ผลของการเสริมซีลิเนียมอินทรีย์ต่อระดับซีลิเนียมในพลาสม่า ลักษณะรูปร่างและการเคลื่อนที่ของตัวอสุจิในสุนัข

นายดิเรก แก้วประภา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF ORGANIC SELENIUM SUPPLEMENT ON SELENIUM LEVEL IN PLASMA, SPERM MORPHOLOGY AND SPERM MOTILITY IN DOGS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Animal Nutrition
Department of Animal Husbandry
Faculty of Veterinary Science
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Thesis Title	EFFECTS OF ORGANIC SELENIUM SUPPLEMENT ON SELENIUM LEVEL IN PLASMA, SPERM MORPHOLOGY AND SPERM MOTILITY IN DOGS.
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ดิเรก แก้วประภา : ผลของการเสริมซีลิเนียมอินทรีย์ต่อระดับซีลิเนียมในพาสม่า ลักษณะรูปร่างและการเคลื่อนที่ของตัวอสุจิในสุนัข. (EFFECTS OF ORGANIC SELENIUM SUPPLEMENT ON SELENIUM LEVEL IN PLASMA, SPERM MORPHOLOGY AND SPERM MOTILITY IN DOGS) อ. ที่ปรึกษา : ผศ. สพ.ญ. ดร.อุตรา จามีกร, อ. ที่ปรึกษาร่วม : รศ. น.สพ. ดร. สุดสรร ศิริไวทยพงศ์, 58 หน้า.

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมซีลิเนียมอินทรีย์ต่อระดับซีลิเนียมในพลาสม่า ลักษณะรูปร่างและการเคลื่อนที่ของตัวอสุจิ และระดับซีลิเนียมที่เหมาะสมต่อการเพิ่มปรับปรุงคุณภาพของน้ำเชื้ออสุจิในสุนัข โดยใช้สุนัขพันธุ์บีเกิ้ล ทั้งหมด 9 ตัว เพศผู้ อายุระหว่าง 4.5-5 ปี น้ำหนักเฉลี่ย 10-13 กิโลกรัม วางแผนการทดลองแบบการสุ่มสมบูรณ์ ก่อนการทดลอง 7 วัน ทำการรีดน้ำเชื้อสุนัขทดลองทุกตัวแล้วนำมาตรวจสอบค่าร้อยละความสมบูรณ์ของอโครโซมและเยื่อหุ้มอสุจิ (Hypo-osmotic swelling test) และการเคลื่อนที่ของตัวอสุจิ (sperm motility test) ด้วยกล้องจุลทรรศน์ โดยคิดเป็นค่าร้อยละ นำค่าร้อยละดังกล่าวของสุนัข 9 ตัว แบ่งเป็น 3 กลุ่มๆ ละ 3 ตัว โดยกำหนดให้แต่ละกลุ่มมีค่าเฉลี่ยร้อยละที่ได้ใกล้เคียงกันมากที่สุดเพื่อลดความแปรปรวนระหว่างกลุ่ม กลุ่มที่ 1 คือกลุ่มควบคุม (ไม่มีการเสริมซีลิเนียม) กลุ่มที่ 2 คือกลุ่มเสริมซีลิเนียมอินทรีย์ที่ระดับ 1 พีพีเอ็ม และ 3 คือกลุ่มเสริมซีลิเนียมอินทรีย์ที่ระดับ 3 พีพีเอ็มในอาหาร อาหารพื้นฐานเป็นอาหารเม็ดสำเร็จรูปทางการค้าที่ตรวจพบว่ามีระดับซีลิเนียมอินทรีย์ในอาหารเท่ากับ 0.03 พีพีเอ็ม ระยะเวลาทดลองทั้งสิ้นนาน 18 สัปดาห์ โดยแบ่งเป็นระยะปรับตัว (สุนัขได้รับอาหารพื้นฐาน 2 สัปดาห์) และระยะทดสอบ (สุนัขได้รับอาหารพื้นฐานพร้อมการเสริมซีลิเนียมอินทรีย์ 16 สัปดาห์)

กลุ่มที่เสริมซีลิเนียมอินทรีย์ที่ระดับ 1 และ 3 พีพีเอ็ม ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($P > 0.05$) ของน้ำหนักตัว ระดับ creatinine และ GPT ในพลาสม่า ระดับ FT3 และ FT4 ในซีรั่ม ลักษณะสีและค่า pH ของน้ำเชื้ออสุจิ แต่พบว่าในสัปดาห์ที่ 12 ของทั้งสามกลุ่ม มีระดับ TT3 ในซีรั่ม เพิ่มสูงขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) และในสัปดาห์ที่ 12 ของกลุ่มที่เสริมซีลิเนียมอินทรีย์ที่ระดับ 1 และ 3 พีพีเอ็ม มีระดับ TT4 ในซีรั่ม สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ในส่วนของลักษณะน้ำเชื้ออสุจิ พบว่าการเสริมซีลิเนียมอินทรีย์ที่ระดับ 1 และ 3 พีพีเอ็ม มีผลให้ค่าเฉลี่ยปริมาตรน้ำเชื้ออสุจิที่รีดได้ ความเข้มข้นของน้ำเชื้ออสุจิ และจำนวนอสุจิทั้งหมดต่อการหลั่งเพิ่มขึ้น ($P < 0.05$) รวมทั้งมีผลให้ค่าร้อยละการเคลื่อนที่ของอสุจิ การมีชีวิตรอดของอสุจิ ความสมบูรณ์ของอโครโซมและเยื่อหุ้มอสุจิ และลักษณะรูปร่างอสุจิที่ปกติเพิ่มขึ้น ($P < 0.05$) และยังมีผลให้ค่าร้อยละของลักษณะรูปร่างอสุจิที่ผิดปกติลดลง ($P < 0.05$) โดยพบว่า การเสริมซีลิเนียมอินทรีย์ที่ระดับ 3 พีพีเอ็ม สามารถปรับปรุงลักษณะน้ำเชื้ออสุจิดังกล่าวได้ดีกว่าการเสริมซีลิเนียมอินทรีย์ที่ระดับ 1 พีพีเอ็ม อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$)

จากผลการทดลองสรุปได้ว่า การเสริมซีลิเนียมอินทรีย์ให้สุนัขโดยการกินสามารถปรับปรุงลักษณะน้ำเชื้ออสุจิได้ดีกว่ากลุ่มที่ไม่มีการเสริมซีลิเนียมอินทรีย์ และพบว่าการเสริมซีลิเนียมอินทรีย์ที่ระดับ 3 พีพีเอ็ม สามารถปรับปรุงลักษณะน้ำเชื้ออสุจิได้ดีกว่าการเสริมซีลิเนียมอินทรีย์ที่ระดับ 1 พีพีเอ็ม อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$)

ภาควิชา	สัตวบาล	ลายมือชื่อนิสิต.....
สาขาวิชา	อาหารสัตว์	ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา	2550	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

#4875555231 : MAJOR ANIMAL NUTRITION

KEY WORDS : SELENIUM/ SPERM/ DOGS

DIRAKE KAEWPRAPHA : EFFECTS OF ORGANIC SELENIUM SUPPLEMENT ON SELENIUM LEVEL IN PLASMA, SPERM MORPHOLOGY AND SPERM MOTILITY IN DOGS. THESIS ADVISOR : ASST. PROF. UTTRA JAMIKORN, D.V.M., M.S., Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. SUDSON SIRIVAIDYAPONG, D.V.M., Ph.D., 58 pp.

The objectives of this study were to evaluate the effect of organic Se on Se level in plasma, sperm morphology, and sperm motility of dogs, and to determine the suitable amount of organic Se supplement which can enhance sperm motility and morphology. Nine healthy male beagle dogs age between 4.5-5 years, have average weight between 10-13 kg. An experimental design was a complete randomized design (CRD). Seven days before the experiment, semen was collected and was examined microscopically to determine the semen quality. The examined parameters were membrane integrity (Hypo-osmotic swelling test) and sperm motility. Then statistical data (percentage) would be calculated and used to pick up nine samples from the entire dogs. The selected samples would be categorized into three groups with three dogs each. This would decrease the variations between the treatment groups. Group 1 was the control group (non supplemented Se), group 2 and 3 were supplemented with organic Se at 1 and 3 ppm, respectively. The dogs received a basal diet that was found to contain Se at 0.03 ppm for 2 weeks. After that, the dogs were fed a basal diet and supplement with various amounts of organic Se for 16 weeks.

Supplementation of organic Se at 1 and 3 ppm had no affect on body weight, color of semen, pH, creatinine, glutamate pyruvate transaminase (GPT), free 3,3',5-triiodothyronine (FT3) and free thyroxine (FT4) in serum. But at week 12th, all three groups had total 3,3',5 triiodothyronine (TT3) in serum greater ($P < 0.05$) than before experimental. Also at week 12th, the group supplemented with organic Se at 1 and 3 ppm had the serum total thyroxine (TT4) greater ($P < 0.05$) than the control group. For the semen characteristics, supplementation of organic Se at 1 and 3 ppm could increase the average ejaculation volumes of semen, sperm concentrations and total sperm concentration ($P < 0.05$). These supplements increase also the percentages of progressive motility, viable sperm, membrane integrity, and normal sperm morphology ($P < 0.05$). The dogs received organic Se supplement at 1 and 3 ppm had decreased percentage of abnormal sperm morphology ($P < 0.05$). In this study, organic Se supplement at 3 ppm could enhance the semen characteristic better ($P < 0.05$) than organic Se supplement at 1 ppm.

In conclusion, oral supplementation of organic Se can enhance the semen characteristic which is important for the male reproduction and can possibly enhance fertility in dogs. Oral supplementation of organic Se at 3 ppm can enhance semen characteristic better ($P < 0.05$) than oral supplementation of organic Se at 1 ppm.

Department :	Animal Husbandry	Student' s Signature :	.....
Field of Study :	Animal Nutrition	Advisor' s Signature :	.....
Academic Year :	2007	Co-advisor' s Signature :	.....

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LIST OF ABBREVIATIONS

BW	body weight
CLIA	chemiluminesecence immunoassay
DM	dry matter
Ds	iodothyronine deiodinases
FSH	follicle stimulating hormone
FT3	free 3,3',5-triiodothyronine
FT4	free thyroxine
GnRH	gonadotropin-releasing hormone
GPX1	glutathione peroxidase activity 1
GPX2	glutathione peroxidase activity 2
GPX3	glutathione peroxidase activity 3
GPX4	glutathione peroxidase activity 4
GPX5	glutathione peroxidase activity 5
GPX6	glutathione peroxidase activity 6
GSH-Px	glutathione peroxidase
H_2O_2	hydrogen peroxide
ICP	inductively coupled plasma
kg	kilogram
LH	luteinizing hormone
mg	miligram
ME	metabolizable energy
Na_2SeO_3	sodium selenite
Na_2SeO_4	sodium selenate
ng	nanogram
ng/dL	nanogram per dillitter
NO	nitric oxide
NOS	nitric oxide-synthase
ppm	part per million

Se	selenium
Se/g	selenium per gram
Se-GSH-Px	selenium-glutathione peroxidase
SD	standard diviation
SGPT	glutamate pyruvate transaminase
SOD	superoxide dismutase
TRs	thioredoxin reductases
TT3	total 3,3',5 triiodothyronine
TT4	total thyroxine
µg	microgram
ug/dL	microgram per dillitter
µg/day	microgram per day
µg Se/g	microgram of Selenium per gram

CHAPTER I

INTRODUCTION AND AIMS

Currently, revolution of dog fertility is extensively spread across the country. Public and private sectors have been supporting the development not only in academic research, but also in business areas. There are a variety of studies in revolution of dog fertility. For example, Morton and Bruce (1989) assessed the quality of fresh and frozen semen, and investigated the effecting factors on efficient use of frozen semen. Another study is Fertility in dogs in relation to semen quality and the time and site of insemination with fresh and frozen by Ferguson et al. (1989). Ponglowhapan et al. (2006) Freezing of epididymal spermatozoa from dogs after cool storage for 2 or 4 days. In the mentioned researches, it is able to see that most researches intended to study in technology of ejaculation and semen collecting process. There are a few more factors affecting the sperm quality, namely Se. At the present, there is a little academic report about the effects of Se supplement on dog reproductive system.

Se is a micro mineral substance that is imperative to living bodies. Se is a component of three enzymes. The first is glutathione peroxidase (GSH-Px) which disposes oxidants. The rest are thioredoxin reductases (TRs) and iodothyronine deiodinases (Ds) (Beckett and Authur, 2005), which associate in thyroid hormone genesis (Beech et al., 1993; Bates et al., 2000; Bianco et al., 2002). Accordingsly, Role and essential of Se depend on processing of these three enzymes. For example, Se is able to reduce processing of cancer and heart disease happening ratio (Combs and Lu, 2001; Ghose et al., 2001). It also supports metabolism process of endocrine gland, immunity system and inflammatory response (Gartner et al., 2002; Gartner and Gasnier, 2003). In reproductive system, Se can increase semen amount, motility rate of sperms as well as effective reproduction ratio (Arthur

et al., 2003; Broome, 2004). Besides, Se is able to help sperm well formation. All of these roles of Se have been reported in human, rats and boars, but there is a little direct research in dogs at this moment.

Studies about Se in dogs are very few. Existing studies about Se in dogs are as follows. Fico et al. (1986) discovered that Se was able to decrease breast cancer probability in dogs. Waters et al. (2005) noticed that Se was able to decrease DNA decomposition and prostate gland cell destruction ratio in dogs. Moreover, Se was able to improve consumption ratio and enhance thyroid hormones in the bloodstream of puppies (Wedekind et al., 2004).

As there is not much study of Se affects on sperms quality in dogs; therefore, this study has been initiated in order to create the output of the study to be basic data for dogs and other researches in the future.

The objectives of this experiment were:

1. To study the effect of organic Se supplement on Se level in plasma, sperm morphology and sperm motility in dogs.

2. To compare the effect organic Se supplement and non supplement in dogs.

3. To determine the suitable level of organic Se supplement on sperm morphology and sperm motility in dogs.

CHAPTER II

BACKGROUND AND INFORMATIONS

Spermatogenesis

Spermatogenesis process in dogs is exactly the same as the process of other animals that begins with spermatogonium (2n) growing to primary spermatocyte. Each spermatocyte will partition itself (meiosis) into two secondary spermatocytes, and the spermatocytes will repeat partitioning themselves (meiosis II) into four spermatid (n) cells. Subsequently, each spermatid cell will develop itself to a complete sperm cell. Overall process takes 56 days to complete in Figure 1.

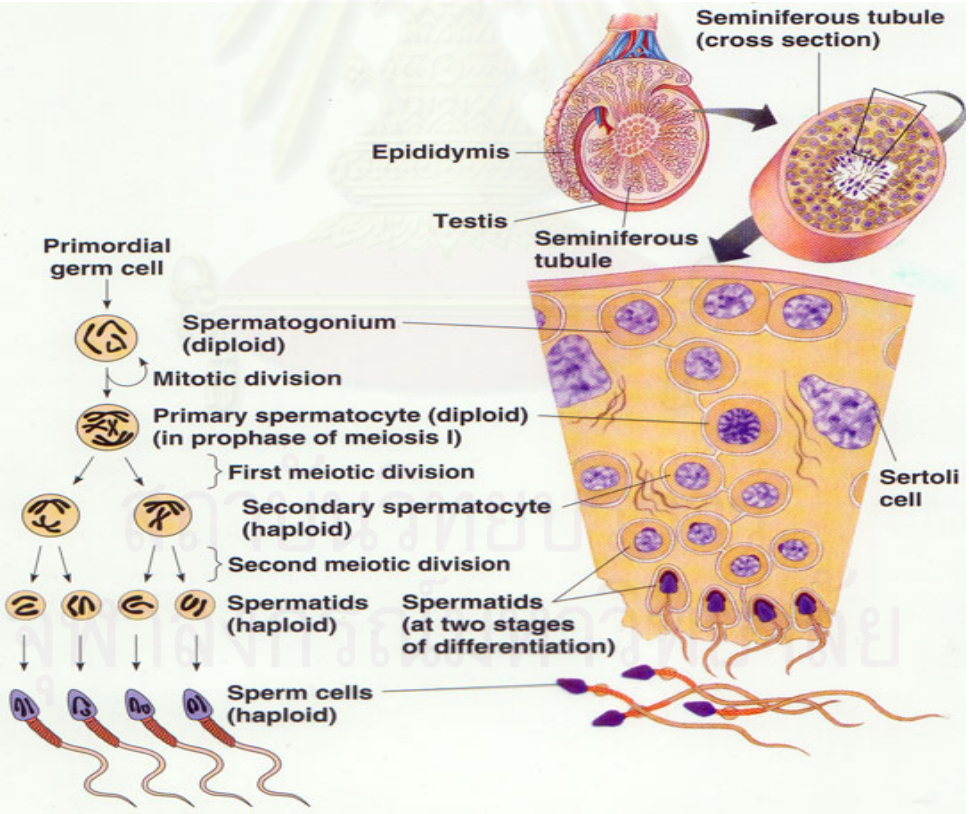

Figure 1. Spermatogenesis process in dogs (Christiansen, 1984)

Components of sperm

1. Head consists of a nucleus and acrosome. The nucleus is inside while acrosome is around the nucleus in front in order to produce enzymes that help to destruct egg membrane.

2. Middle piece comprises two centrioles which are proximal centriole and distal centriole. Inside these centrioles, there are mitochondria performing an energy source for the sperm.

3. Tail is wagged in order to move forward.

An abnormal sperm normally has a morphology form (Johnston et. al., 2001). The morphology form is able to be divided into two types which are primary abnormality as shown in Figure 2. and secondary abnormality is depicted in Figure 3.

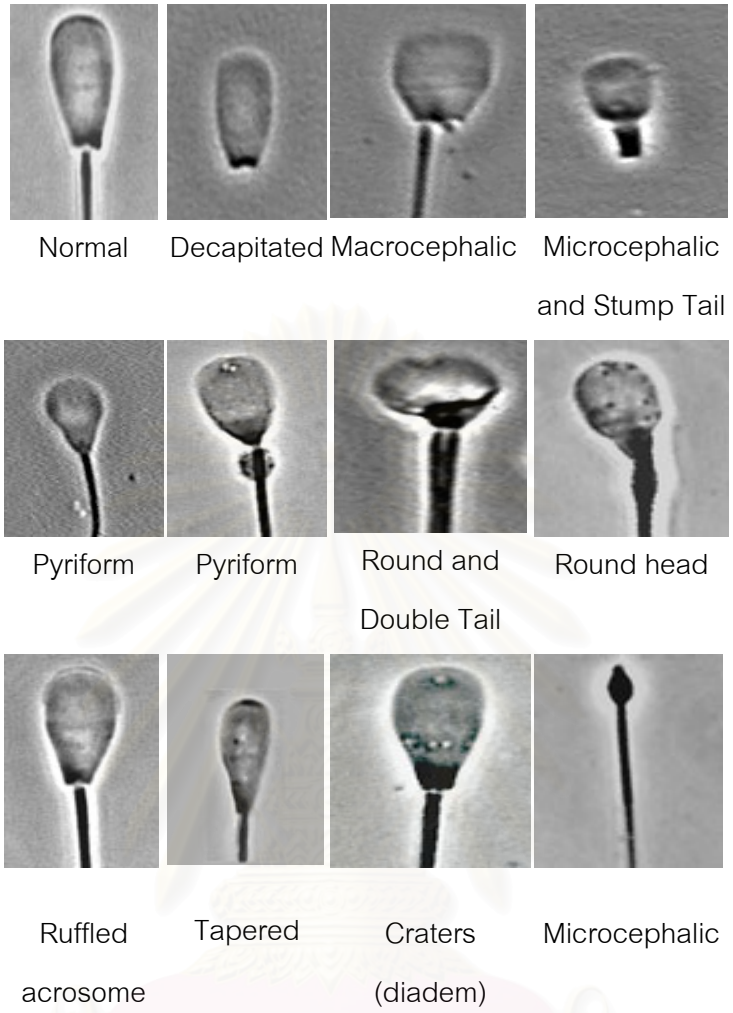

Figure 2. The primary abnormality of the sperm is formed in head and middle piece of the sperm.
www.wisc.edu/repro/lab/procedures/sperm/bovine_abnormals.html [online, 2007]

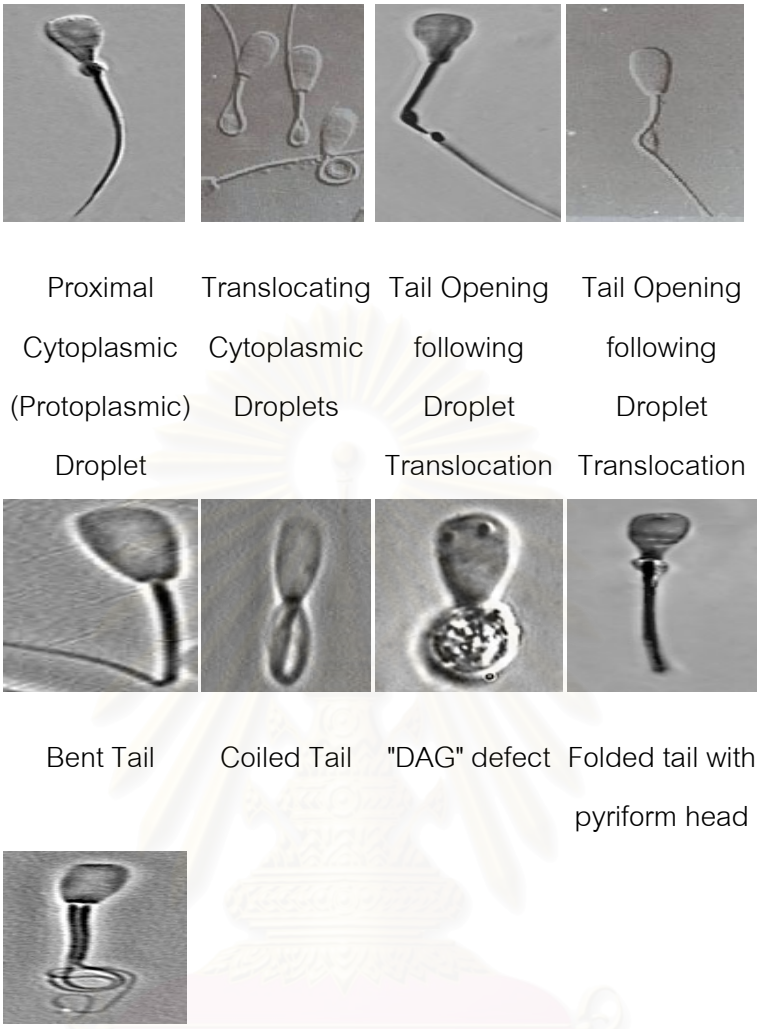

Figure 3. The secondary abnormality of the sperm www.wisc.edu/repro/lab/procedures/sperm/bovine_abnormals.html [online, 2007]

Normal dog ejaculation

The dog ejaculation may be elicited by manual stimulation of the penis in the presence of an estrous teaser bitch. Application to the distending penis of a latex artificial vagina connected to a 12-14 cc calibrated plastic centrifuge tube is recommended to simulate normal copulation. The presence of an estrous teaser bitch has been demonstrated to result in a better quality of semen collected by manual stimulation of the male dog.

Dog semen is ejaculated (Johnston, 2003) in three fractions: (i) the acellular pre-sperm fraction (0.5 to 5.0 cc), which may originate in the prostate; (ii) the sperm-rich fraction (1.0-4.0 cc) which originates in the testes and epididymis; and (iii) the prostatic fluid fraction (2.5-> 80 cc) which also is acellular and originates in the prostate**.** Table 2.1 shows the semen characteristics of normal dogs.

Table 2.1 Semen characteristics of normal dogs (Concannon et al., 2006)

Parameter	Normal range
Acellular pre-sperm fraction	0.5 - 5.0 ml., clear
Sperm-rich fraction	1.0 - 4.0 ml., opalescent
Prostatic fluid fraction	1.0 – 80.0 ml., clear
Ejaculation volume	2.5 – 80.0 ml.
Sperm concentration	4.0 - 400 x 10^6 sperm/cm^3
Total sperm concentration	300 – 2,000 x 10^6 sperm/ejaculation
Progressive motility	> 70%
Normal sperm	> 80%
pH	6.3 – 6.7

Spermatogenesis related hormones

Spermatogenesis process and sexual characteristics response on hormones from an anterior pituitary gland. These indispensable hormones are as follow.

1. Luteinizing hormone (LH)

LH stimulates leydig cells to produce androgen hormone such as testosterone. Androgen hormone controls the primary sex characteristics (ex. sexual organ growth) and the secondary sexual characteristics (ex. libido).

2. Follicle stimulating hormone (FSH)

FSH influences spermatogenesis in seminiferous tubules.

3. Gonadotropin-releasing hormone (GnRH)

GnRH is produced by the bottom part of the brain that is connected to hypothalamus. This hormone has a role in controlling LH and FSH production. A feedback mechanism to GnRH occurs if the level of LH and FSH is high. In the same way, if testosterone is increased, there will be a feedback to impede LH and FSH production.

Thyroid hormone is indirectly relevant to spermatogenesis and libido. It has been discovered that thyroid hormone had an effect on the amount of prolactin hormone controlling in bloodstream to be in instanting level. Prolactin hormone helps testosterone stimulating the prostate gland to produce sperms, to control vas deferens contraction and seminal fluid genesis. Typical level of prolactin hormone supply GnRH secreting of hypothalamus (Navickis et al., 1982; Jana et al., 1996) as show in Figure 4. The thyroid hormones in serum had four type. (i) Free 3,3',5-triiodothyronine (FT3, normal range in dog was 2-4 pg/mL). (ii) Free thyroxine (FT4, normal range in dog was 75-200 ng/dL). (iii) Total 3,3',5 triiodothyronine (TT3, normal range in dog was 1.0-4.0 ug/dL) and (iv) total thyroxine (TT4, normal range in dog was 0.7-3.3 ng/dL) (Beckett and Authur, 2005).

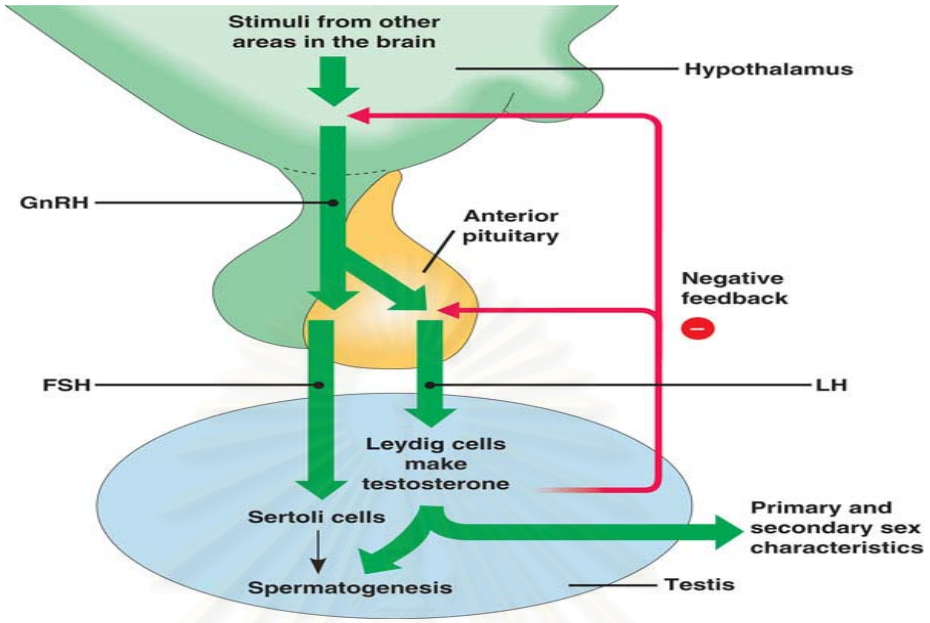

Figure 4. Spermatogenesis process and sex characteristics response on hormones by hypothalamus released the GnRH, after that GnRH stimulating the anterior pituitary gland for released the LH, and LH stimulates leydig cells to produce androgen hormone such as testosterone. Androgen hormone controls the primary sex characteristics (ex. sex organ growth) and the secondary sex characteristics. FSH influences spermatogenesis in seminiferous tubules.
www.zgxl.net/eng/health/humanbody/hormone.html [online, 2007]

Other relevant effecting factors of spermatogenesis process are age, puberty, disease, frequency of ejaculate, method of ejaculation, environment, medicine, and nutrients (Concannon et al. 2006). Various nutrients associate with spermatogenesis process. For example, vitamin C and E act as an anti-oxidant in sperms and in the process (Yousef et al., 2003). Folic acid associates with DNA synthesis as well as is a co-enzyme in RNA synthesis, which is related to genetic substance producing (Tamura and Piccino, 2006). L-carnitine comprising acyl group is acyl-co A substrate in oxidation reaction that originates an energy for the sperm. It also acts as an anti-destruction substance of cells in testis that helps to decrease

germ cell losses (Ming et al., 2004). Arginine is an initiated substance in Nitric oxide (NO) producing process with nitric oxide-synthase (NOS) enzyme. NO affects on vasolidate blood vessels which results to enhance oxygen carrying to sex organ (Balercia et al., 2004). Zinc is a component of superoxide dismutase (SOD) enzyme that is an anti-oxidant in sperms and spermatogenesis (Hendal et al., 2000; Ebisch et al., 2006; Suh et al., 2006).

Currently, there are a numbers of studies about Se, a slight element substance, in human, rats and boars about the benefits of Se with the spermatogenesis and the sperm efficiency. However, a study of Se in dogs has not been conducted at the present.

Generally, Se is contained in food and animal bodies less than 0.01 percentage of the body weight. In addition, Se can be discovered in a diverse organ tissue of the body especially in kidney, pancreas, liver, heart, lungs, muscles, and bloodstream in respectively ordered (Kim and Mahan, 2001). The ability of absorption of Se depends on the type of Se. There are two types of Se which are organic and inorganic Se. Instances of organic Se are selenocysteine, selenoyeast and selenomethionine while sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4) and selenious acid are inorganic Se. It is discovered that organic Se (selenomethionine) has better absorption than inorganic Se (Fortier and Matte, 2005; Schrauzer, 2000).

Se is classified in group six (Authur et. al., 1992) of the periodic table group that is able to have both positive and negative ion from -2 (reduce form) to +6 (oxidative form). For example, selenocysteine and selenomethionine have ion as Se^{-2}, Na_2SeO_3 has Se^{+4} and Na_2SeO_4 has Se^{+6}. Various ion states help in absorption and utilization. This can be stated that if Se is in the reduce form (Se^{-2}), it is able to be more absorbed and used than the oxidative form.

Se is an element of imperative enzymes

1. Glutathione peroxidase (GSH-Px)

GSH-Px acts as an anti-oxidant in a body. A GSH-Px molecule consists of four Se atoms. Beckett and Auther (2005) studies GSH-Px classification and divides GSH-Px into six types which are glutathione peroxidase activity 1 (GPX1), glutathione peroxidase activity 2 (GPX2), glutathione peroxidase activity 3 (GPX3), glutathione peroxidase activity 4 (GPX4), glutathione peroxidase activity 5 (GPX5) and glutathione peroxidase activity 6 (GPX6). GPX types act as anti-oxidants in different parts of the body. For instance, GPX1 is an anti-oxidant in cytocol cell, GPX2 is in digestive tract, GPX3 is in plasma, GPX4 is in tissues and protein structure of sperm cells, GPX5 is unspecified, while GPX6 supports GPX1 work process in cells. Anti-oxidant process is able to be divided into three levels (Guoyao et al., 2004) and there are related anti-oxidant substances as follows.

a. Level 1: catalase, superoxide dismutase and Se-glutathione peroxidase (Se-GSH-Px)

b. Level 2: glutathione, vitamin A, E, C, carotinoids, uric acid and Se-glutathione peroxidase (Se-GSH-Px)

c. Level 3: lipases, proteases and other digestive juices

GSH-Px is able to work in level 1 and level 2 (Rotruck, 1973) by eliminating hydrogen peroxide (H_2O_2) which is originated from metabolism processes. This causes a decrease of the number of H_2O_2 and eventually decreases the number of cells destroyed (Sikka, 2004).

Lasota et al., (2004) studied relation between Se and GSH-Px in plasma and seminal plasma in the boars. The study discovered that Se supplement made the level of Se and GSH-Px high both in plasma and seminal plasma. When it is substituted in a regression of Se and GSH-Px, it displays a high level association ($r = 0.86$) both in plasma and seminal plasma.

2. TRs and Ds enzyme are relevant to thyroid hormones synthesis

2.1 Enzyme TRs is an anti-oxidant in thyroid hormones genesis. This TRs enzyme is divided into three types as follows.

- TR1 acts as an anti-oxidant in liquid inside cells body.
- TR2 is an anti-oxidant in process in the testes.
- TR3 acts as an anti-oxidant in mitochondria of cells body.

2.2 Enzyme Ds works as a stimulator in transforming process of thyroxine (T4) to tri-iodothyronine (T3) as depicted in Figure 5.

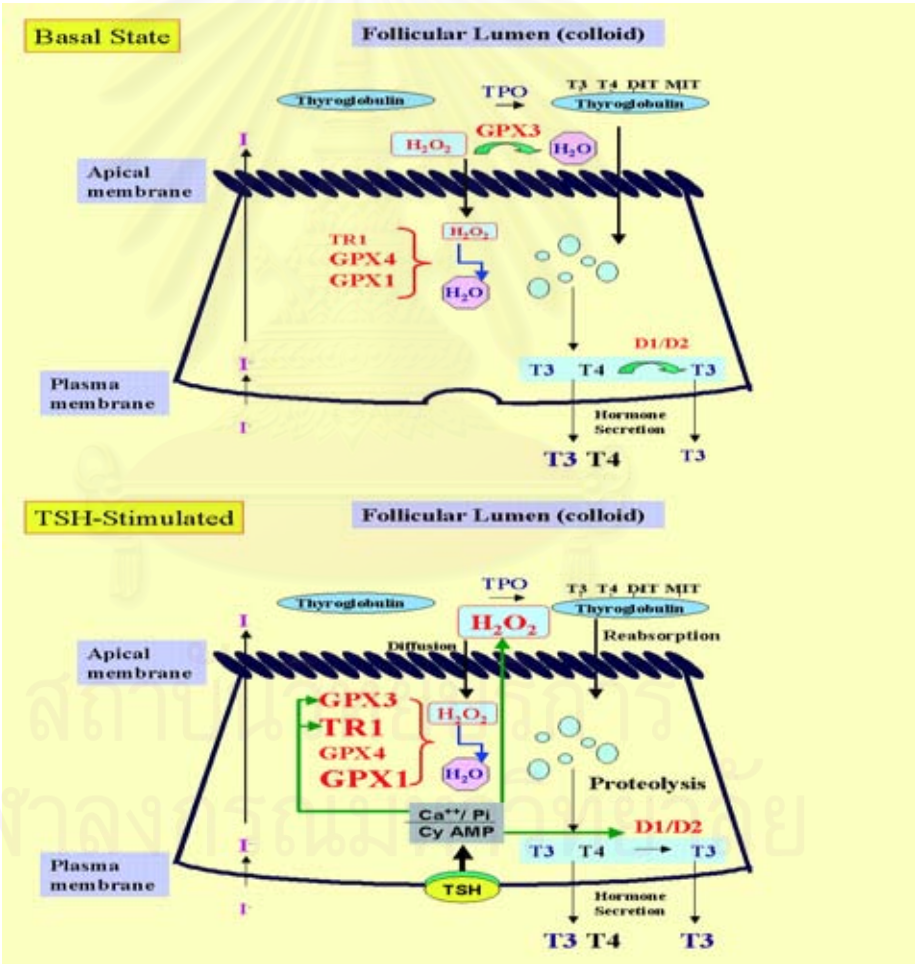

Figure 5. TRs and Ds enzyme are relevant to thyroid hormones synthesis (Beckett and Auther, 2005)

Roles and importance of Se

Since Se is a component of enzyme GSH-Px, TRs and Ds, Se work is dependent on these three enzymes. There are a number of studies about the advantages of Se in medical usage. The examples are the followings.

1. Se helps to prevent various kinds of cancer, for example, skin cancer (200 µg Se/day) (Combs and Lu, 2001; Ghose et al., 2001), prostate gland cancer in humans (100-300 µg Se/day) (Dong et al., 2003; Dong et al., 2004) and in dogs (1-3 µg Se/g diet) (Waters et al., 2005), colorectal cancer in rats (2.0 µg Se/g diet) (Finley et al., 2000) and 0.13-3.0 µg Se/g diet is able to decrease pulmonary metastasis of melanoma cells in mice (Donghua et al., 2004).

2. Se supplement at 2 ppm helps to decrease coronary artery disease in newborn rats (Ostadalova et al., 2006).

3. Se helps to enhance metabolic process of endocrine system (Beckett and Authur, 2005). Currently, 200 µg Se is used in the medical treatment to cure autoimmune thyroiditis in humans (Gartner et al., 2002; Gartner and Gasnier, 2003).

4. Se supplement at the level greater than 10 µg helps to boost the immune system in mice (Arthur et al., 2003; Broome, 2004).

5. Se supplement at 2.5 µg helps to enhance inflammatory response system in mice (Authur et al., 2003). In study of Brown et al., (2000) Se helps to enhance lymphocytes, granulocytes, platelets and erythrocytes cells are significantly increased ($p<0.01$).

The associated roles and necessities of reproductive system

Female reproductive system

Se supplement at 5 ng helps to stimulate estrogen hormone producing which results in decrease oestrus cycle in cows (Basini and Tamanini, 2000).

Male reproductive system

1. Se helps to enhance the reproductive system of male humans (Foresta

et al., 2001) and boars (Marin-Guzman et al., 2000a; Marin-Guzman et al., 2000b; Kolodziej and Jacyno, 2005). The boars received 3-10 ppm Se supplementation, resulted in increased volume of semen. This also improved the percentage and the amount of the normal sperms, the percentage of regular progressive motility as well as the percentage of sperm viability respectively ($p<0.01$).

2. Se supplement at 3-7 ppm could protect or decline protein structure destruction of membrane integrity in boars (Marin-Guzman et al., 2000a). Furthermore, the percentage of sperm viability is increased significantly (with $p<0.01$).

3. Se supplementation at 1-5 ppm helps to raise the percentage of sperm motility (Marin-Guzman et al., 2000b). This results from the working process of Se under the action of GSH, which is an anti-oxidant substance of sperm. This supplement affects mitochondria structures in the tail part of sperm to have an increase of normal arrangement form. As a result, the gaps between mitochondria are dropped off, so that the sperm has an energy source to get better movement, which increases the velocity of the sperm motility significantly ($p<0.01$).

4. Se helps in the development of testes and spermatogenesis, and raise a decrease of oxidative stress in humans (Maiorino and Ursini, 2002), boars (Oldfield, 2003), rats (Olson et al., 2004) and mice (Parminder and Mohinder, 2004). When study the cutting testis through an electron microscope, it is found that Se supplemented group affected increase of the number of sperms in the secondary spermtocyte of the spermatogenesis, for example, the young and mature spermatid cells as show in Figure 2.1.

5. Se supplementation at 0.2-1 ppm helps to boost the number of newborn in mice (Parminder and Mohinder, 2004). This is examined by comparing two groups of male mice. The first group is Se supplemented and the other group is not supplemented with Se. The number of newborn mice from Se supplemented group is twice as many as the number of the newborn from not supplemented group.

Se deficiency

1. GSH-Px (Koller and Exon, 1986), TRs and Ds (Bianco et al., 2002) in plasma decrease.

2. Causes an abnormal immune system or decreases the immune system (Arthur et al., 2003). In human, the Se deficiency leads to be infected by virus easier than the Se supplemented group does (Beck et al., 2003).

3. Leads to an abnormality of thyroid hormones producing since Se is a compound substance of enzyme TRs and Ds, which are associated with thyroid hormones producing.

4. Causes inflammatory bowel diseases in rats (Esworth et al., 2005).

5. Causes fatal myopathy in guinea pigs (Hill et al., 2001).

Se toxicity or selenosis

Se is an element substance that has a low toxic level. Organic Se has lower level of toxicity than the inorganic Se (Kim and Mahan, 2001). Noticing symptoms and illnesses of animals is the method of study toxicity (Mihajlovic, 1992). Toxicity can be separated into three categories as the followings.

1. Subacute selenosis or blind staggers: abnormal signs relating to nervous system such as blindness, ataxia, disorientation and respiratory distress.
2. Acute selenosis: appeared symptoms are lethargy, excessive salivation, vomiting, dyspnea and muscle tremors. Other pathological conditions are congestion in liver and kidneys, fatty degeneration, necrotic tissues in liver, endocarditis and myocarditis.
3. Chronic selenosis or alkali disease: appeared symptoms are hairs falling, weight lose, hoof separation and lameness. In severe cases, liver cirrhosis, atrophy heart and anemia probably occur.

Kim and Mahan (2001) studied the extent of selenosis by comparing the level of Se toxicity of organic and inorganic Se in boars. The results are summarized

by symptoms and processing level of enzyme glutamic pyruvic transaminase (GPT). The results were as follows.

1. Boars supplemented with both types of Se display selenosis at more than 5 ppm supplementation.

2. Organic Se supplemented group displays less selenosis than the group using inorganic Se.

3. The symptoms of toxicity begin with hair falling, lethargy, weight lose, blind staggers and lameness.

4. Hoof separation would be discovered when taking 10 ppm organic Se. But boars taking inorganic Se would display this symptom when receive 5 ppm inorganic Se.

5. The boars that received either organic Se at 10 ppm or inorganic Se at 5 ppm were found that GPT level increased significantly ($p<0.05$).

CHAPTER III

MATERIALS AND METHODS

Experimental design and animals

1. Animals

Nine healthy male beagles, age between 4.5-5 years and average body weight between 10-13 kg were used in this study. Seven days prior to the experiment, semen was collected and examined microscopically to assess the semen quality using the parameters as percentages of intact acrosome, membrane integrity (Hypo-osmotic swelling test), and sperm motility. In order to decrease the variations among the treatment groups, the averages percentages of these parameters were subjected for grouping the animals into three groups. All dogs were housed individually in the metal cages (1.5 x 2.5 x 1.5 meters) with a plastic slat floor and the temperature ranging between 25° and 35 °C. Each individual cage was cleaned twice daily.

2. Feed and Feeding

The economy grade, commercial extruded dog diet that could buy from any convenient store, formulated in accordance with the AAFCO (2000) nutrient guide for adult dog was used as the basal diet. The nutrient compositions of this basal diet were; protein not less than 18 percentage, fat not less than 10 percentage, fiber not greater than 5 percentage, and moisture not greater than 12 percentage. In addition, this commercial diet used selenium yeast as the source of selenium. The three treatments of the current study were as follows.

Table 3.1 Experimental groups

Groups	Description
1	Basal diet
2	Basal diet + 1 ppm Se supplement
3	Basal diet + 3 ppm Se supplement

Dogs were fed in accordance with their energy requirements. Each day, food was weighed and divided into two equal portions and fed to dogs at 8:00 a.m. and at 4:00 p.m. in stainless steel bowls. Body weight of all animals was determined every two weeks and the amount of feed intake was recorded daily. The amount of food was calculated by using standard equations to determine energy requirements of active adult dogs (ME requirement, kcal = 132 x $BW_{kg}^{0.67}$; Case et al., 2000) and adjusted every 2 weeks. In this experiment, tab water was provided all the time through out the period of 16 weeks.

Selenoyeast (Sel-Plex), an organic Se, was used as the source of Se supplementation. The 100 g Sel-Plex consists of 0.1 g organic Se. For practical supplement, Se supplementation was prepared as solution by dissolving 1 g of Sel-Plex with 100 ml of drinking water. The supplementation was divided into 2 times at 15 minutes after each meal. The amount of Se supplement for the dogs in group 2 and 3 were adjusted every two week regarding the change in their body weights.

Sample Collection and Determination

1. Feed

Sampling of basal feed was analyzed for the level of Se by inductively coupled plasma (ICP).

2. Blood

Before noon, after the semen collection, blood collection was performed at week 0^{th}, 4^{th}, 6^{th}, 8^{th}, 12^{th}, and 16^{th}. Five ml of blood from cephalic vein was divided

into two tubes (2 ml in the tube with anti-clot substance and 3 ml in another tube without anti-clot substance) for the measurement of glutamate pyruvate transaminase (GPT), creatinine, Se, total 3,3',5 triiodothyronine (TT3), total thyroxine (TT4), free 3,3',5-triiodothyronine (FT3), and free thyroxine (FT4).

Blood plasma were examined for GPT and creatinine levels on day 0, 28, 56, 84 and 112 (week 0^{th}, 4^{th}, 8^{th}, 12^{th}, and 16^{th}) and were examined for Se levels on day 0, 42, and 84 (week 0^{th}, 6^{th}, and 12^{th}). The Se examination was done by inductively coupled plasma (ICP) method. The levels of TT3, TT4, FT3 and FT4 in serum were determined on day 0, 42, and 120 (week 0^{th}, 6^{th}, and 12^{th}) by chemiluminesecence immunoassay (CLIA) on ACS180 machine.

3. Semen collection , acellular pre-sperm and sperm-rich fractions were collected as the following schedule;

3.1 First 8 weeks: semen collection was performed on week 0^{th}, 2^{th}, 4^{th}, 6^{th}, and 8^{th} of the experiment.

3.2 Last 8 weeks: semen collection was performed on week 9^{th}, 10^{th}, 11^{th}, 12^{th}, 13^{th}, 14^{th}, 15^{th} and 16^{th} of the experiment.

Semen collection was manually ejaculations as described by Johnston (1991) by the same person through the experiment. After manually ejaculations the semen samples for determine the others semen characteristic analysis.

Semen characteristic analysis consists of

1. The color of semen
2. Determination of semen pH using the pH meter
3. Measurement of ejaculation volume of the collected semen
4. Measurement of progressive sperm motility by dropping a drop of semen on a slide which was warmed in an oven at 40^{o}C. The examination was done under a coverslip at x 100 magnification. Progressive motility was graded as percentage which greater than 70% was counted to be good semen quality.

5. Measurement of sperm concentration using Unopett white blood cell/platelet dilutor kits. Ratio of semen:diluted solution was 1:100. A drop of the diluted semen was dropped on a hemacyometer, and covered with a coverglass slip. Then the number of sperms was counted. The concentration of sperm per 1 milliliter of semen would be demonstrated as number of counted sperm x 10^6. The total number of sperms would be calculated as sperm concentration timed the volume of collected semen. This value as the total sperm concentration would be presented as number of sperms x 10^6 per ejaculation.

6. Measurement of viable sperm was inspected by dropping a drop of eosin-nigrosin and a drop of semen on a slide. Under a coverslid, numbers of alive and dead sperms from the total amount of 200 sperms were counted. Living sperms should not be colored while dead sperms should be pink colored. The amounts of live and dead sperms were calculated as percentages.

7. Measurement the amount of normal and abnormal sperms was inspected by staining with the Giemsa stain set (DiffQuick). Stained for five minutes per a kind of solution before cleaning it with distilled water and let it dried. The numbers of sperms with normal and abnormal forms were counted from the total amount of 200 sperms. The amounts of normal and abnormal sperms were calculated and presented as the percentages. These abnormal sperms composed of both the primary and secondary abnormal characteristics (Johnston, 1991).

8. Measurement of membrane integrity was determined by the Host (Hypo-osmotic swelling test) method. The 0.1 ml of semen and 1 ml of 60 milliosmole concentrated fructose were mixed. This mixture was warm at 37 ^{o}C for 45 minutes before counting the number of tail-bending sperms under a microscope. Since low concentrated solution could pass through sperm membrane resulting in swelling and bending sperm (Kumi-Diaka, 1993). This bending was an indicator of the strength of sperm cell membrane (Sanchez, 2002).

Statistical Analysis

All data was expressed as mean $\pm$ SD. These data were analyzed as a completely randomized design using the general linear model and analysis of variance. The ejaculation numbers were presented as number of experimental units. The mean differences between treatments were tested by Duncan's New Multiple Range test and the mean differences between groups were tested by pair T-Test using the commercially computer program. Differences were considered significant when $P < 0.05$.

CHAPTER IV

RESULTS

Effect of organic Se supplement on body weight (BW)

There were significantly differences of an average BW when compared between the treatment groups. For both pre- and experimental period, the control group and group 3 were greater ($P < 0.05$) than group 1 (Table 4.1).

Table 4.1 The average BW of the dogs in all experimental groups[1]

Period	Body weight (kg)		
	Control	1 ppm Se	3 ppm Se
Pre- experimental[2]	11.7 ± 0.8[a]	10.3 ± 0.2[b]	12.2 ± 0.2[a]
Experimental[3]	11.8 ± 0.8[a]	10.5 ± 0.2[b]	12.4 ± 0.2[a]

[1]Mean ± SD

[2]Average value of wk 0th

[3]Average value of wk 4th, 8th, 12th and 16th

[a,b,c]Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

Effect of organic Se supplement on creatinine concentration in plasma

The average creatinine concentrations in plasma were not different when compared between the treatment groups as shows in Table 4.2.

Table 4.2 **The average plasma creatinine concentrations of the dogs in all experimental groups**[1]

Period	Creatinine (U/L)		
	Control	1 ppm Se	3 ppm Se
Pre- experimental[2]	0.8 ± 0.1	1.0 ± 0.3	0.9 ± 0.5
Experimental[3]	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2

[1]Mean ± SD

[2]Average value of wk 0th

[3]Average value of wk 4th, 8th, 12th and 16th

Effect of organic Se supplement on GPT concentration in plasma

There were significant differences of average GPT concentrations in plasma when compared between the treatment groups. For both pre- and experimental periods, the control group had GPT concentrations in plasma significantly lower ($P < 0.05$) than group 2 and group 3. And for the control group, the plasma GPT concentration of the pre-experimental period also was significant lower ($P< 0.05$) than the experimental period (Table 4.3).

Table 4.3 The average plasma SGPT concentrations of the dogs in all experimental groups[1]

Period	SGPT (U/L)		
	Control	1 ppm Se	3 ppm Se
Pre- experimental[2]	41.0 ± 10.2[ad]	55.0 ± 21.9[b]	55.3 ± 20.4[b]
Experimental[3]	49.3 ± 19.4[ae]	56.0 ± 9.6[b]	56.0 ± 18.9[b]

[1]Mean ± SD

[2]Average value of wk 0th

[3]Average value of wk 4th, 8th, 12th and 16th

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

Effect of organic Se supplement on Se concentration in plasma

At week 0th, the average Se concentrations in plasma was not different when compared between the treatment groups. But at week 12th, the dogs in group 2 (0.35 ± 0.05 ppm) and group 3 (0.52 ± 0.08 ppm) had the average Se concentrations in plasma significantly greater ($P < 0.05$) than the control group (0.24 ± 0.03 ppm) (Table 4.4).

Table 4.4 The average plasma Se concentrations of the dogs in all experimental groups[1]

Period	Se (ppm)		
	Control	1 ppm Se	3 ppm Se
Week 0[th2]	0.23 ± 0.01	0.24 ± 0.02[d]	0.23 ± 0.02[d]
Week 12[th3]	0.24 ± 0.03[a]	0.35 ± 0.05[be]	0.52 ± 0.08[ce]

[1]Mean ± SD

[2]Average value of wk 0[th]

[3]Average value of wk 6[th] and 12[th]

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

Effect of organic Se supplement on FT3 concentration in serum

The average FT3 concentration in serum was not difference when compared between the treatment groups at both week 0[th] and 12[th] as shows in Table 4.5.

Table 4.5 The average serum FT3 concentrations of the dogs in all experimental groups[1]

Period	FT3 (pg/mL)		
	Control	1 ppm Se	3 ppm Se
Week 0[th2]	2.5 ± 0.2	2.4 ± 0.7	2.7 ± 0.1
Week 12[th3]	2.8 ± 0.4	2.8 ± 0.6	2.8 ± 0.2

[1]Mean ± SD

[2]Average value of wk 0[th]

[3]Average value of wk 6[th] and 12[th]

Effect of organic Se supplement on TT3 concentration in serum

There were significantly differences of the average TT3 concentrations in serum when compared between the treatment groups. At week 0th, the dogs in the control group (49.3 ± 6.7 ng/dL) and group 2 (46.0 ± 2.6 ng/dL) had the average TT3 concentrations in serum significantly lower ($P < 0.05$) than group 3 (58.0 ± 5.3 ng/dL). At week 12th, the dogs in the control group (65.0 ± 14.7 ng/dL) had the average TT3 concentrations in serum significantly lower ($P < 0.05$) than group 2 (73.0 ± 17.5 ng/dL), and was not differ when compared to group 3 ($P > 0.05$).

For the comparison between week 0th and week 12th, the results demonstrated that the animals in all treatment groups at week 12th had the average TT3 concentrations in serum significant greater ($P < 0.05$) than at week 0th (Table 4.6).

Table 4.6 **The average serum TT3 concentrations of the dogs in all experimental groups**[1]

Period	TT3 (ng/dL)		
	Control	1 ppm Se	3 ppm Se
Week 0th[2]	49.3 ± 6.7^{ad}	46.0 ± 2.6^{ad}	58.0 ± 5.3^{bd}
Week 12th[3]	65.0 ± 14.7^{ae}	73.0 ± 17.5^{be}	71.7 ± 5.5^{abe}

[1] Mean ± SD

[2] Average value of wk 0th

[3] Average value of wk 6th and 12th

^{a,b,c} Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

^{d,e} Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

Effect of organic Se supplement on FT4 concentration in serum

The average FT4 concentration in serum was not difference when compared between the treatment groups as shows in Table 4.7.

Table 4.7 **The average serum FT4 concentrations of the dogs in all experimental groups**[1]

Period	FT4 (ng/dL)		
	Control	1 ppm Se	3 ppm Se
Week 0[th2]	1.0 ± 0.3	1.1 ± 0.3	1.2 ± 0.4
Week 12[th3]	1.1 ± 0.2	1.5 ± 0.5	1.3 ± 0.2

[1]Mean ± SD

[2]Average value of wk 0[th]

[3]Average value of wk 6[th] and 12[th]

Effect of organic Se supplement on TT4 concentration in serum

At week 0[th], the average TT4 concentration in serum was not difference when compared between the treatment groups. However, at week 12[th], the dogs in the control group (1.1 ± 0.5 ug/dL) had the average TT4 concentrations in serum significant lower ($P < 0.05$) than group 2 (2.0 ± 1.1 ug/dL) and group 3 (1.6 ± 1.1 ug/dL) (Table 4.8).

Table 4.8 The average serum TT4 concentrations of the dogs in all experimental groups[1]

Period	TT4 (ug/dL)		
	Control	1 ppm Se	3 ppm Se
Week 0[th2]	1.3 ± 0.2	1.6 ± 0.3	1.7 ± 0.5
Week 12[th3]	1.1 ± 0.5[a]	2.0 ± 1.1[b]	1.6 ± 0.6[b]

[1]Mean ± SD

[2]Average value of wk 0[th]

[3]Average value of wk 6[th] and 12[th]

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

Effect of organic Se supplement on semen characteristic

1. Semen examination by inspection the color of semen. In this study, the dogs in all experimental groups had the white color semen.

2. pH values

The comparison between before and after week 8[th] of the animals in all treatment groups showed no difference ($P > 0.05$) in the pH values of semen (Table 4.9).

Table 4.9 The average pH values of the dogs in all experimental groups[1]

Time	pH values[2]		
	Control	1 ppm Se	3 ppm Se
Week 0th [3]	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Before week 8th [3]	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
After week 8th [4]	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1

[1]Mean ± SD

[2]Normal range in dogs = 6.3-6.7

[3]Average value of wk 0th (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[4]Average value of wk 2nd, 4th, 6th and 8th (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[5]Average value of wk 9th, 10th, 11th, 12th, 13th, 14th, 15th and 16th (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

3. Ejaculation volume (cm^3)

Table 4.10 shows the average ejaculation volumes of the dogs in all experimental groups. At the beginning (week 0th), regarding to the grouping assessment, the average ejaculation volumes were not differ ($P > 0.05$) when compared between the treatment groups. However, at before week 8th, the average ejaculation volume of group 2 (3.7 ± 0.6 cm^3), but not group 3 (3.6 ± 0.5 cm^3), were significantly greater ($P < 0.05$) than the control group (3.4 ± 0.6 cm^3). And at after week 8th, the average ejaculation volume of group 3 (5.3 ± 0.7 cm^3) was the greatest ($P < 0.05$) followed by group 2 (4.4 ± 0.9 cm^3) and control group (3.3 ± 0.4 cm^3), respectively.

For the comparison between week 0th, before week 8th, and after week 8th, the result displayed that the average ejaculation volume at before and after week 8th of both group 2 and group 3 were significantly greater ($P < 0.05$) than at week 0th. These values of both group 2 and 3 also increased as time increased.

Table 4.10 The average ejaculation volumes of the dogs in all experimental groups[1]

Time	Ejaculation volume (cm^3)		
	Control	1 ppm Se	3 ppm Se
Week 0^{th} [2]	3.2 ± 0.3	3.1 ± 0.2^{d}	3.1 ± 0.3^{d}
Before week 8^{th} [3]	3.4 ± 0.6^{a}	3.7 ± 0.6^{be}	3.6 ± 0.5^{abe}
After week 8^{th} [4]	3.3 ± 0.4^{a}	4.4 ± 0.9^{bf}	5.3 ± 0.7^{cf}

[1]Mean ± SD

[2]Average value of wk 0^{th} (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2^{nd}, 4^{th}, 6^{th} and 8^{th} (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9^{th}, 10^{th}, 11^{th}, 12^{th}, 13^{th}, 14^{th}, 15^{th} and 16^{th} (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

4. Progressive motility (%)

Table 4.11 and Figure 6 show the progressive motility percentages of the dogs in all experimental groups. At the beginning (week 0^{th}), regarding to the grouping assessment, the progressive motility percentages were not differ ($P > 0.05$) when compared between the treatment groups.

At before week 8^{th}, the average percentage of progressive motility of the control group (72.4 ± 4.1 %) and group 2 (73.5 ± 2.1 %) were significantly lower ($P < 0.05$) than group 3 (80.3 ± 8.2 %). At after week 8^{th}, the average percentage of progressive motility of group 3 (94.3 ± 1.8 %) was the greatest ($P < 0.05$) followed by group 2 (82.1 ± 6.4 %) and control group (69.7 ± 5.4 %), respectively.

For the comparison between week 0th, before week 8th, and after week 8th, the result displayed that the percentage of progressive motility at before and after week 8th of both group 2 and group 3 were significantly greater ($P < 0.05$) than at week 0th. These values of both group 2 and 3 also increased as time increased.

Table 4.11 The progressive motility percentages of the dogs in all experimental groups[1]

Time	Progressive motility (%)		
	Control	1 ppm Se	3 ppm Se
Week 0th [2]	68.3 ± 2.8	70.0 ± 3.0[d]	70.0 ± 3.0[d]
Before week 8th [3]	72.4 ± 4.1[a]	73.5 ± 2.1[ae]	80.3 ± 8.2[be]
After week 8th [4]	69.7 ± 5.4[a]	82.1 ± 6.4[bf]	94.3 ± 1.8[cf]

[1]Mean ± SD

[2]Average value of wk 0th (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2nd, 4th, 6th and 8th (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9th, 10th, 11th, 12th, 13th, 14th, 15th and 16th (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

5. Sperm concentration (x 10^6 sperm/cm^3)

Table 4.12 and Figure 7 show the average sperm concentration (x 10^6 sperm /cm^3) of the dogs in all experimental groups. At the beginning (week 0^{th}), regarding to the grouping assessment, the average sperm concentration were not differ ($P > 0.05$) when compared between the treatment groups.

At before week 8^{th}, the average sperm concentrations of dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (92.7 ± 9.6, 73.6 ± 9.2, and 62.2 ± 6.1 x 10^6 sperm/cm^3). Also at after week 8^{th}, the average sperm concentrations of dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (115 ± 7.0, 93.9 ± 7.1, and 64.7 ± 15.7 x 10^6 sperm/cm^3).

For the comparison between week 0^{th}, before week 8^{th}, and after week 8^{th}, the result displayed that the average sperm concentration at before and after week 8^{th} of both group 2 and group 3 were significantly greater ($P < 0.05$) than at week 0^{th}. These values of both group 2 and 3 also increased as time increased.

Table 4.12 The average sperm concentration of the dogs in all experimental groups[1]

Time	Sperm concentration (× 10^6 sperm/cm^3)		
	Control	1 ppm Se	3 ppm Se
Week 0^{th} [2]	61.0 ± 11.3	61.6 ± 5.3[d]	63.2 ± 5.3[d]
Before week 8^{th} [3]	62.2 ± 6.1[a]	73.6 ± 9.2[be]	92.7 ± 9.6[ce]
After week 8^{th} [4]	64.7 ± 15.7[a]	93.9 ± 7.1[bf]	115 ± 7.0[cf]

[1] Mean ± SD

[2]Average value of wk 0^{th} (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2^{nd}, 4^{th}, 6^{th} and 8^{th} (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9^{th}, 10^{th}, 11^{th}, 12^{th}, 13^{th}, 14^{th}, 15^{th} and 16^{th} (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

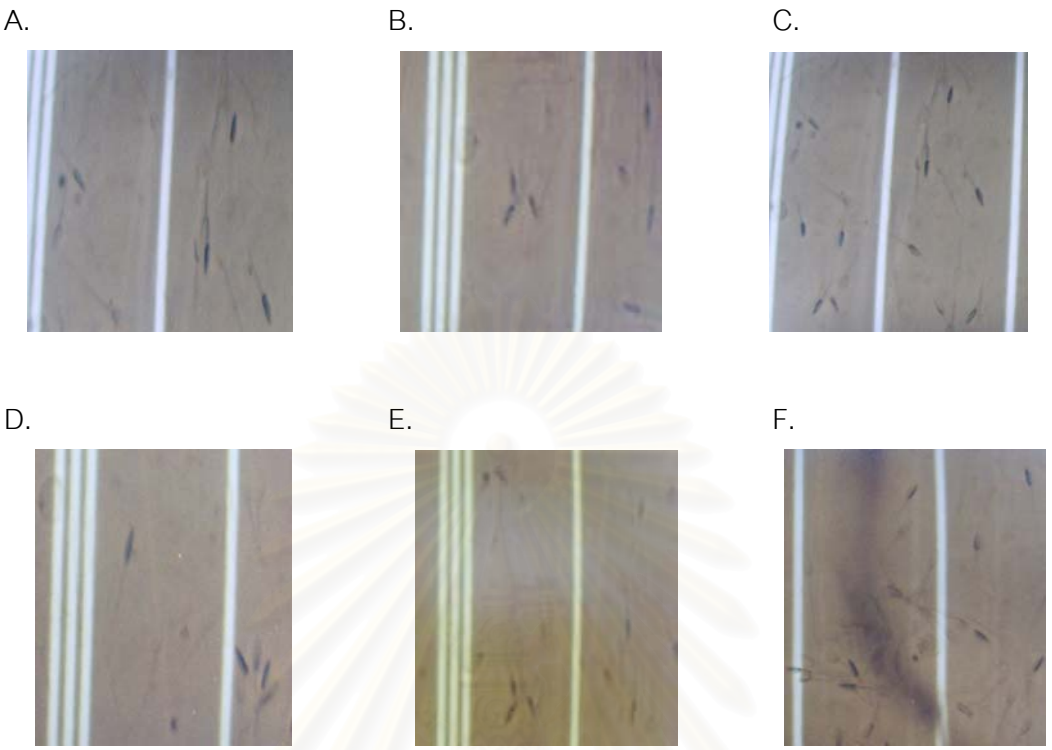

Figure 6. Comparison of microscopy photographs for the average sperm concentration from the dogs in all experimental groups (Magnification x 100); **A, B, C** = at before week 8th of the control, group 2 and 3, respectively, **D, E, F** = at after week 8th of the control, group 2 and 3, respectively.

6. Viable sperm (%)

Table 4.13 and Figure 7 show the percentage of viable sperm of the dogs in all experimental groups. At the beginning (week 0th), regarding to the grouping assessment, the percentage of viable sperm were not differ ($P > 0.05$) when compared between the treatment groups.

At before week 8th, the percentage of viable sperm of dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (80.3 ± 5.6, 75.4 ± 4.2, and 69.1 ± 3.9%). Also at after week 8th, the percentage of viable sperm of dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (87.2 ± 5.1, 76.5 ± 6.7, and 65.0 ± 6.4%).

For the comparison between week 0th, before week 8th, and after week 8th, the result displayed that the average sperm concentration at before and after week 8th of both group 2 and group 3 were significantly greater ($P < 0.05$) than at week 0th. These values of only group 3 also increased as time increased.

Table 4.13 The viable sperm percentage of the dogs in all experimental groups[1]

Time	Viable sperm (%)		
	Control	1 ppm Se	3 ppm Se
Week 0th [2]	62.6 ± 2.7	64.4 ± 1.8[d]	65.3 ± 2.1[d]
Before week 8th [3]	69.1 ± 3.9[a]	75.4 ± 4.2[be]	80.3 ± 5.6[ce]
After week 8th [4]	65.0 ± 6.4[a]	76.5 ± 6.7[be]	87.2 ± 5.1[cf]

[1] Mean ± SD

[2]Average value of wk 0th (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2nd, 4th, 6th and 8th (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9th, 10th, 11th, 12th, 13th, 14th, 15th and 16th (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

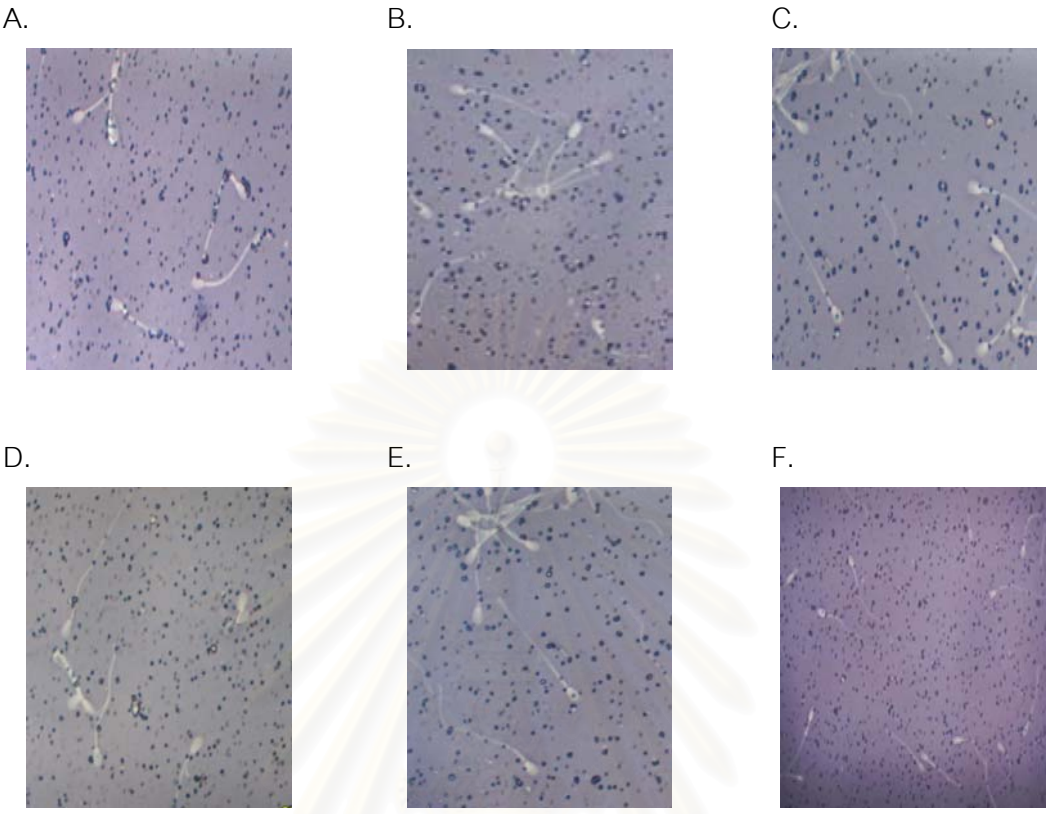

Figure 7. Comparison of microscopy photographs for viable sperm from the dogs in all experimental groups (Magnification x 100); **A, B, C** = at before week 8th of the control, group 2 and 3, respectively, **D, E, F** = at after week 8th of the control, group 2 and 3, respectively.

7. Total sperm concentration (×10^6 sperm/ejaculation)

Table 4.14 shows the average total sperm concentration (x 10^6 sperm /ejaculation) of the dogs in all experimental groups. At the beginning (week 0th), regarding to the grouping assessment, the average total sperm concentration were not differ ($P > 0.05$) when compared between the treatment groups.

At before week 8th, the average total sperm concentrations of dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (334 ± 6, 272 ± 7, and 211 ± 7 x 10^6 sperm/ejaculation). Also at after week 8th, the average total sperm concentrations of dogs in group 3 was the greatest ($P < 0.05$)

followed by group 2 and control group, respectively (611 ± 4, 413 ± 4, and 213 ± 8 x 10^6 sperm/ejaculation).

For the comparison between week 0^{th}, before week 8^{th}, and after week 8^{th}, the result displayed that the average total sperm concentration at before and after week 8^{th} of both group 2 and 3 were significantly greater ($P < 0.05$) than at week 0^{th}. These values of both group 2 and 3 also increased as time increased.

Table 4.14 The total sperm concentration of the dogs in all experimental groups[1]

Time	Total sperm concentration (×10^6 sperm/ejaculation)		
	Control	1 ppm Se	3 ppm Se
Week 0^{th} [2]	195 ± 4	190 ± 3^{bd}	196 ± 4^{bd}
Before week 8^{th} [3]	211 ± 7^{a}	272 ± 7^{be}	334 ± 6^{ce}
After week 8^{th} [4]	213 ± 8^{a}	413 ± 4^{bf}	611 ± 4^{cf}

[1] Mean ± SD

[2]Average value of wk 0^{th} (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2^{nd}, 4^{th}, 6^{th} and 8^{th} (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9^{th}, 10^{th}, 11^{th}, 12^{th}, 13^{th}, 14^{th}, 15^{th} and 16^{th} (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

8. Normal sperm morphology (%)

Table 4.16 and Figure 8 show the normal sperm morphology percentage of the dogs in all experimental groups. At the beginning (week 0^{th}), regarding to the grouping assessment, the normal sperm morphology percentages were not differ ($P > 0.05$) when compared between the treatment groups.

At before week 8th, the percentages of normal sperm morphology were not different when compared between the treatment groups. However, at after week 8th, the percentage of normal sperm morphology of dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (87.8 ± 3.3, 80.5 ± 3.6, and 67.5 ± 4.1%).

For the comparison between week 0th, before week 8th, and after week 8th, the result displayed that the percentage of normal sperm morphology at after week 8th of all treatment group were significantly greater ($P < 0.05$) than at week 0thand before week 8th. In addition, these values were not different when compared between at week 0th and 8th for all treatment groups.

Table 4.16 **The percentage of normal sperm morphology of the dogs in all experimental groups**[1]

Time	Normal sperm morphology (%)		
	Control	1 ppm Se	3 ppm Se
Week 0th [2]	75.9 ± 4.5[d]	74.5 ± 5.1[d]	72.2 ± 6.2[d]
Before week 8th [3]	78.2 ± 6.5[d]	79.2 ± 6.1[d]	79.1 ± 2.9[d]
After week 8th [4]	67.5 ± 4.1[ae]	80.5 ± 3.6[be]	87.8 ± 3.3[ce]

[1] Mean ± SD

[2]Average value of wk 0th (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2nd, 4th, 6th and 8th (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9th, 10th, 11th, 12th, 13th, 14th, 15th and 16th (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)


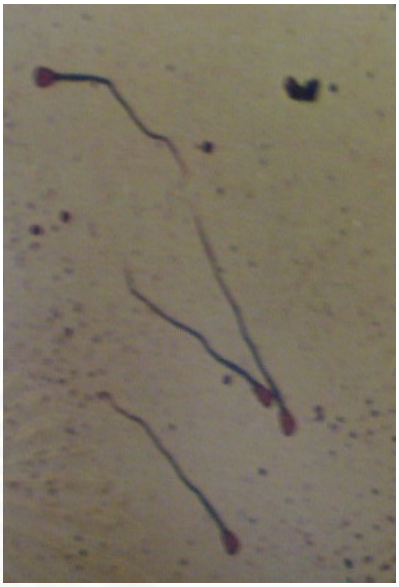

Figure 8. Microscopic photographs of normal sperm morphology from the dogs in the experiment (Magnification x 100).

9. Sperm abnormal morphology (%)

Table 4.17 shows the abnormal sperm morphology percentage of the dogs in all experimental groups. At the beginning (week 0^{th}), regarding to the grouping assessment, the abnormal sperm morphology percentages were not differ ($P > 0.05$) when compared between the treatment groups.

At before week 8^{th}, the percentages of abnormal sperm morphology were not differences when compared between the treatment groups. However, at after week 8^{th}, the percentage of abnormal sperm morphology of dogs in group 3 was the least ($P < 0.05$) followed by group 2 and control group, respectively (12.2 ± 3.3, 19.5 ± 3.6, and 32.5 ± 3.3%).

At after week 8^{th}, the dogs in group 3 had the least ($P < 0.05$) percentage of abnormal sperm morphology when compared to week 0^{th} and before week 8^{th}. For the group 2, both at before and after week 8^{th} had the percentage of abnormal sperm morphology lower ($P < 0.05$) than at week 0^{th}. Figure 10 shows the microscopic photographs of some abnormal sperm morphologies including coil tail,

translocation cytoplasmic droplets, double head, "DAG" defect, decapitated head, and round head.

Table 4.17 The percentage of abnormal sperm morphology of the dogs in all experimental groups[1]

Time	Abnormal morphology (%)		
	Control	1 ppm Se	3 ppm Se
Week 0th [2]	24.1 ± 3.9[d]	25.5 ± 3.2[d]	27.8 ± 3.8[d]
Before week 8th [3]	21.8 ± 2.5[d]	20.8 ± 3.8[e]	20.9 ± 3.5[e]
After week 8th [4]	32.5 ± 3.3[ae]	19.5 ± 3.6[be]	12.2 ± 3.3[cf]

[1] Mean ± SD

[2]Average value of wk 0th (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2nd, 4th, 6th and 8th (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9th, 10th, 11th, 12th, 13th, 14th, 15th and 16th (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

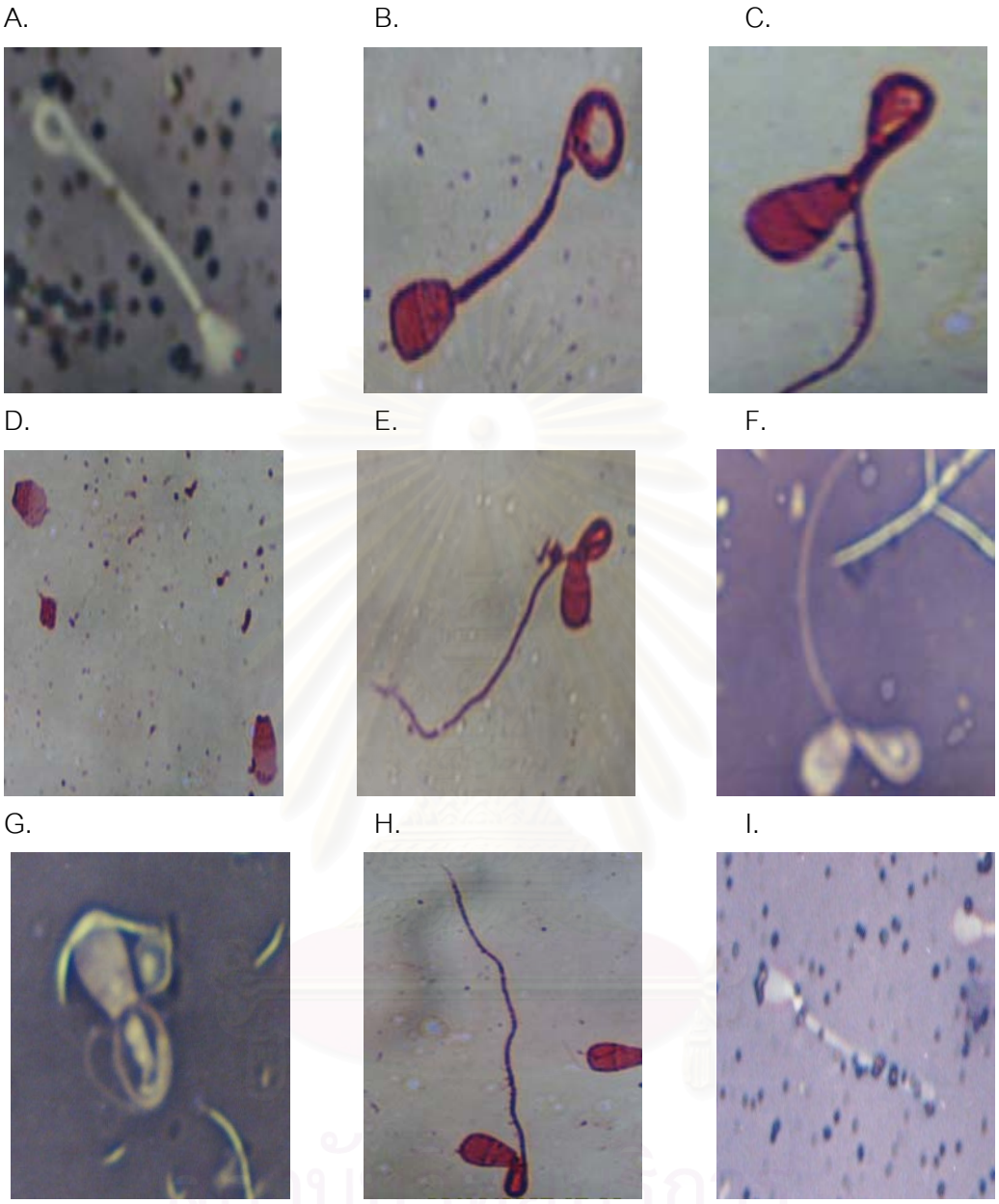

Figure 9. Microscopic photographs of abnormal sperm morphology from the dogs in the experiment (Magnification x 100); **A** and **B**: Coil tail, **C, D and E**: Translocation cytoplasmic droplets, **F**: Double head, **G**: "DAG" defect, **H**: Decapitated head, **I**: Round head.

10. Membrane integrity (%)

Table 4.15 shows the percentage of membrane integrity of sperm from the dogs in all experimental groups. At the beginning (week 0^{th}), regarding to the

grouping assessment, the average total sperm concentration were not differ ($P > 0.05$) when compared between the treatment groups.

At before week 8th, the percentage of membrane integrity of sperm from the dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (82.2 ± 5.3, 77.4 ± 4.8, and 70.9 ± 3.7%). Also at after week 8th, the percentage of membrane integrity of sperm from dogs in group 3 was the greatest ($P < 0.05$) followed by group 2 and control group, respectively (87.9 ± 3.8, 79.7 ± 6.3, and 68.7 ± 5.4%).

For the comparison between week 0th, before week 8th, and after week 8th, the result displayed that the percentage of membrane integrity of sperm at before and after week 8th of group 3 were significantly greater ($P < 0.05$) than at week 0th. These values of only group 3 also increased as time increased.

Table 4.15 **The membrane integrity percentage of the dogs in all experimental groups**[1]

Time	Membrane integrity (%)		
	Control	1 ppm Se	3 ppm Se
Week 0th [2]	69.2 ± 5.1	68.1 ± 6.3[d]	69.2 ± 5.1[d]
Before week 8th [3]	70.9 ± 3.7[a]	77.4 ± 4.8[bd]	82.2 ± 5.3[ce]
After week 8th [4]	68.7 ± 5.4[a]	79.7 ± 6.3[be]	87.9 ± 3.8[cf]

[1] Mean ± SD

[2]Average value of wk 0th (ejaculation number of control = 3, 1 ppm Se = 3, and 3 ppm Se = 3)

[3]Average value of wk 2nd, 4th, 6th and 8th (ejaculation number of control = 6, 1 ppm Se = 7 and 3 ppm Se = 9)

[4]Average value of wk 9th, 10th, 11th, 12th, 13th, 14th, 15th and 16th (ejaculation number of control = 16, 1 ppm Se = 20, and 3 ppm Se = 19)

[a,b,c] Mean ± SD with the same row with differences superscripts differ ($P < 0.05$)

[d,e,f] Mean ± SD with the same column with differences superscripts differ ($P < 0.05$)

CHAPTER V

DISCUSSIONS

This study provided the results on the effect of organic Se supplement on Se level in plasma, sperm morphology and sperm motility in dogs. At the beginning of this study, it is believed that the economy grade, commercial diet should contain Se at the minimum requirement as recommended by NRC (2002) and AAFCO (2000). The minimum requirement of Se for adult dog as recommended by NRC (2002) was 0.35 ppm on DM basis and by AAFCO (2000) was 0.11 ppm. The maximum amount recommended by AAFCO (2000) was 2.0 ppm. Both NRC (2002) and AAFCO (2000) guides did not specify the form of Se whether it should be inorganic or organic mineral (Mahan and Peter, 1996). Now, it is quite well documented that minerals in an organic form including Se can be better utilized by the animals than the inorganic form (Mahan and Peter, 2004). The Se source of the current study was organic Se (Sel-Plex) and basal diet was found to contain only 0.03 ppm Se that it is lowest than the minimum requirement of Se for adult dog as recommended by NRC (2002) and AAFCO (2000). The levels of Se supplement used in the current study were 1 and 3 ppm which excess the minimum requirement as recommended by both NRC (2002) and AAFCO (2000), and also excess the maximum limit as recommended by AAFCO (2000). However, no clinical sign of Se toxicity was observed from any dogs in all experimented groups through out the study. The measurements of blood chemistry as GPT (4-91 U/L) and creatinine (0.6–1.4 U/L) levels of the dogs in all experimental groups were in the normal range (Donald, 1999). No change in the average BW was obtained when compared between at the beginning and the end of the study. The significant differences of the average BW between the treatments at the beginning and the end of the study were not considered the effects of the Se supplementation since the dogs were grouped by percentage of progressive motility and membrane integrity. In study of England et al. (1990) found the age of dogs were between 4.5-5 years, that it is fertility

periods found normal sperm characteristics (ejaculation volumes, total volume of semen, sperm concentration, total sperm concentration, progressive motility, viable sperm, membrane integrity and normal sperm morphology)

No change in plasma Se level for the dogs that received no Se supplement. For the dogs received the Se supplement, it was found as the level of Se supplement increased, the level of Se in plasma increased. These results were similar to the study did by Wedekind et al. (2004) who determined the Se requirement of the puppy.

Selenium is a component of some enzymes important for thyroid hormone synthesis such as GPXs, TRs and Ds (Beckett and Auther, 2005). Increasing amount of Se supplement should result in increasing thyroid hormone concentration. However, Wedekind et al. (2004) reported no differences in serum FT3 and FT4 as increased Se in the diets for puppy. This was in agreed with the current study that the concentrations of FT3 and FT4 in serum were not different for both at the beginning and week 12^{th}. FT3 and FT4 are the active forms of thyroid hormones while TT3 and TT4 are the forms that bound with serum protein and need to be metabolized to active forms prior before use (Heart and Newton,1983). However, the levels of FT3 and FT4 in serum are considered very small as in picogram and nanogram, respectively. These small amounts make them less reliable when performing the assessment for the status of thyroid hormones (Squires, E.J. 2003). Therefore, TT3 and TT4 are the most popular forms for the determination of thyroid hormone status. In this study, change in the concentration of thyroid hormones was observed for only TT3 which the dogs supplemented with Se at 1 ppm had the greatest increasing amount of the serum TT3 concentration. These animals also had the level of serum TT4 greater than the ones that got no Se supplement, but not differ from the ones that got the 3 ppm Se supplement. The increase TT3 as increase Se intake was reported previously in rats (Arthur et al., 1992) and kittens (Wedekind et al., 2003).

The duration of spermatogenesis process of the dog lasts about 56 days (Christiansen, 1984). This current study was designed to collect and compare the data at before and after 8 weeks of organic Se supplementation. It was assumed that the data of semen collection after week 8^{th} to week 16^{th} would represent the sperm characteristics after Se supplement As a result, there were some differences of sperm quantity when compare between before and after week 8^{th} which confirmed the awareness of this study.

Normal range for pH of dog semen is 6.3-6.7 (Concannon et al., 2006), and the semen from all dogs in this study had the normal pH. The pH of semen has direct effect to the living sperm. Either too much acidity or base can reduce the number of live sperm (Concannon et al., 2006)

Semen collection was manually ejaculations as described by Johnston (1991) by the same person through the experiment. In this study, sperm volume seemed to be low (3.1 – 5.3 ml.). This might be cause by the less experience of semen collector. However, the semen volume indicated in this study was the acellular pre-sperm fraction which is in a normal range (Johnston, 2003). The normally manual dog semen is ejaculated by Johnston (2003) in 3 fractions: (i) the acellular pre-sperm fraction (0.5 to 5.0 cc), which may originate in the prostate; (ii) the sperm-rich fraction (1.0-4.0 cc) which originates in the testes and epididymis; and (iii) the prostatic fluid fraction (2.5-> 80 cc) which also is acellular and originates in the prostate.

In this study, supplementation of organic Se at 1 and 3 ppm could significantly increase ($P < 0.05$) the average of ejaculation volumes, total volume of semen, sperm concentrations and total sperm concentration. The trace element Se has multiple actions on characterized selenoenzymes which are the families of GPXs, TRs and Ds. These selenoenzymes are capable of modifying cell function by acting as antioxidants and modifying redox status and thyroid hormone metabolism (Beckett and Auther, 2005). Se has also an important role in spermatogenesis (Mason, 1954; Marin-Guzman et al., 1997) and functions by acting as antioxidants of spermatogenesis and enhancing of development, ultimately and number of sertoli

cell that undergo mitotic divisions in spermatogenesis (Marin-Guzman et al., 2000[a]). An electron microscopic determination of cutting testis found that Se supplementation affected increase the number of sperms in the secondary spermatocyte of the spermatogenesis. This results to increase the average of ejaculation volumes, total volume of semen, sperm concentrations and total sperm concentration (Marin-Guzman et al., 2000[a] and Marin-Guzman et al., 2000[b]).

Supplementation of Se in the current study demonstrated the increase percentage of progressive motility, viable sperm, membrane integrity and normal sperm morphology ($P < 0.05$). Organic Se supplement at 1 and 3 ppm could decrease the percentage of abnormal sperm morphology ($P < 0.05$). In the study of Watanabe and Endo (1991) and Behne et al. (1996) found low sperm production and poor sperm quality including impaired motility with flagella defects localized primarily to the midpiece have been a consistent feature in Se-deficient animals. This trace mineral helps in the development of testes and spermatogenesis, and raise a decrease of oxidative stress in humans (Maiorino and Ursini, 2002), boars (Oldfield, 2003), rats (Olson et al., 2004) and mice (Parminder and Mohinder, 2004).

Marin-Guzman et al. (2000b) reported that organic Se 1-5 ppm helps to raise the percentage of sperm motility. By working under the action of GSH, an anti-oxidant substance of sperm, Se supplement affects mitochondria structures in the tail part of sperm to have an increase of normal form arrangement. Accordingly, gaps between mitochondria are dropped off, so that the sperm has an energy source to get better movement, which increases the velocity of the sperm motility significantly. As a result, spermatozoa had greater ATP concentration causing the increase percentage of progressive motility. In addition, Se represents an integral component of GSH-Px, this enzyme protects internal structures of cell against free radicals and also acts as antioxidant for cellular membrane lipids peroxidations (Kolodziej and Jacyno, 2005). Therefore, increase viable sperm, membrane integrity (Kendall et al., 2000) and normal sperm morphology were recognized. In the study by Marin-Guzman et al. (2000a) and Marin-Guzman et al. (2000b), supplementation of Se at 3-10 ppm to the boars resulted in increase amount of semen. These

supplement also improved the percentage and the amount of normal form sperms, the percentage of regular progressive motility as well as the percentage of sperm viability to respect ($P < 0.01$).

In addition, the result demonstrated that the Se supplementation directly affected the average sperm concentration of dogs. In other words, the more Se supplements the greater average of sperm concentration. It could be seen that the average sperm concentration in semen of group 3 was significantly greater than group 1 ($P < 0.05$). The percentages of sperm abnormal morphology of the controlled group, group 1 and group 3 were 32.5 ± 3.3 %, 19.5 ± 3.6 % and 12.2 ± 3.3 % respectively, ($P < 0.05$). This can be seen that the percentage of abnormal morphology of the controlled group was markedly greater ($P < 0.05$) than group 1 and group 3 ($P < 0.05$). And group 3 had lower ($P < 0.05$) percentage of sperm abnormal morphology than that in group 1. These results showed that the concentration of Se supplementation is inversely proportional to the percentage of sperm abnormal morphology in dogs.

In conclusion, supplementation of organic Se may be able to enhance the spermatogenesis process resulting in improvement of semen characteristics which are important for the male reproduction and enhance fertility in dogs. Supplementation of organic Se at 3 ppm can enhance semen characteristics better ($P < 0.05$) than supplementation at 1 ppm. However, supplementation of organic Se at 3 ppm excesses the maximum amount for maintenance dog as recommended by NRC (2002) and AAFCO (2000). Furthermore, the increase level of Se supplementation may have the indirect effect on the thyroid hormones that benefit the sperm formation. However, the sperm characteristic improvement should be further investigated by breeding or insemination with female dog and pregnancy rate should be evaluated.

REFERENCES

AAFCO. 2000. Association of American Feed Control Officials: Official Publication. The Association, Atlanta, GA.

AOAC. 1990. Method of analysis. Association of Official Analytical Chemistry. Washington, DC.

Arthur, J.R., Mckenzie, R.C. and Beckett, G.J. 2003. Se in immune system. J.Nutr. 133: 1457-1459.

Arthur, J.R., Nicol, F., Beckett. G.J. 1992. Selenium metabolism and bioavibility. Biol.Trace.Elem.Res. 33: 37-42.

Balercia, G., Moretti, S., Vignini, A., Magagnini, M., Mantero, F., Boscaro, M., Ricciardo-Lamonica, G. and Mazzanti, L. 2004. Role of nitric oxide concentrations on human sperm motility. J Androl. 25 (2): 245-249.

Basini, G. and Tamanini, C. 2000. Se stimulates estradiol production in bovine granulosa cells:possible involvement of nitric oxide. Domest. Anim.Endocrinol.18: 1-17.

Bates, J.M., Spate, V.L., Morris, J.S., Germain, D.L. and Galton, V.A. 2000. Effects of Se deficiency on tissue Se content, deiodinase activity, and thyroid hormone economy in the rat during development. Endocrinology. 141: 2490-2500.

Beck, M.A., Levander, O.A. and Handy, J. 2003. Se deficiency and viral infection. J. Nutr. 133: 1463s-1467s.

Beckett, G.J. and Arthur, J.R. 2005. Se and endocrine systems. J. Endocrinol.184: 455-465.

Beech, S.G., Walker, S.W., Dorrance, A.M., Arthur, J.R., Nicol, F., Lee, D. and Beckett G.J.1993. The role of thyroidal type-I iodothyronine deiodinase in tri-iodothyronine production by human and sheep thyrocytes in primary culture. J. Endocrinol. 136: 361-370.

Behne, D., Kyriakopoulos, A., Weiss-Nowak, C., Kalcklösch, M., Westphal, C., and Gessner, H., 1996, Newly found selenium-containing proteins in the tissues of the rat, Biol. Trace Elem. Res. 35: 99–110.

Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J. and Larsen P.R. 2002. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr. Rev. 23: 38-89.

Broome, C.S. 2004. An increase in Se intake improves immune function and poliovirus handling in adults with marginal Se status. Am. J. Clin. Nutr. 80: 54-162.

Brown, K.M., Pichard, K., Nicol, F., Beckett, G.J., Duthie, G.G. and Arthur, J.R. 2000. Effects of organic and inorganic Sesupplementation on selenoenzyme activity in blood lymphocytes, granulocytes, platelets and erythrocytes. Clin. Sci. 98: 593-599.

Case, L.P., Carey, D.P., Hirakawa, D.A. and Daritotle, L. 2000. Canine and feline nutrition : a resource for companion animal professionals. 2nd ed. St. Louis: Mosby, pp. 592.

Christiansen, I.J. 1984. Reproduction in the Dog and Cat. Bailliere Tindall, London. pp. 90–91.

Combs, G.F. and Lu, J. 2001. Se as a cancer preventative agent. In Se: Its Molecular Biology and Role in Human Health, Edited by Hatfield. Boston: Kluwer Academic Publishers. P 205–219.

Concannon, P.W., England, G., Verstegen, J. and Linde, C. 2006. Assessment of reproductive problems in the male dogs : http://www.ivis.org/advances/Concanon/gradil/chapter.asp?LA=1

Donald, C. P. 1999. Veterinary drug handbook 3rd ed. White bear lake, Minnesota. pp. 706.

Dong, Y., Zhang, H., Hawthorn, L., Ganther, H.E. and Ip, C. 2003. Delineation of the molecular basis for Se-induced growth arrest in human prostate cancer cells by oligonucleotide array. Cancer Res. 63: 52–59.

Dong, Y., Lee, S.O., Zhang, H., Marshall, J., Gao, A.C. and Ip, C. 2004. Prostate specific antigen expression is down-regulated by Se through disruption of androgen receptor signaling. Cancer Res. 64: 19–22.

Donghua L., George, L.G., Yee, J.A. and Yan L. 2004. Dietary supplementation with high-Se soy protein reduces pulmonary metastasis of melanoma cells in mice. J. Nutr.134: 1536-1540.

Ebisch, I.M.W., Thomas, C.M.G., Peters, W.H.M., Braat, D.D.M. and Steegers Theunissen, R.P.M. 2006. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum. Reprod. Update. 10: 1093-1098.

England, G. C.W., Allen, W.E., Middleton, D.J. 1990. An investigation into the origin of the first fraction of the canine ejaculate. Res. Vet. Sci. 49: 66-70.

Esworthy, S.R., Yang, L., Frankel, P.H. and Chu, F.F. 2005. Epithelium-specific glutathione peroxidase, Gpx2, is involved in the prevention of intestinal inflammation in Se-deficient mice. J. Nutr. 135: 740-745.

Ferguson, J.M., Renton, J.P., Fartad, W. and Douglas, T.A.1989. Insemination of beagle bitches with frozen semen. J. Reprod. Fertil. Suppl. 39: 293-298.

Fico, M.E., Poirer, K.A., Watrach, A.M. and . Milner, J. A. 1986. Differential effects of Se on normal and neoplastic canine mammary cells. Cancer Res. 46: 3384-3388.

Finley, J.W., Davis, C.D. and Yi, F. 2000. Se from high Se broccoli protects rats from colon cancer. J. Nutr. 130: 2384-2389.

Foresta, C., Flohe, L., Garolla, A., Roveri, A., Ursini, F. and Maiorino, M. 2002. Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase. Biol. Reprod. 67: 967–971.

Fortier, M.E. and Matte, J.J. 2005. Dietary organic (Sel-plex) and inorganic Se for hyperovulatory first-parity sows:antioxidant status, hormone response, embryo development and reproductive performance. In: Nutritional biotechnology in the feed and food industries. Proceedings of altech's 21st annual symposium. Edited by T.P. Lyons and K.A. Jacques: Notingham university pres. P 175-183.

Gartner, R. and Gasnier, B.C. 2003. Se in the treatment of autoimmune thyroiditis. Biofactors. 19: 165–170.

Gartner, R., Gasnier, B.C., Dietrich, J.W., Krebs, B. and Angstwurm, M.W. 2002. Se supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. J. Clin. Endocrinol. Metab. 87: 1687–1691.

Ghose, A., Fleming, J. and Harrison, P.R. 2001. Se and signal transduction: roads to cell death and anti-tumour activity. Biofactors. 14: 127–133.

Guoyao, W., Yun, Z.F., Sheng, Y., Lupton, J.R. and Turner, N.D. 2004. Glutathione metabolism and its implications for health. J. Nutr. 134: 489-492.

Heart, I.R. and Newton, R.W. 1983. The new medicaine a integrated system of study V2 endrocrinology. Parkpress Baltomore. Published in USA and Canada. pp. 22-26.

Hill, K.E., Motley, A.K., Xia, L., May, J.M. and Burk, R.F. 2001. Combined Se and vitamin E deficiency causes fatal myopathy in guinea pigs. J. Nutr. 131: 1798-1802.

Jana, B., Dusza, L. and Sobczak, J. 1996. Effect of exogenous porcine prolactin (pPRL) on LH plasma concentration and on the release of GnRH in vitro from the stalk median eminence in prepubertal gilts. Anim. Reprod. Sci. 4 (3-4): 295-304.

Johnston, S.D. 1991. Performing a complete canine semen evaluation in small animal hospital. Vet. Clin. North. 21: 545-555.

Johnston, S.D., Root Kustritz, M.V., Olson, P.N. 2001. Clinical approach to infertility in the male dog. In canine and feline theriogenology, W.B. Saunders Co., Philadelphia, PA, 2001.

Johnston, S.D. 2003. Clinical approach to azoospermia in dogs. [online]. Available from : http://www.vin.com/proceedings/Proceedings.plx?CID=WSAVA2003&PID=6685&O=Generic [2008, Feb 10]

Kendall, N.R., McMullen, S., Green, A. and Rodway,. R. G. 2000. The effect of a zinc, cobalt and Se soluble glass bolus on trace element status and semen quality of ram lambs. J. Anim. Reprod. Sci. 62: 277-283.

Kim Y.Y. and Mahan D.C. 2001. Comparative effects of high dietary levels of organic and inorganic Seon Se toxicity of growing-finishing pigs. J. Anim. Sci. 79: 942-948.

Koller, L.D. and Exon, J.H. 1986. The two faces of Se deficiency and toxicity are similar in animal and mammary. Can. J. Vet. Res. 50: 297-306.

Kolodziej, A. and Jacyno, E. 2005. Effect of Se and vitamin E supplementation on reproductive performance of young boars. J. Pol. Agric. Univ. 48: 68-75.

Kumi-Diaka, J. 1993. Subjecting canine semen to hypo-osmotic test. Theriogenology. 39: 1279-7289.

Lasota, B., Blaszczyk, B., Seremak, B. and Udala, J. 2004. Se status and GSH-Px activity in semen and blood of boars at different ages used for artificial insemination. Reprod. Dom. Anim. 39: 309-314.

Mahan, D.C. and Parret, N. A. 1996. Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine. J. Anim. Sci. 75: 2994-3003.

Mahan, D.C. and Peter, J.C. 2004. Long-term effects of dietary organic and inorganic selenium sources and levels on reproducing sows and their progeny. J.Anim.Sci. 82: 1343-1358.

Maiorino, M. and Ursini, F. 2002. Oxidative stress, spermatogenesis and fertility. Biol. Chem. 383: 591–597.

Marin-Guzman, J., Mahan D.C., and Pate, J.L. 2000[a]. Effect of dietary Se and vitamin E on spermatogenic development in boars. J. Anim. Sci. 78: 1537-1543.

Marin-Guzman, J., Mahan, D.C. and Whitmoyer, R. 2000[b]. Effect of dietary Se and vitamin E on the ultrastructure and ATP concentration of boar spermatozoa, and the efficacy of added sodium selenite in extended semen on sperm motility. J. Anim. Sci. 78: 1544-1550.

Ming, C., Blackman, M.R., Wang, C. and Swerdloff, R.S. 2004. The role of carnitine in the male reproductive system. Ann. N.Y. Acad. Sci. 1033: 177–188.

Morton, D.B. and Bruce, S. G. 1989. Semen evaluation, cryopreservation and factors relevant to the use of frozen semen in dogs. J. Reprod. Fert. Suppl. 39: 311-316.

Mihajlovic, M. 1992. Se toxicity in domestic animals. Glas. Srp. Akad. Nauka. 42: 131-144.

Navickis, R. J., Jones, B.C and Hsueh, J.W.1982. Modulation of prolactin receptors in cultured rat granulosa cells by FSH, LH and GnRH. Mol. and Cel. Endcrin. 27 (1): 77-88.

NRC. 2002. Nutrient requirements of dogs and cats. Washington, D.C. National Academies Press. pp. 398.

Oldfield, J.E. 2003. Some recollections of early swine research with Se and vitamin. J. Anim. Sci. 81: E145-E148.

Olson, G.E., Virginia P.W., Hill, K.E. and Raymond, F.B. 2004. Sequential development of flagellar defects in spermatids and epididymal spermatozoa of Se-deficient rats. J.Reprod. 127: 335-342.

Ostadalova, I., Vobeckyl, J., Chvojkova, Z., Mikova, D., Hample, V. and Osadal, B. 2006. Se increases tolerance of the neonatal rat heart to ischemia/reperfusion injury. J. Mol. and Cel. Cardiol. 40: 955-956.

Parminder, K. and Mohinder, P. 2004. Effect of Se-induced oxidative stress on the cell kinetics in testis and reproductive ability of male mice. J Nutr. 21: 351-357.

Ponglowhapan, S., Chatdarong, K., Sirivaidyapong S. and Lohachit, C. 2006. Freezing of epididymal spermatozoa from dogs after cool storage for 2 or 4 days. Theriogenology. 66: 1633-1636

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. 1973. Se: Biochemical role as a component of glutathione peroxidase. J. Sci. 179: 588–590.

Sanchez, A. and Rubilar, G. 2002 . Evalution of fresh and frozen canine semen by hypoosmotic swelling test. J. Arch. Med. Vet. 34: 487– 498.

Schrauzer, G.N. 2000. Selenomethionine : A review of its nutritional significance, metabolism and toxicity. J.Nutr. 130: 1653-1656.

Sikka, S.C. 2004. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. J.androl. 25: 5-16.

Squires, E.J. 2003. Applied animal endocrineology. CABI publishing, Walling ford, London, UK. pp. 96-99.

Suh, M., Merrells, K., Hosea, H., Jamieson, J. and Taylor, C. 2006. Relationship between abnormal sperm morphology induced by dietary zinc deficiency and lipid composition in testis of growing rats. FASEB J. 20: A626-A635.

Tamura, T. and Picciano, M.F. 2006. Folate and human reproduction. J Clin Nutr. 83: 993-1016.

Watanabe, T. and Endo, A. 1990. Biotin Deficiency Per Se Is Teratogenic in Mice. J. Nutr. 121: 101-104.

Waters, D.J., Shen, S., Glickman , L.T., Cooley, D.M., Bostwick , D.G., Qian , J., Gerald, F., Combs, J.R. and Morris, S.J. 2005. Prostate cancer risk and DNA damage: translational significance of Se supplementation in a canine model. Carcinogenesis. 26: 1256-1262.

Wedekind, K.J., Yu, S. and Combs, G.F. 2004. The Se requirement of the puppy. J. Anim. Phys. and Anim. Nutri. 88: 340-349.

Yousef, M.I., Abdallah, G. A. and Kamel, K. I. 2003. Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits. J.androl. 76 (1-2): 99-111.

www.faculty.sunydutchess.edu/scala/Bio102/default.htm [online, 2007]

www.wisc.edu/repro/lab/procedures/sperm/bovine_abnormals.html [online, 2007]

www.zgxl.net/eng/health/humanbody/hormone.html [online, 2007]

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