**	**pH**	**normal**
fraction 1 (persperm)		(1-2 ml)		(clear)		
fraction 2 (spermrich)		(5-10ml)		(opalescent)		(6.3-6.7)
fraction 3 (prostate fluid)		(5-20 ml)		(clear)		(6.0-7.4)

Microscope					
Artifact		(+1 - +4)	Morphology (%)		
Progressive motility		(>70%)	Normal		(>80%)
spermatozoa/ ml		(× 10^6)	Primary abnormality		(< 20%)
spermatozoa/ejaculate		(300-2,000 ×10^6)	Secondary abnormality		(< 20%)
Semen culture :					
Result					
CFU					
Seminal cytology (epithelial cell, RBC, WBC. bacteria) :					

Appendix Figure 6 Data collection form (created from this study)

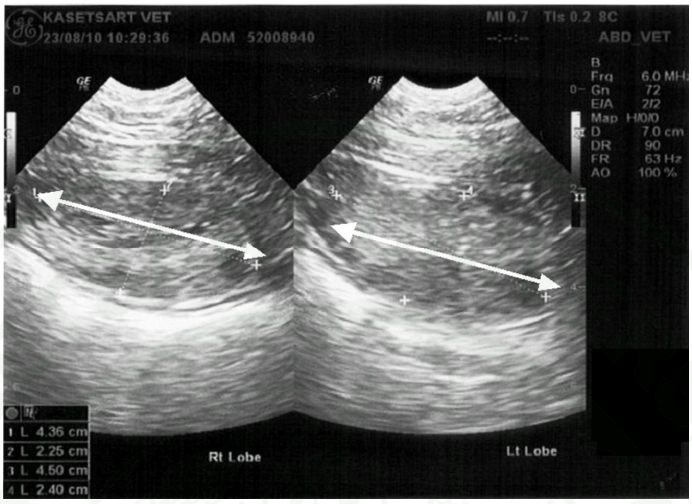
Appendix Table 1 Normal parameters for canine ejaculation.

Parameter	value
pH	6.3- 6.7
Volume:	1-30 mL
1 st fraction	1-12 mL
2 nd fraction	1-2 mL
3 rd fraction	up to 20 mL
Progressive motility	> 70%
Normal sperm morphology	> 80%
Abnormal sperm morphology	< 20%
Total sperm / ejaculation	>200 X106
WBCs	< 2000/mL
Alkaline phosphatase (IU/l)	5000-40,000

Source : Keenam (1998)

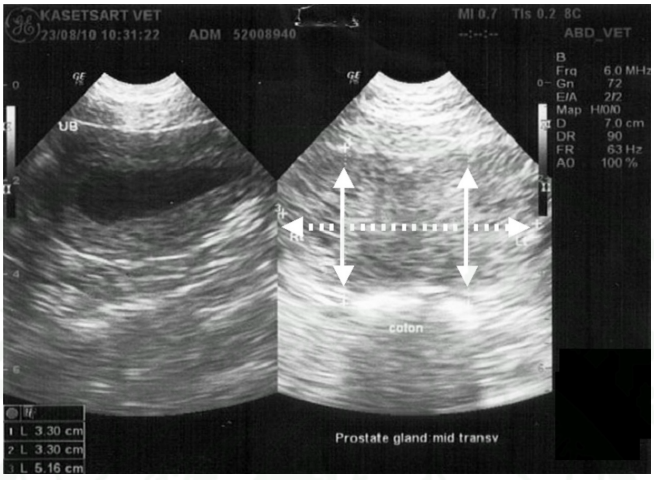
Appendix C

Prostatic volume calculation



Appendix Figure 7 Showing a saggital plain from right and left lobes of prostate gland for length (L) measurement.

L right	=	4.36 cm
L left	=	4.50 cm
L average	=	(L right + L left)/ 2
	=	(4.36 + 4.50)/2
	=	4.43 cm (L)



Appendix Figure 8 Showing a transverse plain of prostate gland for width (W) (dot arrow) and depth (D) measurements for right and left lobes.

W	=	5.16 cm (W)
D right	=	3.30 cm
D left	=	3.30 cm.
D average	=	(D right + D left)/2
	=	(3.30+3.30)/2
	=	3.30 cm (D)
Prostate volume	=	**(1/2.6×L× W × D) + 1.8**
	=	(1/2.6 × 4.43 × 5.16 × 3.30) + 1.8
	=	**30.81 mL**

(Sirinarumitr *et al*., 2001)

Appendix D

Hormone concentrations and validations

Hormone validation was reported only T measurement because DHT measurement details were inadequate from Laboratory. Precision are separated in intra-assay and inter-assay. The repeated measurement of a single sample in a single assay run is intra-assay. The repeated measurement of a single sample in a repeat assay on different days is inter-assay. Reasonable precision is a variability of less than 15% for both intra-assay and inter-assay (Robert, 1998).

Appendix Table 2 Intra assay of T measurement by Immulite™ Test kit.

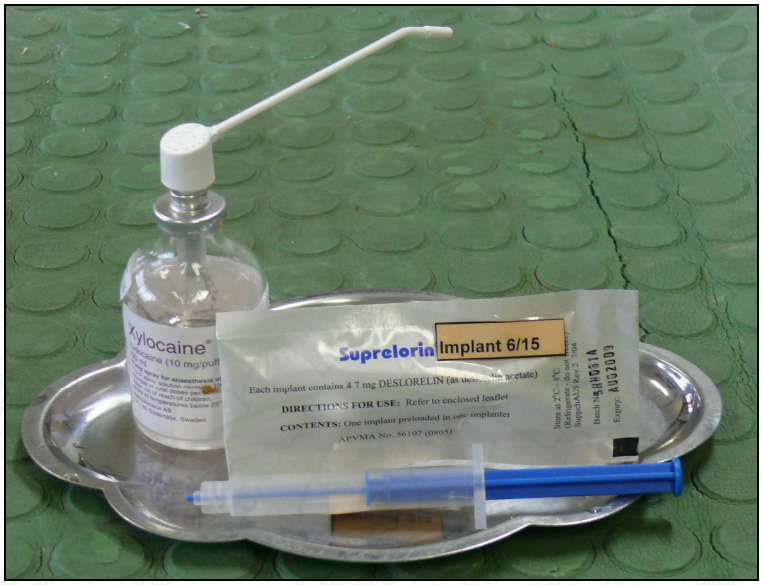
Pool serum level	Results					Mean	SD	Coefficient of variation (%)
	1	2	3	4	5			
Low	3.30	3.37	3.59	3.09	3.18	3.31	0.19	5.80
Medium	4.02	3.46	4.21	3.80	3.94	3.89	0.28	7.22
High	8.68	9.74	9.32	10.07	10.40	9.64	0.67	6.95

Appendix Table 3 Inter assay of T measurement by Immulite™ Test kit.

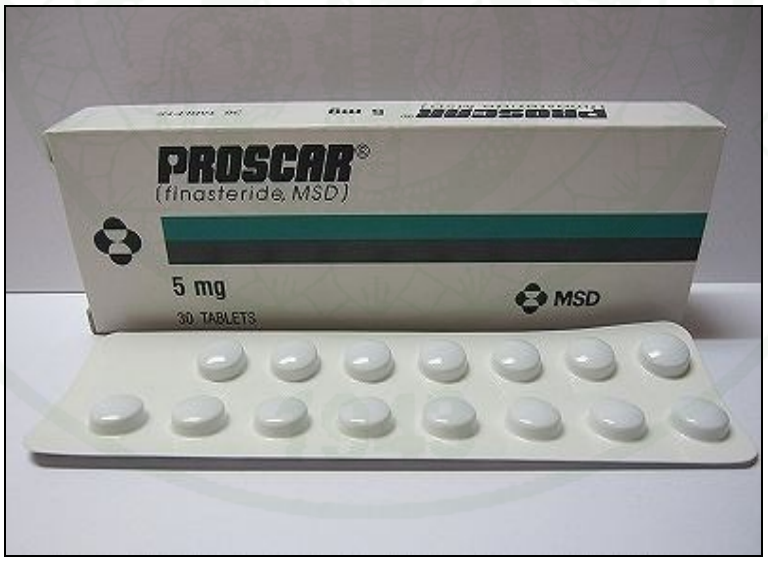
Pool serum level	Results					Mean	SD	Coefficient of variation (%)
	1	2	3	4	5			
Low	1.15	1.34	0.59	0.64	0.60	0.86	0.35	40.95
Medium	4.26	5.29	3.21	2.81	2.75	3.66	1.09	29.80
High	15.39	15.13	8.00	8.76	7.36	10.93	3.99	36.48

Appendix D

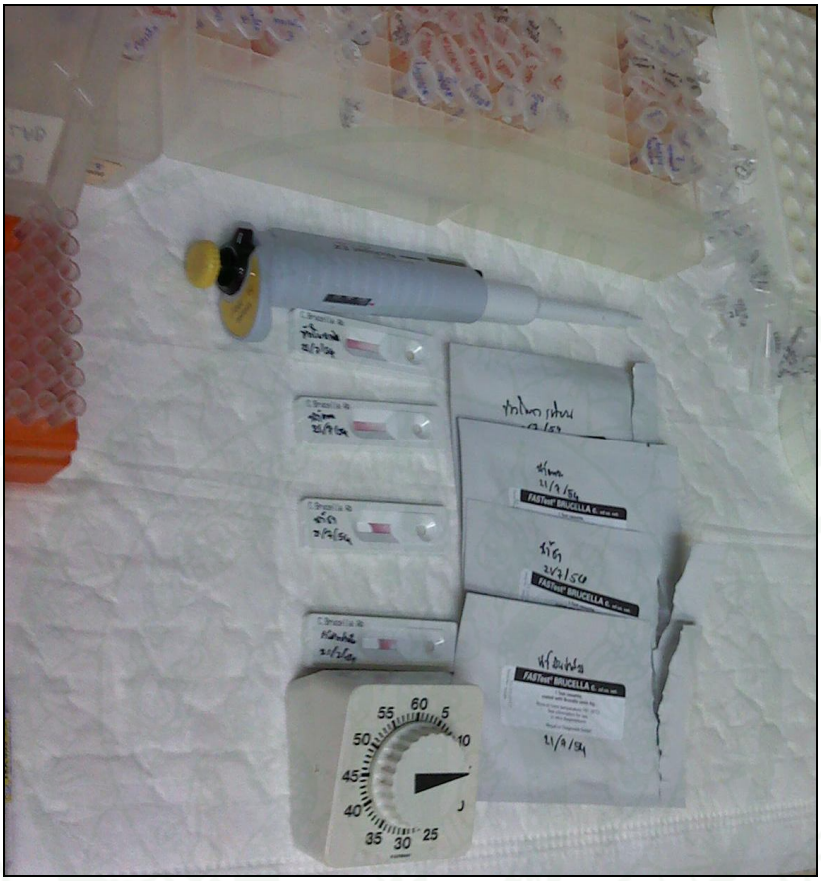
Experimental figures



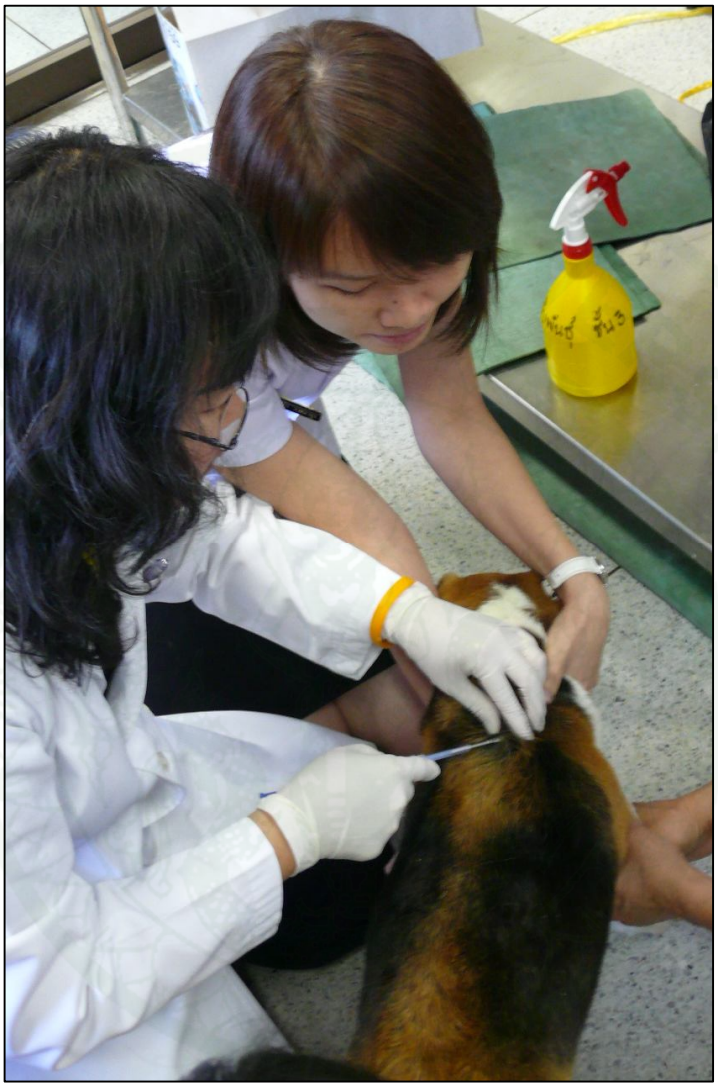
Appendix Figure 9 4.7 mg deslorelin (Suprelorin™) a single implantation



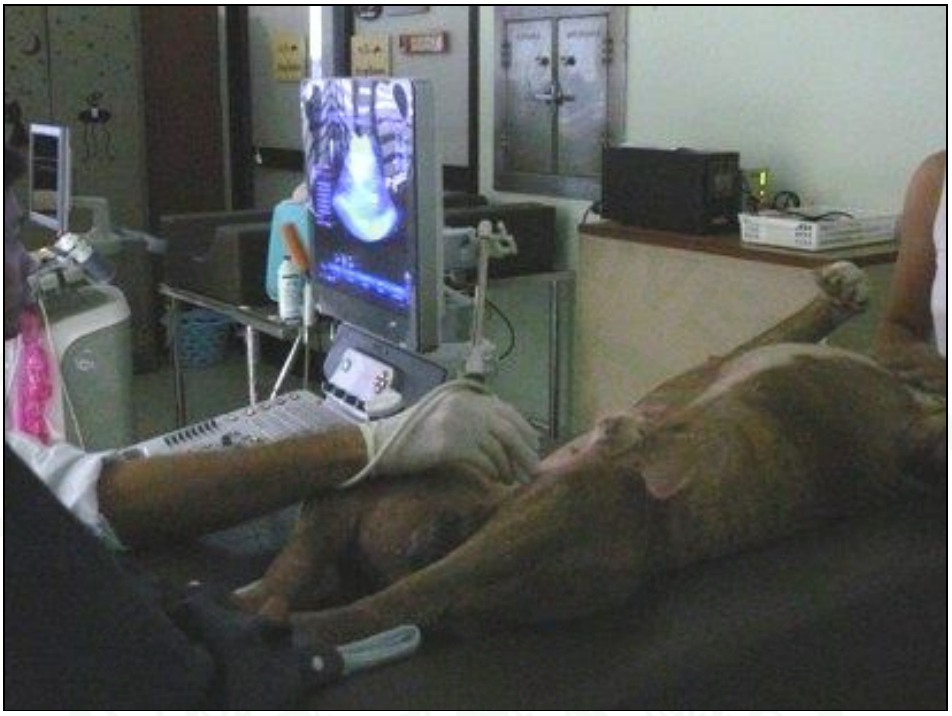
Appendix Figure 10 5 mg finasteride (Proscar™)



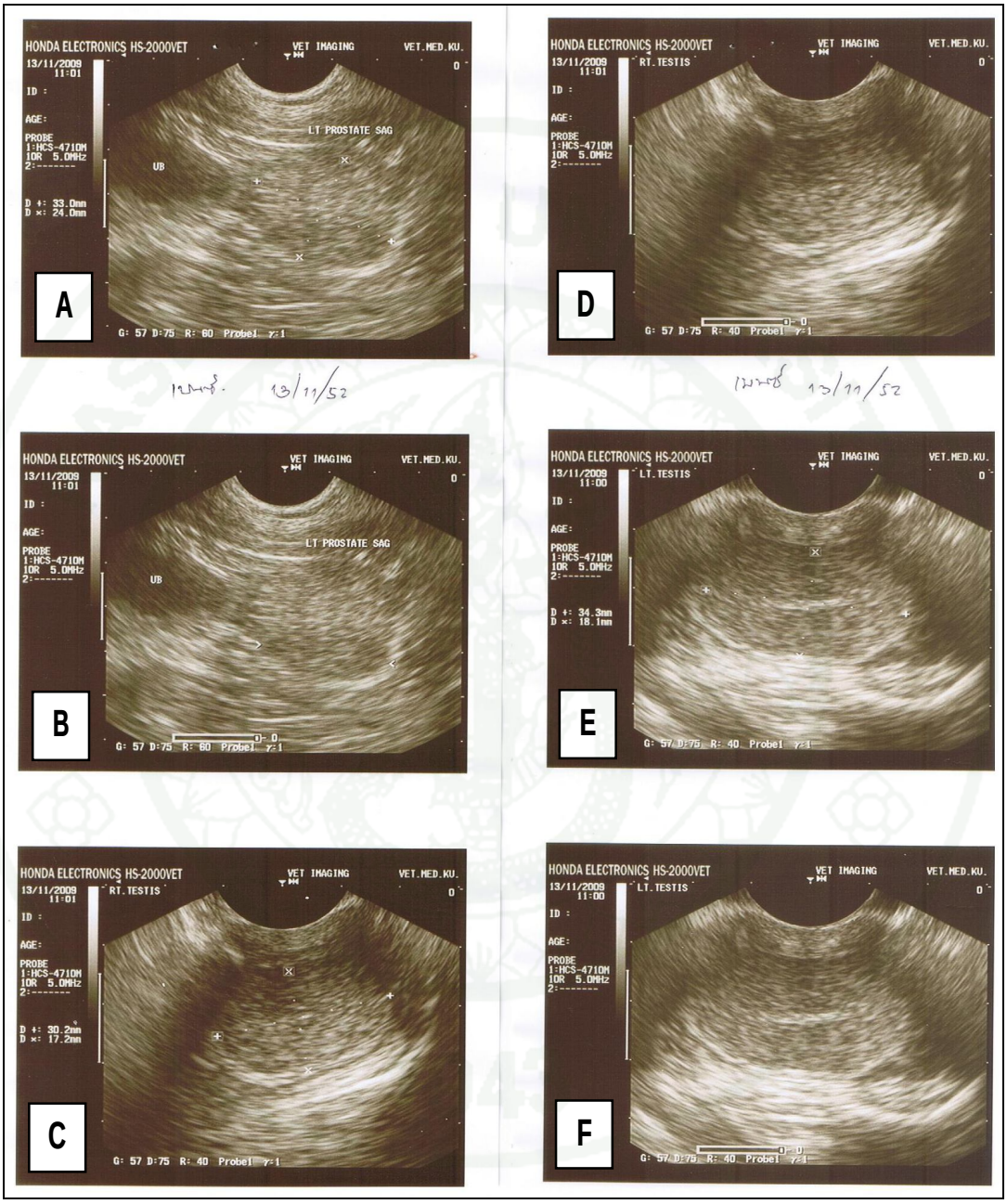
Appendix Figure 11 All dogs had to screen for Canine brucella antibody test (FASTest ™ BRUCELLA c.) before treatment.



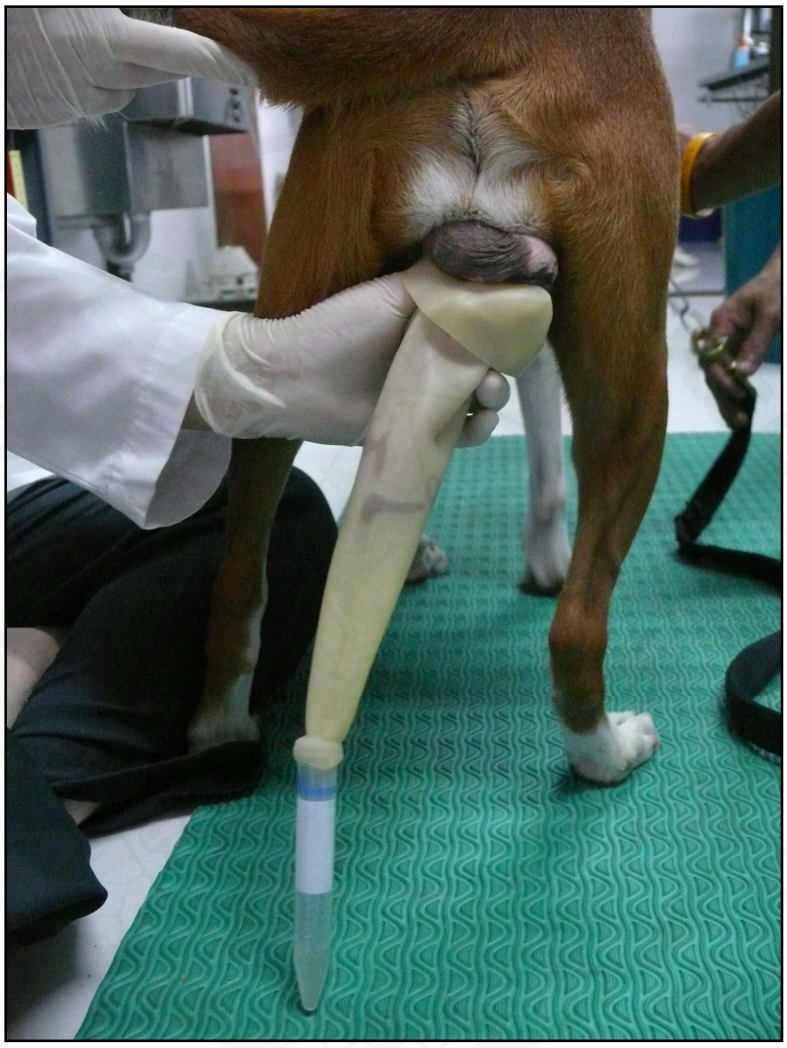
Appendix Figure 12 Dog was implanted with a single dose 4.7 mg of deslorelin (Suprelorin™, Peptech Animal Health), subcutaneous injection between base of scapular area.



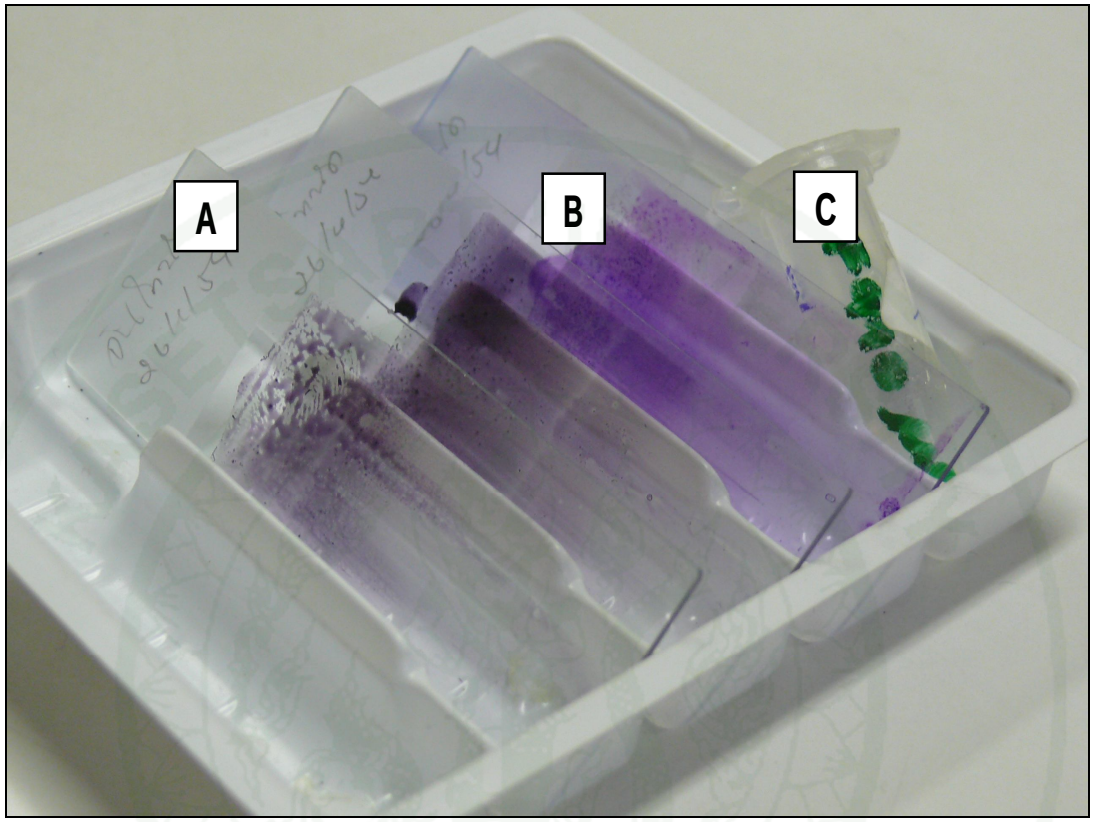
Appendix Figure 13 Transabdominal ultrasonography for prostatic size and parenchyma.



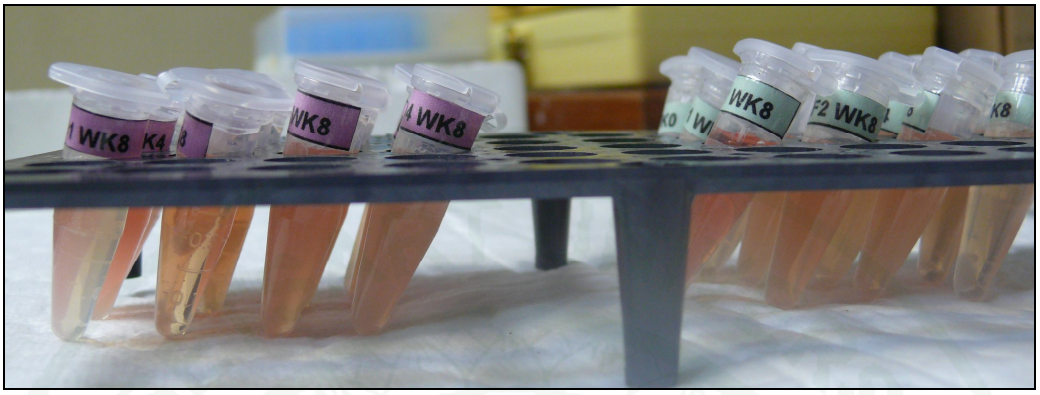
Appendix Figure 14 Transadominal ultrasonography to display 1. Urinary bladder (A,B) 2.Prostatic parenchyma and prostatic measurement at saggital plane (A, B) and transverse plane (C, D) 3.Testis (E, F).



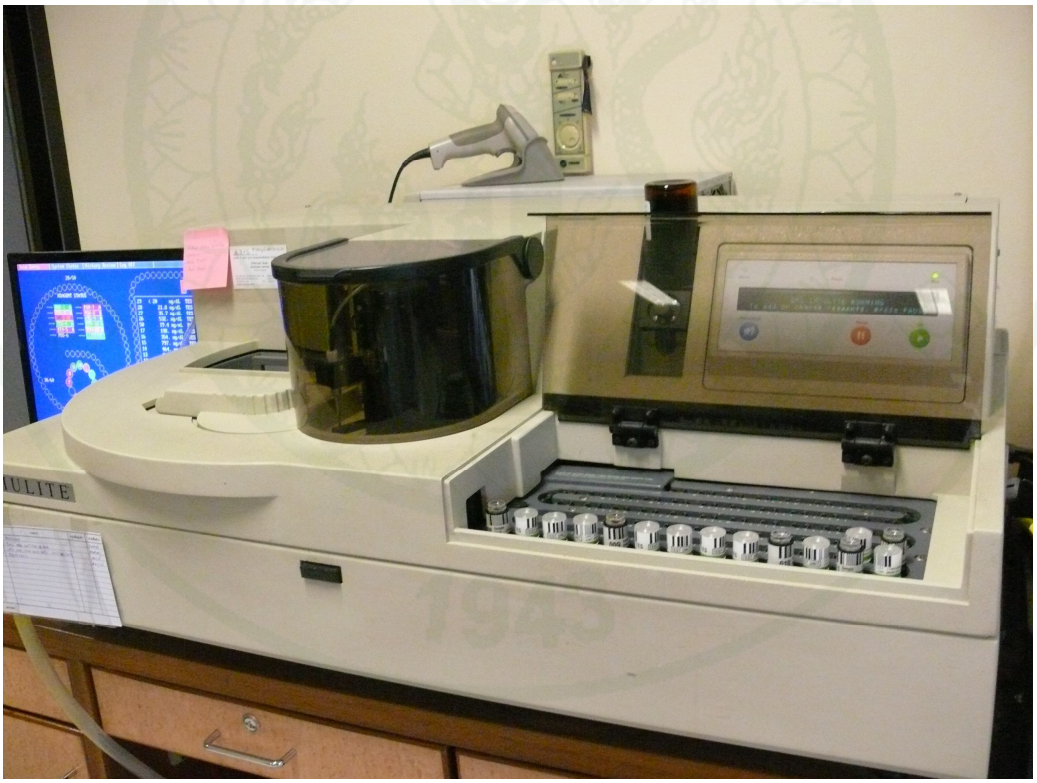
Appendix Figure 15 Manual semen collection.



Appendix Figure 16 Semen evaluation for sperm morphology with dip quik stain (A), percentage of dead and alive with eosin- nigrosin stain (B), and semen concentration (C).



Appendix Figure 17 Pool serum samples for hormone assay.



Appendix Figure 18 Immulite™ machine for chemiluminescence hormone assay.

Appendix Figure 19 Immulite™ test kit for serum T measurement by chemiluminescence method.

CURRICULUM VITAE

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