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# THE EFFECT OF A SLOW RELEASE IMPLANT CONTAINING THE GnRH AGONIST DESLORELIN ON PITUITARY AND TESTICULAR FUNCTION IN MATURE MALE DOGS

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**Abstract**

The effect of chronic treatment with a slow release implant containing 6 mg of the GnRH agonist deslorelin on pituitary and testicular function was studied in mature male dogs. Four dogs were implanted with 6 mg deslorelin implant (group 1). Group 2 (n=4) were used as control dogs and they were implanted with implant without deslorelin (blank implant). In group 1, plasma LH and testosterone concentrations were undetectable on day 20.5 ± 0.4 and 26.5 ± 2.5 after implantation, respectively. Detectable then normal concentrations occurred 50.8 ± 3 and 51 ± 3.7 weeks after implantation, respectively. Testes volume dropped to 35 % after 13.5 ± 7.4 weeks and no ejaculate could be obtain after 5.5 ± 2.5 weeks after implantation. Complete recovery of semen characteristics were achieved 58.5 ± 2.9 weeks after implantation. Histological findings on the testes and prostate after the dogs recovery showed similar with the control dogs. This study demonstrate that implantation using a slow release implant containing 6 mg of the GnRH agonist deslorelin in dogs is effective in long term suppression of the reproductive function in male dogs and that the effect are reversible.

Key word: GnRH agonist, deslorelin, testosterone, LH, testes, ejaculate, prostate.

# EFEK DARI PEMBEBASAN PERLAHAN IMPLAN YANG MENGANDUNG GnRH AGONIS DESLORELIN PADA FUNGSI PITUITARI DAN TESTIKULER ANJING JANTAN DEWASA

**Abstrak**

Penelitian tentang efek pemberian kronik pembebasan perlahan implan yang berisi 6 mg GnRH agonis deslorelin pada fungsi pituitari dan testis telah dilakukan pada anjing jantan dewasa. Empat anjing diberi implan yang berisi 6 mg deslorelin (grup 1). Grup 2 (n=4) digunakan sebagai anjing kontrol dan diberi implan tanpa deslorelin (implan kosong). Pada grup 1, konsentrasi LH dan testosterone plasma tidak terdeteksi secara berurutan yaitu pada hari ke 20,5 ± 0,4 dan 26,5 ± 2,5 sesudah pemberian implan. Terdeteksi dan kemudian konsentrasi normal terjadi pada minggu ke 50,8 ± 3 and 51 ± 3,7 sesudah pemberian implan. Volume testes drop sampai 35% sesudah minggu ke 13,5 ± 7,4 dan tidak ada ejakulat yang bisa didapat pada minggu ke 5,5 ± 2,5 sesudah pemberian implan. Semen karakteristik kembali ke normal pada minggu ke 58,5 ± 2,9 sesudah pemberian implan. Hasil pemeriksaan histologi pada testes dan prostata sesudah anjing rekoveri sama seperti pada anjing kontrol. Penelitian ini menunjukkan bahwa implantasi dengan menggunakan pembebasan perlahan implan yang berisi 6 mg GnRH agonis deslorelin pada anjing efektif untuk menekan fungsi reproduksi dalam jangka panjang pada anjing jantan dan efeknya bersifat reversibel.

Kata kunci: GnRH agonist, deslorelin, testosterone, LH, testes, ejaculate, prostate.

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## Introduction

The administration of agonistic analogue of GnRH has been shown to be effective in suppressing testosterone secretion and spermatogenesis in a wide range of species including the rat (Fraser *et al.*,1982; Rivier *et al.*, 1979; Sandow *et al.*, 1980; Vickery *et al.*, 1985), Hawaiian monk seal (Atkinson *et al.* 1993), boar (Xue *et al.*, 1994), stallion (Boyle *et al.* 1991), ram (Lincoln *et al.*, 1986) and dog (Inaba *et al.*, 1996; Paramo *et al.*, 1993; Vickery *et al.*, 1984). These workers reported a great variation in the response to administration of GnRH agonists in dogs, ranging from severe impairment of spermatogenesis (Rivier *et al.*, 1979) to less drastic reductions in testicular weight with only partial impairment of spermatogenesis and no inhibitory effect on fertility (Sandow *et al.*, 1980). In dogs, Vickery *et al.*, (1985) reported that in acute trials, the potency of effect of the agonist depended on the dose rate, with the higher doses tending to produce a more rapid down regulation of the hypothalamo-pituitary axis. Their investigations indicated that the response of dogs to injection with a GnRH agonist is variable. Although Inaba *et al.*, (1996) showed that the effect of GnRH agonists is reversible in dogs, the long term suppression of testicular function requires investigation and definition.

This experiment was designed to provide information on: (1) whether a slow release implant of the GnRH agonist deslorelin (Peptide Technology Pty Ltd, Sydney, Australia) could be used to suppress the testicular function of male dogs; (2) if so, how long this suppression lasted and; (3) was the effect fully reversible.

### Materials and Methods

***Animals***. Eight male adult dogs ranging in age from 2 to 3 years were used in this experiment. The animals were housed indoors. During the day they were outdoors for 2 to 6 hours in large shaded sandy runs. All dogs were fed with biscuits (approximately 600 g per dog per day) and canned meat (approximately 400 g per dog three times a week) (Pedigree® PAL®, Uncle Ben's of Australia) and had access to water ad libitum.The dogs were randomly assigned into two groups. Group 1 (n=4) each received a 6 mg deslorelin implanted subcutaneously. Group 2 (n=4) each received an implant without deslorelin (blank implant).

Semen was collected by hand manipulation without a teaser bitch as described by Seager (1986) at weekly intervals. Only second sperm-rich fraction was collected. Immediately after collection, sperm concentration and motility were determined by haemocytometer count and microscopic examination of a drop of semen, respectively. A nigrosin/eosin preparation was made for subsequent assessment of the precentages of live spermatozoa and the percentage of abnormal spermatozoa, a total of 100 spermatozoa being counted on each slide.

Testes volume was estimated using caliper (Mitutoyo, Japan) as described by Love *et al.*, (1991). The length, width and height of both testes were measured. Each measurement was taken 3 times and the values were averaged to give the recorded measurement. The volume of an ellipsoid (Volume = $4/3\pi abc$; a= height/2 ; b= width/2 ; c= length/2 ) was used to estimate testicular volume.

Blood samples were collected at 20 minutes intervals for 2 hours after introduction of an indwelling intravenous cannula R 16 G (Cavafix®, B. Braun Medical, SA Barcelona) and before insertion of the deslorelin implant, to determine the normal physiological concentrations and pattern of release of testosterone and LH. After insertion of the deslorelin implant, blood was sampled at 20 minutes interval for 4 hours, then hourly for 6 hours and then daily for 5 days. After 5 days, blood samples were generally collected twice weekly for the duration of the experiment. The catheter was filled with heparin solution (10 i.u/ml) in 0.15 M NaCl. Blood samples (4 ml) were taken by aspirating the heparin solution, collecting the blood sample, and then refilling the catheter with heparin solution after the sample had been withdrawn. The blood sample was placed into lithium heparin tubes and immediately centrifuged at 3000 rpm for 10 minutes at 4° C. The plasma was separated and stored in 2 separate 5ml plastic vials at - 20° C until assayed for

testosterone and LH.

Immediately after the animals had been killed, tissue samples from testes, prostate and epididymis were collected and fixed in Bouin's solution for 24 hours, embedded in paraffin wax, and four pieces of each of the fixed tissues were sectioned and stained with hematoxylin/eosin.

***GnRH agonist deslorelin implant.*** The GnRH agonist used in this experiment was deslorelin ( D-Trp$^{6}$-Pro$^{9}$-des-Gly$^{10}$-LHRH ethylamide). This agonist was prepared and supplied by Peptech Animal Health Pty Ltd, Sydney, Australia. The implant was formulated into bioimplants that were 0.23 X 15.2 mm and contained a 6 mg of deslorelin. The in vitro release rate of deslorelin was approximately 50 µg per 24 hours, as determined by HPLC and UV absorbence at 278 nm (Peptide Technology Limited, Sydney, Australia). Implants were prepackaged in 13 gauge needles and were injected subcutaneously in the neck between the shoulder blades under aseptic conditions.

***Hormone assays.*** **LH assay.** The antiserum polyclonal used in the assay was antiserum to canine Luteinizing Hormone (cLH), the antiserum was raised in a rabbit, AFL 8311890 kindly provided by Dr. AF. Parlow (Director, Pituitary Hormones and Antisera Center, Harbor-UCLA, Medical Center, 1000 West Carson Street, Torrance, California). An aliquot of 20 – 30 µg, solubilized in PBS at a concentration of 100 µg cLH per ml was used for radio iodination procedure and reference. The assay diluent was 0.05 M phosphate/0.01 M disodium EDTA pH 7.5, containing 0.1 % azide and 0.1 % filtered egg white. This buffer was used for dilution of samples, standard and radiolabelled cLH. The standard curve included triplicate tubes for total counts and NSB, 9 replicates of zero standard, 3 replicates of each standard and 6 replicates each of three quality control pools.

The limit of detection of the standard curve was 0.23 ± 0.12 ng/tube and the non-specific binding was always less than 6 %. Six replicates of three pooled plasma samples were included in each assay. They contained LH at concentrations of 0.25 ± 0.3 ng/ml, 0.48 ± 1.4 ng/ml, and 0.7 ± 0.5 ng/ml and were used to estimate variation within and between assays. The within assay coeficients of variation were 11.7±4.2 %, 6.9±2.3 %, and 13.6±1.8% respectively. The between-assays coeficients were 11.7 %, 13.4 %, and 9.4 %).

**Testosterone assay**. Plasma testosterone was measured using a non-extraction radioimmunoassay developed in the animal science laboratory of the University of Western Australia by Mrs. MA Blackberry. Standards made by serial dilution of a stock containing 10 µg/ml 4-androsten-17ß-ol-3-one (Sigma) to 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 ng/ml in castrated ram plasma. The radiolabelled testosterone (1,2,6,7-3H-testosterone; Amersham; specific activity 90 Ci/mmol) was diluted in gelatin-phosphate buffer. The antiserum (R3) was raised in rabbit against testosterone-3-CMO-HSA using an antigen donated by Dr R.I. Cox (CSIRO, Prospect, NSW, Australia) and was diluted to 1:1000 in gelatine phosphate buffer (GPB) and saturated with BSA before storage stored at -20 °C. It was used at a working dilution of 1:70,000 (final dilution 1:280,000) and cross-reacted primarily with dihydrotestosterone (70%) and androstenedione (3.7%). Cross reactions with progesterone, oestradiol-17ß, oestrone and oestriol were all less than 0.05%. Each assay included one standard curve, and up to 300 unknown samples in duplicate. The standard curve included triplicate tubes for total counts and non-specific binding (NSB), 9 replicates of zero standard, (Bo) 3 replicates of each standard and and 6 replicates each of three quality control pools.

The limit of detection was 0.6 ± 0.2 ng/ml. The NSB was 5.3 ± 1.7 % of total counts. Included in each assay were six replicates of three pooled plasma samples containing 0.63 ng/ml, 1.76 ng/ml, and 4.47 ng/ml . They were used to estimate the coefficients of variation within assays (21.7 ± 1.07 %, 11.0 ± 1.6 %, and 13.6 ± 2.8%) and between assays (20.4 %, 10.1 %, and 10.6 %).

***Statistical analyses.*** Hormone concentrations of testosterone and LH are shown as mean ± SEM. Differences in mean semen volume, sperm concentrations, LH and testosterone concentrations within and between animals were evaluated by ANOVA, followed by pairwise comparisons of means TUKEY (HSD) using Statistix

version 4.1 © 1994, (Analytical Software). The level of significant was set at $P < 0.05$.

## Results

The insertion of the deslorelin implants resulted in a marked rise in LH concentrations within 40 minutes after implantation (Fig.1a). The LH concentration remained above the pretreatment values for up to 6 hours. A decline to concentrations below the pretreatment levels was apparent by nine days, and concentrations fell to levels below the sensitivity of the assay (0.2 ng/ml) by 20 days after implantation. Plasma LH concentrations remained markedly suppressed and undetectable for about 42 to 56 weeks (Fig.1b), before returning to normal, pre-treatment concentrations. There was no significant change in the plasma LH concentrations in control dogs over the period of blood sampling (Fig.1a and Fig. 1b).

Plasma testosterone concentrations had a profile similar to that of plasma LH concentrations throughout the experimental period (Fig. 1c & Fig. 1d). The concentration of plasma testosterone peaked at the 60 minutes after implantation and remained elevated for up to 4 days. After 26 to 40 days of treatment the concentrations of testosterone were undetectable for about 42 to 56 weeks of implantation. Plasma concentrations then rose above assay sensitivity and returned to normal concentrations over several weeks . There was no significant change in plasma concentrations of testosterone over the period of sampling in the control dogs (Fig. 1c and Fig. 1d).

During the first 5 to 6 weeks after implantation all semen characters in treated dogs changed markedly. After 6 weeks of implantation, no ejaculates were produced (Fig. 2). Prior to this time there was a progressive increase in the percentage of abnormal spermatozoa with cytoplasmic droplets in the ejaculate (Fig. 2). Before the cessation of ejaculation a reduction in the motility and sperm concentrations was observed. Detectable ejaculate volumes were again achieved by 48 weeks after implantation and these ejaculates increased in volume until normal concentrations and motility of spermatozoa in the ejaculate were observed, around 3 weeks later (Fig.2).

The testicular volume of the dogs prior to and following injection of the 6mg deslorelin implant are presented in Fig. 3. The mean testicular volume of each dog dropped significantly ($P<0.05$) after 5 weeks of implantation, with the low volume being maintained for 42 weeks for dog no. 40; 43 weeks for dog no. 47; 46 weeks for dog no.79 and 56 weeks for dog no. 46 (Fig. 3).

After recovery, seminiferous tubules had a normal histological appearance. Spermatozoa were observed in seminiferous tubules and in lumens of the ductus epididymidis. A normal pseudostratified columner epithelium was seen with numerous stereocilia in the ductus epididymidis as control. Prostate showed tubulo acinar structure with secretory granule activity, as control.

## Discussion

Implantation of dogs with a slow release implant containing the GnRH agonist deslorelin (D-Trp6-Pro9-des-Gly10-LHRH ethylamide) with an in vitro release rate approximately 50 µg/24 h resulted in an acute increase in concentrations of circulating LH and testosterone. This acute response is similar to that observed in the ram following a continuous infusion of GnRH agonists (Lincoln *et al.*, 1986), in dogs following daily injections of GnRH agonist nafarelin (Vickery *et al.*, 1984) and following treatment with a sustained release formulation of the GnRH agonist leuproride acetate (Inaba *et al.*, 1996). The concentrations of plasma LH peaked 40 minutes after implantation (measured at 20 minutes intervals). Plasma testosterone concentrations peaked 20 minutes later. This lag time between peak concentrations of LH and testosterone was in agreement with a previous report that peak testosterone values occure 15 to 105 minutes after the LH peak (Guenzel-Apel *et al.*, 1994). The acute elevations of both hormone concentrations were also noted at 2 hours and 4 hours after injection of GnRH agonist (Vickery *et al.*, 1984). The faster response in the present study may due to the fact that bioimplant containing deslorelin has a high release rate up to 100 µg/24h for the first 2 - 3 days.

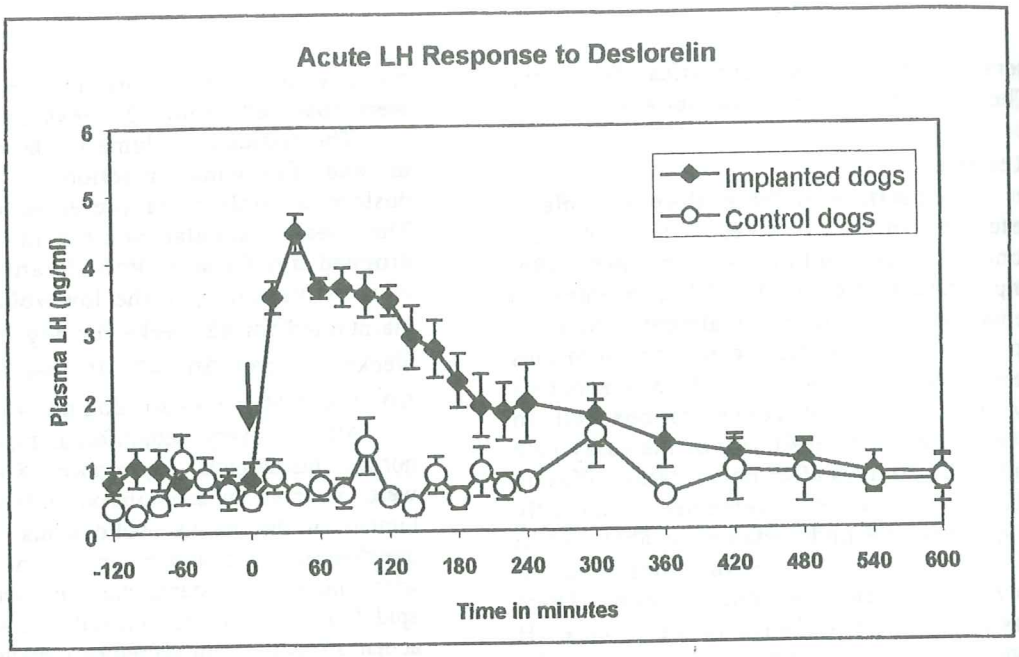


**Figure 1a.** Acute plasma LH response to s.c. injection of a slow release implant containing the 6 mg deslorelin in male dogs (◆, 6 mg deslorelin; O, control). Values are plotted as mean ± SEM (n=4).

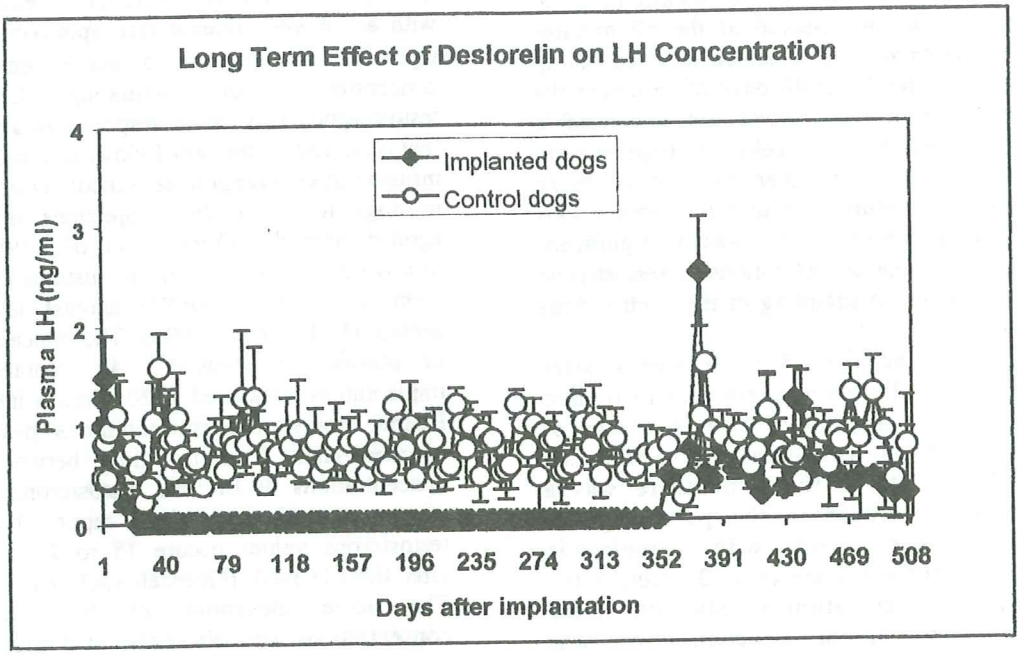


**Figure 1b.** Long term effect of a slow release implant containing 6 mg deslorelin on plasma LH concentrations in male dogs (◆, 6 mg deslorelin; O, control). Values are plotted as mean ± SEM, n = 4).

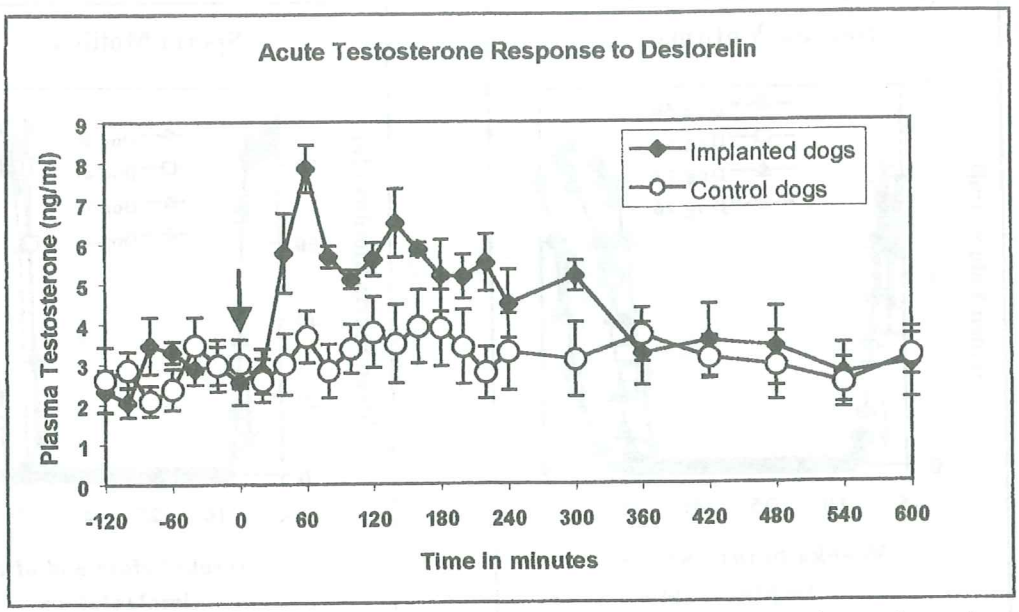


**Figure 1c.** Acute plasma testosterone response to s.c. injection of a slow release implant containing the 6 mg deslorelin in male dogs (◆, 6 mg deslorelin; O, control). Values are plotted as mean ± SEM (n=4).

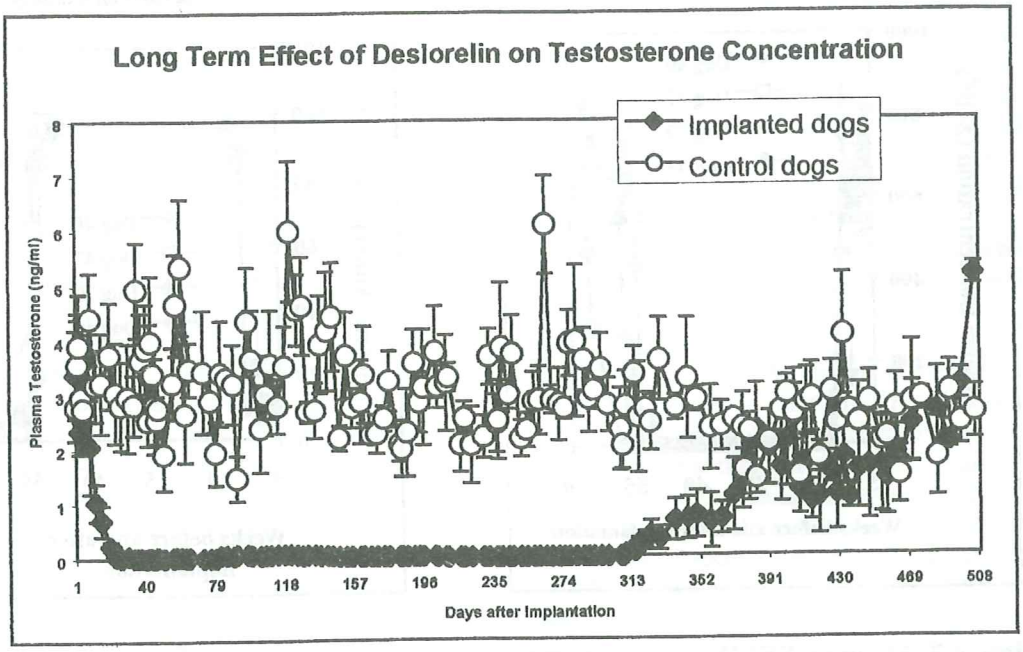


**Figure 1d.** Long term effect of a slow release implant containing 6 mg deslorelin on plasma testosterone concentrations in male dogs (◆, 6 mg deslorelin; O, control). Values are plotted as mean ± SEM, n = 4).

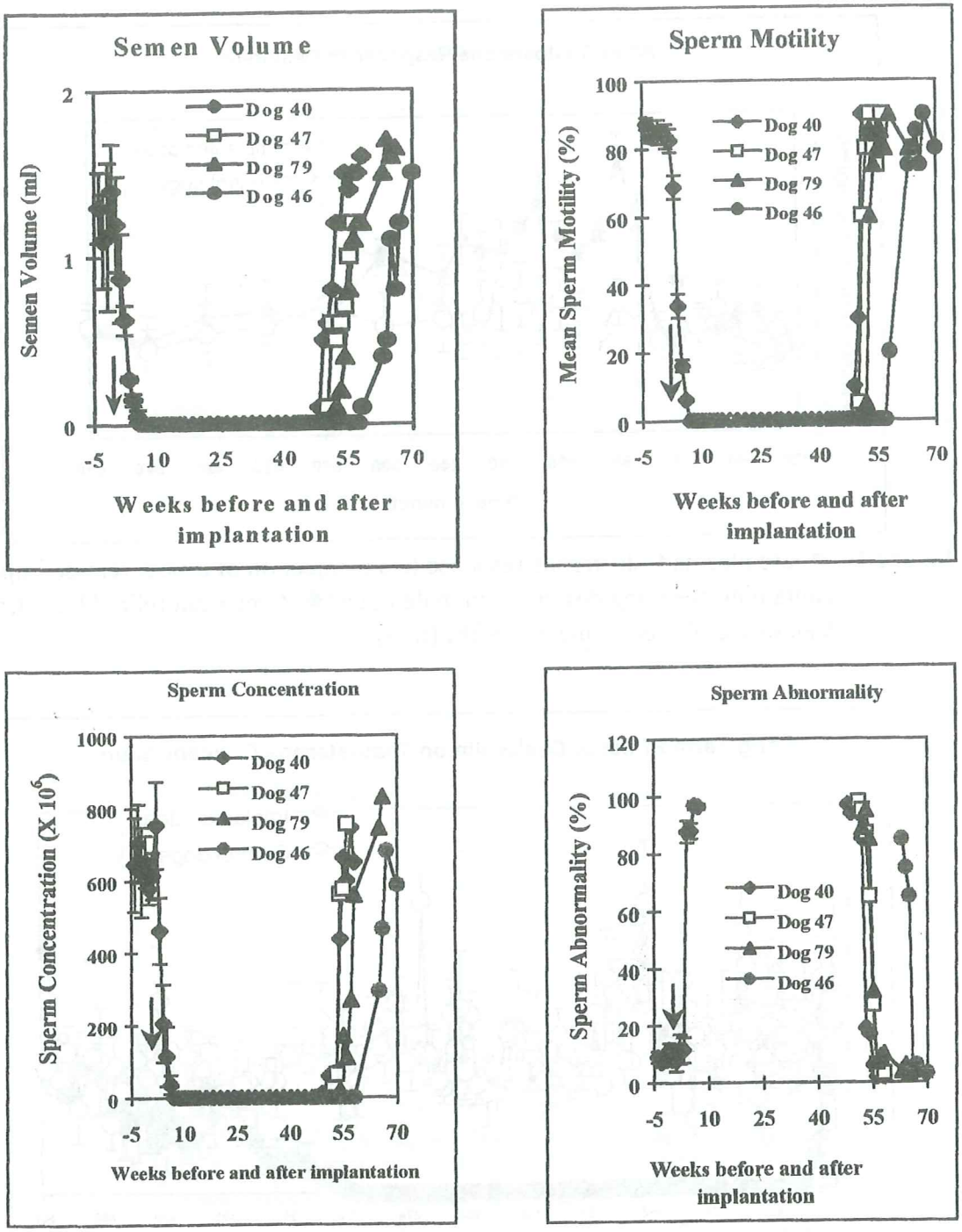


**Figure 2.** Mean (± SEM) semen volume, sperm motility, sperm concentration and sperm abnormality for male dogs receiving s.c. injection of a slow release implant containing 6 mg deslorelin (from week -5 to 47: n = 4). From week 48 to 70 values are plotted as individual dogs.

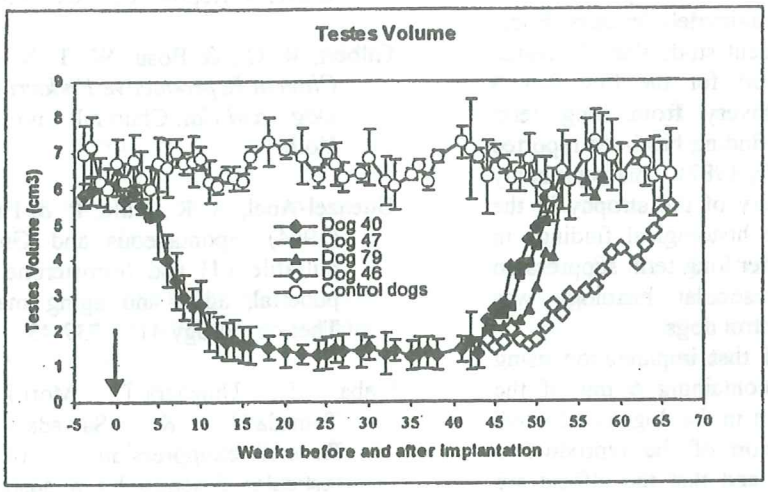


**Figure 3.** Testes volume for male dogs receiving s.c. injection of a slow release implant containing the 6 mg deslorelin (from week -5 to week 44values are plotted as mean ± SEM, n = 4). From week 45 to 66 of treatment dogs values are plotted as testicular volume for individual dogs.

Following the acute response, plasma concentrations of LH and testosterone paradoxically fell progressively and became undetectable after 21 days. This progression was also found in the previous studies in dogs using other GnRH agonists (Inaba *et al.*, 1996; Vickery *et al.*, 1984) and in other species such as ram (Lincoln *et al.* 1986). This phenomenon was also noted by Duello *et al.*,(1983) who concluded that the GnRH agonist initially stimulated LH release, followed by a down-regulation of the pituitary GnRH receptor. This loss of GnRH receptors leads to the suppression of gonadotropin release (Loumaye & Catt, 1983).

The decrease in plasma testosterone concentrations was followed by a progressive decrease in the ejaculate volume, accompanied by a decline in the motility and maturity of spermatozoa in the ejaculate. In the dog, testosterone controls the prostate secretion and is also required for maintenance of spermatogenesis (Gilbert & Bosu, 1987).

The suppression of testosterone secretion from the testes may due to a loss of testicular LH receptors in response to the high dose of deslorelin. Dube *et al.* (1987) found that an increase in endogenous LH after injection GnRH agonist is followed by a marked and sustained loss of testicular LH receptors and a marked atrophy of Leydig cells. Moreover, McLachlan *et al.*, (1995) explained that LH receptors are only found on Leydig cells and the action of LH is through the stimulation of testosterone secretion by Leydig cells. The decrease in testosterone observed in dogs treated chronically with GnRH agonist is likely to be due to the decrease in plasma concentrations of LH and possible reduction in testicular LH receptor.

The inhibitory effect of long term treatment with the slow release implant containing deslorelin was fully reversible in the dogs as judged by the increase in the plasma concentrations of LH and testosterone and recovery of testicular function. The plasma LH and testosterone concentrations recovered to be within the normal range after 50.7 ± 2.9 and 51.2 ± 3.7 weeks of implantation, respectively. The reversibility of GnRH agonist has been reported in a number of species including dogs ( Inaba *et al.*, 1996; Vickery *et al.*, 1987). Reversibility of testicular suppression after long term implantation was assessed using semen characteristics, measured testicular volume and examination of histological sections of the testes. Ejaculate volume was in the normal range at about 55.0 ± 3.5 weeks of implantation. Normal semen characteristics were achieved 58.5 ± 2.9 weeks of implantation. The recovery of plasma testosterone concentration and the appearance of

normal sperm in the ejaculate approximately 8 weeks later is consistent with the spermatogenic cycles in the dogs, approximately 56 days (Foote *et al.*,1972). In the present study that all treated dogs showed discomfort for the first 2 - 3 ejaculations after recovery from long term suppression. A similar finding has been reported by (Vickery & Nestor Jr. 1987). This is probably due to the slow recovery of the atrophy in the reproductive tract. The histological findings in all dogs showed that after long term suppression of testosterone the testicular histology was similar to that in the control dogs.

It can be concluded that implantation using slow release implant containing 6 mg of the GnRH agonist deslorelin in the dogs is effective in long term suppression of the reproductive function in male dogs and that the effects are reversible.

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