CHAPTER 3

Materials and methods

1. Animals

Ten healthy adult mongrel sexually intact male dogs weighing between 10 to 20 kg were used in this study. Prior to inclusion in the study, dogs underwent a complete physical examination, including general health appearance; blood samples were submitted for a CBC and serum biochemical analyses, prostatic palpation per rectum, semen samples were collected and submitted for microscopic evaluation. The dogs were housed individually in cages in an animal room and fed premium quality commercial dog food ,and water was available *ad libitum*. To evaluate the effects of deslorelin on general health of animals, their general condition was observed once a day and weight was recorded weekly through the trial period. Dogs were randomly assigned to a treatment group (n = 8) to recieve a 10 mg deslorelin implant or a control group (n = 2) to recieved an implant without deslorelin. Dosage of deslorelin in the treated dogs ranged between 0.5-1 mg/kg BW. The interscapular region of each dog was clipped and cleansed, and deslorelin-containing or placebo implants were inserted aseptically under the skin. Reaction at the site of implantation was observed daily for two weeks. Skin reactions, inflammatory signs or behavioural changes were observed.

2. Chemicals

The potent long-acting GnRH agonist deslorelin prepared as a biocompatible cylindrical implant (3.6mm long x 2.3mm in diameter) was supplied by Peptech Animal Health Pty Limited, NSW, Australia. Each implant contained 5mg of active ingredient deslorelin. Two implants were preloaded in a disposable syringe-like implanter and kept dark in an individual sterile package at 4°C before use.

3. Study design

The study was conducted as a placebo-controlled trial. The observation period was 48 weeks.

4. Reproductive function was evaulated by the following parameters

All following parameters were performed once begining one week before implantation and, every second week after implantation for the first 12 week period, and every forth week for the second 36-week period.

4.1 Semen characteristics

The sperm-rich fraction was collected by digital manipulation in a calibrated plastic vial as described by Linde-Forsberg (1991) and analyzed to determine its volume, motility, sperm concentration and pH. The percentage of motile spermatozoa moving progressively across the field was estimated by subjective microscopic examination on wet smear at a magnification x 200 using a phase contrast microscope, and the sperm concentration was determined using an improved Neubauer haemocytometer counting chamber.

4.2 Serum testosterone concentrations

Two-ml blood samples were collected from the cephalic vein at the same time of day each time of blood collection. Serum testosterone concentrations were measured by use of the commercially available electrochemiluminescence immunoassay "ECLIA" with 0.020 ng/ml sensitivity which was intended for use on the Boehringer Mannhelm Elecsys 1010 and 2010 immunoassay analyzers. Values below the detection limit are reported as < 0.020 ng/ml.

4.3 Scrotal circumference measurements

Measurement was carried out when dogs were in standing position. The position of greatest testis diameter in the transverse plane of the scrotal sac was measured as a scrotal circumference (cm).

4.4 Testicular volume

In vivo testicular volume measurements were evaluated using a real-time B-mode ultrasound machine with a convex array transducer as described by England (1991). Dogs were placed in a position of dorsal recumbency without any anesthesia. A 5.5-7.5 MHz curved linear array transducer was moved slowly over the surface of each testis in three planes; ventro-dorsal (sagittal and transverse) and caudo-cranial (dorsal). Testicular volume was calculated from the ultrasound measurements using the formula for the volume of an ellipse;

$$V = \pi/6\ abc\ (cm^3)$$

where a=transverse diameter in cm; b=sagittal diameter in cm and c=dorsal diameter in cm. Total testicular volume was calculated from the volume of both testicles.

4.5 Prostatic volume

In vivo prostatic volume was determined by the method using ultrasonographic measurement as described by Kamolpatana et al. (2000). Each dog was positioned in dorsal recumbency without any anesthesia, and a 5.5-7.5 curved convex array transducer was placed on the abdominal wall and positioned for longitudinal and transverse images through the prostate. The greatest craniocaudal (length;L), transverse (width;W) ,and dorsoventral (depth;D) diameters of the prostate were measured in cm. Measured prostatic volume was calculated using the formula;

$$V=[1/2.6\ (LxWxD)] + 1.8\ (cm^3)$$

5. Statistical analyses

Percentage changes in serum testosterone concentrations, scrotal circumference, total testicular volume and prostatic volume were calculated by comparing values with pretreatment values. All tests were accessed by using SAS statistical package (SAS Institute, 1989). All statistical analyses of serum testosterone concentrations, scrotal circumference, total testicular volume and prostatic volume were performed on mean values for the treatment group and the control group. Differences in mean values were tested by using analysis of variance (general linear model procedure). A value of $P<0.05$ was considered statistically significant. Descriptive statistics will be used to determine the variation on semen quality.