UNIVERSITY OF VETERINARY MEDICINE BUDAPEST

The use of GnRH analogues in dogs

Literature review

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Abstract

This study summarizes the different experiments done on the use of GnRH analogues in dogs. The differences between antagonists and agonist. Specifically focusing on deslorelin as a slow releasing implant and the effect it has on the reproductive endocrinology and the reproductive function in male dogs, and their efficacy as a long-term chemical contraceptive, with the potential of reversibility or retreatment and its therapeutic use in the different aspects of veterinary medicine. The journal of animal reproduction and the journal of theriogenology of animal reproduction were the main sources used to collect all the data and information. The results were promising proving the efficacy of deslorelin implant as a long-term reversible contraceptive in companion animals. Showing initially a marginal improvement of semen quality and possibly fertility due to an increase in hormone levels (testosterone, LH and FSH). It later induces a long-term depression of the hypothalamic pituitary axis leading to infertility due to its effect on spermatogenesis and steroidogenesis. As well it has shown promising results in its use in androgen dependent diseases and other aspects of veterinary medicine.

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1. INTRODUCTION

Surgical castration has been the preferred method for many years in veterinary medicine. In 1960 with the development of human oral contraceptive specifically low dose estrogens or estrogen progesterone combination, more efforts have been put to control the reproduction in dogs and cats [40].

Many chemical contraceptive methods have been researched and used. For example, testicular/ epididymal sclerosing agents such as zinc gluconate neutralized by arginine given as an intra testicular injection was unsuccessfully marketed in the United States in 2003. Similarly, calcium chloride dihydrate administered as well as an intra testicular injection and provides permanent sterility but it's use is extremely limited. They lead to inflammation which subsequently leads to atrophy or fibrosis of spermatogenic tissue.

As well there has been growing concerns about surgical castration due to surgery pain, and the risk of complications after anesthesia. A recent study in Brazil showed that the main concerns for surgical castration of adopted shelter dogs were 56.5% that were against the procedure for compassion and ethical reasons and 11.4% considered the procedure unnecessary [1].

A Study was as well conducted in the UK toward routine neutering of companion animals and found that the predominant view >80% supported routine castration and around 10% disagreed and thought that it should only be done for medical reasons [63].

The suppression of fertility in animals using GnRH analogues has been studied thoroughly over the years. A new drug delivery formulation has been introduced which encouraged the commercialization of veterinary products and normalizing the use of contraceptive in veterinary medicine [57]. The application of the new formulation of the GnRH analogues has been approved in different countries and its use has been expanded into different aspects but not limited to suppress fertility.

The European medicines agency has authorized the use of Suprelorin (deslorelin acetate) in July 2007 for use in male dogs and male ferrets to suppress fertility [11]. The aim of this literature review is to summarize the present knowledge about the effect of GnRH analogues on dogs, mechanism of action, and it's use in veterinary medicine while focusing on deslorelin acetate.

2. LITERATURE REVIEW

2.1 GENERAL INFORMATION ABOUT GONADOTROPHIC RELEASING HORMONES.

GnRH is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2), that is produced in the hypothalamus and acts on the pituitary gland. The synthesis of GnRH is influenced by many factors such as body condition score, age, sex, and the concentration of different hormones. GnRH has a short half-life 2-5 mins due to rapid cleavages by proteases.

GnRH is released in a pulsatile manner from the hypothalamus in the brain and travels down to the anterior portion of the pituitary gland through the hypophyseal portal system and binds to the transmembrane receptors on the secretory cells of the adenohypophysis. It leads to a major endocrine response from the pituitary gland which in turns releases heterodimeric glycoprotein in a pulsatile manner. Luteinizing hormone (LH) every 2-4 hours for about 10-20 minutes and follicle stimulating hormone (FSH) which exercise their effect on the testicles through the specific transmembrane receptors FSH-r and LH-r. The FSH receptors are found on the Sertoli cells in the seminiferous tubules at the testicular level and the LH receptors on the interstitial Leydig cells. Both FSH and LH exercise an effect on spermatogenesis through the regulation of the Sertoli cell factor. There is an endocrine response to the FSH and LH stimulation from the testicles. Steroid hormone Testosterone is then excreted in a pulsatile manner by Leydig cells in response to the pulsatile excretion of LH. Testosterone excretion starts 30 minutes after the LH peak and is precisely regulated by negative feedback to the pituitary and subsequent decrease in serum concentration of LH. The non-steroid inhibin is excreted in a non-pulsatile manner by the Sertoli cells in response to FSH and is postulated to control the secretion of FSH by exhibiting a negative feedback, as well as androgen binding protein, which binds to testosterone in the seminiferous tubules, maintaining the high testosterone concentration required for spermatogenesis These gonadal hormones are the main feedback to maintain the hypothalamic pituitary axis [51].

Testosterone is required for spermatogenesis, male libido, maturation of the spermatozoa, function of the accessory glands, masculine secondary characteristics, male behavior, negative feedback of LH secretion.

Secretion of testosterone is pulsatile so serum concentration of testosterone measure as a single sample, can vary from 0-4-10 ng/ml. The center of spermatogenesis occurs within the blood

testes barrier (BTB), which consists of the basement membrane, the seminiferous tubules, the Sertoli cells.

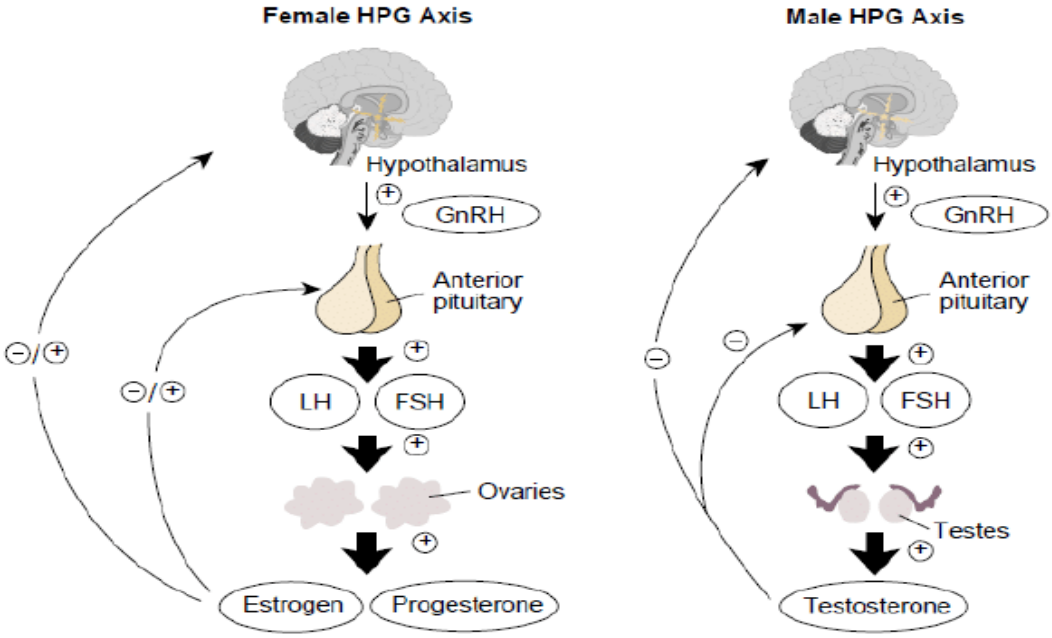

FIGURE 1 HYPOTHALAMIC PITUITARY GONADAL AXIS [35]

2.2 GENERAL INFORMATION ABOUT GNRH ANALOGUES

The major difficulties in the veterinary application of GnRH analogues have been of ease of use, biocompatibility, long term release of sufficient amount of drugs, and cost effectiveness. GnRH analogues which include agonists and antagonists, have been synthesized by substitutions of amino acids in the original GnRH molecule. GnRH was first isolated and identified in 1971 and more then 3000 analogues have been developed. They have a low oral bioavailability (0.1) since they are susceptible to gastrointestinal peptidase degradation, therefore oral administration is unsuitable, and they require parenteral administration. The main target in synthetizing GnRH analogues was to achieve greater potency and longer half-life by increasing receptor affinity and decreasing degradation elimination [47]. The primary structure of mammalian GnRH Fig.2. An ultimate feature of agonists is the substitution of L isomers with D isomers. Receptor binding and activation (agonists) are properties of the NH2 and COOH terminal domains [47]. There are three ways GnRH can be used to suppress fertility , first it can be prevented from reaching its pituitary receptors by neutralization of GnRH in the hypophyseal portal blood by antibodies. GnRH receptors can be blocked by chemical

antagonists of GnRH or GnRH agonist can exhibit an inhibitory effect when given long term [16].

$$pGlu^{1}–His^{2}–Trp^{3}–Ser^{4}–Tyr^{5}–Gly^{6}–Leu^{7}–Arg^{8}–Pro^{9}–Gly^{10}–NH_2$$

FIGURE 2 PRIMARY STRUCTURE OF MAMMALIAN GNRH [47]

To date two GnRH agonists have been used to modify testicular function deslorelin and azagly-nafarelin. Their structures are presented in Fig3. They both have a structure correlated to the sequence of native GnRH with alterations in three of their amino acids on position 1 pGlutamin, position 6 Glycerin, and on position 9 proline, and deletion of glycerin at position 10. The purpose of these structural changes is to reduce sensitivity to proteolysis and increase biological activity and potency [9]. Although this study will focus on the use of deslorelin in dogs.

Amino acids 1, 6, and 10 are the ones modified to generates GnRH agonists used in dogs.

Native GnRH: pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly.

Deslorelin acetate: H-Pyr-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-NHEt.CH_3CO_2H.

Azagly-nafarelin: H-Pyr-His-Trp-Ser-Tyr-D-2Nal-Leu-Arg-Pro-$NHNHCONH_2$.

FIGURE 3 COMPARATIVE SEQUENCE OF NATIVE GNRH, DESLORELIN ACETATE, AND AZAGLY-NAFARELIN.[9]

GnRH agonists	**Potency**
Histrelin and nafarelin	200
Lutrelin	150–180
Deslorelin	150
Azagly nafarelin	50–100
Buserelin	15–30
Leuprolide	15

FIGURE 4 RELATIVE POTENCIES DESCRIBED FOR SOME GNRH AGONISTS IN DOGS [19].

2.3GnRH antagonist

The edge between GnRH agonist potency, dose and duration of treatment determines whether pro or anti fertility effects are produced. They initially stimulate the production and release of gonadotropins from the pituitary with varying potency. Prolonged use of agonists acts through desensitization of the receptors which will lead to their down regulation. To achieve sterility long term agonists, need to be used either through continuous administration or by long term release formulation. At the beginning of the treatment an increase in gonadotropins is detected (increase in FSH and LH), this is called the flare up effect which can be seen as a shortcoming since it delays gonadal suppression by a some weeks [19],[4].

GnRH antagonists are synthesized to have high affinity to the receptors without activating them, with a low histaminergic effect and a resistance to enzymatic degradation. Contrary to agonist they do not cause a flare up and they lead to a rapid desensitization [19]. There are limited data available for the use of GnRH antagonists in dogs. The first molecule available was about two decades ago and this is when it was first described in dogs. "The first-generation GnRH antagonist had a limited duration of action, and therefore, they had to be administered daily to be bioactive. Large doses were also required to obtain adequate suppression of the GnRH receptor. They were hydrophobic with solubility limitations inducing nodule formation at the site of injection. They also had a tendency to produce allergic local and systemic side effects" [17]. Detilirix a 2nd generation GnRH antagonist suppressed ovulation in the female dog. In male dogs, it caused a fast decline in testosterone within 2 hours after a first dose (4 microgram/ kg to 2 mg/kg). 3rd generation antagonist Acyline, cetrorelix, ganirelix, teverelix. Acyline being the most potent and having the longest duration of action. A single subcutaneous injection of acyline (330 microgram/kg) suppressed semen quality in six dogs. This was observed for 60 days and no clinical, hematological or serum biochemical side effects were found. Male sexual behavior was unaltered during this period. The use of a combination of agonist and antagonist is possible. The combination of a short acting GnRH antagonist could prevent the flare up effect of a long term agonist [19].

A. Endocrine effect of GnRH antagonist Acyline

An experiment to study the endocrine effect of GnRH antagonist was done by [18] on 7 intact male dogs of reproductive age, they were given a single dose acyline injection 330 microgram/kg that was made in a lyophilized powder and suspended in sterile water and the levels of gonadotropic hormones and testosterone were evaluated. the dogs were followed up for 30 days, blood sampling was done the day before , then 30 ,60,90 minutes after treatment, then again 3,6,9,12 and 24h and 3,6,9,14,22,29 days after. The blood was centrifuged and stored at -20 degree Celsius until the hormone assay was done.

Serum concentration of FSH was measured using homologous immunoradiometric assay. Serum LH and testosterone using radio immunoassay.

Serum concentration of FSH and LH were below pretreatment values 60 minutes after administration of the injection, while testosterone decreased below treatment value 90 minutes post injection. All three hormones kept decreasing until day 9.

Serum concentration of all hormones started increasing on day 14, but by day 29 serum concentration of FSH and Testosterone were above baseline value, which indicates a rebound hyperstimulation effect due to decreased negative feedback. A second dose should be given again before the increase in the hormone level happens (around day 10). No side effects or allergic reactions were observed in these dogs [18].

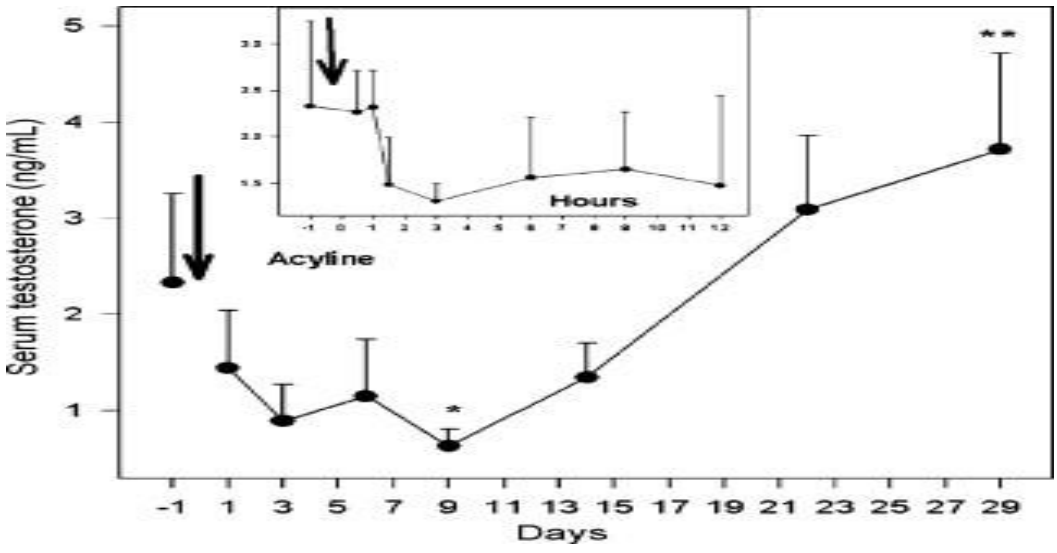

Figure 5 Serum testosterone concentrations before and after a single subcutaneous administration of 330 mg/kg acyline (arrows) in five male dogs. Asterisks represent concentrations that differ significantly. The inset shows the first 12 h after injection [18].

B. Effect of Acyline on testicular characteristics.

In another study the effect of acyline were studied on male canine testicular characteristics. 7 dogs were used in this experiment, follow up was done weekly. During the follow up physical exam was performed, scrotal diameter was measured, testicular consistency was examined, libido and erection ability were also examined. Libido was assessed according to erection, ejaculation and thrusting movements. Second and third fraction of ejaculate were also collected. Sperm concentration with progressive motility was examined under 400x microscope view, as well an improved Neubauer hemacytometer chamber was used to examine sperm morphology (one drop of semen on glass with giemsa staining). Hematological and biochemical tests were done as well to determine possible systemic side effects.

Statistical analysis was done according to the scrotal width, volume of the second fraction, spermatozoa concentration, morphological abnormalities, and motility.

The results of this experiment proved that a single high dose of GnRH antagonist acyline would seriously decrease semen quality and testicular volume. Complete absence of libido was observed in 4 of the 7 dogs during the first or second week after treatment. The volume of the second and third fraction of ejaculate were reduced the first month after treatment. Sperm motility decreased during this period which might suggest impaired epididymal function, sperm abnormalities were increased, azoospermia was not detected but rapid severe oligozoospermia. The author had different ideas as to why this would happen possibly secondary to an impaired libido, decrease in hormone levels playing an effect on spermatogenesis, or a direct suppression of spermation. In this study as well no adverse allergic side effects were observed [59]. Further research are needed for the new generation GnRH antagonist in male dog before they could be widely be endorsed for clinical, and contraceptive use [17].

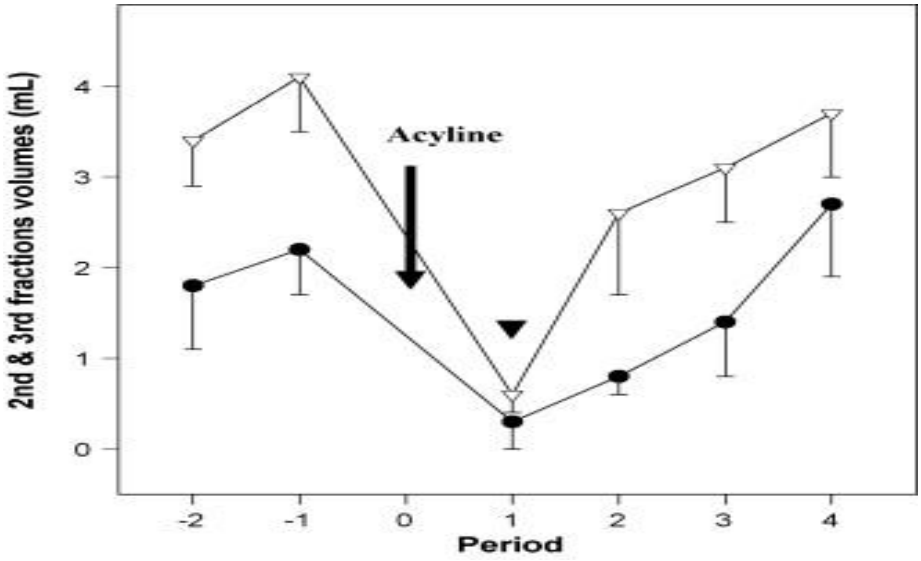

FIGURE 6 SECOND AND THIRD FRACTIONS OF THE EJACULATE OF SEVEN DOGS GIVEN ACYLINE (330 MG/KG SC) AT THE END OF TWO, 2-WEEK PERIODS (PERIODS -2 AND -1, BLACK ARROW), WITH FOLLOW-UP FOR ANOTHER FOUR, 2-WEEK PERIODS (PERIODS 1, 2, 3, AND 4). SYMBOLS INDICATE BIOLOGICALLY SELECTED SIGNIFICANT DIFFERENCES. [59]

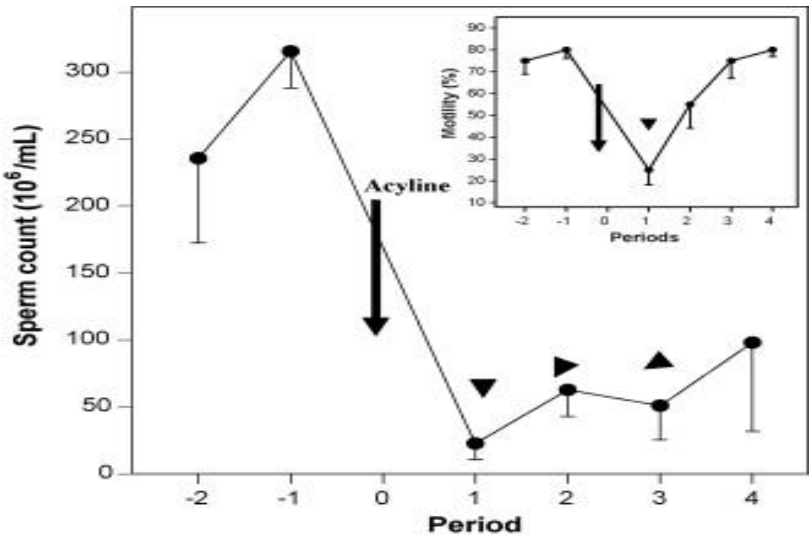

FIGURE 7 SPERM COUNT OF THE SAME DOGS AND PERIODS OF FIG.6 INSET: PERCENTAGE OF SPERM MOTILITY OF THE DOGS. IN BOTH PANELS, SYMBOLS INDICATE BIOLOGICALLY SELECTED SIGNIFICANT DIFFERENCES.[59]

3. MATERIALS AND METHODS

The present research paper will analyze the different research and experiments done to understand the mechanism of action of GnRH analogues, their effect on male fertility and reproduction and their off-label use and effectiveness in different aspects of veterinary medicine. To collect all the research, I used the library data base and google scholar searching specifically for the use of GnRH analogues in dogs. Most of the research I collected are from the journal of animal reproduction and the journal of theriogenology of animal reproduction. I collected a few articles and focused on the suppression of fertility and male sexual characteristics in dogs after the use of GnRH analogues. Specifically I focused on the use of the implant deslorelin since it is the mostly used GnRH analogue in the veterinary industry and it has been well studied in dogs. Each reference and source I used had a different method and analysis to get to their results. I focused on the experiments that I thought were most relevant especially the ones done in the aspect of suppression of male fertility where the research was focused on understanding the mechanism of action and the efficiency of the long-term releasing implant and the effects and structural changes it causes in the reproduction system of dogs. I tried to gather all the experiments that would describe all the different effects deslorelin could have first on the endocrine system, on the reproductive function and more. Most references and articles I found go back to Junaidi [28–31] as comparison of their results and as sources for their conclusion which is why I think they are the leading researchers for that topic. For each part of my thesis, I used a different selection criterion for example in the off-label use of GnRH I have found many experiments, I focused on the ones I thought were most interesting and relevant for today's clinical work and thought they would be beneficial for any veterinarian reading my thesis. I would use one reference that would describe the experiment and show promising results and then looked for references that could confirm the findings and even add value to them or I would discard the experiment if I found contradicting research. For example, in the reference I used for the study of the use of deslorelin for dogs with BPH they used the experiment done by [29] where he proved the atrophy of the prostate gland in healthy dogs after administration of the implant by sampling the tissue and studying it under the microscope, which added value to the experiment and results found on dogs with BPH that received deslorelin implant as a treatment. When comparing results of different research, I sometimes noticed inconsistencies in results and realized these were due to different methods used in the research for example differences in frequency of sampling and evaluation, so I tried to pay attention to the latter.

4. Results

4.1GnRH agonist – Deslorelin

Deslorelin is a synthetic GnRH that was formulated to suppress fertility in male dogs and reduce aggression and unwanted male behavior. It's been manufactured as a slow releasing device called deslorelin releasing implant or DRI. It is placed beneath the skin between the dog's shoulder blades, it is not necessary to prepare the administration site. The product can be repeatedly dosed to extend the period of infertility. The implant is packed in a single use sterile syringe implant device. It has a 3-year shelf life and should be stored in a refrigerator.

Multiple experimental studies have been done to study the effects of this formulation on the endocrine function, prostate and testicular characteristics, dose response evaluation and more.

In this part of my thesis, I will summarize the different experiments about the use of deslorelin. The deslorelin releasing implant was developed in Australia and is marketed by Virbac France. The 4.7 mg implant which is labelled to suppress fertility was approved for sale in Australia in 2007, the European union in 2008, and later in 2020 it was launched in China and Mexico. The 9.2 mg implant which is labeled to suppress fertility for at least 12 months is approved in Australia and in the European union [9].

A. Effect of Deslorelin on the reproductive endocrine function

The new drug delivery formulation of the GnRH deslorelin was studied for its effectiveness as a long-term reversible contraceptive in male dogs and its effects on the endocrine function. It's important to note that to be acceptable contraceptives for male dogs need to have certain criteria as to be able to reduce the output of fertile gametes, interfere with endocrine control of libido and secondary sexual characters.

The results of the study showed that In the control group no changes were detected in the hormonal assay. In the group that received the deslorelin 6mg implant, LH concentration showed to have increased rapidly within 20 minutes and peaked at 40 minutes and then decreased gradually. The curve for testosterone was similar to LH [30, 31]. On the long-term plasma concentration of LH decreased to a level below pretreatment values by day 9 and remained lower then control values from 20 days till week 49 after implantation. The concentration then started increasing by week 50 and returned to similar pretreatment values. Similar effect was seen for testosterone. It was not detectable again until 44 weeks post implantation, then plasma concentration began to increase, approximately 6 weeks earlier than LH concentration, and returned to post treatment values 52 weeks after implantation. In a study

performed on tomcats a similar testosterone increase was noted in male cats receiving a 4.7 mg deslorelin implant although it was not significant compared to the results seen in dogs. It started 40 minutes after implantation peaking at 1 hour and the rise lasted less than what is seen in dogs [20]. This shows the effect GnRH agonist deslorelin as a slow-release implant has on the endocrine function and the sexual hormones on the short term as a flare up effect by an

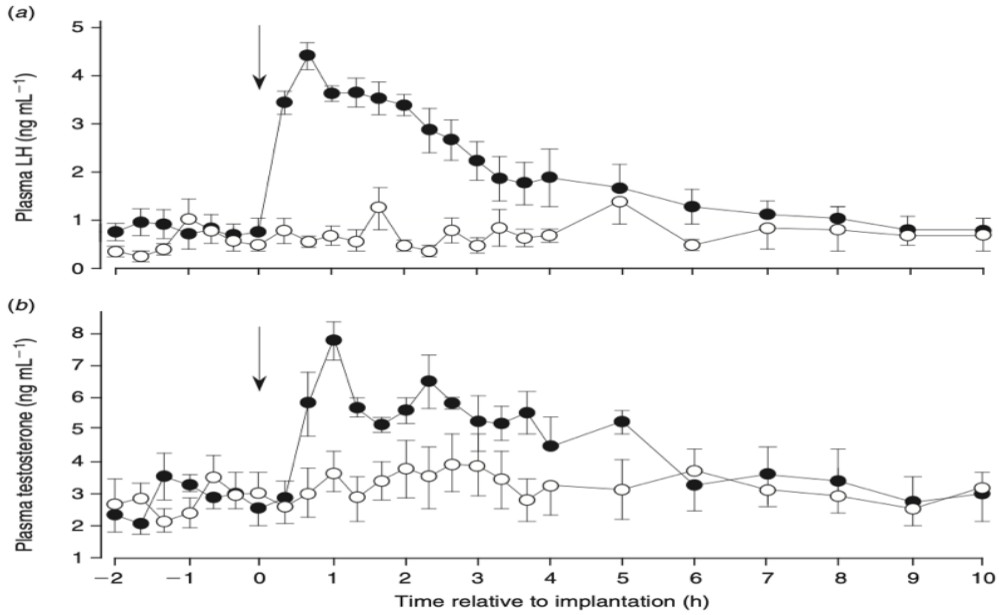

FIGURE 8 SHORT TERM RESPONSE IN THE PLASMA CONCENTRATION OF LUTEINIZING HORMONES (LH) AND TESTOSTERONE FOLLOWING THE IMPLANTATION OF THE SUBCUTANEOUS SLOW- RELEASE IMPLANT 6MG DESLORELIN IN MALE DOGS. [30]

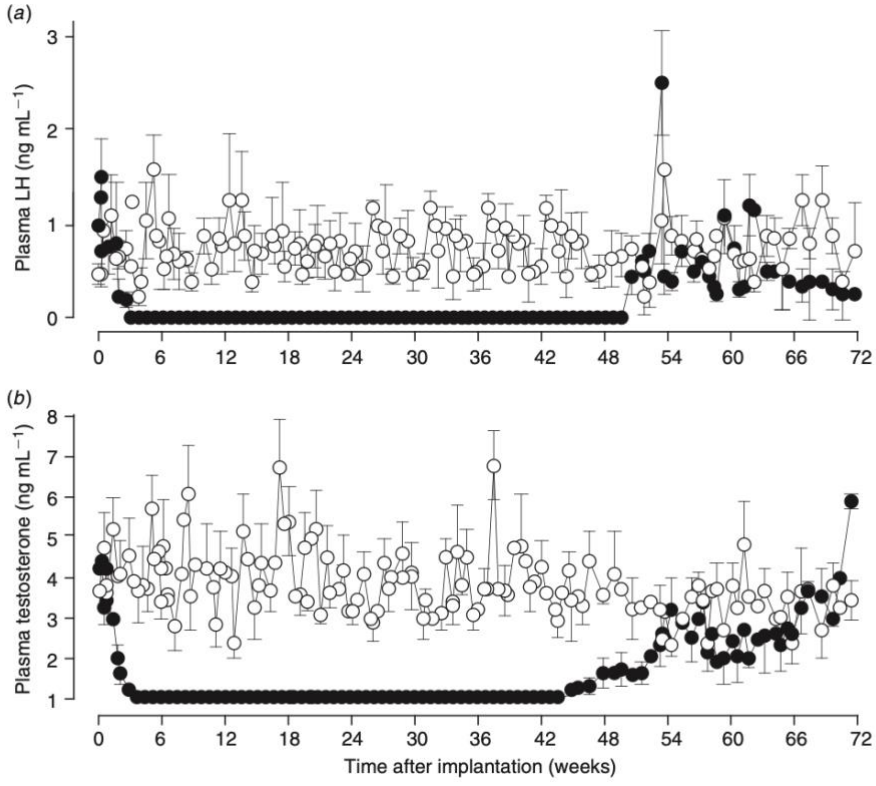

FIGURE 9 LONG TERM RESPONSE IN THE PLASMA CONCENTRATION OF LH AND TESTOSTERONE FOLLOWING THE IMPLANTATION OF THE SUBCUTANEOUS SLOW-RELEASE IMPLANT 6 MG DESLORELIN IN MALE DOGS.[30]

In a study the responses of the reproductive endocrine axis were challenged with native GnRH and bovine LH after implantation of deslorelin. For that experiment 5 groups of 4 dogs each were used. Control group received a blank implant while the other 4 groups received a 6 mg deslorelin implant. Native GnRH decapeptide was given on days 15,25,40 and 100 after implantation. Bovine LH was injected on days 16,26,41 and 101 after implantation. To determine the response after the injections of GnRH and LH blood sampling was done at -40,-20,-10 and 0 minutes before injection and then every 10 minutes for 90 minutes and every 20 minutes to 150 minutes after injection. The response of LH and testosterone to exogenous native GnRH was examined and compared between control dogs and dogs that received deslorelin. In the control group an LH peak was detected after 20 minutes of intra venous native GnRH injection and a peak in testosterone concentration 20 minutes later. In the group of dogs that received deslorelin implant, LH and testosterone values were significantly decreased by day 15 and no response was detected for either LH or testosterone following GnRH challenge at 100 days after implant administration. Next is the comparison of the testosterone response to LH between control groups and deslorelin groups. In the control group the testosterone concentration increased, while in deslorelin group the response was significantly lower on all days tested. Which proves that the long-term GnRH agonist led to a downregulation of the pituitary gland.

Day after implantation	Treatment	n	Baseline	Response	Recovery
			LH (ng mL^{-1})		
15	Control	4	0.64 ± 0.06^{a}	1.32 ± 0.23^{a}	0.41 ± 0.06^{a}
	Deslorelin I	4	0.06 ± 0.03^{b}	0.17 ± 0.02^{b}	0.13 ± 0.04^{b}
25	Control	4	0.72 ± 0.08^{a}	2.17 ± 0.33^{a}	0.65 ± 0.07^{a}
	Deslorelin II	4	0.04 ± 0.02^{b}	0.05 ± 0.02^{b}	0.04 ± 0.02^{b}
40	Control	4	0.41 ± 0.04^{a}	1.23 ± 0.20^{a}	0.55 ± 0.11^{a}
	Deslorelin III	4	0.07 ± 0.02^{b}	0.03 ± 0.02^{b}	0.07 ± 0.01^{b}
100	Control	4	0.72 ± 0.08	1.83 ± 0.27	0.65 ± 0.07
	Deslorelin IV	4	UD	UD	UD
			Testosterone (ng mL^{-1})		
15	Control	4	1.33 ± 0.08^{a}	2.62 ± 0.28^{a}	1.03 ± 0.20
	Deslorelin I	4	0.75 ± 0.05^{b}	1.07 ± 0.16^{b}	0.88 ± 0.04
25	Control	4	1.38 ± 0.20^{a}	3.62 ± 0.38^{a}	3.22 ± 0.11^{a}
	Deslorelin II	4	0.33 ± 0.02^{b}	0.45 ± 0.04^{b}	0.32 ± 0.02^{b}
40	Control	4	1.31 ± 0.15^{a}	3.57 ± 0.31^{a}	3.01 ± 0.39^{a}
	Deslorelin III	4	0.06 ± 0.01^{b}	0.14 ± 0.01^{b}	0.09 ± 0.01^{b}
100	Control	4	1.02 ± 0.09	3.59 ± 0.35	2.77 ± 0.25
	Deslorelin IV	4	UD	UD	UD

[a,b]Values within each control–deslorelin-treated group with different superscript letters differ significantly ($P < 0.05$).

TABLE 1 MEAN PLASMA CONCENTRATION OF LUTEINIZING HORMONE AND TESTOSTERONE FOLLOWING AN INJECTION OF NATIVE GONADOTROPIN RELEASING HORMONE IN CONTROL DOGS AND GROUP OF DOGS TREATED WITH GONADOTROPHIN RELEASING HORMONE SUPER AGONIST DESLORELIN IMPLANT [31].

Day after implantation	Treatment	n	Testosterone (ng mL^{-1})		
			Baseline	Response	Recovery
15	Control	4	1.31 ± 0.15^{a}	3.45 ± 0.29^{a}	3.03 ± 0.24^{a}
	Deslorelin I	4	0.77 ± 0.06^{b}	1.42 ± 0.22^{b}	0.44 ± 0.09^{b}
25	Control	4	1.13 ± 0.06^{a}	2.03 ± 0.26^{a}	0.95 ± 0.09^{a}
	Deslorelin II	4	0.58 ± 0.04^{b}	0.68 ± 0.09^{b}	0.29 ± 0.02^{b}
40	Control	4	1.29 ± 0.10^{a}	3.63 ± 0.30^{a}	0.98 ± 0.03^{a}
	Deslorelin III	4	0.17 ± 0.02^{b}	0.40 ± 0.03^{b}	0.28 ± 0.05^{b}
100	Control	4	1.19 ± 0.04	2.30 ± 0.29	1.46 ± 0.30
	Deslorelin IV	4	UD	UD	UD

[a,b]Values within each control–deslorelin-treated group with different superscript letters differ significantly ($P < 0.05$).

TABLE 2 MEAN PLASMA CONCENTRATION OF TESTOSTERONE FOLLOWING AN INJECTION OF BOVINE LUTEINEIZING HORMONE IN CONTROL DOGS AND GROUPS OF DOGS TREATED WITH A GONADOTROPHIN RELEASING HORMONE SUPER AGOSNIT DESLORELIN IMPLANT [31].

A similar study was done in 5 tom cats where they received a 4.7 mg deslorelin implant and GnRH stimulation tests are performed to measure the testosterone increase. They were challenged with buserelin at 4 weeks intervals afterward until week 20. The results showed that the GnRH stimulation did not cause an increase in testosterone and because of the loss of stimulatory effect of the short term GnRH agonists we know that the application of the long term GnRH agonist leads to a down regulation of the pituitary gland as was mentioned above [22].

Different dosages of deslorelin 3mg, 6mg, 12mg were tried on different dogs of different breeds and body weight. In all the groups that received deslorelin 20 minutes after receiving the implant, a fast increase in LH was detected and it lasted 80 minutes, it declined to pretreatment values after around 3 days. 12 days after implantation the levels of LH were undetectable. Testosterone followed a similar wave with a small delay, fast increase of plasma testosterone concentration was seen 60 minutes after implant till 120 minutes. At day 6 levels declined to pretreatment values and at day 12 they were undetectable. This shows that there is no difference between dosages for the timing of the LH peak. Although at the 5-10 h level (300-600 minutes after implantation) the higher doses of deslorelin (6,12 mg implants) showed higher values then in the 3mg implant group.The long term effect on hormone concentration was similar in all groups that received deslorelin, starting with an increase in concentration later declining to pretreatment values and then undetectable values. After a few months the concentration came back to normal values. They observed a difference in the time of recovery. For the 3 mg group plasma LH concentration reached nomal values between 198 and 474 days. For the 6 mg group plasma LH concentration reached normal between 349 and 686 days and for the 12 mg group plasma LH concentration reached normal value between 487 and 800 days. Similar effect was observed for testosterone, with the 12 mg group taking the longest for recovery [28].

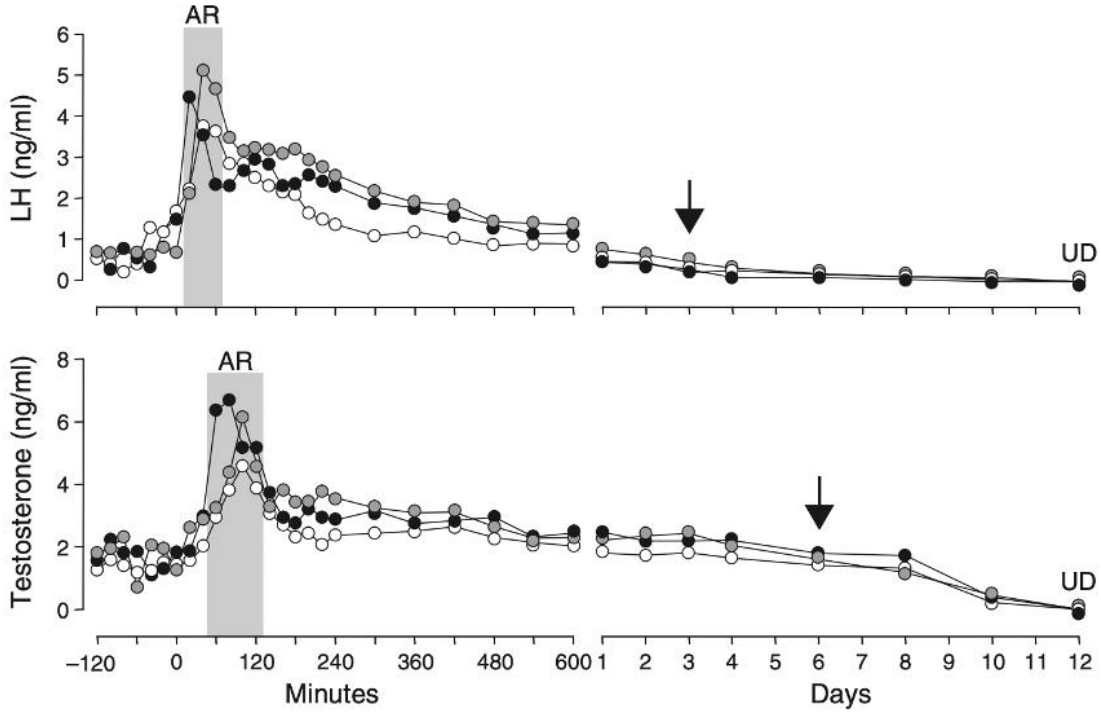

FIGURE 10 ACUTE RESPONSE IN THE PLASMA CONCENTRATION OF LH AND TESTOSTERONE FOLLOWING THE IMPLANTATION OF 3MG (WHITE), 6 MG (GREY) AND 12 MG (BLACK) DESLORELIN IN MALE DOGS. AR ACUTE RESPONSE, UD UNDETECTABLE.[28]

B. THE EFFECT OF DESLORELIN ON THE PROSTATE GLAND AND TESTICLES

Testicular volumes in the 6mg deslorelin treated group fell below control values by week 5 after implantation and kept decreasing after. A lower and stable level was recognized at week 10 and was sustained for 40-45 weeks. The testicular volume decreased in all the deslorelin (3mg, 6mg, 12mg) treated group by 35% compared to pretreatment values [28, 30]. Similar results were observed in a study done on cats using a 4.7 mg deslorelin implant where testicular volume decreased by 25% on week 4, 60% on week 12 and 73.5 % on week 36 [20]. Furthermore with the azagly-nafarelin 18.5 mg implant they observed a reduction of testis size by 80% on week 17 and prostate size by 46% on week 5 in dogs [41].

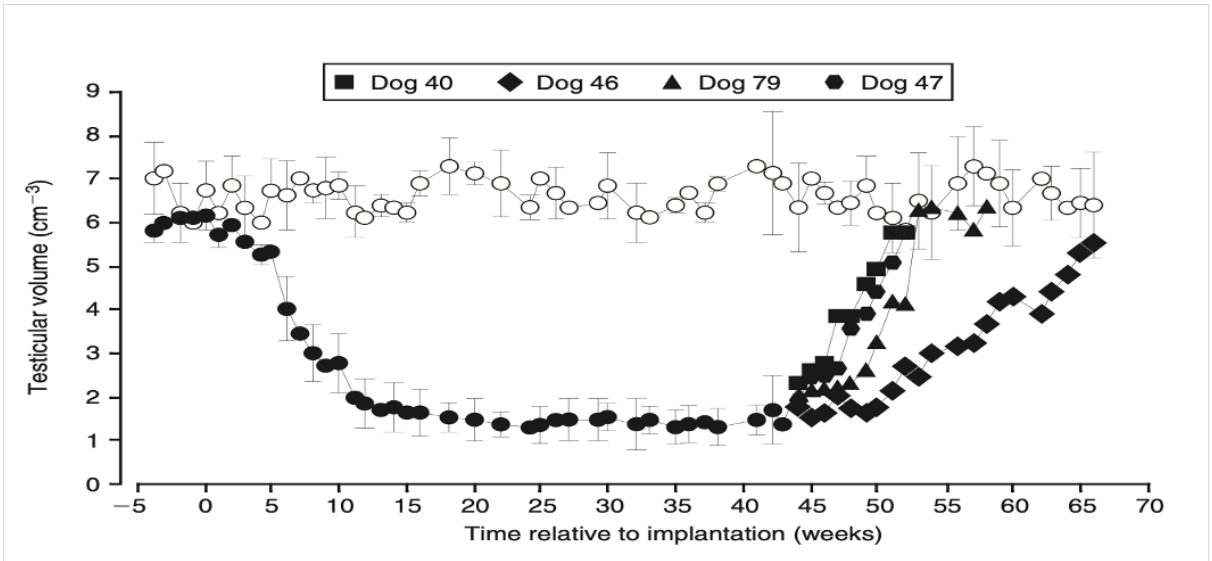

FIGURE 11 CHANGES IN THE VOLUME OF THE RIGHT TESTICLE IN CONTROL DOGS VS. DOGS GIVEN THE IMPLANT. [30]

The reproductive variable in dogs that received different dosages of deslorelin 3,6 and 12mg showed that the 12 mg group had the most significant effects than the smaller dosage group. Such as the time of minimal testicular volume (35% less then pretreatment volume) and the time of absence of ejaculte.

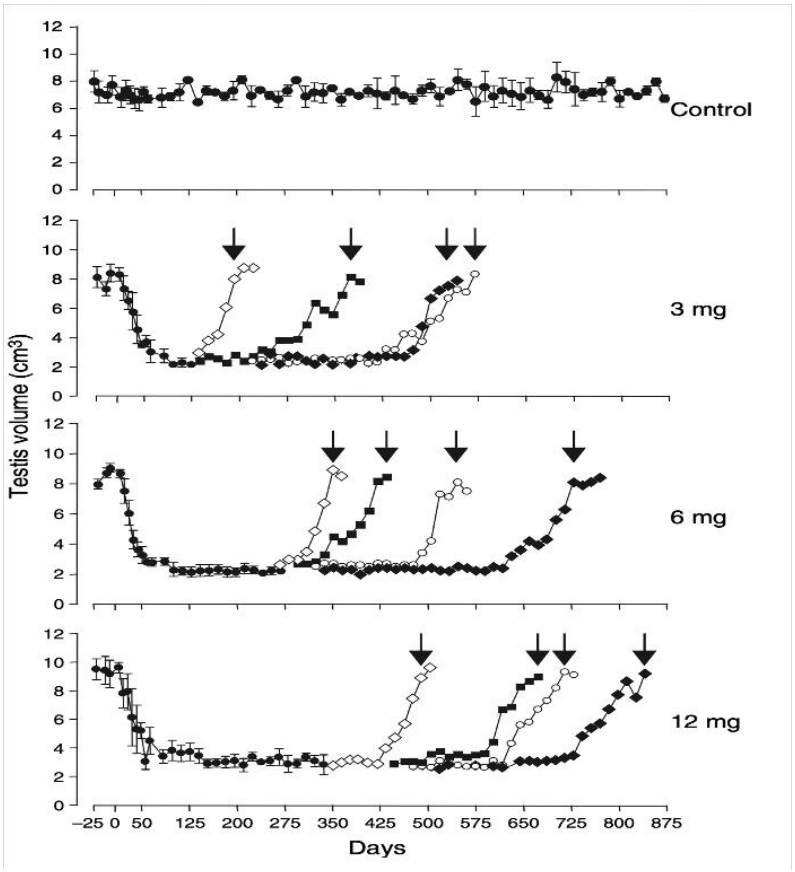

FIGURE 12 TESTICULAR VOLUME IN MALE DOGS FOLLOWING THE IMPLANTATION OF A BLANK IMPLANT OR A DESLORELIN IMPLANT CONTAINING 3,6 OR 12 MG DESLORELIN. THE TIME OF IMPLANTATION WAS 0 ON DAY 0. RETURN OF VOLUMES TO PRE TREATMENT VALUES ARE INDICATED BY ARROWS.[28]

Testicular tissue and prostatic tissue were studied under the microscope for dogs that received deslorelin, they saw that on day 41 after implantation 89.8 % of the slides showed atrophied and aspermatogenic seminiferous tubules, and complete atrophy of the glandular epithelium of the prostate gland with an appearance of excessive connective tissue. On day 101 99.8% of the slides showed epithelial atrophy of the seminiferous tubules. Day 41 and day 101 being the days of maximal suppression. Smaller Sertoli cells were detected in dogs that received the deslorelin implant with smaller nucleoli and Leydig cells showed atrophied nucleoli compared to the control group. On day 41 the regressions in the testis prostate and epididymis are complete, Leydig cells are atrophied, the glandular epithelium of the prostate gland is non secretory. Concurring with previous endocrine results by day 41 testosterone and LH are undetectable which correlates with what is observed here. There is also a lack of ejaculate production due to the prostate atrophy and testicular volume is minimal. The atrophy of Sertoli cells and the loss of spermatogenic activity is also confirmed. The changes observed in the prostatic tissues showed atrophy of the nuclei and epithelium appearing non secretary is identical to the changes found in dogs that have undergone a gonadectomy [29]. Similar results

were seen in a study done on dogs when they received GnRH agonist azagly-nafarelin 18.5 mg. Prostate size decreased from 4.8 cm2 pretreatment to 2.8 cm2 8 weeks post implant. Testicular sized decreased from 7.33 cm2 to 3.23 cm2. Leydig cells showed nuclear changes and the mean area of cell nuclei was smaller than in control groups following the decrease in testosterone levels [24].

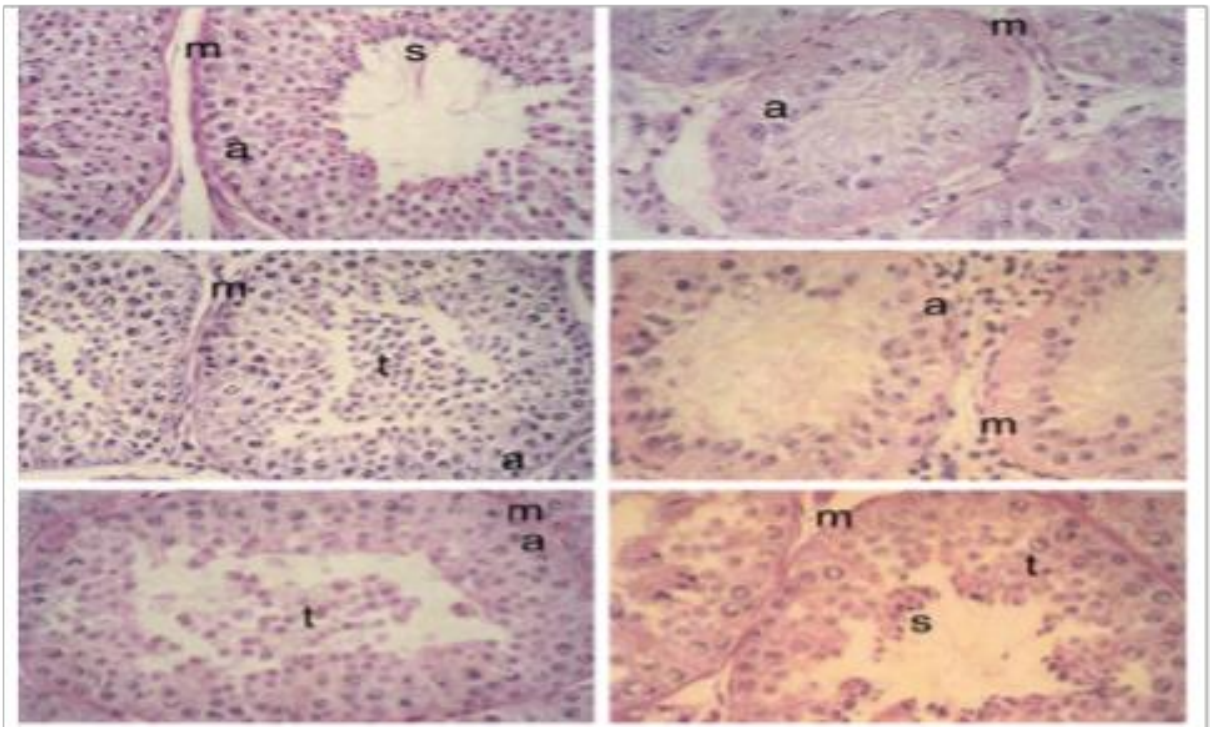

FIGURE 13 "LIGHT MICROGRAPHS OF SEMINIFEROUS TUBULES FROM (A) A CONTROL DOG SHOWING ACTIVE SPERMATOGENESIS AND SPERMATOZOA IN THE LUMEN (HES ·100); (B) A DESLORELIN-TREATED DOG 15 DAYS AFTER IMPLANTATION, SHOWING EARLY SPERMATIDS (T) SHED INTO THE LUMEN (HES ·80); (C) A DESLORELIN-TREATED DOG 25 DAYS AFTER IMPLANTATION, SHOWING EARLY SPERMATIDS (T) SHED INTO THE LUMEN (HES ·100); (D) A DESLORELIN-TREATED DOG 40 DAYS AFTER IMPLANTATION, SHOWING DEFINITE SPERMATOGENIC ARREST, WITH THE SEMINIFEROUS TISSUE DOMINATED BY TYPE A SPERMATOGONIA (A) AND FEW STAGES BEYOND PRIMARY SPERMATOCYTE; LEYDIG CELLS ALSO APPEAR ATROPHIC (HES ·80); (E) A DESLORELIN-TREATED DOG 100 DAYS AFTER IMPLANTATION, WITH ATROPHIC AND ASPERMATOGENIC SEMINIFEROUS TUBULES, AND ATROPHIC LEYDIG CELLS (HES ·128); (F) A DESLORELIN-TREATED DOG AFTER RECOVERY FROM TREATMENT, WITH ACTIVE SPERMATOGENESIS IN THE SEMINIFEROUS TUBULES (HES ·100). ABBREVIATIONS: A, TYPE A SPERMATOGONIUM; M, BASEMENT MEMBRANE; S, SPERMATOZOON; T, SPERMATID"[29].

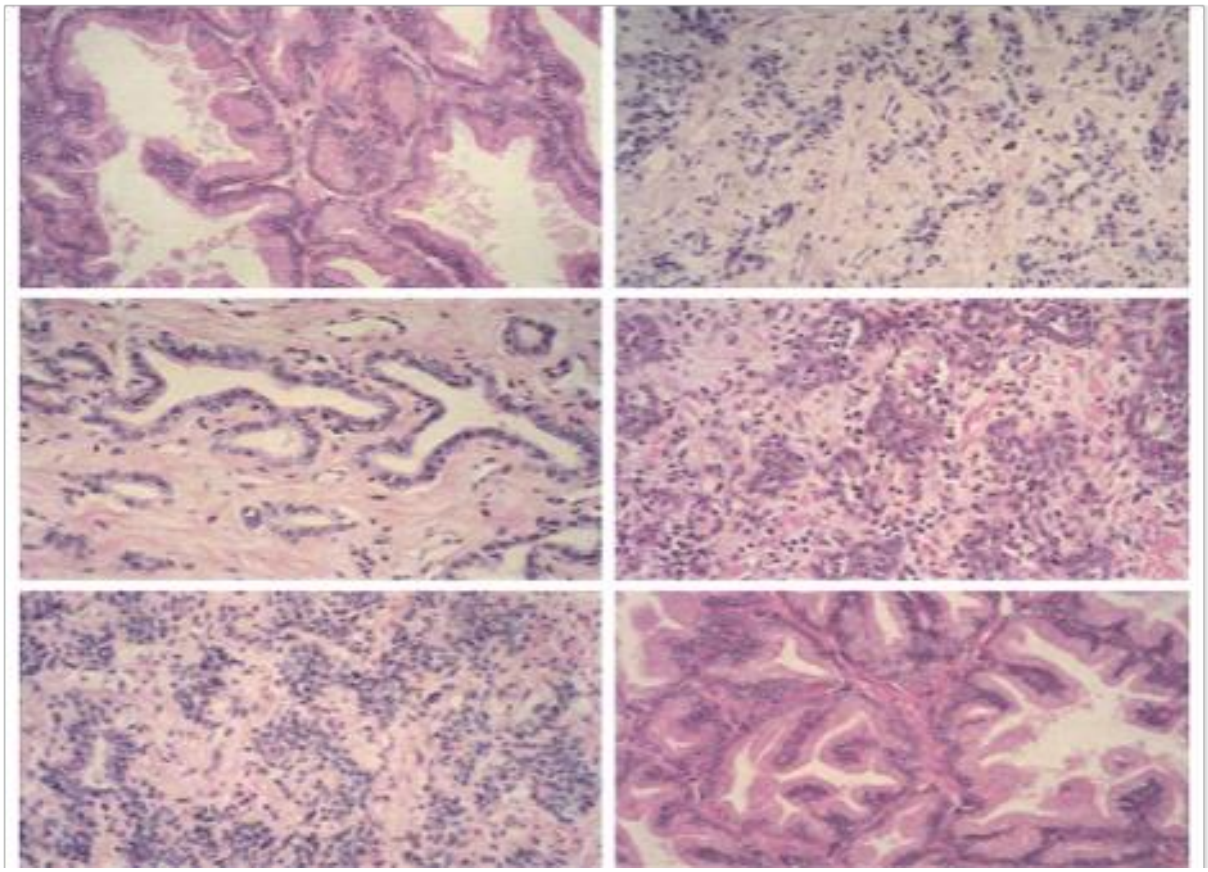

Figure 14 "Light micrograph of the section of prostate gland of (a) a control dog showing a predominance of glandular structures comprising irregular acini with typical projections inside the lumen (HES ·80). (b) a deslorelin-treated dog 15 days after implantation, showing atrophic tubular glandular structures, with tubules lined by cuboidal, non-secretory epithelium (HES ·80); (c) a deslorelin-treated dog 25 days after implantation, also showing atrophic tubular glandular structure and tubules lined by cuboidal, non-secretory epithelium (HES ·128); (d) a deslorelin-treated dog 40 days after implantation, showing atrophic tubular glandular structures and non-secretory epithelium (HES ·80); (e) a deslorelin-treated dog 100 days after implantation, with complete atrophy of glandular epithelium and relative increase of the amount of otherwise unchanged connective tissue (HES ·80); (f) a deslorelin-treated dog after recovery from treatment, with a normal predominance of glandular structures comprising irregular acini with typical projections inside the lumen (HES ·80)"[29].

C. The effect of deslorelin on semen quality

After semen evaluation in dogs that received deslorelin implant it was noted that motility and sperm concentration both decreased and higher abnormality percentage was detected [57]. The most common secondary abnormalities were proximal and distal cytoplasmic droplets, coiled tails, bent tails. From week 6 semen could not be collected anymore due to lack of ejaculate up till week 48. By week 60 the general values started improving and going back to pretreatment levels. Although there was some considerable variation between individuals in the recovery process of ejaculate volume and semen quality [30].

It was observed that at the beginning semen motility increased and later on from day 23-32 decreased in all dogs. From day 9-17 total number of sperm was initially stable then showed a peak at day 23-32 before decreasing rapidly. Semen volume increased until day 23-32 and then

decreased gradually. The results supports the report of onset of sterility between 36 and 48 days. Semen parameters show a decrease by day 28-35 after implantation. Semen quality might be improved at the beginning of the treatment, or be unaffected during the first month. During the 2nd and 3rd month of their study a rapid decline of most seminal parameters, sperm morphology was unaffected and all dogs became aspermic [28, 52]. As I mentioned before at around day 41 post treatment 90% of of seminephrous tubules are atrophied which could explain the aspermia [29]. Some dogs showed to be still fertile according to semen parameters at day 59 and day 70 post implantation which proves the variability between indivudual responses, so veterinarians should warn to keep the dogs away from bitches in estrus for at least 2 months after implant since fertility might improve in that period of time [52]. First the return of motile sperm, later normal morphology and last the total sperm count will be seen. Probably due to a progressive reactivation of the testicular tubular compartments [23].

From day 35-42 there was no ejaculation but thrust and erection was still observed which means that the libido was not affected. In dogs that received the 3 mg implant ejaculation was abscent for 60 weeks, in the 6 mg implant ejaculation was abscent for 68 weeks and the 12 mg implant for 92 weeks [28].

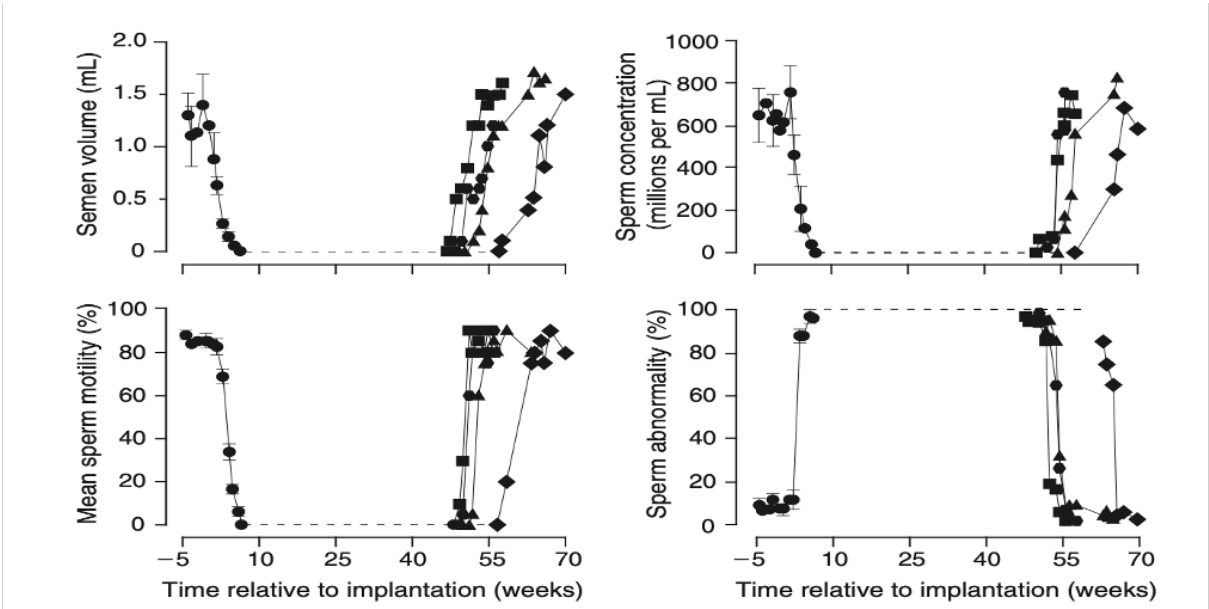

FIGURE 15 MEAN SEMEN VOLUME, SPERM MOTILITY, SPERM CONCENTRATION AND SPERM ABNORMALITY IN MALE DOGS GIVEN A SUBCUTANEOUS SLOW-RELEASE IMPLANT CONTAINING 6MG DESLORELIN. [30]

D. REVERSIBILITY AND REAPPLICATION

All induced effects of GnRH slow releasing implant are found to be fully reversible, with all hormone levels FSH,LH and testosterone reaching pretreatment concentrations within a few weeks after the implant is expired or after implant removal [24, 30, 31].

Research on the morphology of the testis and the prostate gland after administering the 6 mg deslorelin implant observed recovery of normal seminiferous tubules with a normal epididymal duct and physiological prostate tissue, the author proved here by observing the tissue sample under the microscope that histological and endocrine function and structure is back to normal. Although in this study the progress of Leydig and Sertoli cells is not described after recovery, it can be assumed that they regain normal morphology as well [29]. As well testicular volume goes back to pretreatment size both in dogs and cats [21, 28].

Semen quality showed to be back to physiological range at about 25 weeks after the end of treatment in dogs that received azagly-nafarelin 18.5 mg. As compared to dogs who received deslorelin where normal ejaculate would appear at around 46 and 54 days after normal testosterone levels are detected. This variance could be due to the difference in the active substance used leading to differences in pharmacodynamic and pharmacokinetic profiles [23]. Recovery of ejaculate for the dogs that received the 3mg deslorelin implant was at 62 weeks, for the 6mg group at 76 weeks and for the 12 mg group at 102 weeks. It was apparent as well that there were variations between animals in duration and time of recovery. The 6 and 12 mg doses showed to be the most consistent in times. These results prove that the dosage of deslorelin does not affect the extent of fertility suppression but rather the duration of suppression. It was observed that suppression was maximal in all groups with the different doses. The only difference that was perceived was in the timing till recovery [28].

In the dose response study, the hormonal concentration came back to normal values. They observed a difference in the time of recovery. For the 3 mg group plasma LH concentration reached nomal values between 198 and 474 days. For the 6 mg group plasma LH concentration reached normal between 349 and 686 days and for the 12 mg group plasma LH concentration reached normal value between 487 and 800 days. Similar effect was observed for testosterone concentration, with the 12 mg group taking the longest for recovery [28]. That being said there probably are differences between individuals of different body weight as they would receive a different dosage the smallest bodyweight taking the longest to recover once the efficacy of the implant is done. It could also be said that the restart of spermatogenesis correlates first with the increase of hormone levels. The return of normal sperm appearance about 9 weeks after the

recovery of testosterone concentration is consistent with the 56-day spermatogenic cycle of dogs [15, 46].

Dose of deslorelin	Duration of complete suppression (days)			
	Undetectable LH, testosterone	Testicular volume < 35%	No ejaculate produced	
	Mean	Mean	Mean	Range
3 mg	367 ± 64[a]	366 ± 77[a]	416 ± 88[a]	280–504
6 mg	419 ± 72[b]	472 ± 74[b]	476 ± 83[b]	350–728
12 mg	607 ± 69[c]	634 ± 59[c]	644 ± 67[c]	560–840
	Complete recovery of function (days after implantation)			
	Normal LH, testosterone	Testicular volume normal	Normal ejaculate produced	
3 mg	394 ± 65[a]	408 ± 77[a]	440 ± 66[a]	294–524
6 mg	484 ± 72[b]	514 ± 74[b]	538 ± 83[b]	366–724
12 mg	668 ± 47[c]	676 ± 59[c]	716 ± 67[c]	568–836

Values are days, mean ± SEM (n = 4). Means with different superscripts in the same columns are different.
[a,b]$p < 0.05$, [b,c]$p < 0.01$, [a–c]$p < 0.001$.

TABLE 3 THE DURATION OF COMPLETE SUPPRESSION AND DELAY TO COMPLETE RECOVERY OF REPRODUCTIVE FUNCTION (RETURN TO NORMAL, PRETREATMENT VALUES) IN DOGS AFTER IMPLANTATION WITH DIFFERENT DOSES OF DESLORELIN.

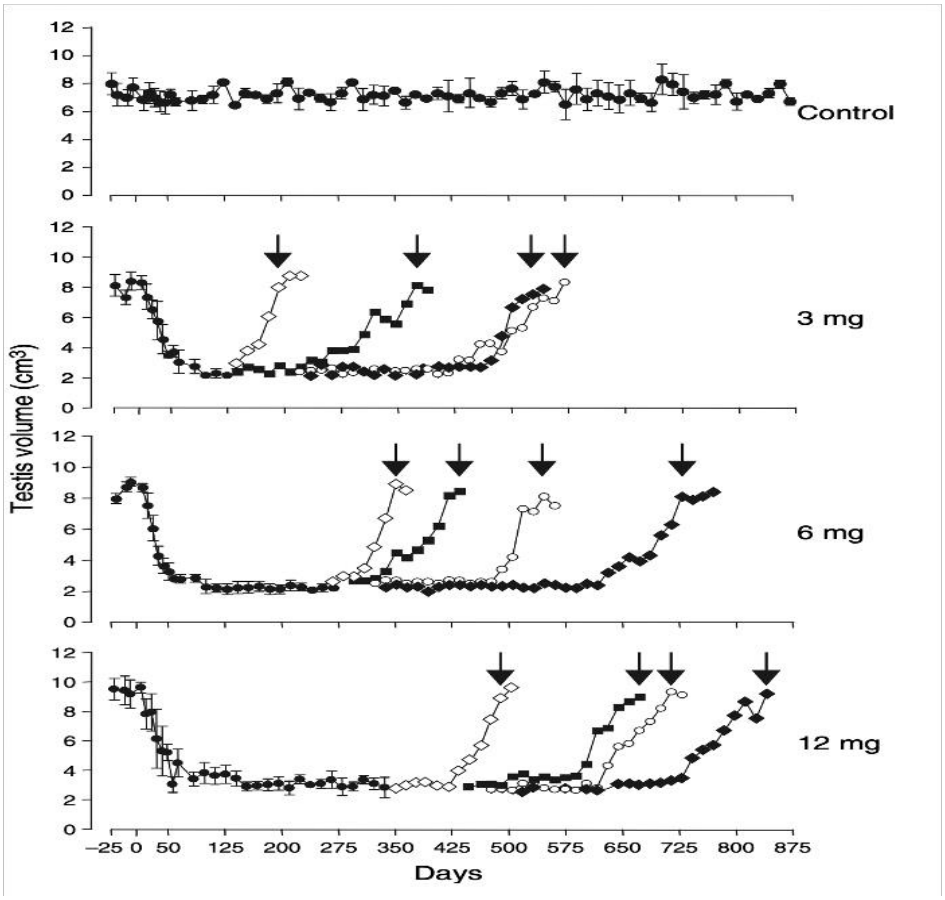

FIGURE 16 TESTICULAR VOLUME IN MALE DOGS FOLLOWING THE IMPLANTATION OF A BLANK IMPLANT OR A DESLORELIN IMPLANT CONTAINING 3,6 OR 12 MG DESLORELIN. THE TIME OF IMPLANTATION WAS 0 ON DAY 0. RETURN OF VOLUMES TO PRE TREATMENT VALUES ARE INDICATED BY ARROWS.[28]

Repeated implantation with deslorelin showed that the effects could be reintiated and prolonged in dogs that were already treated.

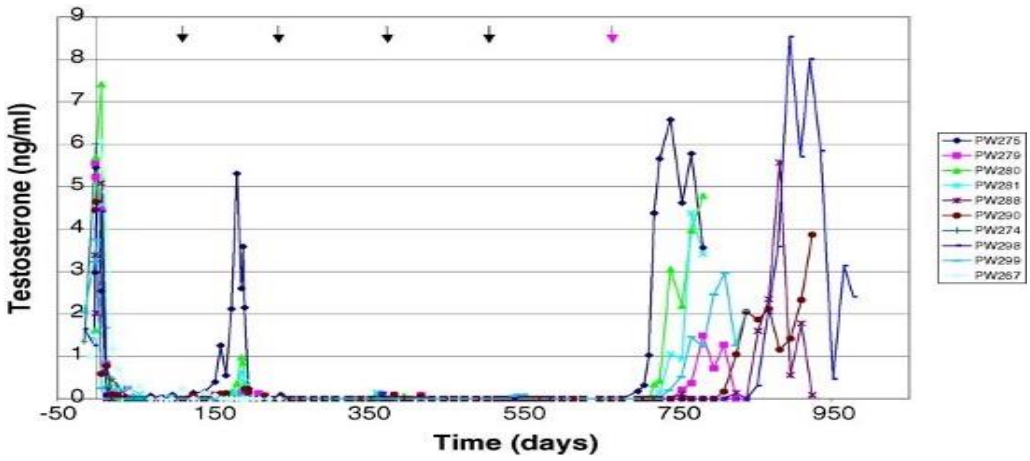

FIGURE 17 TESTOSTERONE CONCENTRATION (NG/ML) FOLLOWING SERIAL IMPLANTATIONS WITH A 4.7 MG DESLORELIN IMPLANT IN MALE DOGS (INDIVIDUAL VALUES FROM DOGS REIMPLANTED AFTER RECOVERY FROM PRIOR IMPLANTATION). IMPLANTATION ARROW FURTHEST TO THE RIGHT INDICATES THE TIME WHEN THE IMPLANT WOULD HAVE BEEN GIVEN. [58]

4.2 OFF LABEL USE OF DESLORELIN

A. BENIGN PROSTATIC HYPERPLASIA (BPH)

The size and volume of the prostate gland is androgen related. It is a common disease in older intact male dogs, more then 80% of dogs over 5 years old [49], and 95 % over 9 years old have some sort of prostate enlargement [65]. In dogs the main circulating androgen is testosterone, it is produced by Leydig cells of the testicles and converted by type II alpha reductase into dihydrotestosterone in the prostate, seminal vesicles, epididymis, skin, liver and brain [44]. Today the treatment of choice for BPH is castration. When this is not an option other treatment methods have been tried. For example finasteride and ostarone acetate are good options. Nonetheless new studies have proven that the GnRH analogue deslorelin is a good treatment choice for bening prostatic hyperplasia. As I mentioned above in my thesis multiple clinical trial proved that deslorelin reduces prostatic size as proven in the study done by Junaidi A [29]. One clinical trial specifically compared the use of deslorelin acetate and ostarone acetate in the treamtment of BPH [44]. In that study 4 groups of dogs were used in total 45 dogs. Group I was

made of 10 healthy dogs. Group II was made of dogs diagnosed with BPH but they didn't receive any treatment. Group III was made up of 15 dogs that recevied deslorelin implant, and group IV was made of 10 dogs that received osaterone acetate. The dogs were carefully chosen according to physical examination, history, clinical signs, and confirmed diagnosis of BPH with ultra sound and fine needle aspiration of the prostate to confirm BPH by cytologic charactersitics. Most common clinical signs in BPH are droppings of serosanguineus fluid from the penis (unrelated to urination), rectal tenesums, ribbon shaped feces, hemospermia, hematuria [43]. Testicles are the main source of testosterone, therefore the removal of testicles would decrease the testosterone level and concequently shrink the prostate gland. This process takes about 9 weeks after castration and the prostate becomes no more then 25-30 % of it's pre castration size [27]. The most common reason why castration would not be performed are anesthesia and surgery risks in older dogs, stud dogs that are high value, or working dogs as testosterone is essential for their best performance. Osaterone acetate is marketed as oral tablets, relieving clinical symptoms for five months after a 7-day treatment course. For dogs allocated in group III and IV confirmation of BPH was based on " reported clinical signs, symmetrical enlargement of the prostate by rectal examination, and the results of the prostate biopsy. The objective score system for grading BPH by Zambelli was used in the study [64].

Anorexia	Weight Loss	Defecation	Dysuria	Urinary Incontinence	Urinary Leakage			Hematuria		
					Amount	Frequency	Duration	Amount	Frequency	Duration
1: absent	1: absent	1: normal	1: normal	1: absent	1: absent	1: absent	1: absent	1: absent	1: absent	1: absent
2: for 1 day	2: mild	2: regular with tenesmus	2: flow weaker or interrupted very	2: present	2: few drops	2: once in a year	2: one week	2: few drops	2: once in a year	2: one week
3: 1 to 7 days	3: moderate	3: regular with tenesmus	2: flow weaker or interrupted very		3: urine	3: 2-3 times per year	3: two week	3: pinkish drops	3: 2-3 times	3: two week
4: > 7 days	4: severe	4: absent for last a few days	4: urinary retention		4: copious	4: > 3 times per year	4: > 15 days	4: red urine	4: >3 times per year	4: > 15 days

TABLE 4 OBJECTIVE SCORE SYSTEM FOR BENIGN PROSTATIC HYPERPLASIA SIGNS GRADING, BY ZAMBELLI [64].

On day 0 group III after performing the prostate biopsy received a 4.7 mg deslorelin implant. On day 0 group IV after performing the prostate biopsy were started on osaterone acetate tablets 0.25-0.5 mg/kg every 24 hours for 7 days as recommended by the manufacturer. The dogs were examined on day 0, 7,14,21, week 8,12,16,20,24 and three months after the end of the

trial on week 36. Clinical symptoms were present in all dogs from group II throughout the whole study. In group III 40% of dogs had no symptoms and 60% by week 8. On day 7 five dogs in group III had a flare up effect, with aggravated clinical signs. In group IV on day 7 80% of the dogs had no symptoms and by day 14 a 100% showed no symptoms. On week 24 , 40% of the dogs in group IV showed signs of relapse with clinical signs reappearing. The testosterone levels were measured. In group III on day 7 a rise was observed. By week 8 40% of dogs showed undetectable testosterone levels. By week 16 100% of the dogs showed undetectable levels. In group IV the lowest testosterone level was observed on week 16, however as ocaterone acetate doesn't affect the testosterone concentration the difference is negligible. In week 36, 3 month after the end of the trial none of the dogs from group III showed symptoms related to benign prostatic hyperplasia, while 75% of examined dogs in group IV were showing clinical signs such as sanguineous discharge from the prepuce or urethra and hemospermia. The results of that clinical trial showed that both drugs are effective for the treatment of BPH with some small differences, osaterone acetate providing a faster resolution of clinical signs compared to deslorelin (first 14 days) and there is no flare up effect, but relapse with enlargement of the prostate gland and reappearance of clinical signs happens faster (week 24).

Deslorelin on the other hand, took longer for clinical signs to disappear 8 weeks after inserting the implant. Additionally the flare up effect lead to worsening and aggrevation of the clinical signs before they were completely resolved. The authours recommended the use of both GnRH agonist and an androgen receptors blocker as osaterone acetate during the first few days of therapy to prevent the flare up effect and the worsening of the patient's clinical signs. Although deslorelin lasted longer 36 weeks with no relapse showed in the group of dogs that received it [44].

In another research done by the same authors they examined the findings of the prostate gland and testes in dogs receiving deslorelin acetate or osaterone acetate. The results of their study proved the importance of B mode and doppler imaging technique for the diagnosis and progess assement in dogs with BPH. They realised that the blood flow kinetics demonstrated a time association between the blood flow changes registered in the prostatic artery and the succeeding volumetric and sonographic improvement of the prostate parenchyma. The flow indices were reduced much earlier then a signigicant reduction in prostate volume was noticed, suggesting that the regulation of the prostate blood flow is a primary target of androgen action. Concequently, they presumed that the sonographic recovery of the prostate parenchyma, occurs secondary to the regression of the prostate vascular system. The comparison of both ostarone

acetate and deslorelin acetate showed some difference in the observed sonographic movement. Deslorelin reduced prostate volume slower, but the reduction level was higher and its effect lasted longer (up to 36 weeks) than for ostarone acetate (effects lasted 20 weeks) [45].
All this being said, deslorelin proves to be a good option for the treatment of BPH as the author recommended it should be used with a combination of androgen receptor blocker for the first few days to prevent the flare up effect of GnRH agonists. Studies have been conducted to evaluate the concomitant use of GnRH agonists and antiandrogenes drugs such as flutamide[38], or steroidogenesis inhibitors such as ketoconazole and aminoglutehimide [37]. They observed that dogs receiving the combination with flutamide showed the same hormonal changes as dogs treated only with GnRH, indicating that this compound does not alter the agonist effect and could be used to prevent the concequence of the flare up effect [13].

B. The use of GnRH analogues for the treatment of leishmaniosis

It is believed that male and female mammals have different immune responses, it has been proved in many studies. Most parastic, viral or bacterial diseases including lesihmania infections frequently show more severe clinical signs in males then females. In one study done they have proved that "Differences in susceptibility to intravenously inoculated Leishmania major were observed in male and female mice. In all cases, males had significantly higher liver parasite burdens than females. Orchidectomy of BALB/c males resulted in a 20% decrease in the number of parasites in the liver compared with either normal or sham-gonadectomized controls. Additionally, testosterone treatment of female BALB/c mice resulted in an 88% increase in the number of liver amastigotes. These results suggest that the hormone testosterone can modulate systemic Leishmania major infections in BALB/c mice" [7]. It has as well been proved by many authors that testosterone has an immunosuppressive effect [14] [3][25]. In research, they were able to prove that dogs infected with Leishmaniosis that received a combination of Deslorelin, meglumine antimoniate and allopurinol showed a better clinical improvement compared to the dogs that received only meglumine antimoniate and allopurinol. All of that being said GnRH analogue deslorelin could be used in the treatment of canine leishmaniosis [50].

C. The use of GnRH for the treatment of Alopecia X

Alopecia X or hair cycle arrest is a non-inflammatory non pruritic skin disorder commonly seen in Nordic dogs. The pathomechanism of this disease is not perfectly understood which makes treatment and diagnosis challenging. Two experimental studies evaluated the use of deslorelin implant (GnRH Agonist) in dogs with confirmed hair cycle arrest. The first study treated two intact male keeshonden with confirmed hair cycle arrest diagnosis with a 4.7 mg deslorelin implant administered subcutaneously. 3.5 months later there was extensive regrowth of hair in all previously affected areas. Skin punch biopsy was done before treatment and showed excessive trichilemmal keratinization and moderate to severe follicular hyperkeratosis. There was mild hair follicle dysplasia. No inflammation was seen in any of the sections examined. These findings are accurate for the diagnosis. A biopsy was repeated and showed anagen follicles predominated, with one in five of the follicular units remained in kenogen phase or telogen with retained hair shafts surrounded by moderate follicular hyperkeratosis. Over the next few month owners reported that the dog's fur continued to improve. And 14 months after administration owners reported that the dog's coat remained full. No adverse effects were reported [39].

In the next study 20 dogs with confirmed hair cycle arrest were studied. 16 intact males, 3 neutered females, 1 neutered male. Each dog was treated with a subcutaneous sterile implant containing 4.7 mg deslorelin. Dog who responded were re-implanted 6 months later to achieve a 1-year pharmacological exposure and 1 year follow up.

Hair regrowth was visible at 3 months from the first examination in 12 out of 16 intact males (75%). Although it was not seen in any of the neutered female dogs. The overall efficacy noted by the authors was 60% in the 20 dogs. At 6 months after implantation dogs that experienced hair growth at the 3rd month had a fully recovered coat and there were no change in the non-responder dogs [2].

FIGURE 18 EVOLUTION OF THE EFFECT OF DESLORELIN IN TWO SELCTED CASES A,D TIME OF FIRST EXAMINATION AND WHEN DESLORELIN TREATMENT WAS STARTED, B,E THREE MONTH FROM T0, C,F SIX MONTHS FROM T0.

As deslorelin acts via inhibition of gonadotrophin production and none of the neutered females responded to the treatment, the authors speculated that hair regrowth was mediated by inhibition of gonadal hormone not present in neutered animals. The latter could be explained by the fat that canine hair follicles have been shown to express sex steroid receptors and their cells were shown to metabolize sex steroids [5, 6]. As this study was done for the period of 1 years, relapse could still happen once the dogs are no longer exposed to deslorelin as its effects are reverisble. According to another study done on 35 pomerian dogs diagnosed with hair cycle arrest and treated by gonadectomy. 42.9 % of the pomeranian responded [26]. Therefore a 60% success in deslorelin is to be expected. Given that the pathogenesis of this condition is still unclear other factors related to the hair follicle behavior might be involved. Deslorelin is an attratctive simple and cost friendly solution for the treatment of hair cycle arrest in intact dogs [2].

D. PREPUBERTAL AND NEONATAL USE OF DESLORELIN

In a study done by Sirivaidyapong S, the implantation of deslorelin at early prepubertal age and it's role in delaying puberty was studied. They used 6 male beagles from two litters, and litter of five mongrel dogs at the age of 4 months were put into two groups. The first group was implanted with 4.7 mg subcutaneous deslorelin implant, the other group with 9.4 mg deslorelin and three dogs from each litter were implanted with a placebo. Sexual behavior and testicular size were monitored after the treatment. Semen collection and semen evaluation were performed at 2 years post implantation in dogs that showed well developped testicular size.

The results were the following two year post implantation, 3 male dogs that received the 4.7 mg deslorelin began to show male sexual behavior. Testicles were well developped with firm consistency upon palpation and semen was collectable. In the other 5 implanted dogs they showed only mild male sexual behavior, with small testicles, low libido and no collectable semen at ages of 2-5 years and 3.2 years. The control dogs showed normal reproductive performances with normal sperm quality. No difference were observed for growth, size, height, figure or behavior between the control and implanted groups except for the testicular development. Following these results it was shown that the 4.7 mg deslorelin could be effective in postponing puberty for less than two years and the 9.4 mg deslorelin implant tend to last for longer [55].

In another study done by Faya M, they assessed the efficiency and clinical safety of postnatal administation of GnRH agonist on canine puberty postponement. Sexual steroids and histological gonadal changes were described. 24 puppies were divided into two groups, 12 received deslorelin, 12 received placebo postnatally. The dogs were followed up till puberty when they were castrated and their gonads were histomorphometrically studied. The results showed that the deslorelin implanted dogs reached puberty at 72.7 weeks while the placebo dogs reached it at 35.8 weeks on average. There were difference between individual dogs 9 of the dogs that received the deslorelin implant reached puberty on average at 72 weeks, while 3 dogs at 108 weeks still hadn't reach puberty. Their were no differences in the two group at the age of cessation of growth all animals in this experiment cessed growing at 29 weeks aproximately independetly of their pubertal status. Additionally neither withers, hieght or body weight differed between the same groups at puberty. The non pubertal male dogs suffered from bilateral cryptorchydism. Libido appeared normal in all the dogs. In male dogs scrotal volume at puberty did not differ from the placebo group. All the bitches ovulated during their pubertal estrus cycle and 8 females in the deslorelin and placebo group were mated and pregnancies in both groups appeared to be normal. No adverse side effects were observed in this study. To

conclude the use of GnRH agonist deslorelin does not affect growth in neonatal puppies, it succesfully delays puberty. The long juvenile or neonatal hormone deprivation did not appear to have a negative effect on the dogs by the time they reached puberty. Normal reproductive function was noted which proves the reversability of the contraceptive effect [12].

In dogs major growth occurs between 3 and 6 months and usually epipheasal growth plates close betweent the age of 4 and 12 months depending on the size and breed. It is a known fact that jevenile gonadectomy leads to a delay in growth plate closure. In a study done by Kaya D, they observed that the growth plate closure was delayed until 20 months of age however without any clinical impact. Dogs treated had a normal growth and withers size. However it is believed that the delay in epipheseal growth plate closure can lead to bone and joint abnormalities [33]. Moreover, in a study done with a large number of dogs the results showed that there is an increase in the incidence of hip dysplasia in dogs that are gonadectomized at an early age [54, 56]. In a study with 24 dogs evaluating the effects of deslorelin implants in prepubertal female short and long term observed hip dysplasia in two of the dogs that were treated with an deslorelin implant in a silican hound breed which usually does not have a hereditary predisposition[42]. .

Multiple studies were done [33, 53, 55, 58] and they all showed similar results the use of a GnRH agonist implant effectivetly and safely delays puberty in dogs with no significant side effects observed except the appearance of juvenile genitalia and the increase in estrogen levels the first ten days after implantation which leads to a rapid cornification of the vaginal intermediate cells and an increase in estrogen [42]. Although the risk of the delay of the epipheal plate closure should be kept in mind.

E. Use of deslorelin for anal adenoma in intact males

Perianal gland tumors are one of the most common neoplastic disease in male dogs. Some breeds show a higher prevalence to developping these tumors as the cocker spaniel, beagle, bulldog and samoyed [8]. The most frequent type of tumor seen is the adenoma. Perinal glands are non secretory modified sebaceous glands that occur normally around the anus in dogs but can be found in other areas like the skin of prepuce, skin, loin, groin, posterior parts of hindlimbs, abdomen, head and neck. It is usually diagnosed on the basis of physical, cytological and histopathological examination. It is believed that it is a hormone dependant tumor [32]. Adenomas of the gland develop about 4.5 times more often then carnicomas. Castration without excision of the growth has shown to reduce the neoplasm without recurrence [62]. In a experimental study they examined the prevalence of androgen receptors on perianal glands in

normal tissues, in hyperplastic tissue, in neoplasm specifically adenomas, epithelioma and carcinoma. They found that there was an increase in percentage of androgen receptors in hyperplastic tissue and an slight increase of androgen receptor in all of the neoplasms mentioned above [48]. It is believed that anal adenoma are stimulated by androgenic hormones and suppresed by estrogenic hormones. Older intact males are at highest risk but it seems females that received an ovariohysterectomy showed a higher prevalence as well probably due to the low estrogen that could not suppress the growth [34]. In a study, antihormonal treatments were used for the treatment of perianal tumors using andorcur an anti androgenic drug or amoxifen an antiestrogeneic drug depending on the hormonal levels. The treatment was given for a month. 100% of adenomas responded to the therapy with no recurrence in the next 6 months, but the antihormone therapy was less efficient for epithelioma where reucurrence was observed and even less efficient for carcinoma [8].

I believe that deslorelin could as well potentially work, since it is anticipated that perianal adenomas are hormone dependant neoplasm, and the use of anti hormone therapy specifically anti androgen therapies in intact males showed good results with the regression of the tumor and no recurrence, similar results could be seen with the deslorelin implant, although due to the initial increase in hormone levels in the first phases the tumor might increase in size or seem worse. I believe that similarly to cases with benign prostatic hyperplasia a combination of anti androgenic and deslorelin may be better. Deslorelin has shown to succesfully decrease testosterone, FSH and LH levels to undetectable levels and shows to have a long term efficacy up to 12 month with the 9.8 mg implant. All that being said I deem deslorelin could be a good option for the treatment of hormone dependent perianal adenomas.

5. Discussion and conclusion

In conclusion GnRH analogues today are widely used in veterinary medicine. In Europe the deslorelin releasing implant is indicated for the use as a contraceptive in mature male dogs and male ferrets. A lot of research has been done in the aspect of its use as a contraceptive in male dogs. They all prove its effectiveness in suppressing fertility by inhibiting the pituitary gonadal axis for a long time and the sure reversibility of the contraceptive with spermatogenesis and sexual behavior returning to normal once the implant is aged. Although variations between individuals are seen in the time of suppression of fertility and time to recovery the results are idem. As I mentioned before, ethical questions are being raised about performing a gonadectomy in companion animals with more and more people leaning against it. Furthermore

with a bigger overpopulation of stray dogs. This could be a non-risky noninvasive solution to control the growth of street dog population. It could as well be very useful in dogs that are at high risk for anesthesia and so are not candidates for surgical removal of the gonads. It also seems promising for the eradication of unwanted male behavior as sexual, territorial, dominant and aggressiveness. In addition, it could be used as a preview for the pet owner as to how surgical castration would affect their pet's behavior. Testosterone concentration and sperm output were successfully decreased to undetectable values and after a 12-month period a complete recovery was observed. Plasma concentration of LH and testosterone are reduced to undetectable values within 4 weeks and lead to infertility within 6 weeks. As well testosterone and LH concentration and semen quality returned to normal by week 60 after implant depending on the dosage used [36]. Compared to an injection of GnRH elevation of LH and testosterone were noted much later 2-4 hours compared to the implant [61]. It is believed that this is due to the high rate of release in the first 2-3 days. It is alleged that testosterone level decreases due to the lack of LH to stimulate the Leydig cells or the loss of testicular LH receptors [10]. The decrease in plasma testosterone concentrations and gonadotrophins, in addition to the prostate atrophy and the decrease in testicular volume explains the decrease in ejaculate volume, decline in the sperm motility and maturity of the spermatozoa and the general decrease in semen quality. The return of normal sperm appearance about 9 weeks after the recovery of testosterone concentration is consistent with the 56 day spermatogenic cycle of dogs.

The atrophy of Sertoli cells and the loss of spermatogenic activity is also confirmed. The changes observed in the prostatic tissues, atrophy of the nuclei and the epithelium appearing non secretary is identical to the changes found in dogs that have undergone a gonadectomy.

For male dogs the inhibition of gonadal function with deslorelin will potentially completely desensitize Leydig cells to LH, leading to an androgen deprivation. This is usually a desirable consequence in companion animals to reduce unwanted behaviors specifically sexual , aggressive, and territorial behavior [31].

The effect of deslorelin on semen quality supports the report of onset of sterility between 36 and 48 days and semen parameters show a decrease by day 28-35 after implantation. Semen quality might be improved at the beginning of the treatment, or be unaffected during the first month. During the 2nd and 3rd month a rapid decline of most seminal parameters is seen, and all dogs became aspermic.

The dosage of deslorelin does not affect the extent of fertility suppression but rather the duration of suppresion. It was observed that suppression was maximal in all groups with the different doses. The only difference that were perceived was in the timing till recovery.

In the other aspect of its clinical use, it has shown promising results in the treatment of benign prostatic hyperplasia in intact male dogs. It shrinks the prostate gland with long term results and improvement of clinical signs, although it would be recommended to combine it in the first phase of the treatment with an anti-androgenic drug to prevent the exacerbation of clinical signs. It was also shown that dogs infected with Leishmaniosis that received a combination of Deslorelin, meglumine antimoniate and allopurinol showed a better clinical improvement compared to the dogs that received only meglumine antimoniate and allopurinol. All of that being said GnRH analogue deslorelin could be used in the treatment of canine leishmaniosis [50]. Moreover its use isn't necessarily confined for the treatment of leishmania, but it could be helpful in other kind of diseases requiring a boost of the immune system. Although more extensive research would need to be done. Deslorelin could be a good solution for the treatment of alopecia x in intact males as it acts via inhibition of gonadotrophin production. It was seen that none of the neutered females responded to the treatment. The authors speculated that hair regrowth was mediated by inhibition of gonadal hormone not present in neutered animals. Deslorelin is an attratctive simple and cost friendly solution for the treatment of hair cycle arrest in intact dogs[2]. Although not always 100% successful more research is needed to get a better understanding of the pathogenesis of the disease and the effect deslorelin could have for treatment.

I also discussed it's used in prepubertal and neonatal dogs which according to the results of the experiments conducted I think should be avoided. It successfully delays puberty, and doesn't affect growth rate. The only side effect observed is the appearance of juvenile genitalia and the increase in estrogen levels the first ten days after implantation which leads to a rapid cornification of the vaginal intermediate cells and an increase in estrogen [42]. While it delays the closure of the epiphyseal plate, which some authors believe could with time lead to joint disease such as hip dysplasia. The mentioned disease was found in one of the experiments done in 2 silican hounds that are not a breed predisposed to developing this disease. It correlates with the study done in early castration of dogs that showed a higher prevalence of hip dysplasia [56]. These findings could be relevant as well in the case of GnRH use

The use of deslorelin for perianal adenoma could as well be successful. Although no studies were done in the matter, it is known that perianal adenoma is a hormone dependent tumor, and it has shown to respond to anti hormonal therapy. Hypothetically the role deslorelin plays in decreasing testosterone, LH and FSH to undetectable levels could help regress the tumor and prevent its reoccurrence in the long term.

In all the studies done and discussed here no adverse effect were observed. Which makes it a safe alternative to surgical castration. According to all the research I have collected and described deslorelin implant will successfully suppress fertility in male dogs, by decreasing semen quality, sperm motility, spermatozoa concentration and morphology, and by causing an atrophy of the seminiferous tubules and the prostate gland. What I also found was that there are relevant variations between individuals and that should be kept in mind when using deslorelin. These variations could be due to many reasons, but it is believed that individual absorption of the active substance varies between animals. As for GnRH 3rd generation antagonist I believe could have a great impact in the veterinary industry as they don't lead to a flare up effect and work faster compared to agonists. There is very limited research about their use although some were done with a high dose single injection of the agonist acyline which showed good results. I believe more studies should be done exploring the different dosages, possibly a higher dosage could suppress fertility for longer and exploring potential adverse effects . As well as their use in combination with GnRH agonist for the prevention of the flare up effect.

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65. Serum biochemical and hematological parameters in dogs with benign prostatic hyperplasia (BPH)

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