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Chronic use of Deslorelin in Dogs: Six cases (2005-2022)

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Acronyms and abbreviations

ALT: alanine transaminase

AST: aspartate transaminase

BPH: benign prostatic hyperplasia

CMA: chlormadinone acetate

CPSE: canine prostatic specific esterase

CT: computerized tomography

DMA: delmadinone acetate

FSH: follicle stimulating hormone

GGT: gamma-glutamyl transferase

GnRH: gonadotropin-releasing hormone

HPG: hypothalamus-pituitary-gonadal

LH: luteinizing hormone

MA: megestrol acetate

MPA: medroxyprogesterone acetate

OHE: ovariohysterectomy

OSS: ovary-sparing spay

OVX: ovariectomy

PGS: proligestone

T: testosterone

UI: urinary incontinence

US: ultrasonography

USMI: urethral sphincter mechanism incompetence

Riassunto

Il deslorelin, un agonista dell'ormone rilasciante gonadotropine, è attualmente registrato per l'induzione dell'infertilità temporanea nei cani maschi, nei gatti maschi, nei furetti maschi e anche nelle cagne prepuberi, ma vari studi hanno dimostrato la sua utilità anche per altre condizioni che richiedono un trattamento cronico. L'efficacia e la sicurezza, nonché il ritorno alla fertilità di questo metodo di sterilizzazione non chirurgica sono ben noti dopo un singolo utilizzo, ma i dati disponibili sul suo uso prolungato sono molto limitati. Per dare ulteriore contributo all'uso cronico di questo farmaco, abbiamo pubblicato un articolo scientifico che presenta sei casi di cani trattati cronicamente con deslorelin tra il 2009 e il 2022. I cani presentati in questo lavoro sono stati trattati per 2-9 anni consecutivi a scopo anticoncezionale (un caso) e per condizioni non riportate sul foglietto illustrativo del farmaco: iperplasia prostatica benigna e malattia della ghiandola perineale (un caso), controllo del comportamento riproduttivo nei cani maschi (due casi) e incontinenza urinaria in cagne adulte sterilizzate (due casi). Tutti gli animali erano in buona salute durante il trattamento e non hanno presentato effetti collaterali a breve termine. Reazioni di flare-up (aumento del comportamento riproduttivo per 1-3 settimane dopo il trattamento) sono state osservate in 1/4 dei maschi interi e non sono state osservate nelle cagne incontinenti sterilizzate. Il deslorelin è stato efficace in tutti i cani trattati. La fertilità è stata immediatamente recuperata in un cane maschio che ha prodotto una cucciolata quando il proprietario ha tardato l'inserimento di un nuovo trattamento, necessario per mantenere e prolungare l'effetto. Due cani hanno sviluppato una neoplasia: una cagna sterilizzata trattata per 3.5 anni per incontinenza urinaria ha sviluppato un carcinoma ipofisario e un cane maschio intero trattato per 9 anni per il controllo della fertilità ha sviluppato un carcinoma vescicale. Entrambe le neoplasie non risultano avere alcun rapporto con il trattamento a base di deslorelin. Il valore scientifico di questo lavoro è costituito da case reports su nuove applicazioni e l'uso cronico del deslorelin. Dopo quasi 20 anni di utilizzo del farmaco, è essenziale riportarne tutti gli effetti sospettati o confermati, poiché questi dati son fondamentali per l'uso sicuro del farmaco e per problemi legati alla gonadectomia e le sue alternative. Questo lavoro conclude che gli impianti di deslorelin possono essere considerati un'alternativa sicura alla castrazione chirurgica in patologie specifiche mediate dagli ormoni riproduttivi e in situazioni in cui la castrazione chirurgica non è un'opzione, come nel caso di animali affetti da condizioni cardiovascolari o altre malattie sistemiche che rendono l'anestesia non sicura. Nonostante la limitata numerosità campionaria, possiamo affermare che il trattamento cronico con deslorelin è considerato presumibilmente sicuro quando l'animale viene trattato per un tempo illimitato e, potenzialmente, a vita, ma che sono necessarie ulteriori ricerche per confermare questa ipotesi.

Abstract

Deslorelin, a long-acting gonadotropin-releasing hormone agonist, is currently registered for the induction of temporary infertility in male dogs, male cats, male ferrets, and also prepubertal female dogs, but research has shown its usefulness for other conditions requiring chronic treatment. Efficacy and safety as well as return to fertility of such non-surgical neutering methods are well known following a single use but little if any data is available on prolonged use. To offer further support to this claim, we published a paper presenting six cases of dogs chronically treated with deslorelin between 2009 and 2022. The dogs presented in this paper were treated for 2 to 9 consecutive years for ensuring failure to reproduce (one case) as well as for conditions which are not found on the drug leaflet: benign prostatic hyperplasia and perineal gland disease (one case), control of reproductive behavior in male dogs (two cases) and urinary incontinence in spayed adult bitches (two cases). All animals were in good health during treatment and presented no short-term side effects. Flare-up reactions (an increase in reproductive behavior for 1–3 weeks after treatment) were observed in 1/4 intact males and were not observed in the spayed incontinent bitches. Deslorelin was effective in all treated dogs. Fertility was immediately regained in one male dog who sired a litter when his owner forgot to come back for re-treatment at the right time to maintain and prolong its effect. Two dogs developed a neoplasia: a spayed bitch treated for 3.5 years for urinary incontinence developed a pituitary carcinoma, and an intact male dog treated for 9 years for the control of fertility developed a bladder carcinoma. Both neoplasia were considered unrelated to the treatment. This paper provides valuable clinical information on new applications and chronic use of deslorelin. After almost 20 years of using the drug, it is essential to report all suspected or confirmed effects as these data are critical for the safe use of the drug and for the problems related to gonadectomy and its alternatives. Based on this work, deslorelin implants can be considered as a safe alternative to surgical castration in specific pathologies mediated by reproductive hormones and in situations where surgical castration is not an option, such as animals suffering from cardiovascular conditions or other systemic diseases which make anesthesia unsafe. Despite the limited sample size, we can state that chronic treatment with deslorelin is considered potentially safe when an animal is being treated for an unlimited time period and, potentially, for life, but that further research is necessary to investigate and confirm this hypothesis.

1. Introduction

Managing reproduction is a common request from owners and a recurrent conversation whenever there is a newly acquired dog. Today many options for fertility suppression are available, but surgical sterilization is still often the only one offered and the most commonly used in many countries, although important limitations are posed by the cost, the need of surgical environment and the surgical risk and/or anesthetic complications (ACC&D, 2021)

However, interest in contraception as a reversible, temporary and nonsurgical approach to reproduction control is growing. A wide array of pharmaceutical products is available that has been demonstrated to safely inhibit estrus in females and spermatogenesis in males of dogs for varying periods of time.

In fact, surgical castration has been prohibited as an elective procedure in Finland and Norway among other northern European countries and has limited application in other countries (Fossati, 2022), indicating a growing need for alternative methods to manage reproduction of pets.

These contraceptive methods include the use of steroid hormones such as estrogens, progestogens (e.g. megestrol acetate, medroxyprogesterone acetate and proligestone) and androgens (e.g. testosterone esters), or the use of protein hormones like gonadotropin-releasing hormone agonists (e.g. deslorelin, nafarelin and buserelin) and antagonists (e.g. acyline and prolactine) (Kutzler, 2018[a]). Even though studies on these drugs have been underway for decades, they are not available in many countries' markets (Kustritz 2018) and there is a need of further studies to prove their safety for clinical use.

1.1 Physiology of reproduction

The functionality of the reproductive apparatus is guaranteed by the interaction of three endocrine organs which make up the hypothalamus-pituitary-gonadal (HPG) axis. The hypothalamus synthesizes gonadotropin-releasing hormone (GnRH), which is transferred by the hypophyseal portal system to the adenohypophysis (the endocrine part of the pituitary gland) where it induces the release of luteotropic hormone (LH) and follicle stimulating hormone (FSH). These two hormones are also known as gonadotropins since they play a role in the development of the reproductive organs of both females and males.

Secretion of these hormones is regulated by a positive and negative feedback. When GnRH is released by the hypophysis it affects the hormones further down the HPG axis by also increasing their release and consequently their concentration, operating a positive feedback. On the other

hand, after GnRH stimulates the release of gonadotropins and consequently of estrogens, progesterone and/or androgens, these in turn decrease the secretion of the GnRH via a long loop mechanism and of LH and FSH via a short loop mechanism, resulting in a negative feedback that regulates their own concentration (Sjaastad et al., 2013).

It's of more recent discovery that the control of reproductive hormone's production is also regulated by the peptide kisspeptin, which binds to the receptors of neurons secreting GnRH and is involved in the mechanisms initiating GnRH release at puberty (Sjaastad et al., 2013).

1.1.1 Reproductive physiology of the female dog

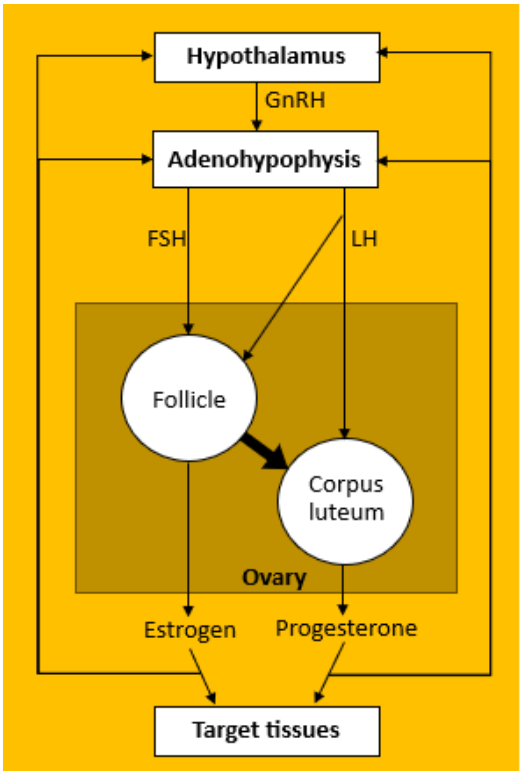
In the ovaries, LH stimulates the thecal cells to bind cholesterol and use it to biosynthesize testosterone, which induces the synthesis of estradiol precursors (Noakes et al., 2001). Both estrogens and testosterone have a retroactive action on the HPG axis, since they entail a negative feedback on the hypophyseal release of FSH and LH and the hypothalamic release of GnRH, which itself also causes a decrease in the release of FSH and LH (England, 2010[a]).

On the other hand, FSH induces ovarian granulosa cells to proliferate and produce estrogen, through the conversion operated by aromatase on testosterone released by the thecal cells (Sjaastad et al., 2013). As the follicles develop, the granulosa cells also become sensitive to LH and gain the capacity to produce estrogens directly from cholesterol. The interactions between theca and granulosa cells thus make it possible to achieve estradiol levels necessary for both ovulation induction and luteinization of granulosa cells, resulting in the formation of the corpus luteum.

Estrogens regulate estrus and follicular development, hence their relevance in the female reproductive physiology. They are at the base of the development of female secondary traits and accessory reproductive organs as well as male receptivity, in addition to the cyclic growth of the mammary gland and duct and stimulation of the myometrium for spermatozoa transport and parturition (Sjaastad et al., 2013). Estrogens also carry out a regulatory action on the HPG axis, which however is dependent on the concentrations of progesterone as well: when progesterone is abundant a negative feedback on GnRH occurs, on the contrary if its blood concentration is below 1.0 ng/mL there is a positive feedback (Sjaastad et al., 2013).

Besides the hormones and feedback loops whose involvement in the control of GnRH release has already been mentioned, an important regulation is also carried out by opiates, dopamine, melatonin and prolactin. More specifically, dopamine increases GnRH release and has a folliculogenic effect (Okkens and Kooistra, 2006), while melatonin's effect depends on whether the species has inductive or inhibiting photoperiod (Bittman et al., 1983; Grubaugh et al., 1982).

Figure 1.1 Schematic presentation of the mechanisms involved in the hypothalamic-pituitary-gonadic axis. The thick arrow demonstrates the evolution of the follicle to corpus luteum and the change in its receptiveness and production of hormones, while thin arrows document positive feed-back effects when going downwards and negative feed-back effect when pointing upwards. For clarity of the graph, activin and inhibin regulations within the HPG axis have been omitted.



Among females the aforementioned hormones play a central role in the regulation of the reproductive cycle, which differs greatly among various domestic species, but is usually composed of four stages: proestrus, estrus, diestrus and anestrus (England, 2010[a]).

Conventionally the beginning of the cycle is considered to coincide with the onset of proestrus, when follicles are developing while the corpus luteum from the previous cycle starts degenerating and therefore producing less progesterone, to the point where its blood concentration is less than 1.0 ng/mL, when, as previously mentioned, this results in a positive feedback on the release of GnRH and, consequently, gonadotropins. This feedback allows the FSH increase to complete the ovarian follicle development and, as the granulosa cells become more and more receptive to LH, the LH increase stimulates the steroidal synthesis in both thecal and granulosa cells (Sjaastad et al., 2013). As the follicles grow and enter the selection phase, they begin producing inhibin, which selectively inhibits the secretion of FSH from the adenohypophysis, but doesn't affect LH secretion, which avoids the growth of new follicles and produces follicular atresia (Sjaastad et al., 2013). The resulting increase in estrogens in the blood stream and consequent positive long loop feedback cause the GnRH secretion to become pulsatile and, in turn, a pulsatile release of LH. As

the LH pulses get increasingly frequent, the peaks fuse into a final LH surge and ovulation occurs (Pawson and McNeilly, 2005).

Ovulation occurs a few days after the estrogen peak and marks the concurrent initial increase in progesterone. At this stage, proestrus has already ended and estrus has begun, a period when the female is receptive towards the male and expresses species-specific mating behaviors due to high estrogen levels (England, 2010[a]). During this stage, estrogens and gonadotropins gradually decrease while progesterone keeps increasing as the follicle ruptures and transforms into a corpus luteum. In diestrus progesterone is the prevalent hormone, until the non-gravid corpus luteum encounters luteolysis and progesterone production drops, this is followed by anestrus of variable (3-10 months) duration, devoid of any estrous or reproductive activity and during which internal and external genitalia are at their smallest observed size (Weems et al., 2006).

The first two stages constitute the follicular phase of the cycle, since they center around the development of the ovarian follicles, which are recruited in a group, grown until an appropriate group is selected and these follicles become dominant.
Diestrus and anestrus on the other hand constitute the luteal phase, as the corpus luteum substitutes the ovarian follicle and produces progesterone to maintain a hypothetical pregnancy (England, 2010[a]).

Bitches are non-seasonal monoestrus species (Kutzler, 2018[b]), which means that they usually have one or two reproductive cycles per year with each one lasting on average 7 months and occurring usually in spring and autumn. However, in most cases, smaller breeds tend to have more reproductive cycles a year, hence shorter ones, when compared with larger breeds (Christie and Bell, 1971).

The interestrus interval lasts an average of 31 weeks, ranging typically between 16-56 weeks (Kutzler, 2018[b]).
The length of each stage results grossly unaltered by the season or a successful conception and is averagely (Concannon, 2010):

- Proestrus: 9 days (range: 5-20 days)
- Estrus: 9 days (range: 6-11 days)
- Diestrus: 60 days (range: 45-70 days)
- Anestrus: 5-7 months (range: 3-10 months)

Proestrus can easily be identified through a clinical evaluation since it's characterized by serosanguineous discharge from an edematous vulva (enlarged and reddened) due to the increased vascularity of the uterus and external genitals promoted by estrogens (Sjaastad et al., 2013). In

addition, an ethologic assessment may detect significant behaviors ascribable to the hormonal levels in proestrus, as, during this stage, the bitch attracts the male dog but is not receptive to mating. Additionally, she may show increased urine marking and roaming (England, 2010[a]).
In estrus, bitches are receptive to mating and show inviting behavior: standing to be mated, upward winking of the vulva, spinal lordosis and vertical deviation of the tail in response to tactile stimulation of the skin to the vulva's side (Romagnoli, 1992). A cytologic exam on a vaginal smear will reveal > 90% of cornified epithelial cells. Progesterone serum concentrations reach 10-25 ng/mL in bitches by the end of the estrus (England, 2010[a]).

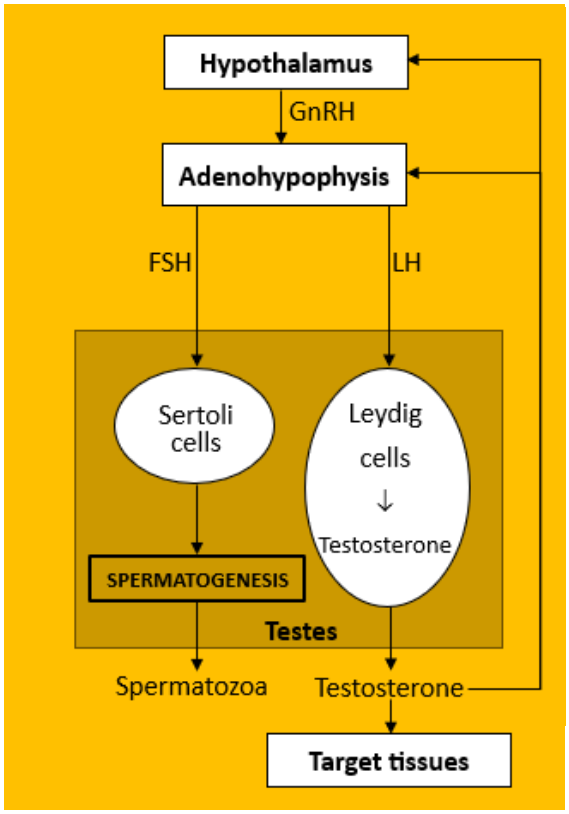
As the dog is a polytocous species, usually more than one follicle becomes dominant and then ruptures during ovulation and it's unlikely for them to be synchronized, which is why ovulation is usually considered complete 3 days after the LH surge and fertilization of the oocytes in the oviduct to occur around 3 days after that. This additional temporal window is due to the fact that the bitch ovulates immature oocytes, which cannot be fertilized immediately and need to carry out the first meiotic division and become secondary oocytes (England, 2010[a]). This timeline is averagely valid, however can extend to a longer fertilization period since oocytes remain viable in the reproductive tract for an average of 5 days and may begin degeneration as late as 14 days after ovulation (Noakes et al., 2001). Oocytes may therefore be fertilized a few days later, still maintaining maximum fertility, before this begins declining rapidly as the cells start degenerating and the cervix closes.

1.1.2 Reproductive physiology of the male dog

The male reproductive system is also subject to the endocrine control of the HPG axis: GnRH regulates the release of the aforementioned gonadotropins from the anterior pituitary gland. A negative feedback controls the release of GnRH at hypothalamic level and gonadotropin release at pituitary level, as all the hormones down the HPG axis in consequence to GnRH action, including FSH, LH testosterone and its active metabolites (estradiol and dihydrotestosterone) also take part in these loops (England, 2010[b]).

FSH has a stimulating effect on the Sertoli cells (the nutritional cells of the testes) which convert testosterone in estrogens and stimulate germ cells to activate spermatogenesis. This effect is supported by the testosterone produced thanks to the LH-stimulated endocrine activity in the Leydig cells (Sjaastad et al., 2013).

Figure 2.2 Schematic presentation of the mechanisms involved in the hypothalamic-pituitary-gonadic axis. The arrows document positive feed-back effects when going downwards and negative feed-back effect when pointing upwards. For clarity of the graph, inhibin regulation within the HPG axis has been omitted.



The brief schematization of the main functions of the cell types in the seminiferous tubules in Figure 1.2 already introduces the two main functions of the male reproductive system: the production of sperm (spermatogenesis) and the production of steroid hormones (steroidogenesis) (Noakes et al., 2001[b]).

Spermatozoa are produced in the seminiferous tubule, originating from the basal zone where spermatogonia begin dividing by mitosis and differentiating, as they move towards the adluminal zone, into primary spermatocytes, secondary spermatocytes and spermatids (Sjaastad et al., 2013). Once the stage of spermatid production has been reached, Sertoli cells start producing inhibin to promote a negative feedback that inhibits FSH release selectively, without affecting that of LH. In addition, Sertoli cells form junctional complexes separating the basal and adluminal zones and effectively producing a barrier between blood and testis (England, 2010[b]).

Meanwhile, steroid hormones are produced by the Leydig cells in the interstitial tissue surrounding the seminiferous tubules. These cells have specific LH receptors that initiate and regulate steroidogenesis. Testosterone is an important androgen, essential for its role in the development of secondary male traits and behavior, function of the accessory glands, production of male gametes, descent of testes in the scrotal sac and the regulation of libido (England, 2010[b]).

1.2 Control of reproduction in the female

Control of reproduction in the bitch is a common request for a vetierinary clinician. The pet owner may seek a temporary or permanent solution to avoid hormone-dependent behavioral changes or physiological events occurring during proestrus or estrus, which range between attempts to escape, aggressiveness towards other animals and/or humans, disobedience, attractiveness to males and vulvar bleeding (Romagnoli and Sontas, 2010). The request may also be intended to prevent undesired pregnancies, dystocia, postpartum diseases and/or hormone-dependent pathologies, such as vaginal hyperplasia or uterine diseases (Reichler, 2009).

The veterinarian may suggest a surgical or non-surgical method, with the former dominated by ovariectomy and ovariohysterectomy as the most common techniques and the latter using parenteral administration of steroidal or nonsteroidal agents as the preferred approach.

The medical approach to prevent breeding is based on administration of synthetic analogues of progesterone or androgens such as testosterone (for their negative feedback on the HPG axis) or long-acting GnRH agonists.

1.2.1 Sterilization

Sterilization is permanent cessation of reproduction via surgical removal of the gonads. It produces complete cessation of fertility, associated with disappearance of reproductive behaviors. This feature may be sought by owners as our society considers some aspects of reproductive behaviors in pets annoying (such as serosanguinous vulvar discharge during bitches' estrous cycle) or undesirable (mounting from male dogs) (Cheryl, 2018).

Surgical methods for sterilization in bitches are ovariohysterectomy (OHE), ovariectomy (OVX), and more rarely, hysterectomy and tubal ligation.

OVX is generally the most practiced as, compared to OHE, it is technically less complicated, surgery is less invasive, therefore usually shorter in duration, with less morbidity and pain (Van Goethem et al., 2006).

Gonadectomy in bitches is associated with various advantages: decreased incidence of mammary gland and uterine diseases such as neoplasia (Priester, 1979; Dorn et al., 1968, Misdorp, 1988), pyometra and mastitis; complete absence of ovarian diseases such as neoplasia and cysts; complete absence of progesterone (i.e. false pregnancy and pyometra) or estrogen-related diseases (i.e. vaginal hyperplasia or prolapse) and pregnancy or parturition-related diseases, like abortion, dystocia, uterine prolapse and subinvolution of placental sites (Romagnoli and Sontas, 2010).

However, the surgical approach is an irreversible method and includes disadvantages: generic surgical risk; some behavioral abnormalities; obesity (McGreevy et al., 2005); increased risk of non-reproductive cancers such as osteosarcoma (Hart et al., 2016; Ru et al., 1998; Cooley et al., 2002; de la Riva et al., 2013), hemangiosarcoma (Ware et al., 1999; Prymak et al., 1988; Gruntzig et al., 2016), lymphoma (Zink et al., 2014; Hart et al., 2014) and cutaneous mast cell tumors (Hart et al., 2014; White et al., 2011); orthopedic disorders like hip dysplasia (Spain, 2004; de la Riva et al., 2013; Hart et al., 2016), patellar luxation (Kustritz et al., 2017) and cranial cruciate ligament injury (Slauterbeck et al., 2014; Kustritz et al., 2017); osteoporosis (Burrow et al., 2005); coat changes (Scott, 1990) and atopic dermatitis (Spain et al., 2004; Sundburg et al., 2016); chronic vaginitis (Verstegen-Onclin and Verstegen, 2006), urethral sphincter mechanism incompetence leading to urinary incontinence (Okkens et al., 1981; Spain et al., 2004; Thrusfield et al., 1998; Byron et al., 2017; Forsee et al., 2013) and urolithiasis (Okafor et al. 2013; Wisener et al., 2010).

In addition, ovarian surgery can produce some complications: in the short-term bleeding at the ovarian pedicles may occur after OVX or OVH, producing significant postoperative hemorrhage (van Goethem et al., 2006), while in the long-term pyometra or persistent reproductive behaviors may result from ovarian tissue remnants after OVX or OVH (Pearson, 1973).

Recent research has been highlighting the potential role of LH in the insurgence of these side effects. Removal of the ovaries results in a decreased estrogen production, preventing the negative feedback on the pituitary and hypothalamus. As a result, LH maintains elevated concentrations, affecting the reproductive tract but also all the tissues presenting LH receptors: thyroid and adrenal glands, pancreas, gastrointestinal tract, urinary tract, joints and ligaments, lymphocytes and several neoplastic tissues (Kutzler, 2020). Although the role of these receptors throughout the body is not completely known, their role in the etiology of increased incidence of several sterilization complications is gradually unfolding. The possibility that LH receptor activation plays an important role in these mechanisms continues to be investigated (Kutzler, 2020).

Hysterectomy, or ovary-sparing spay (OSS), has been rarely performed in the past and has been given more attention in the last decade. Dogs that have undergone this procedure continue their estrous cycle but should not have any vulvar discharge, however both short-term and long-term concerns are reported, such as possible abnormalities in length of estrous cycle, increased signs of false pregnancy, ovarian cysts and fibrosis, inflammation of the uterine stump and mammary neoplasia (Hoveler et al., 1987; Hoffman et al.1992; Kaszak et al., 2016). The preservation of ovaries in OSS preserves hormonal production and allows the HPG negative feedback to remain

intact, resulting in advantages in limiting the incidence of potentially LH-mediated diseases after sterilization (Kutzler, 2020).

1.2.2 Progestogens

Synthetic analogues of progestogens have long been used for temporary or prolonged delaying or suppression of estrus. Their mechanism of action involves acting as progesterone agonists on their receptors and inhibiting the release of GnRH from the hypothalamus, hence suppressing the pituitary-ovarian communication and resulting in lowered gonadotropin release. Responsiveness to progestogens determines duration of effect, which increases progressively with anestrus (Max et al., 2014).

Various compounds are currently available on the market: medroxyprogesterone acetate (MPA), megestrol acetate (MA), delmadinone acetate (DMA), chlormadinone acetate (CMA) and proligestone (PGS) (Romagnoli and Concannon, 2003).

MPA is a long-acting progestogen available as an oral or depot formulation; the latter is possible since the molecule is metabolized slowly by the liver and therefore effective concentrations can be maintained for various months after a single injection (Jordan, 1994). Suggested dosages in bitches are 2.5-3 mg/kg every 4-5 months via an intramuscular or subcutaneous single injection (Romagnoli, 2002). The drug should be administered in anestrus to maximize its progesterone-agonist effect and avoid the pulsatile secretion of GnRH necessary for estrus, resulting in an effective and prolonged postponement of ovulation (Jackson, 1984).

MA is a short-acting progestogen, making it more indicated for temporary estrus delay. It is available as an oral and parenteral formulation. Oral MA dosages vary according to the stage of the estrous cycle with a prolonged administration at lower dosages in anestrus and shorter administration at higher dosages in pro-estrus (Burke and Reynolds Jr, 1975).

DMA and CMA may be used for prolonged postponement of the estrous cycle, but scarce information is currently available in bitches (Romagnoli and Sontas, 2010).

Proligestone is the newest form of long-lasting progestogen commercially available and has been studied for a more selective action on the HPG axis and less progestogenic activity compared to other synthetic progestogen analogues, hence less side effects (Selman et al., 1996).

These compounds act on all target organs of progesterone, therefore inducing increased endometrial growth and secretion, cervical closure and proliferation of mammary parenchyma. Side effects are usually negligible when progestogens are administered with the correct posology

and for the right period of time in healthy females (Romagnoli and Sontas, 2010). Therefore, incidence of adverse effects appears to be associated with overdosing, wrong choice in patients (in case of concomitant diseases or pregnant females) and/or administrating outside of anestrus (Jochle, 1991), which commonly cause increased appetite, increased thirst, weight gain, restlessness and lethargy (Burke and Reynolds Jr, 1975; Sawada et al., 1992). Synthetic formulations vary in their affinity for receptors for other steroid hormones, such as androgens (Kloosterboer et al., 1988) and glucocorticoids (Fekete and Szeberenyi, 1965; Selman et al., 1996), which determines potential side effects.

High dosages may increase the frequency of uterine and mammary diseases (Anderson et al., 1965; Brodey, 1966), as well as adrenocortical dysfunction (Selman et al., 1997; McCann et al., 1987), immunosuppression (Turcotte et al., 1968), Cushing syndrome (Selman et al., 1997), acromegaly (Eigenmann et al., 1983; Selman et al., 1991), insulin resistance and diabetes mellitus (Sloan and Oliver, 1975; Selman et al., 1997; McCann et al., 1987), dermal lesions (Bell et al., 1993; Scott and Concannon, 1983) and altered behavior (Beijerink et al., 2007; Knol and Egberink-Alink, 1989). This may result in hair loss and skin discoloration at the injection site (Scott and Concannon, 1983), formation of mammary nodules (Misdorp, 1988; Misdorp, 1991), cystic endometrial hyperplasia (Kim and Kim, 2005), pyometra (Nelson and Kelly, 1976), fetal developmental defects (e.g. masculinization of female fetuses) (Wilkins, 1960), lactation arrest and delayed parturition if administered to pregnant females (although they may be used to support pregnancies in case of luteal insufficiency and to prevent risks of abortion) (Romagnoli and Sontas, 2010).

1.2.3 Androgens

Similarly to progestogens, androgens operate a negative feedback on the HPG axis and suppress the release of gonadotropins. In addition, the presence of androgen receptors in estrogen-target tissues may, after binding, reduce the functional response to estrogen (England, 1997).

Treatment with androgens, in veterinary practice, usually relies on the administration of testosterone and its esters or mibolerone in the first half of anestrus (Romagnoli and Sontas, 2010). Mibolerone is a synthetic androgen used for long-term estrus suppression in bitches as an oral preparation given daily (Wiebe and Howard, 2009), but its use is not recommended for a period longer than 2 years (Romagnoli and Sontas, 2010). Mibolerone was taken off the veterinary market in North America in the mid-1990's. Small animal practitioners still occasionally prescribe it as a compounded formulation, however research-based data on the clinical use of mibolerone in the dog are very scant.

Side effects after administering androgens at excessively high dosages, for too long or to the wrong patient include: vaginitis, hypertrophy of the clitoris, atrophy of the endometrial lining, cervical closure, thickening of cervical dermis, atrophy of mammary parenchyma, lactational arrest, fetal development arrest or masculinization of female fetuses, increased appetite and bodyweight, increased libido and aggressiveness, anabolic effects, urinary incontinence and/or spraying, skin discoloration, epiphora and growth of anal hepatoid glands (Burke et al., 1977; Burke, 1982; Maenhoudt et al., 2014).
Androgens are not usually recommended in bitches intended for future breeding, younger than 7 months old, pregnant, with androgen-dependent neoplasms or animals with known hepatic or renal conditions (Romagnoli and Sontas, 2010).

1.2.4 Gonadotropin-releasing hormone agonists

GnRH analogs, including both agonists and antagonists, are produced by amino acid substitutions within the original GnRH molecule resulting in greater potency and a longer effect.
GnRH agonists activate the GnRH receptor determining temporary increased secretion of FSH and LH, however, after this initial stimulation, all the reserves of gonadotropins are depleted and their production is decreased due to the downregulation of the receptors the GnRH agonists interact with. The sensitivity of the pituitary gland to GnRH and the indirect response of the ovary to it changes from early to late anestrus, with a significant increase in pituitary sensitivity to GnRH (as shown by circulating LH concentrations) and an increase in ovarian responsiveness to LH and FSH (Kutzler 2018[b]). This interaction effectively desensitizes the adenohypophysis to the stimulatory effects of GnRH, resulting in suppression of gonadotropin receptors or intermediary enzymes involved in the steroidogenic pathway in the ovaries. This effect is reversible if the treatment is interrupted but can be maintained with chronic administration of the medication (Herbert and Trigg, 2005).

The interval between the treatment and onset of receptor downregulation amounts to 3-4 weeks (Fontaine and Fontbonne, 2011; Duygu and Serhan Serhat, 2017), during which ovarian activity is usually stimulated as the GnRH agonist mimics GnRH. This initial flare-up effect may also result in the induction of a fertile estrus (Borges et al., 2015) 4 to 8 days after implantation (Trigg et al., 2001). This induced estrus is comparatively shorter than a natural estrus (Fontaine et al., 2011) and is characterized by normal estrous signs if the bitch is treated in anestrus, while administration in diestrus produces a surge in progesterone secretion as most common effect (Romagnoli and Sontas, 2010).

GnRH agonists are used in veterinary medicine with different applications depending on the formulation. Long-acting GnRH agonists such as deslorelin or azagly-nafarelin remain active over a long period of time and can be used for reproduction management (estrus induction, delay and suppression), while short-acting GnRH agonists (e.g. gonadorelin, goserelin, lecirelin, peforelin, buserelin and fertirelin) take effect rapidly and are used especially to treat diseases such as ovarian cysts, endometriosis and endometrial hyperplasia, as well as disorders due to GnRH deficiency (Hardman and Limbird, 2001).

Deslorelin acetate made new progress in the control of bitches' reproduction as a long-acting GnRH agonist. After subcutaneous application, low doses of the drug are continuously released, thereby masking natural GnRH pulses from the hypothalamus (Romagnoli et al., 2009) and ultimately suppressing the HPG axis. Studies have been conducted with bitches that show the subcutaneous biodegradable implants to be effective if administered in diestrus (Trigg et al., 2001) and can also be repeated to determine a long-lasting suppression of the estrous cycle. Nonetheless, deslorelin is currently only authorized for temporary infertility in adult male dogs and ferrets, male cats and prepuberal bitches; hence its use in post-puberal bitches is to be regarded as 'off-label' (Amaral et al., 2023).

Side effects reported following deslorelin implant administration include: persistent estrus (including ovarian cysts), endometrial proliferation and secretion, proliferation of mammary parenchyma during the first 2-4 weeks after treatment, cervical closure, possible atrophy of mammary parenchyma, induced lactation and/or lactational arrest, pyometra, weight gain, coat and behavioral changes (Fontaine and Fontbonne, 2010; Arlt et al., 2011; Palm and Reichler, 2011). In addition to these, after prolonged GnRH treatment in intact females some studies report coat changes, hip dysplasia, false pregnancy, urinary incontinence, endometrial hyperplasia, and ovarian tumors (Brandli et al., 2021). The more serious side effects were reported after later onset of treatment (Palm, 2011). A comparative assessment for many of these major side effects that are in common with gonadectomy is currently not possible due to lack of research on chronic use of deslorelin in bitches, where the use is off label but not unheard of (Borges et al., 2015; Romagnoli et al., 2009; Körber et al., 2013).

Currently the 'flare-up' effect is still the major limitation for the use of deslorelin implants as a method for contraception in adult female dogs and is especially crucial if population control is to be addressed. However, in case of privately owned dogs, it remains a valid alternative to surgical methods, as an effort can be made to avoid mating during this critical temporal window. In addition, treatment can be appropriately timed, as studies show that when deslorelin is

administered to some diestrous bitches (with serum progesterone levels > 5.0 ng/mL), the initial flare-up is suppressed (Trigg et al. 2001; Wright et al., 2001). Another limitation to this approach to estrus suppression is that the duration efficacy of deslorelin seems to be dose related and with great individual variation within the same dose (Trigg et al., 2001). A solution to this issue would be multiple serial implant administration, which has shown good results, without adverse effects or diminished efficacy in male dogs (Trigg et al., 2004), but requires further investigations in bitches. Nonetheless, reports show that both female and male dogs that have received implants have regained normal fertility after termination of the implants' efficacy (Herbert and Trigg, 2005; Stempel et al., 2022; Borges et al., 2015; Lucas, 2014).

1.2.5 Gonadotropin-releasing hormone antagonists

These are synthetic peptides that compete with GnRH at the receptor level but are devoid of stimulatory activity and block GnRH action, leading to an immediate, dose-dependent, pituitary suppression without an initial stimulation of the HPG axis. The resulting suppression of gonadotropin release (Heber et al., 1982) can be used for estrus prevention (Noakes et al., 2019) or effective early pregnancy termination (Vickery et al., 1989). Due to their high costs, their use is currently more widespread in human medicine (Fontbonne, 2010), while in regard to dog reproduction the information is scarce and further work is required before they can be recommended.

While the development of GnRH agonists progressed quickly, the antagonists lagged behind, due to their high cost of production and the first generations of GnRH antagonists being too weak and exhibiting major side effects (Heber et al. 1982). Specifically, GnRH antagonists may induce degranulation of mast cells, hence a significant histamine release resulting in anaphylactic reactions, which, in addition to being short-term formulations and having local solubility limitations, adversely affected the widespread usefulness of the early generations of antagonists (Fieni et al., 1998).

More recently, a new series of potent, acceptably long-acting, water-soluble and low histamine-release third-generation GnRH antagonists were developed (Jiang et al., 2001; Broqua et al., 2002), with cetrorelix, abarelix and ganirelix already on the human pharmaceutical market, and antarelix, teverelix, degarelix, ozarelix, ornirelix, azaline B and acyline undergoing clinical trials in the last decade (Gobello, 2012). The rapid suppression of the pituitary achieved by the antagonists, without an initial stimulatory effect, is the main advantage of these compounds over the agonists. This effect may even be used to prevent the 'flare-up' effect in GnRH agonist-implanted bitches, by associating the implants with a single acyline injection (Gobello, 2012).

1.3 Control of reproduction in the male

Prevention of fertility in male dogs is a commonly requested procedure from pet owners and there are different ways a veterinarian can provide this service effectively and safely. The most common and widely used techniques are surgical sterilization such as orchiectomy or vasectomy, intra-epididymal or intratesticular sclerosing agents, immunosterilization (which uses the immunological system with vaccinations against GnRH, the luteinizing hormone LH receptor and the zona pellucida proteins) and medical suppression of spermatogenesis. Each available alternative presents different advantages and possible side effects (Maenhoudt et al., 2014).

In male dogs, surgical castration is the most common sterilization technique (Trevejo et al., 2011). Orchiectomy consists in surgical removal of the testes and is completely effective and permanent. It offers benefits such as prevention of testicular neoplasia and androgen-dependent diseases including benign prostatic hyperplasia (Rhodes, 1996), prostatic neoplasia (Bryan et al., 2007), chronic prostatitis (Reichler, 2009), perianal adenomas (Wilson and Hayes Jr, 1979) and perineal hernias (Maute et al., 2001). In addition, gonadectomy in males is often associated with a decline in reproductive behaviors (Trevejo et al., 2011).
On the other hand, the medical approach is based on hormonal downregulation and involves different classes of drugs to achieve contraception: progestogens, androgens, anti-androgens and gonadotropin-releasing hormone agonists.

1.3.1 Sterilization

In male dogs, orchiectomy is the most common method of contraception, however, postoperative complications are also to be expected in many cases, these vary from short-term problems such as hemorrhage from the pedicle of the spermatic cord and scrotal bruising, to long-lasting effects like the development of obesity and urinary incontinence (Maenhoudt et al, 2014). In addition, castration is correlated with an increased incidence of scirrhous cords, transitional cell carcinoma and prostatic neoplasia in male dogs (Kustritz et al., 2017). Similarly to what was already described in spayed female dogs, castrated male dogs also present an increased risk of joint disorders (Hart et al., 2014), hemangiosarcoma (Ware et al., 1999; Prymak et al., 1988), osteosarcoma (Ru et al., 1998) and lymphoma (Zinc et al., 2014).

Recent research on the role of sustained supraphysiologic LH concentrations in gonadectomized dogs is showing a potential causative link with many long-term health complications of neutering. Removal of the testes results in a decreased testosterone production, preventing the feedback on the HPG axis. As a result, LH maintains elevated concentrations, affecting the reproductive tract

but also joints, thyroid and several neoplastic tissues presenting LH receptors. Although precise etiology is still unknown, an increase in LH concentrations could play a central role in endocrinological and musculoskeletal diseases, as well as in contributing to neoplastic growth after gonadectomy (Kutzler, 2020).

Another surgical approach is vasectomy, which involves bilateral removal and/or occlusion of a portion of the vas deferens, which renders the animal infertile by preventing sperm from being ejaculated during copulation (Pérez-Marín et al., 2006). This procedure doesn't interfere with the androgen production, which means male secondary sex characteristics and reproductive behaviors, as well as androgen-dependent diseases, are not prevented (Zhang et al., 2012). In addition, overtime it appears to be associated with seminiferous tubules degeneration and possible formation of spermatoceles or sperm granulomas (Pérez-Marín et al., 2006; Zhang et al., 2012). Given its scarce benefits to the animal, vasectomy has been rarely performed in the past but has been the object of attention from clinicians and researchers lately due to the above-mentioned side effects of gonadectomy.

Studies have shown testosterone-dependent behaviors, such as mounting, roaming, inter-male aggression and urine-marking, to be reduced by about 50% to 70% but not necessarily eliminated with castration, resulting in another possible presenting complaint for some dog owners (Hopkins et al., 1976; Neilson et al., 1997; Maarschalkerweerd et al., 1997).

1.3.2 Progestogens

This class of compounds acts by mimicking the effect of progesterone, therefore exogenous administration operates a negative feedback on the HPG axis. The consequent suppression of GnRH and gonadotropin secretion inhibits spermatogenesis.

Progestogens may be used to reduce reproductive behaviors attributed to testosterone (Knol and Egberink-Alink, 1989) as well as for contraception (England, 1997), but they are more commonly used in females.

Progestogens such as megestrol acetate and medroxyprogesterone acetate are synthetic progestogens. MA is metabolized rapidly when administered orally in male dogs and exhibits a half-life of 8 days. Studies show that low dosages (2 mg/kg for 7 days) don't always produce changes in semen quality and higher doses produce minor secondary sperm abnormalities (Kutzler, 2010). Hence, it may be effective in suppressing reproductive behaviors but not sufficient to block spermatogenesis and ensure contraceptive efficacy (Asa, 2018). This is not coherent with the effect

of MA in bitches, where the low dose treatment is usually effective in interrupting the estrous cycle.

On the contrary, MPA is a long-acting injectable form which has been demonstrated to be a more effective contraceptive compared with MA. It produces a rapid response, while significantly decreasing the number of spermatozoa ejaculated and their percentage with normal morphology and motility (England, 1997). The change in semen quality occurs a week after treatments and only high doses of MPA appear to produce deleterious effects on sperm quality (Maenhoudt et al., 2014). No changes in libido and/or sexual behavior were observed following MPA treatment, even after high doses of MPA (England, 1997).

However, this form of contraception used chronically may cause side effects such as adrenocortical suppression, dermatological alteration (ex. alopecia, hair discoloration, skin thinning), weight gain, lethargy and mammary nodules or hypertrophy (Maenhoudt et al., 2014).

1.3.3 Androgens

Androgens operate a negative feedback that produces a suppressive effect on the release of LH, therefore causing a decrease in serum and intratesticular testosterone concentrations and consequent reduction in spermatogenesis.

A subcutaneous injection of 5 mg/kg of testosterone esters (proprionate, phenylpropionate, isocaproate or decanoate) in a male dog produces an important decrease in the spermatozoa's motility within 3 weeks from the treatment, which persists for 3 months (Kutzler, 2010).

The main limitation to this form of treatment is that exogenous administration of testosterone analogues increases predisposition to androgen-dependent diseases. In addition, it may directly stimulate libido and prostatic growth (Romagnoli, 2009)

1.3.4 Anti-androgens

Several groups of compounds have been described to have anti-androgenic properties through different mechanisms of action, such as progestins, receptor binding anti-androgens, competitive enzyme inhibitors, aromatase inhibitors, GnRH analogues, estrogens and anti-estrogens (Bamberg-Thalén and Linde-Forsberg, 1992). More specifically, receptor binding anti-androgens include steroidal and non-steroidal compounds that interact with androgen receptors, forming a complex that, although unstable and transitory, blocks the effect of androgens that may otherwise occupy the site and affect their target cells (Kutzler, 2010). Unlike androgens, these compounds do not induce androgen-dependent gene transcription and protein synthesis.

Flutamide and nilutamide are both non-steroidal receptor binding anti-androgens, the former inhibits androgen uptake and nuclear binding by binding to the androgen receptor in the target tissues (Neri, 1989), while the latter is characterized by a longer plasma half-life that allows higher concentrations to be reached, leading to an extended inhibition of androgen binding (England, 1997). In dogs, administration of either type of anti-androgens has shown to affect spermatogenesis only slightly.

The main clinical application of anti-androgens in dogs is benign prostatic hyperplasia, in addition to management of testosterone dependent behavioral problems and reversible suppression of fertility (Gobello, 2006).

1.3.5 Gonadotropin-releasing hormone agonists

GnRH agonists cause transient increases in serum LH and testosterone concentrations, which are followed by a massive decrease once downregulation of anterior pituitary GnRH receptors ensues. This causes testosterone levels to drop to basal concentration due to the decrease in gonadotropic support (Maenhoudt et al., 2014), triggering lack of spermatogenesis in most seminiferous tubules (Goericke-Pesch et al. 2009) and consequent oligozoospermia or azoospermia within 1-2 months after drug administration (Junaidi et al. 2009). Reduced semen quality is also due to reduced semen volume and massive increase in tail abnormalities of the spermatozoa produced (Driancourt and Rhodes, 2018). This cascade leads to shrinkage of the testicles and prostate, with testicles commonly reducing to <50% of the pre-treatment volume (Junaidi et al., 2009).

Inoculation of a 4.7 mg slow-release subcutaneous implant of deslorelin reduces LH and testosterone concentrations in plasma to undetectable levels within 4 weeks and ceases semen production and libido within 5-6 weeks (Trigg et al., 2006). Onset of complete suppression of fertility with this implant may typically occur between 1 and 2 months after treatment (Driancourt and Briggs, 2020). By 26 days after implantation, atrophy of the testes and prostate gland is induced, explaining the reported loss of ejaculate and arrest of sperm output (Junaidi et al., 2009).

Studies have shown that consecutive administration of multiple implants at 6-month intervals are well tolerated and do not result in adverse effects or diminished efficacy (Trigg et al., 2006), therefore offering an option to generate permanent efficacy. Systematic re-implantation of long-term release GnRH agonists could also offer an option for dogs diagnosed with hormone-dependent diseases that have unacceptable risk factors for anesthesia or surgery.

The duration of fertility suppression of the deslorelin implants is variable, with the minimum duration of efficacy reaching 180 days (4.7 mg implant) and 400 days (9.4 mg implant) (Trigg et

al., 2006), but the median duration of efficacy of the 4.7 mg implant reaching 300–400 days (large and small dogs respectively) (Trigg et al., 2006).

Depending on the administered implant's dosage, recovery of normal testosterone levels and normozoospermia also varied in time (Romagnoli et al. 2012). Complete return of semen quality to pre-treatment values was observed by 62 (3 mg) to 102 (12 mg) weeks post-implantation (Junaidi et al. 2009). Important individual variations are also to be expected.
Continuous administration or long-term release formulations of GnRH agonists reversibly suppressed reproductive function in male dogs (Inaba et al., 1996). This is also demonstrated at a histological level, in accordance with data on endocrine variables and semen production (Junaidi et al. 2009).

Deslorelin has undergone studies to prove its effectiveness as a contraceptive in male dogs, where it is labelled as having a 98% efficacy for at least 6 months and complete reversibility (Herbert and Trigg, 2005), however duration of the implant's effect is unpredictable due to significant individual variability (Maenhoudt et al., 2014).

During the first days post-implantation, until desensitization to GnRH is achieved, there is a short-lived step during which exposure to the GnRH agonist has stimulatory effects on the pituitary gonadal axis. During this "flare-up phase," an acute increase in serum testosterone occurs and may cause transitory changes in behavior, specifically increase in reproductive behavior and aggression toward other male dogs (Driancourt and Briggs, 2020). This may constitute a major limitation to use of deslorelin in dogs with sociopathic disorders related to aggression (EMA, 2018) due to possible heightened behavior response.

1.3.6 Gonadotropin-releasing hormone antagonists

GnRH antagonists occupy the GnRH receptors on gonadotrope cell membranes, making them unavailable and causing a dose-dependent, suppression of the HPG axis. Unlike GnRH agonists, the effect is immediate and no flare-up effect occurs (Kutzler, 2010).
In male dogs, a single subcutaneous dose of acyline (a third-generation GnRH antagonist) safely and reversibly decreased serum gonadotropins and testosterone concentrations for 10 days and prevented physiological response of the HPG axis to agonistic challenge for up to 14 days (García Romero et al., 2012). The same protocol reversibly impaired spermiogenesis, spermatocytogenesis and semen quality (sperm count, morphology and total motility), in addition to libido and erection which were also affected during the first month of the follow-up period (Gobello, 2012).

1.4 Deslorelin

As already mentioned, deslorelin is a synthetic analogue of gonadotropin releasing hormone, approved for use in veterinary medicine. It is currently licensed in certain countries for long-term suppression of fertility or as a reproduction-control drug in dogs and ferrets. Moreover, deslorelin acetate is used in reproductive management across various species and stops the production of sex hormones.

1.4.1 History

Deslorelin acetate's initial use was as a drug promoting ovulation within 48 hours in mares in preparation for artificial insemination, thanks to the initial flare-up effect on the pituitary gland and its associated surge of LH secretion (Oglesby, 2005).
It was first developed by the UK company Dechra and commercialized as Ovuplant™ (Dechra) in a biocompatible sustained-release subcutaneous implant. Its registration by the FDA was achieved in June 1998 for sales in the United States (Peptech Animal Health, 1998), becoming the first hormone approved for inducing ovulation in mares in the country. It was demonstrated that short-term deslorelin implants induced ovulation comparably to 3000-5000 IU of hCG, confirming its efficacy (Bradecamp, 2007). Although hCG costs approximately ⅙ of Ovuplant price it was well-received as an alternative to mares presumed to be refractory to hCG administration due to its inclination to induce antibody formation (Squires and Simon, 2011).

During the second breeding season after Ovuplant's commercialization, reports presented an increase in the number of mares with prolonged interovulatory intervals had they failed to conceive in the cycle. In 2000 and 2001 this effect was traced back to the fact that after the initial surge in LH, deslorelin acetate causes downregulation of GnRH receptors and consequent drop in gonadotropins, effectively inducing prolonged interestrus in cycling mares. The recommendation was to remove the implant once ovulation was confirmed to avoid deslorelin's later effects, so methods to remove the implant after 48 hours of injection in the mare were developed (Farquhar et al., 2002).

The molecule's applications widened as Virbac started marketing Deslorelin acetate as Suprelorin™ in 2007 (EMA, 2010), a slow-releasing deslorelin acetate subcutaneous implant officially used to control fertility in male dogs. It became legal for male dog contraception in Australia and New Zealand the same year and in the European Union in 2008 (EMA, 2010). In 2020, the 4.7mg implant was launched in China and Mexico.

1.4.2 Molecule

Deslorelin is an analogue of the natural decapeptide GnRH which binds to specific G protein-coupled receptors on pituitary gonadotropes. Their activation leads to phosphoinositide breakdown with production of inositol 1,4,5-trisphosphate (Ins(1,4,5)P3) and diacylglycerol. These compounds act as second messengers and initiate Ca2+ release from intracellular stores and activation of protein kinase C, thereby increasing both the synthesis and secretion of gonadotropins (Hardman and Limbird, 2001).

Prolonged activation of GnRH receptors on the pituitary cells by GnRH, after a brief stimulatory period, triggers down-regulation, as the GnRH receptors are internalized and the intracellular signaling cascade is deactivated (Driancourt and Briggs, 2020). Pituitary desensitization leads to suppressed gonadotropin secretion, which is the primary mechanism of action of all analogues of long-acting GnRH agonists.

Deslorelin differentiates from endogenous GnRH for the amino acid in the sixth position of the sequence, since the glycine of the gonadotropin-releasing hormone is substituted with tryptophan, and for the loss of the tenth amino acid, a glycine (Hardman and Limbird, 2001). Hence, the name deslorelin corresponds to the following sequence: (6-D-tryptophan-9-[(N-ethyl-L-prolinamide]-10-desglyci-namide) (National Center for Biotechnology Information, 2023).

Thanks to the structural changes from the original molecule, deslorelin is a potent analogue of GnRH, with relative potency around 150 (considering the original molecule as the unit) (Hardman and Limbird, 2001) and is more resistant to proteolysis (Furman, 2017). This results in an enhanced biological activity as well as prolonged action.

Deslorelin is also known as an LHRH agonist since it works by stopping the production of gonadotropins such as the luteotropic hormone (D'Occhio and Aspden, 1996), even if the inhibiting effect is not limited to LH and it blocks the synthesis and release of FSH too.

The low and continuous dosage of deslorelin effectively reduces the functionality of the male reproductive organs, libido and spermatogenesis, lowering the plasmatic levels of testosterone and achieving infertility within approximately 6 weeks after the treatment (EMA, 2008), although the interval from treatment to complete infertility may be as long as 70 days (Romagnoli et al., 2012).

The metabolism of the drug is fast and it remains almost entirely bioavailable. No pharmaceutical interactions with other medicinal products or other forms of interaction are known (EMA, 2008).

1.4.3 Vehicle

The veterinary drug known as Suprelorin™ (Virbac) is an implant containing 4.7mg or 9.4mg of deslorelin acetate commercialized by Virbac. The European Medicines Agency (EMA) (EMA, 2008) indicates the recommended dose to be a single implant per dog, regardless of its size.

The implant is cylindrical, with color varying from white to pale yellow and is intended for subcutaneous use. The injection should occur in the loose skin in the back, between the shoulder blades. Adipose tissue should be avoided since the release of the drug might be altered in areas of low vascularization (EMA, 2008).

The formulation proposed by Virbac contains the active ingredient and 3 excipients: hydrogenated palm oil, lecithin and sodium acetate anhydrous (only present in the 9.4 mg deslorelin formulation). The implant is packed in a single use sterile syringe implant device (Driancourt and Briggs, 2020).

1.4.4 Implant

The implant of the medication has to follow a specific step-by-step procedure (EMA, 2008):

1. Remove the Luer Lock from the injector for the implant.
2. Connect the actioner to the injector using the Luer Lock connection.
3. Lift the loose skin between the scapulas. Insert the entire length needle subcutaneously. It is not necessary to prepare the implantation site.
4. Press the injector's plunger fully into the actioner and, at the same time, slowly extract the needle.
5. Press the skin at the insertion site while the needle is withdrawn and maintain pressure for 30 seconds.
6. Examine the syringe and needle to make sure that the implant has not remained inside the syringe or needle and that the distancer is visible. The implant might be palpable in situ.

The injection should be repeated every 6 months or 12 months, according to the dosage used, to maintain the effect.

The implant is biocompatible and therefore doesn't need to be removed; however, should it be deemed necessary to interrupt the treatment, the implants can be removed surgically by a vet after localization via ultrasonography (EMA, 2008), although identifying the implant is not always an easy feat in clinical practice.

The deslorelin releasing implant is placed beneath the skin between the dog's shoulder blades, however choosing a different implantation site such as the navel area of the abdomen may prove useful should removal of the implant become necessary later on.

1.5 Off-label uses of deslorelin

Deslorelin, as the veterinary medicinal product Suprelorin™, is currently registered for the induction of temporary infertility in male dogs, male cats, male ferrets, and also prepubertal female dogs. Clinical application should involve healthy and intact patients.

However, it has been used off-label and is currently under study for different purposes in dogs:

- Control of exaggerated libido and aggressiveness in males (Ström et al., 2010).
- Benign prostatic hyperplasia (Ponglowhapan and Lohachit, 2010; Romagnoli, 2006; Jurczak et al., 2010; Ström et al., 2010).
- Control of reproduction in females (Romagnoli et al., 2009).
- Urinary incontinence in spayed females (Reichler et al., 2003; Reichler et al., 2006).
- Estrus induction in females (Kutzler et al., 2002).
- Estrus suppression in females (Fontaine and Fontbonne, 2010).
- Delay of puberty (Sirivaidyapong et al., 2012).
- Alopecia X (Albanese et al., 2014).

Anecdotal use of deslorelin is also reported for treatment of perianal gland adenoma and improvement of semen quality.

1.5.1 Benign prostatic hyperplasia

Benign prostatic hyperplasia (BPH) is a condition characterized by the abnormal non-cancerous growth and glandular hyperplasia of the prostate of intact male adult dogs. Its prevalence increases with aging, with over 90% of intact dogs at 8 years of age showing signs of BPH (Christensen, 2018). Prostatic growth and secretion are hormonally driven as they're modulated by 5-alpha-dihydrotestosterone, an active metabolite of testosterone; a shift in the balance between rate of production and removal of this steroid in the prostate may increase the number of prostatic cells, determining BPH (Barsanti and Finco, 1995). This may result in the formation of cysts accumulating urine, blood and prostatic fluid, which predisposes the prostate to develop infection from bacteria ascending the urethra or from hematic circulation.

The enlargement of the prostate may lead to various clinical signs, including straining to urinate, hematuria, pollakiuria, strangury, discharge from the penis and constipation or difficulty defecating (Christensen, 2018). The diagnosis of BPH is based on a demonstration of an enlarged prostate in the absence of any other reproductive or general abnormalities; the assay of canine prostatic specific esterase (CPSE), a marker for prostatic growth, is helpful in confirming the diagnosis;

although measuring high CPSE value is not very specific and may also be due to prostatitis or prostatic neoplasia (Bell et al., 1995).

Registered treatments for canine BPH are antiandrogens like osaterone acetate or progestogens like delmadinone acetate (Nizanski et al., 2014). Dogs of reproductive value may also be treated (off-label) with a competitive inhibitor of steroid 5alpha-reductase such as finasteride or flutamide (Barsanti and Finco, 1995).

Finasteride is a synthetic steroidal anti-androgen, which does not function as a receptor binding anti-androgen, but rather inhibits type II 5-a-reductase enzyme, found in the prostate and reproductive organs. This enzyme is responsible for transforming testosterone into its potent metabolite di-hydro-testosterone, hence this drug may be used to selectively lower DHT in the prostate, without altering serum testosterone concentrations, to treat prostatic diseases like BPH (Cohen et al., 1995). It may be used to induce remission of clinical signs of BPH and/or to keep the condition under control with repeated treatments (Sirinarumitr et al., 2001). Finasteride may be a good treatment choice for short-term use in breeders since it doesn't alter spermatogenesis and is well known to produce a dose-dependent decrease in prostatic size in dogs; however, it's currently only commercialized for use in men. It is well tolerated in dogs, its effect sets in slowly and clinical signs may take up to 3-4 weeks to disappear. However, the prostate starts growing again in size as soon as treatment is discontinued and clinical signs tend to reappear within a few weeks of treatment withdrawal (Iguer-Ouada and Verstegen, 1997).

For safety or practical reasons, it may be preferable to use deslorelin, particularly for chronic use given its slow-release formulation (Junaidi et al., 2009). As prostatic growth is testosterone-dependent, GnRH agonists can be used to effectively reduce the size of the prostate gland and consequently alleviate clinical signs of BPH (Junaidi et al., 2009). Different studies have been conducted to monitor deslorelin's efficacy in treating dogs with BPH, resulting in the prostates shrinking steadily to minimal values (20-25% of their initial volumes) within 16 weeks and clinical signs progressively vanishing during the 8 weeks post implant (Driancourt and Briggs, 2020). However, deslorelin should not be used in complicated BPH cases where prostatic enlargement is causing colonic impaction or dysuria (Ferré-Dolcet and Sussan, 2021). Moreover, it is important to note that initially, due to the "flare-up" effect of GnRH agonists, it is possible that clinical signs associated with BPH may increase for the first weeks after treatment (Driancourt and Briggs, 2020), hence why ideal patients for deslorelin treatment of BPH are not symptomatic.

1.5.2 Urinary incontinence

Urinary incontinence (UI) is the involuntary leakage of urine, typically occurring during recumbency or standing (Arnold et al., 2009). While this condition may occur in dogs of any age or sex, 75% of adult cases are documented in spayed females (Thrusfield et al., 1998), particularly within larger breeds with a body weight over 20 kg and especially prominent in Boxers (Arnold et al, 1989).

This is primarily attributed to the prevailing etiology of acquired UI, notably urethral sphincter mechanism incompetence (USMI), wherein the act of spaying contributes to a weakening of the external urethral sphincter's capability for closure (Augsburger and Cruz-Orive, 1998). The reported incidence of post-spaying USMI varies between 5% and 20% in bitches (Arnold et al., 2009).

Historically, the underlying mechanism of USMI has been attributed to decreased levels of estrogens post-spaying, leading to a subsequent reduction in the sympathetic tone of the smooth musculature of the external urethral sphincter (Romagnoli and Sontas, 2010). On the contrary, more recent studies have supported an alternative theory, implicating the incompetence to elevated levels of GnRH and LH consequent to the absence of gonadal feedback. This hormonal imbalance leads to decreased contractility of the smooth muscle in the lower urinary tract (Reichler et al., 2013).

Clinical management of UI due to USMI is typically achieved using steroids or sympathomimetic drugs (Romagnoli and Sontas, 2010), employed singularly or concomitantly, to leverage the capacity of estrogens in increasing the expression of α-agonist receptors (Arnold et al., 2009). Treatment with alpha-adrenergic agonists results in continence in 75% of incontinent bitches (Arnold et al., 2009). Recently, the administration of GnRH agonists has emerged as a promising alternative, demonstrating effectiveness in approximately 50% of afflicted cases, with continence lasting 50-575 days after treatment (Reichler et al., 2006). Where medical treatment fails, continence can be obtained with surgical therapy.

1.5.3 Control of reproductive behavior

The management of fertility and undesired reproductive behavior is a recurring presenting concern in male dogs. Owners often seek resolution for issues such as inadvertent matings, heightened libido, mounting behavior, inter-male aggressiveness, authoritative dominance directed at owners, excessive territorial urine marking and proclivity for wandering (Hart and Eckstein, 1997). These manifestations

not only disrupt harmonious cohabitation but also have implications for animal welfare and community interactions.

Male canine reproductive behavior is intricately regulated by the concentration of serum testosterone (T) and is profoundly influenced by the formative experiences the animal undergoes during its growth and developmental stages. Consequently, conditions rooted in male behavior attributed to testosterone secretion can be addressed through castration, the administration of antiandrogens or GnRH-agonists as well as behavioral training (Knol and Egberink-Alink, 1989; Kutzler, 2010). Castration executed prior to puberty causes a lack of development of the aforementioned male reproductive behaviors. Conversely, if castration is performed after puberty, particularly in instances where the male has interacted with or mated with females in estrus, the efficacy of mitigating these behaviors is notably diminished (Maarschalkerweerd et al., 1997).

GnRH-agonists, functioning through the downregulation of the HPG axis, lead to a reduction in serum testosterone concentration (Romagnoli et al., 2012; Lucas, 2014). Their application has demonstrated commendable success in attenuating a spectrum of male reproductive behaviors, including mounting, excessive libido, hypersexuality, intermale conflict and excessive territorial urine marking (Driancourt and Briggs, 2020), effectively contributing to enhanced behavioral control. However, it is important to acknowledge the nuanced nature of aggressiveness - exhibiting both hormonal and behavioral dimensions - rendering it less amenable to control through either surgical or medical neutering methods (Hart and Eckstein, 1997). This intricate interplay underscores the imperative of comprehensive understanding and tailored interventions for managing complex behavioral aspects in male canines.

2. Materials and methods

The study presented in this thesis includes 6 client-owned dogs that were treated chronically with deslorelin (4.7 mg and 9.4 mg implants, Suprelorin™, Virbac) for diverse complaints. They were selected by consulting with Professor Stefano Romagnoli and examining the clinical records of the Veterinary Teaching Hospital of the University of Padua. Prior to deslorelin treatment, all owners were informed of possible side effects and off-label use of deslorelin. They all consented to treatment and disclosure of their dogs' cases in a scientific publication.

For all dogs, data from their clinical records was used, including hematology, urinalysis, hormonal assays and medical imaging. When possible, dogs were examined, assessing general health, reproductive behavior and possible implant side effects. In addition, their owners were either interviewed by phone call or email to obtain complete clinical records of the patients. Information was collected on the reason for treatment, the patient's clinical history, the success and duration of the treatment, and the occurrence of side effects after treatment with deslorelin. All available data were collected prior to September 2022 and included in the article published on Animals.

Production of the article involved conducting a literature review related to reproduction management in dogs, the different methods available as well as their advantages and disadvantages. An in-depth research on the use of deslorelin, especially regarding its off-label and chronic use, was conducted to identify the gaps in current knowledge and determine the significance of our research.

The draft of the article was written using the knowledge obtained from the literature review in combination with the data regarding the selected dogs. Patients were divided depending on the reason for treatment with deslorelin implants and a brief introduction was provided for every complaint. Each patient's clinical history was presented, with special attention to their reproductive health and the records on the deslorelin implants received. The authors then offered a comment on what information could be gathered from the use of deslorelin in that patient, as well as a final conclusion on the study.

After careful revision of the final draft and consultation with fellow coauthors, the manuscript was tailored for Animals and submitted for peer review. Experts in the field provided feedback and suggested improvements, which allowed to revise the manuscript and increase its validity, clarity, accuracy and significance. Once the manuscript met Animals' standards, it was submitted for publication and finally accepted.

After the article was published in January 2023, the owners of the dogs were contacted again to follow up on the patients' clinical condition and the effect of the implants. Interviews by phone call were conducted until August 2023 to provide complete overviews of the treatments and offer a complete picture to date.

3. Results

3.1 Publication on Animals

animals

MDPI

Case Report

Chronic Use of Deslorelin in Dogs: Six Cases (2005–2022)

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Simple Summary: This paper presents six different cases of dogs treated repeatedly with deslorelin, a drug responsible for a six- or twelve-month block of the reproductive system which is registered for use in adult male dogs and ferrets, male cats and prepuberal bitches. Efficacy and safety as well as return to fertility of such non-surgical neutering methods are well known following a single use but little if any data is available on prolonged use. The dogs presented in this paper were treated for 2 to 9 consecutive years for ensuring failure to reproduce (one case) as well as for conditions which are not found on the drug leaflet (benign prostatic hyperplasia and perineal gland disease (one case each) and control of reproductive behavior in male dogs and urinary incontinence in spayed adult bitches (two cases each). All animals were in good health during treatment and presented no short-term side effects. Flare-up reactions (an increase in reproductive behavior for 1–3 weeks after treatment) were observed in 1/4 intact males and were not observed in the spayed incontinent bitches. Deslorelin was effective in all treated dogs. Fertility was immediately regained in one male dog who sired a litter when his owner forgot to come back for re-treatment at the right time. Deslorelin implants can be considered as a safe alternative to surgical castration in specific pathologies mediated by reproductive hormones in situations in which surgical castration is not an option such as animals suffering from cardiovascular conditions or other systemic diseases making anesthesia unsafe.

Abstract: Deslorelin is currently registered for the induction of temporary infertility in male dogs, male cats, male ferrets, and also prepubertal female dogs, but research has shown its usefulness for other conditions requiring chronic treatment. This paper presents six cases of dogs chronically treated with deslorelin for indications such as benign prostatic hyperplasia, control of fertility, abnormal reproductive behavior and urinary incontinence. All animals were in good health during treatment. Treatment duration was 2–9 years. No short-term side effects were observed except for flare-up reactions, which were observed only in 1/4 intact males. Two dogs developed a neoplasia: a spayed bitch treated for urinary incontinence developed a pituitary carcinoma, and an intact male dog implanted for control of fertility developed a bladder carcinoma. While the pituitary carcinoma seems unlikely to be related to deslorelin, the bladder carcinoma could be due to the neutered condition of the dog (which was treated for 9 years) as urinary tract neoplasia is more common in dogs following gonadectomy. Chronic treatment with deslorelin is regarded as safe when an animal is being treated for life. The possibility that a pause in the treatment might be helpful for the animal should be investigated.

Keywords: benign prostatic hyperplasia; chronic treatment; control of reproductive behavior; deslorelin; dog; urinary incontinence.

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1. Introduction

Controlling reproduction in small animals is becoming increasingly important both for pets as well as breeding animals. Historically, reproduction control has been achieved using progestogens until early in this century when new and challenging options became available, such as the long-acting agonists of GnRH. Progestogens have been largely misused due to poor attention to scientific evidence, as shown by the growing number of case reports documenting side effects due to excessive dosing [1], which have produced a generalized fear about their use.

Long-acting GnRH agonists initially cause a pituitary stimulation leading to the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), followed by increased production of the respective gonadal hormones. Such continued release may cause a temporary increase in reproductive function (called flare-up reaction), which is observed in females [2] as induction of estrus and may become manifest in males as increased libido. This initial phase (which is often silent) is then followed by a downregulation of pituitary GnRH receptors with a long-lasting block of pituitary function, which interrupts all reproductive functions. There is currently one single long-acting GnRH agonist approved for veterinary use in the European Union: deslorelin. The other long-acting GnRH agonist, azagly-nafarelin [3], despite successful application, has never been launched and is now withdrawn from use in the European Union. The former has been the object of relevant clinical research activity internationally since the early 2000s [4–8]. In August 2005, the University of Padova was granted permission by the Italian Ministry of Health to import 10 samples of a deslorelin-based drug (Suprelorin™, Peptech Animal Health, Macquarie Park, New South Wales, Australia), which was marketed at that time in Australia and New Zealand as a veterinary compound for use in male dogs (document DGVA-III/29534IP dated 29 august 2005). The request for import had been made to study potential clinical applications of deslorelin for the treatment of benign prostatic hyperplasia. In 2007, deslorelin (Suprelorin™, Virbac, Carros, France) started being distributed on the European market with an indication for induction of temporary infertility in male dogs. Soon after its launch, it became evident that deslorelin was potentially effective for several other indications, such as urinary incontinence in spayed bitches, as well as for the control of reproductive behavior in male dogs. As these conditions often require a chronic treatment, we started using deslorelin chronically in selected canine patients with the above presenting complaints. This paper reports on the chronic use of the 4.7 mg and 9.4 mg deslorelin implants in dogs treated at the University of Padova for a variety of different indications.

2. Materials and Methods

Clinical records of canine patients being seen as first-opinion or referral cases at the Veterinary Teaching Hospital of the University of Padova, Italy (UNIPD) were searched using deslorelin as a keyword. The records of patients being treated for at least 2 years (4 or 2 consecutive treatments with the 4.7 mg or 9.4 mg deslorelin implant, respectively) or longer were selected and case histories as well as results of hematobiochemical, urinalysis, diagnostic imaging, histopathology and necropsy exams were collected and analyzed. Animal owners were contacted by telephone and the history of each case was reviewed, cross-checking the time sequence of events and the accuracy of the data reported in clinical records.

3. Results

The following cases of six different dogs treated for ≥2 consecutive years with deslorelin were retrieved. The clinical conditions for which chronic deslorelin treatment was used were benign prostatic hyperplasia (1), urinary incontinence in spayed bitches (2) the control of fertility (1) and control of abnormal reproductive behavior in intact males (2). All owners had given their informed consent prior to the

use of deslorelin in their dogs. The cases are grouped based on the indications for their treatment with deslorelin, and each case is briefly commented at the end.

Benign prostatic hyperplasia in intact male dogs

Benign prostatic hyperplasia (BPH) is a condition characterized by the abnormal growth and glandular hyperplasia in the prostate of intact male adult dogs. Its prevalence increases as the dog ages, with > 90% of intact dogs at 8 years of age showing signs of BPH [9]. Prostatic growth and secretion are modulated by 5-alpha-dihydrotestosterone; a shift in the balance between rate of production and removal of this steroid in the gland may increase the number of prostatic cells, determining BPH [10]. This may result in the formation of cysts accumulating urine, blood and prostatic fluid, which predisposes the prostate to develop infection from bacteria ascending the urethra or from blood circulation. The diagnosis of BPH is based on a demonstration of an enlarged prostate in the absence of any other reproductive or general abnormalities; the assay of canine prostatic specific esterase (CPSE), a marker for prostatic growth, is helpful in confirming the diagnosis. Registered treatments for canine BPH are antiandrogens like osaterone acetate or progestogens like delmadinone acetate [11]. However, for safety or practical reasons, it may be preferable to use deslorelin, particularly for chronic use [12]. Deslorelin should not be used in complicated BPH cases in which prostatic enlargement is causing colonic impaction or dysuria [13]. Dogs of reproductive value may also be treated (off label) with a competitive inhibitor of steroid 5alpha-reductase such as finasteride or flutamide [10].

3.1. Case 1. Benign Prostatic Hyperplasia in an Intact German Shepherd Dog

An 8-year-old, 33.7 kg body weight (BW), intact male German shepherd dog of the Arma dei Carabinieri was referred to the VTH of UNIPD as an emergency case for difficult defecation and ear problems, which had led to a reluctance to perform his duties as an anti-drug dog.

On the day of referral, the dog's general conditions were normal, although he was underweight due to poor appetite, had normal skin, normal subcutis and mucosal membranes, normal lymph nodes, and showed local alopecia and purulent ear discharge from his left ear. Abdominal palpation was unremarkable. On ultrasound, the dog's prostate was increased in size and a few small cysts and a large (3.1 × 3.2 cm diameter) prostatic cyst were observed (Figure 1).

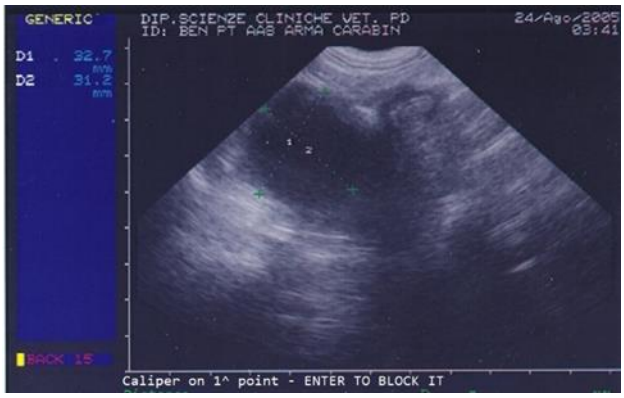

Figure 1. Abdominal ultrasound of a German shepherd dog before deslorelin implant. The scan shows the ultrasonographic appearance of the prostate. The largest prostatic cysts' perpendicular diameters are measured as D1 = 32.7 mm and D2 = 31.2 mm.

Benign prostatic hyperplasia (BPH) and purulent otitis were diagnosed, and an antibiotic treatment was prescribed. Two weeks after treatment, the dog's conductor reported that the dog's skin and ear condition as well as his ability to work had improved but appetite was still poor and defecation was still difficult. The prostate

was not painful on palpation. A blood sample was collected for a complete blood count (CBC) and serum biochemistry evaluation. CBC revealed mild eosinophilia while biochemical parameters were normal except for high total proteins (88 g/L) and high albumin (36 g/L). Urinalysis revealed the presence of erythrocytes (250 cell/L), leucocytes (25 cell/L) and proteins (25 mg/dL) in urine. On prostatic ultrasound, the prostate was regarded as similar to the previous exam (Figure 2).

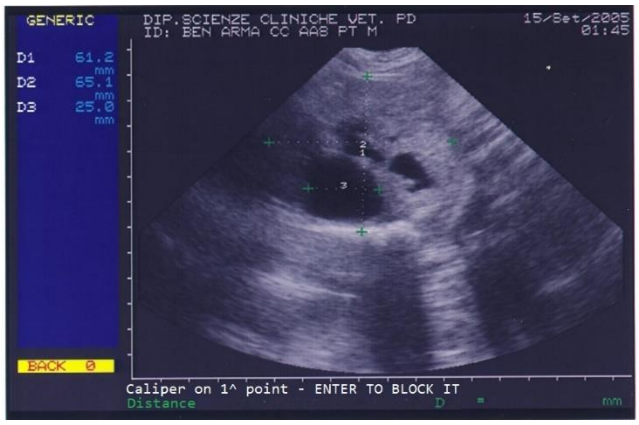

Figure 2. Abdominal ultrasound of a German shepherd dog before deslorelin implant. The scan shows the ultrasonographic appearance of the prostate and the presence of different-sized cysts. The prostate's perpendicular diameters are D1 = 61.2 mm and D2 = 65.1 mm. The largest prostatic cysts' diameter is measured as D3 = 25 mm.

A 4.7 mg deslorelin implant (Suprelorin™, Virbac, Carros, France) was administered in the subcutaneous tissue between the shoulder blades.

The dog returned for monthly follow-ups to monitor the implant's action during the first 6 months, at which times a clinical exam, which included a prostatic ultrasound and a gonadotropin-releasing hormone (GnRH) stimulation test, was performed. The dog's conditions improved remarkably already at the first monthly check when the dog showed no more difficult defecation and a normal appetite, and the ultrasound showed a progressive reduction in prostatic size and disappearance of all prostatic cysts. Upon reimplantation in March 2006, the dog's prostate was very small (2.9 × 2.8 cm) and showed a normal texture (Figure 3).

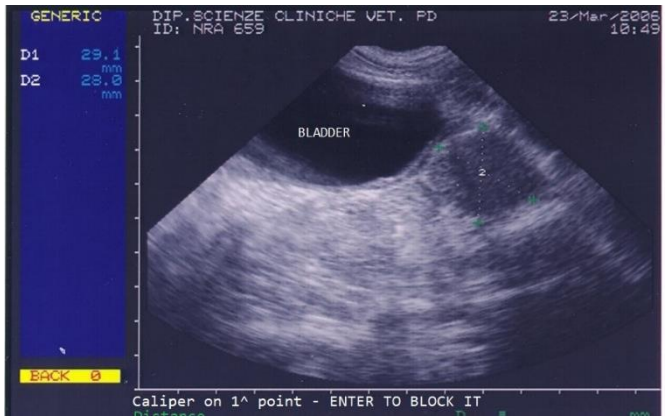

Figure 3. Abdominal ultrasound of a German shepherd dog after a 4.7 mg deslorelin implant. The scan shows the ultrasonographic appearance of the bladder and prostate. The prostate's perpendicular diameters are D1 = 29.1 mm and D2 = 28 mm.

Figure 4 shows changes in testicular size as well as testosterone (T) concentration during the 6 months follow-up (October 2005–March 2006) after the first deslorelin treatment. Administration of the 4.7 mg implant was repeated three times in October 2005, March 2006 and September 2006, while in March 2007 a 9. 4 mg deslorelin implant was administered. The dog continued to work normally

throughout these years and never lost his ability to detect drugs. He was retired from his duties in 2008 and adopted from a local family we had no contact with. He lived well until his death for natural reasons in 2010.

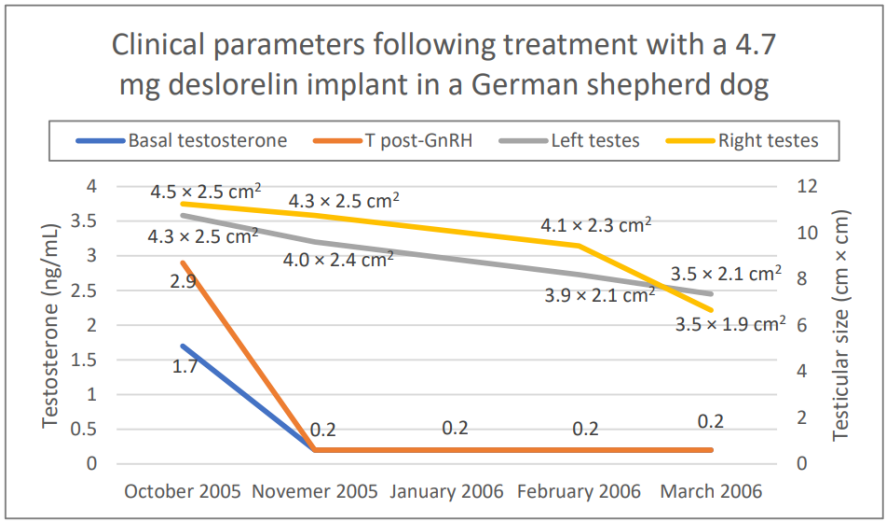

Figure 4. Clinical parameters (testicular size, serum testosterone concentration before and one hour after a stimulation test with 50 μg gonadorelin SC) of a German shepherd dog treated with a 4.7 mg deslorelin implant. Testosterone concentration was measured with chemiluminescence immunoassay. Testicular size was measured during ultrasonographic examination.

Comment—The active principles suggested for BPH treatment are inhibitors of steroid 5alpha reductase such as finasteride and flutamide, or molecules such as osaterone acetate (not yet marketed at that time) or delmadinone acetate which compete with androgens for their receptors. This case shows that deslorelin may be used as a treatment for BPH in dogs for which fertility is not a priority or is not immediately required. Following the removal of testosterone from the general circulation, the prostate quickly decreases in dimensions reaching by the end of treatment a size comparable to that of the prostate of a surgically castrated dog. In addition, the prostatic cysts disappeared completely within one month from implantation. However, due to the flare-up phase, a worsening of the clinical situation may be observed when deslorelin is used as a first line treatment for BPH. This medical option should be avoided if the prostate is inflamed or extremely enlarged. The duration of action of deslorelin, or rather the relevant frequency of implant administration in case of BPH, is difficult to determine as prostatic growth is a slow process: once the prostate has become almost atrophic at the end of a deslorelin treatment it may take weeks or a few months before it starts giving problems due to BPH. The GnRH simulation test was performed in order to assess the status of the hypophyseal–pituitary–gonadal (HPG) axis as this was the first case in which deslorelin was ever used at our institution; such a test is not usually necessary in practice when using deslorelin. The assay of CPSE was not yet marketed at the time this dog was examined.

Urinary incontinence following spaying

Urinary incontinence (UI) is the involuntary leakage of urine, typically occurring during recumbence or standing [14]. The problem may occur in dogs of any age or sex; however, 75% of adult cases are reported in spayed females, mostly of large sized breeds [15]. This is due to the most common cause of acquired UI being urethral sphincter mechanism incompetence (USMI), where after spaying the sphincter is weakened leading to reduced urethral closure [16]. Although in the past USMI was attributed to loss of estrogens and a consequent reduced sympathetic tone

of the urethral sphincter, more recent studies attribute the incompetence to elevations of GnRH and LH (due to absent gonadal feedback) leading to decreased contractility of smooth muscle in the lower urinary tract [17]. The incidence of post-spaying USMI is between 5% and 20% in bitches. Medical treatment of UI due to USMI is normally achieved using steroids or sympathomimetic drugs, individually or combined, to take advantage of the estrogens' action in increasing the expression of α-agonist receptors [14,18]. Treatment with GnRH agonists is a more recent alternative that is proving to be useful in approximately 50% of cases [19].

3.2. Case 2. Urinary Incontinence in a Spayed Boxer Bitch

A 4-year-old, 35 kg BW, spayed Boxer bitch was referred to the VTH of UNIPD in early 2002 with a presenting complaint of urinary incontinence following spaying. The bitch was ovariohysterectomized at 2 years of age. Two weeks after spaying, the owner started to find small amounts of urine on the floor. The bitch was initially successfully treated by the referring veterinarian with a human drug Temporinolo™ (Sanofi, Paris, France) SID (35 mg phenylpropanolamine + 8 mg chlorphenamine) and later referred to the VTH at UNIPD when the drug was taken off the market.

On clinical examination on the day of referral, the bitch was healthy and in good body conditions, and all her clinical parameters as well as hematology and serum biochemistry were normal. Abdominal ultrasonography showed a normal appearance of the urinary system. A neurological exam revealed no abnormalities. Based on the dog's history and absence of any clinical abnormality, a diagnosis of urinary incontinence (UI) due to urethral sphincter mechanism incompetence (USMI) was tentatively made (pending urinanalysis results). A 1 mg/day dose of estriol (Ovestin™, Organon, Oss, The Netherlands, the veterinary product was not yet available at that time) was prescribed and, after some attempts at tapering down the dosage, a 0.5 mg/day dosage administered early in the morning proved successful. Over the following 3 years, the bitch was re-examined annually. She remained clinically normal, continent, presented no side effects and her hematology, serum biochemistry and urinalysis remained normal.

Three years after the start of estriol administration, deslorelin was suggested as an alternative. In November 2005, a 4.7 mg deslorelin implant (Suprelorin™, Virbac, Carros, France) was administered in the subcutaneous tissue between the shoulder blades. The owners reported that urinary continence was restored 2 days after implant administration. The estriol protocol was suspended 2 months after deslorelin treatment without any relapse of signs of UI. The bitch remained continent without additional treatments for a total of 6.5 months (except for a single episode of UI occurring at 4 months when her family moved, which required estriol administration) and a new deslorelin implant was applied.

The second 4.7 mg implant (June 2006) restored urinary continence, with the exception of an important episode of incontinence in correspondence with the return of the bitch's owners from a 3-day trip. The owner administered estriol for the following month, until the signs of UI subsided. During the medical follow-ups in the six months of efficacy of the implant, the owner reported normal eating, drinking and interactive behavior and good appetite.

In December 2006, a third 4.7 mg deslorelin implant was applied. Upon a clinical and blood check prior to the following deslorelin administration the bitch showed normal hematology and an increase in Creatine Kinase (CK), cholesterol and albumin on serum biochemistry.

A fourth 4.7 mg deslorelin implant was applied in June 2007. Multiple incontinence episodes occurred 1 month after this implant, but they were controlled with estriol. The owner reported overall normal behavior, except for an increase in water intake.

The next implant was scheduled for December, but the owner delayed the procedure by a month and during this time more involuntary micturition episodes

occurred. In January, a 9.4 mg deslorelin implant was suggested to extend the efficacy window of the treatment to 12 months. During the clinical follow-up scheduled 3 months after the 9.4 mg deslorelin implant insertion, the owner reported that the bitch drunk about 5 L of water a day, particularly at night, had frequent episodes of voluntary micturition of transparent urine, and showed increased appetite, pica and coprophagy. In addition, she was lazier, and unwilling to take walks or engage in other activities. A clinical follow-up was scheduled for the following month when the bitch still manifested a sluggish and depressed attitude and extreme muscle fatigue. Polyuria and polydipsia were persistent and her appetite had decreased. Episodes of epistaxis and labored breathing had also occurred. The bitch's conditions continued to worsen and a month later she died suddenly. Upon necropsy, a Cushing's syndrome was diagnosed due to a pituitary carcinoma.

Comment—In this case, post-spaying USMI was successfully treated both with a human estriol-based compound (the veterinary formulation was not commercially available at that time) as well as repeated deslorelin implants. Urinary continence was restored about 2 days after implant administration. Despite minor variations, the duration of effect of each implant reflected the 6-month period of efficacy in male dogs. Major stressful events appeared to impair the treatment in this bitch, as a decrease in the implant's efficacy was shown shortly after the bitch's family moved or during her owner's absence.

Pituitary carcinomas are rare, slow-growing tumors mostly reported in older dogs [20,21], which tend to develop from the corticotroph cell line, therefore inducing hypercortisolism [22]. Hypercortisolemia was unfortunately not investigated in this bitch; however, her clinical signs of polyuria/polydipsia are clear indications of a derangement of pituitary corticotroph function. Corticotroph carcinomas and pluri-hormonal pituitary adenomas and carcinomas (where a single tumor consists of two different hormone-secreting cell types and therefore expresses multiple hormones) have been reported in two dogs and three cats [22] with invasive pituitary neoplasms composing only 11.5% of secondary intracranial neoplasms in dogs. The complex tumoral condition encountered upon necropsy, with multiple coexisting neoplasms, is often encountered in Boxers, where intracranial neoplasia is especially prevalent [23]. The pathogenesis and cause of these tumors is still unknown, yet their development could theoretically be related to increased positive feedback signals or decreased negative feedback signals relatively to one of the different hormone-secreting cell types [22]. The possibility that prolonged downregulation of pituitary GnRH receptors caused by deslorelin favored the development of a pituitary neoplasia in this dog deserves attention. However, it should be underlined that the target of deslorelin action is the gonadotroph and not the corticotroph cells and that a correlation between treatment with deslorelin and the development of pituitary carcinomas has never been reported. However, deslorelin has not been on the market for too long and the few cases in which it has been used chronically do not allow us to rule out the existence of links between the prolonged downregulation of pituitary gonadotroph receptors and the development of pituitary neoplasia. Reimplanting with deslorelin after more than 6 (or 12, depending on the implant) months following the previous implant causes the occurrence of the initial period of gonadotropin release, which is due to pituitary stimulation by deslorelin, something which in this bitch occurred twice. The effect of the repeated flare-ups between the prolonged periods of downregulation should be further investigated.

3.3. Case 3. Urinary Incontinence in a Spayed Rhodesian Ridgeback

A 3-year-old, 36.5 kg BW, spayed Rhodesian Ridgeback bitch was referred to the VTH at UNIPD with a presenting complaint of UI. The bitch was ovariohysterectomized at 2 years of age. Two months later, the dog began presenting signs of UI. A general physical examination showed that the patient was healthy, with a palpable abdomen and normal clinical parameters. A sample of urine was collected via spontaneous micturition to rule out cystitis, urine biochemistry was unremarkable and the urinary protein and urinary creatinine ratio was 0.02. The incontinence continued untreated for 6 months before examining the bitch. The owner reported that the leaking episodes occurred with variable incidence, both during recumbency and during movement without voluntary signs of micturition. A blood sample was collected for a CBC and serum biochemistry evaluation. CBC was unremarkable, while blood chemistry revealed high alanine aminotransferase (ALT = 424 U/L), high globulins (49 g/L) and high total proteins (80.67 g/L). Urinalysis was unremarkable and urinary creatinine ratio was 0.03. An abdominal ultrasound evaluation revealed no abnormalities.

A diagnosis of USMI was made and a pharmacological therapy with estriol (Incurin™, MSD, Rathway, New Jersey, USA) 1.0 mg SID was started. Urinary continence was restored for 3 months, after which the patient started leaking urine again. The signs of UI only lasted for the summer months and disappeared during fall. At the start of winter, UI began occurring more than once a day; therefore, the bitch was seen again. Clinical examination revealed that the dog was in health and her clinical parameters were normal. Her abdomen was palpable. Body weight was 35.2 kg. The vulva appeared to be slightly enlarged, vulvar mucosa was normal and a drop of urine was visible. A vaginal smear was performed, which revealed low cellularity, the presence of non-keratinized cells and moderate neutrophilia. Urinalysis was unremarkable and urine culture showed no bacterial growth. Estriol treatment was withdrawn and substituted with phenylpropanolamine (Propalin™, Vétoquinol, Magny-Vernois, France). Urinary continence was restored for 6 months, after which signs of UI recurred and the treatment was suspended. At this time (July 2020), deslorelin was suggested as a possible treatment, and a 4.7 mg deslorelin implant (Suprelorin™, Virbac, Carros, France) was administered in the subcutaneous tissue between the shoulder blades. The treatment was effective for about 19 weeks. During the 20th week, signs of UI recurred and the owner reported occasional tenesmus, a prolonged defecating position and soft feces. The dog's general health was normal. Rectal exploration revealed the presence of formed feces and presence of a bloody-purulent secretion from the left perianal gland, which was manually expressed. An antibiotic therapy of metronidazole-spiramycin (Spiroxan™, Ceva, Libourne, France) associated with the non-steroidal anti-inflammatory drug meloxicam (Meloxoral™, Dechra, Northwich, England, UK) 1 mg/kg SID for 7 days was prescribed to treat the anal sacculitis, which was suspected to be caused by the chronically softened feces. A 9.4 mg deslorelin implant was applied (December 2020). Eight months later, the bitch was seen again because the owner had to move and wanted to treat the dog again even though the effect of deslorelin had not disappeared yet. The bitch appeared to be in good general conditions. The owner reported that sporadic episodes of tenesmus had occurred over the previous days. A general physical examination showed normal clinical parameters (pulse, respiration and rectal temperature, auscultation, abdomen palpation, skin and subcutis). Body weight was 34.5 kg. A new 9.4 mg deslorelin implant was administered (August 2021) in the subcutaneous tissue between the shoulder blades. A blood sample was collected for a CBC and serum biochemistry evaluation. CBC revealed low platelet count (186 10^3/μL); blood biochemistry reported low calcium (Ca = 8.57 mg/dL), low glycemia (77 mg/dL) and low azotemia (20 mg/dL). Serum protein electrophoresis revealed a low portion of albumins and high portion of α1-proteins. Urinalysis was unremarkable and urine culture showed no bacterial growth. Based on a follow-up

call 8 months after administration of the last implant, the bitch was in good health and lively, her weight had increased to 37.5 kg and she had remained continent throughout the time.

Comment—Post-spaying USMI in this bitch was treated with estriol and phenylpropanolamine with some success, although relapses were noticed following prolonged use of both drugs. Such "delayed" treatment failures are occasionally observed in incontinent bitches treated with both the above drugs. Deslorelin administration in this bitch has proven effective in the long run and the treatment is currently being successfully continued without any negative side effects. Treatment with deslorelin allowed the signs of UI to disappear within 1–3 days after implant administration. The short duration of effect of the 4.7 mg implant in this bitch might have been due to the inflammatory condition, which had developed on her anal glands causing tenesmus thereby presumably increasing pelvic contractility and bladder instability. In this patient, the duration of effect of the 9.4 mg implant for the management of UI seems to be at least 8 months (as the second one was administered when the first one was still active), if not longer. More cases however are needed to confirm this observation. Variations in body weight appear to be unrelated to use of deslorelin but rather due to the bitch being stressed by having to move frequently with her owner between Padova and Rome; once she settled in Rome in the last year her weight returned to normal.

Controlling fertility and abnormal reproductive behavior in male dogs

The control of fertility as well as abnormal reproductive behavior is a common presenting complaint in male dogs as owners may be concerned about their male dogs mismating bitches as well as displaying strong libido and mounting, inter-male aggressiveness, aggressive dominance towards owners and roaming behavior [24,25]. Male reproductive behavior depends on serum T concentration and is influenced by the animal's experience during growth and development; therefore, male behavioral conditions that are thought to be due to testosterone secretion can be treated with castration or the administration of antiandrogens or GnRH-agonists as well as behavioral training [26,27]. A lack of development of the above features of male reproductive behavior occurs when males are castrated prior to puberty, while if castration is performed after puberty and particularly when a male has been exposed to or has even bred female/s in heat its effectiveness in controlling annoying male behaviors is greatly reduced. GnRH-agonists downregulate the HPG axis thereby decreasing serum T concentration [7,8]; their use has proven effective in decreasing all aspects of male reproductive behavior except for aggressiveness—which has a dual connotation (hormonal and behavioral), therefore cannot always be controlled by either surgical or medical neutering.

3.4. Case 4. Controlling fertility in a Male Maremma Shepherd Dog

A 2-year-old, intact, 34.5 kg BW, male Maremma shepherd was presented for pharmacological neutering due to a history of breeding with his sister producing a litter of eight normal pups. His vaccination and heartworm prevention programs were current and he did not have any health issues.

On the day of referral all clinical parameters were normal including prostatic size on rectal palpation. Blood was collected for hematological evaluation and testosterone levels, with results being unremarkable. In March 2006, a 4.7 mg deslorelin implant (Suprelorin™, Virbac, Carros, France) was administered in the subcutaneous tissue between the shoulder blades. Six months later, the dog was clinically normal, his testicles had a soft consistency and a size (measured with a caliper) of 2.3 × 1.5 cm (right) and 3 × 1.2 cm (left) and serum T concentration after GnRH stimulation was < 0.2 ng/mL. In September 2006, the dog was implanted with another 4.7 mg implant.

The dog was then rechecked three times at 2-month intervals and found to be always in good general conditions, active, lively and with good appetite. In May 2007 (8 months following the previous treatment), his testicles were still soft in consistency and with a size (measured with a caliper) of 2.7 × 1.5 cm (right) and 2.9 × 1.4 cm (left) and serum testosterone concentration was still < 0.2 ng/mL; the dog was treated with a third 4.7 mg deslorelin implant.

In February 2008 (8.5 months later), the dog was seen again and this time a 9.4 mg implant was administered. This treatment was repeated in May 2009, then in May 2010 the dog was seen again for another deslorelin treatment. At this time, the dog's owners asked for the dog to be treated with the 4.7 mg implant as they were convinced that the dog was less lively and active and with a stronger tendency to gain fat when treated with the 9.4 mg implant.

From this time onward, the dog was treated quite regularly for the following 5 years (always with the 4.7 mg implant) during which time he remained in normal clinical conditions based on regular clinical checks. The timely sequence of all deslorelin treatments and measures of each testis due to the effect of the different deslorelin implants are summarized in Table 1.

Table 1. Date of implantation and variations in testicular size of a Maremma shepherd dog treated continuously with deslorelin between 2006 and 2015. Measures were taken at most clinical checks prior to reimplantation and reflect size of the right and left testes as measured during clinical examination using a stainless-steel caliper.

Date	Deslorelin Implant	Right Testicle	Left Testicle
8 February 2006	4.7 mg		
14 September 2006	4.7 mg	3 × 1.2 cm	2.3 × 1.5 cm
24 May 2007	4.7 mg	2.9 × 1.4 cm	2.7 × 1.4 cm
6 February 2008	9.4 mg	3.5 × 1.5 cm	3 × 1.5 cm
20 May 2009	9.4 mg		
22 May 2010	4.7 mg		
9 September 2010		3.2 × 2 cm	3.8 × 2.1 cm
24 November 2010		2.2 × 4 cm	2.1 × 4 cm
22 December 2010	4.7 mg	2.4 × 4 cm	2.2 × 4.1 cm
17 May 2011	4.7 mg		
23 November 2011	4.7 mg		
27 April 2012	4.7 mg		
18 March 2013	4.7 mg	4.2 × 2.2 cm	4.2 × 2 cm
6 February 2014	4.7 mg		
23 July 2014	4.7 mg		
20 January 2015	4.7 mg		
23 July 2015	4.7 mg	1.8 × 3.1 cm	1.8 × 3.1 cm

Testis consistency remained soft and with a good mobility. The prostate was also small and normal, except for one US examination in November 2010, which revealed a mild increase in volume and hyperplasia, cranial repositioning and slight asymmetry compared to previous measurements. The prostate returned to a normal appearance after a new implant.

The fall 2012 appointment was delayed as the owner forgot about it and the dog was treated on 20 March 2013. At this time, testicular size (right testis 4.2 × 2.2 cm, left testis 4.2 × 2.0 cm) and normal consistency indicated that the dog had presumably regained full fertility by the time he was implanted. The prostate remained normal in size, consistency and symmetry. The dog's sister came in heat less than a month later and breeding between them resulted in pregnancy with birth of a litter of 3 pups.

Since 2014, the implant was placed in the subcutaneous tissue of the periumbilical area. The dog remained healthy and with small, soft testes and a small prostate during the 2 years following February 2014. During the Spring 2015, he developed dysuria, hematuria and difficult defecation. Survey abdominal X-ray and US showed the presence of a mass at the level of the bladder neck. A CT exam showed the mass to be originating from the inner aspect of the bladder. Surgical removal was attempted but only a biopsy was made as the mass had already involved both ureters. A histological diagnosis of bladder adenocarcinoma was

made, then the dog was treated with chemotherapy for a few months and euthanized in December 2016.

Comment—This case shows that deslorelin is effective in maintaining male dogs in a permanent state of sterility and fertility can be easily regained as soon as the treatment is discontinued. Semen quality following the 6-month treatment duration is reported to be restored between 3 and 5 months after implant removal [28]. This seems to be the case for most deslorelin treated dogs in which the implant is not removed although in a few cases a dog may take up to 1 or more years to regain full fertility [29]. Repeated deslorelin implant administration in this dog caused prolonged sterility. Testicular volume could not always be determined accurately in this dog as his owner would commonly refuse testicular ultrasonography on financial grounds; however, testicular size and consistency was reduced during treatment based on clinical assessment and increased quickly as soon as treatment was discontinued. Deslorelin treatment allowed to maintain this dog in a continuous sterile condition for 7 years and, when the owner forgot to bring him back for a treatment, he bred his sister again and three pups were born. Except for this short gap in 2013, the dog was treated for a total of 9 years and his health remained normal throughout this time. The incidence of bladder carcinoma in dogs is low (about 2% of all canine tumors) [30]. Some breeds such as Scottish terriers, Shetland sheepdogs and West Highland white terriers are at higher risk [31]; however, there is no data on its incidence in Maremma shepherds. Incidence is reported to be affected by neutering [31,32]. The possibility that prolonged chemical neutering caused by deslorelin acted similarly to surgical neutering in favoring the development of bladder neoplasia in this dog cannot be ruled out and should be considered. The duration of effect of the 4.7 mg implant in this dog was frequently longer than 6 months while duration of the 9.4 mg implant could not be assessed as the dog was reimplanted always at the end of the 12th month. The belief that the 9.4 mg implant was causing the dog to be less active and gain more weight is not supported by any objective fact. Such a complaint has never been reported to the VTH of UNIPD by any other owner of dogs being treated with the 9.4 mg deslorelin implant. In this dog, no side effects directly due to deslorelin and no flare-up reaction following any treatment were ever observed.

3.5. Case 5. Controlling Hypersexuality in an American Staffordshire Male Dog

A 2-year-old, 23.5 kg BW, male, intact American Staffordshire dog was referred to the VTH of UNIPD for hypersexuality and benign prostatic hyperplasia. Soon after puberty, he had started showing an excessive amount of energy in all his behaviors both at home with continuous mounting of the legs of his owners followed by ejaculation as well as when taken out with aggressiveness against other dogs and a continuous pulling very strongly on his leash, which made him very difficult to control. The dog also had pain in his lower abdomen, perianal gland pain and would occasionally show hematuria at the beginning of spontaneous micturition. Owners also reported that feces were ribbon-like and pasty, with a final bloody spray a few days after stimulation from females in heat.

Upon referral (May 2015), clinical examination revealed that all clinical parameters were normal except for his perianal glands, which were slightly swollen and painful and his prostate, which on ultrasound appeared increased in size and with one large 3 × 2 cm diameter cyst in the right lobe and a few smaller cysts on both lobes. CBC was unremarkable, while blood chemistry revealed high magnesium (Mg = 2.51 mg/dL), high ALT (115 U/L), high aspartate aminotransferase (AST = 49 U/L), high globulins (45 g/L) and high total proteins (74.6 g/L). Basal testosterone concentration was 5.17 ng/mL. Since his owners were very keen in avoiding surgical castration and because of the dog's high liver enzymes, it was decided to try a treatment course with a short acting drug such as finasteride 0.5 mg/day orally

(Finasteride Teva Generics, Teva, Tel Aviv, Israel), and then to re-evaluate the dog after one month to consider the option of using deslorelin. At the following check-up, one month later, the dog was healthy and in good body conditions, he was not showing perianal gland pain and only a single episode of blood in urine had been noticed by his owners. However, the prostatic cysts were still present. An additional 15 days of finasteride treatment (same protocol as above), paired with 11.5 µg/kg megestrol acetate (Estropill, MSD, Rathway, New Jersey, USA) were prescribed (MA is a short-acting compound and its very low dosage makes it a safe option for dogs with questionable liver function as the treatment can be stopped at any time). At this time the dog owners declined to retest serum biochemistry on financial grounds.

The following month (July 2015), the dog was examined once more, all his clinical parameters were normal and owners reported that body functions were also normal again, with normal colored urine and well-formed feces. Perianal glands did not appear to be swollen or painful. Upon ultrasonography, the dog's prostate appeared to have decreased in size, from 5 cm in the last measurement to 3.5 cm, and there were fewer cysts, with only 2 medium-large-sized cysts (1.8 × 1.3 cm). Again, the dog owners declined to retest serum biochemistry on financial grounds. Therefore, the dog was administered a 4.7 mg deslorelin implant together with a 50 mg oral dose SID of cyproterone acetate (Androcur™, Bayer, Leverkusen, Germany) during the initial 2 weeks following implantation in an effort to avoid a worsening of prostatic conditions due to the flare-up reaction. The dog had a worsening of his behavior for about 3 weeks but then gradually improved and became a very easy-to-handle, calm and affectionate dog.

In December, the owners reported that the dog had greatly improved: he had stopped mounting other bitches and was only playing with them, had normal urine and feces and only showed a very mild irritation of the perianal gland. All his clinical parameters were normal and US confirmed that the prostate had improved and measured 3 × 4 cm with a single cyst in the left lobe measuring 0.5 cm (>2 cm 6 months prior). At this time, the dog was administered another 4.7 mg deslorelin implant.

In May 2016, the 4.7 mg implant was repeated after confirming general health, normal behavior and the normal ultrasonographic appearance of the prostate. The dog had had a gastrointestinal problem during the summer for which he had been admitted to a veterinary clinic for 2 days, at which time his blood (hematology and biochemistry) as well as US tests were normal. The dog recovered uneventfully. At the following appointment the owners came in late in December 2016 and by that time the dog was already showing increased testicular size and recurrence of the perianal gland problem. This was considered to be due to the loss of the effect of the previous implant and, when a new 4.7 mg deslorelin implant was administered, the dog showed a 3-week flare-up reaction (Table 2). Four months later, a 9.4 mg deslorelin implant was voluntarily administered in advance to avoid risking a new flare-up. Since then, the 9.4 mg deslorelin treatment was repeated yearly.

Table 2. Date of implantation, type of deslorelin implant, lot number, expiration date and presence of a flare-up reaction following treatment of an American Staffordshire intact male dog continuously treated with deslorelin between 2015 and 2021. A flare-up reaction occurred in December 2016 as the dog was reimplanted after more than 6 months following the previous 4.7 mg treatment.

Date	Deslorelin Implant	Lot Number	Expiration Date	Flare-Up Reaction
23 July 2015	4.7 mg	data	October 2016	Yes
22 December 2015	4.7 mg	SLV308B21	October 2016	No
19 May 2016	4.7 mg	SDW322D21	March 2017	No
1 December 2016	4.7 mg	SJW333F21	August 2017	Yes, 3 weeks
30 March 2017	9.4 mg	TDX353B21	March 2018	No
5 March 2018	9.4 mg	TDX353B21	March 2018	No
2 April 2019	9.4 mg	THY394C21	July 2019	No, but effect lasts until November 2019
9 December 2019	9.4mg	TGZ427D21	June 2020	Yes, 10 weeks, effects last until June 2020
6 October 2020	9.4 mg	TAB46721	December 2022	Yes, 4 weeks, treated for BPH
6 September 2021	9.4 mg	TAB46721	December 2022	No

A year later (March 2018), clinical examination revealed all the dog's clinical parameters were normal. A new 9.4 mg deslorelin implant was administered in the periumbilical region.

The following year, the implant was administered in April and only remained effective until November, when testicles increased in size, frequent ejaculations occurred and the dog's behavior became erratic and very agitated again. For this reason, the following 9.4 mg implant was administered before the 12th month, in December 2019. This implant's effectiveness was also shorter, with renewed problems beginning in June 2020: increased testicular size, frequent ejaculations and mounting of his owners.

In October 2020, the 9.4 mg implant was repeated after confirming general health and a US showing increased prostate size and two large cysts. Another 9.4 mg implant was administered 11 months later in September 2021.

Comment—This dog is very unusual because of his extreme vigor, libido and restlessness, which make him a difficult dog to manage. His behavior is probably the result of a very strong testosterone production or an alteration of its receptors, which is also causing his prostatic and perianal gland problems. This dog would obviously benefit from removal of his gonads as demonstrated by his improvement during periods in which he was being treated with deslorelin. In a case like this, it is fundamental to avoid a flare-up reaction. When his owners forgot to bring him back in November 2016 and treatment was delayed a few weeks, the dog's testicles started to increase in size immediately and the dog resumed his libido-related behavior making life very difficult for his owners. In order to avoid flare-up reactions in cases like this dog, it is important to schedule an appointment prior to the end of the previous implant's action in order to allow the dog's gonads to remain constantly under the effect of deslorelin. The two flare-up reactions of 2019 and 2020 were unexpected. As no testosterone measurements were performed, a shorter duration of efficacy of the implant cannot be confirmed. Possible explanations could be some product defects or incorrect technique of administration. A different mechanism of action of the implant for the management of behavior other than the one involved in the control of the synthesis and release of testosterone cannot be ruled out. Interestingly, the fact that the last implants used in this dog had the expected normal duration of efficacy proves that the dog remained sensitive to the action of deslorelin.

3.6. Case 6. Controlling Hypersexuality in a Mixed-Breed Male Dog

A 3-year-old, 5.8 kg BW, mixed-breed, intact male dog was referred to the VTH of UNIPD for pharmacological neutering. The dog had a history of aggressiveness towards other dogs but not humans, and a strong libido, which led him to the display of frequent masturbation and attempts to mount his owner, as well as a tendency to develop inflammation of the perianal glands. The dog lived indoors and, despite being fed dry food ad libitum, had recently lost 800 g during the previous 2 months. The owner also reported the dog as being hyperactive and having a voracious appetite and polyuria/polydipsia. On clinical examination, the dog was alert and normally responsive, his clinical parameters (pulse, respiration and rectal temperature) were normal and his testicles were normal in size, shape and consistency. Testicular size was assessed with a caliper: the right testis measured 3.24 cm^3 (applying volume formula L × W × H × 0.71 [33]) and the left measured 3.2 cm^3. As the dog could not be restrained manually, he was sedated with a premedication of 0.2 mg/kg of butorphanol and 3 μg/kg of dexmedetomidine to carry out clinical procedures. A blood sample was collected for a CBC and serum biochemistry evaluation, which were unremarkable except for a slight neutropenia (3870/μL) and decrease in red blood cells (RBCs) and platelet distribution width (PDW), low total proteins (59.33 g/L), high C-reactive protein (CRP = 2.3 mg/dl) and low globulins (27 g/L). Urinalysis revealed alkaline urine (pH = 8) and a modest increase in specific gravity (1.050); the urine culture showed no bacterial growth. In October 2020,

deslorelin was suggested as a potential treatment and a 4.7 mg deslorelin implant was administered in the subcutaneous tissue between the shoulder blades. The clinical follow-up 3 months later (January 2021) revealed that a flare-up effect had not taken place. The owner reported that the libido had diminished, but the aggressiveness towards other dogs had not. In addition, little if any effect on the perianal glands was observed, as the dog continued licking his perineal area and scratching it by dragging his posterior on the ground. Clinical examination revealed that the dog was alert and normally responsive and his clinical parameters were normal. He gained 0.5 kg since the last examination. US showed pharmacologically induced prostatic hypotrophy, since the prostate measured 1.12 cm^3, while a volume of 5.59 cm^3 was expected (BW = 7 kg); in addition, the right testis measured 1.28 cm^3 (applying volume formula L × W × H × 0.71 [33]) and the left 1.74 cm^3.

A second follow-up occurred in March 2021, and the owner reported an improvement in appetite (the dog weighed 7.2 kg) and perianal glad inflammation, along with a reduction in libido, decreased leg humping and masturbation. The testes were measured with a caliper (volume was calculated as before: right testis measured 0.74 cm^3 & left testis measured 0.96 cm^3) and US (right testis 2.33 cm × 0.62 cm and left testis 0.8 cm × 0.69 cm).

The following month (April 2021) marked 6 months from the implant and the dog was examined again. The owner reported that the dog had been in good health and active, with a normal general state. In the last 10 days, 2–3 mounting episodes had occurred. Clinical examination showed good general conditions and a BW of 7.1 kg. CBC revealed mild lymphocytosis (2920/μL) and monocytopenia (270/μL). Blood biochemistry reported low total proteins (61.45 g/L), high albumins (33.93 g/L) and low globulins (28 g/L) resulting in a high albumin/globulin ratio (1.23). The dog was assessed as being presumably at the end of the function of the deslorelin implant. He was sedated with 0.2 mg/kg of butorphanol and 3 μg/kg of dexmedetomidine and a new 4.7 mg deslorelin implant was inserted.

A follow-up examination 2 months later (June 2021) showed that the dog was healthy, with no signs of flare-up effect and serum testosterone was undetectable. The dog was re-examined 5.5 months after the deslorelin implant. The owner reported an efficacy of treatment for 5 months and an increase in mounting episodes in the last 2 weeks. They initially occurred once or twice per week and then increased in frequency to daily occurrences. Upon examination, clinical parameters were normal and the testes did not appear to have increased in size. A blood sample was collected under sedation (same drugs as above), CBC revealed neutropenia (4690/μL), low platelet count (74 × 10^3 /μL) and presence of platelet aggregates, and high RBC (7.7 × 10^6/μL), Hgb (18 g/dl) and Hct (52.7%). Biochemistry reported low total proteins (61.45 g/L), high albumins (33.93 g/L) and low globulins (28 g/L) resulting in a high albumin/globulin ratio (1.23). In September 2021, a 9.4 mg deslorelin implant was inserted in the subcutaneous tissue at the level of T12. In March 2022, on clinical examination, the dog weighed 6.8 kg and owners reported he had been well, eating with appetite and with normal general health. He had not shown interest in bitches in heat and reported few episodes of masturbation. A blood collection could be performed without sedation; CBC was unremarkable and blood chemistry parameters fell within the reference intervals. Serum testosterone following a GnRH stimulation test (carried out with 50 μg gonadorelin administered SC 1 h previously) was 0.7 ng/mL.

Comment—In this dog, repeated deslorelin treatments were effective in reducing libido and masturbation and normalizing his dominant attitude with an improvement in his relationship with his owner as well as an increase in weight and body conditions. There were no treatment-related negative effects; however, deslorelin was not effective for aggressiveness towards other dogs as this is evidently a behavioral condition requiring an appropriate behavioral approach. The 4.7 mg implant was effective for behavior management for about 5 months and clinical signs

due to strong libido would gradually recur from the start of the 5th month post-treatment, while only 6 months have elapsed since administration of the 9.4 mg implant and therefore it is too early to assess the duration of this implant in this dog. Testicular US could not be reliably performed in this dog to calculate testis volume due to costs.

4. Discussion

Deslorelin should be regarded as a very safe drug. The majority of short-term side effects due to deslorelin have been reported in intact bitches (a category which was not featured in our study) such as persistent estrus, uterine disease, ovarian cysts, pseudopregnancy, UI, cystitis, increased weight and behavioral as well as coat changes [6,34–36]. Weight gain and a change in temperament were observed in case n. 4, the Maremma shepherd dog treated for 9 years, although such effects were specifically reported following the use of the 9.4 mg implant and not following the use of the 4.7 mg implant. This difference in weight gain and behavior between the two types of deslorelin implants is hard to explain and might be due to a coincidence with another subclinical condition/s affecting the dog. Unwanted pregnancy is also a possible side effect following a deslorelin-induced heat as such pregnancy is typically followed by a mid-term abortion in bitches [6]. The occurrence of heat is very difficult to avoid in intact post-pubertal bitches treated with deslorelin. In bitches, the administration of a deslorelin implant during diestrus will drastically reduce the incidence of heat [6] but some bitches may still develop flare-up signs and occasionally ovarian cysts and pyometra, particularly when treated during a luteal phase [37]. When compared to other drugs to achieve a long-lasting control of reproduction and reproductive behavior such as progestins, deslorelin is certainly advantageous as it achieves a block of the HPG axis following a prolonged pituitary stimulation leading to pituitary exhaustion and ultimately resulting in the absence of all pituitary-controlled reproductive hormones from the organism. Progestins instead block the HPG axis thanks to a feed-back mechanism obtained by the administration of exogenous hormones. Although progestins should be considered as safe drugs (provided that they are used only in the right patient for the correct amount of time and at an appropriately low dosage), it is undisputable that their use constitutes extra work for the liver and kidneys. Therefore, progestins have some limitations connected with the duration of treatment and health conditions of the patient. Deslorelin has no such limitations and in fact it should be safely used in animals with renal insufficiency who cannot undergo surgical neutering.

Long-term problems following the use of deslorelin have been reported in intact bitches [37] but have not been observed yet in other categories of patients (intact male dogs and spayed bitches). We observed two cases of neoplasia in our patients n. 2 and 4. The case of pituitary carcinoma developing in the Boxer bitch with urinary incontinence (case n. 2) deserves further investigation. It appears unlikely to be related directly to the use of deslorelin as it presumably developed from the adrenocorticotroph-secreting cells, which are not a target of deslorelin action. However, abnormal pituitary stimulation has been proposed as a cause of this type of tumors [22]. Deslorelin produces a prolonged rather than abnormal pituitary stimulation, which does not cause any pituitary derangement in normal, young or adult animals, although it remains to be established whether or not such a prolonged stimulation may be a problem for elderly animals, in which flare-up effects should probably be avoided. The effect of the prolonged use of GnRH agonists on the pituitary gland and consequent repeated initial stimulation should be further investigated.

Similarly, no direct connection can be established between deslorelin administration and the bladder carcinoma that developed in the Maremma Shepherd dog (case n. 4). GnRH receptors can be found in many organ systems including the urinary tract of dogs [38], and bladder function in spayed bitches is directly

influenced by GnRH agonists [39]. However, the role of GnRH agonists on the urinary function of intact male dogs, if any, is unknown. Neutered dogs are at a higher risk of developing urinary tract and prostatic neoplasia [40–43]. As the Maremma shepherd dog was treated throughout most of his life with deslorelin (except for a short lap of a few months in 2013), his long-lasting (chemically) neutered condition might have played a role in the development of his bladder neoplasia. Perhaps a short pause (3–6 months or longer) in deslorelin treatment might be beneficial for patients undergoing chronic treatments, allowing gonadal hormones to exert their function in stimulating the immune system.

The flare-up reaction is an interesting phenomenon: a) it can be useful for some clinical situations (as for estrus induction in intact females); b) it can be irrelevant for conditions, such as benign prostatic hyperplasia, as time for reimplantation can be decided by monitoring the patient and acting when clinical or diagnostic imaging signs indicate that the condition is worsening again; or c) it can be dangerous in the case of patients treated for excess libido such as case n. 5. Considering the potential negative effect of gonadectomy on the immune system and general health of dogs [44], the usefulness of letting dogs undergoing chronic deslorelin treatment go through short (3–6 months or longer) periods of times without treatment should be investigated.

5. Conclusions

The results of this study indicate that the long-term use of deslorelin may be effective in diseases such as prostate hyperplasia, reproductive behavior disorders or urinary incontinence. In addition, according to the results from these case studies, deslorelin is a safe drug to choose for a chronic treatment, where repeated implants are administered for years of the dog's life. The possibility of a correlation between the long-term use of deslorelin and the development of pituitary carcinomas should be further investigated. Moreover, as surgical neutering has been associated with the occurrence of urinary tract and prostatic neoplasia, the possibility exists that side effects of the prolonged absence of gonadal hormones may also occur during a chronic deslorelin treatment.

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Institutional Review Board Statement: Ethical review and approval were waived for this study since the administered veterinary drugs are approved for use in the treated dogs. The drug used in our clinical cases (deslorelin) is marketed for use in male dogs, while for UI in castrated females there is a large enough body of literature to justify its use, without requiring owners' signed consent and an ethical review.

Informed Consent Statement: Informed consent was obtained from all animal owners involved in the study whenever necessary.

Data Availability Statement: The data that support the findings of this study are available on request from stefano.romagnoli@unipd.it. The data are not publicly available due to privacy or ethical restrictions.

Conflicts of Interest: Stefano Romagnoli's research on deslorelin is funded by Virbac; however, all cases that were objects of this paper were first-opinion clinical cases for which the clients paid all expenses including cost of the implant. In addition, he has received honoraria in the past from Virbac for speaking at symposia. Chiara Milani has received support for her clinical activities on deslorelin from Virbac. Lluis Ferrè-Dolcet has received honoraria in the past from Virbac for speaking at symposia. Christelle Fontaine was a Virbac employee. Alice Diana declares no conflicts of interest.

References

1. Romagnoli, S.; Lopate, C. Reproductive Endocrinology and Breeding Management of the Bitch. In *The Textbook of Veterinary Internal Medicine*, 8th ed.; Ettinger, S., Feldman, E., Cote, E., Eds; Elsevier: Amsterdam, The Netherland, 2015; Volume 2, Section XXII, pp. 1845–1859.
2. Borges, P.; Fontaine, E.; Maenhoudt, C.; Payan-Carreira, R.; Santos, N.; Leblond, E.; Fontaine, C.; Fontbonne, A. Fertility in Adult Bitches Previously Treated with a 4.7 mg Subcutaneous Deslorelin Implant. *Reprod. Domest. Anim.* **2015**, *50*, 965–971. https://doi.org/10.1111/rda.12616.
3. Rubion, S.; Driancourt, M.A. Controlled delivery of a GnRH agonist by a silastic implant (Gonazon) results in long-term contraception in queens. *Reprod. Domest. Anim.* **2009**, *44*, 79–82. https://doi.org/10.1111/j.1439-0531.2009.01415.x.
4. Junaidi, A.; Williamson, P.E.; Cummins, J.M.; Martin, G.B.; Blackberry, M.A.; Trigg, T.E. Use of a new drug delivery formulation of the gonadotrophin-releasing hormone analogue Deslorelin for reversible long-term contraception in male dogs. *Reprod. Fertil. Dev.* **2003**, *15*, 317–322. https://doi.org/10.1071/rd03039.
5. Trigg, T.E.; Doyle, A.G.; Walsh, J.D.; Swangchan-uthai, T.A review of advances in the use of the GnRH agonist deslorelin in control of reproduction. *Theriogenology* **2006**, *66*, 1507–1512. https://doi.org/10.1016/j.theriogenology.2006.02.037
6. Romagnoli, S.; Stelletta, C.; Milani, C.; Gelli, D.; Falomo, M.E.; Mollo, A. Clinical use of deslorelin for the control of reproduction in the bitch. *Reprod. Domest. Anim.* **2009**, *44*, 36–39. https://doi.org/10.1111/j.1439-0531.2009.01441.x.
7. Romagnoli, S.; Siminica, A.; Sontas, B.H.; Milani, C.; Mollo, A.; Stelletta, C. Semen quality and onset of sterility following administration of a 4.7-mg deslorelin implant in adult male dogs. *Reprod. Domest. Anim.* **2012**, *47*, 389–392. https://doi.org/10.1111/rda.12058.
8. Lucas, X. Clinical use of deslorelin (GnRH agonist) in companion animals: A review. *Reprod. Domest. Anim.* **2014**, *49*, 64–71. https://doi.org/10.1111/rda.12388.
9. Christensen, B.W. Canine Prostate Disease. *Vet. Clin. Small Anim. Pract.* **2018**, *48*, 701–719. https://doi.org/10.1016/j.cvsm.2018.02.012.
10. Barsanti, J.A.; Finco, D.R. Medical management of canine prostatic hyperplasia. In *Current Veterinary Therapy, XII*; Bonagura, J.D., Kirk, R.W., Eds; Saunders: Philadelphia, PA, USA, 1995; pp. 1033–1034.
11. Nizanski, W.; Levy, X.; Ochota, M.; Pasikowska, J. Pharmacological Treatment for Common Prostatic Conditions in Dogs–Benign Prostatic Hyperplasia and Prostatitis: An Update. *Reprod. Domest. Anim.* **2014**, *49*, 8–15. https://doi.org/10.1111/rda.12297.
12. Junaidi, A.; Williamson, P.E.; Trigg, T.E.; Cummins, J.M.; Martin, G.B. Morphological study of the effects of the GnRH superagonist deslorelin on the canine testis and prostate gland. *Reprod. Domest. Anim.* **2009**, *44*, 757–763. https://doi.org/10.1111/j.1439-0531.2008.01066.x.
13. Ferré-Dolcet, L.; Sussan, B. Acute colonic impaction and faecaloma due to canine benign prostatic hyperplasia—Case report. *Acta Vet. Hung.* **2021**, *69*, 9–13. https://doi.org/10.1556/004.2021.00011.
14. Arnold, S.; Hubler, M.; Reichler, I. Urinary incontinence in spayed bitches. New insights into the pathophysiology and options for medical treatment. *Reprod. Domest. Anim.* **2009**, *44*, 190–192. https://doi.org/10.1111/j.1439-0531.2009.01407.x.
15. Thrusfield, M.V.; Holt, P.E.; Muirhead, R.H. Acquired urinary incontinence in bitches: Its incidence and relationship to neutering practices. *J. Small Anim. Pract.* **1998**, *39*, 559–566. https://doi.org/10.1111/j.1748-5827.1998.tb03709.x.
16. Augsburger, H.R.; Cruz-Orive, L.M. Influence of ovariectomy on the canine striated external urethral sphincter (M. urethralis): A stereological analysis of slow and fast twitch fibres. *Urol. Res.* **1998**, *26*, 417–422. https://doi.org/10.1007/s002400050079.
17. Reichler, I.M.; Hubler, M.; Jöchle, W.; Trigg, T.E.; Piché, C.A.; Arnold, S. The effect of GnRH analogs on urinary incontinence after ablation of the ovaries in dogs. *Theriogenology* **2003**, *60*, 1207–1216. https://doi.org/10.1016/s0093-691x(02)01368-7.
18. Holt, P.E. Urinary incontinence in the bitch due to sphincter mechanism incompetence: Prevalence in referred dogs and retrospective analysis of 60 cases. *J. Small Anim. Pract.* **1985**, *26*, 181–190. https://doi.org/10.1111/j.1748-5827.1985.tb02099.x.
19. Reichler, I.M.; Jochle, W.; Pichè, C.A.; Roos, M.; Arnold, S. Effect of a long-acting GnRH analogue or placebo on plasma LH/FSH, urethral pressure profiles and clinical signs of urinary incontinence due to sphincter mechanism incompetence in bitches. *Theriogenology* **2006**, *66*, 1227–1236. https://doi.org/10.1016/j.theriogenology.2006.03.033.
20. Rosol, J.T.; Gröne, A. Endocrine Glands. In *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*, 6th ed.; Maxie, G., Ed.; Saunders: Philadelphia, PA, USA, 2015; Volume 1, pp. 276–290.
21. Song, R.B.; Vite, C.H.; Bradley, C.W.; Cross, J.R. Postmortem evaluation of 435 cases of intracranial neoplasia in dogs and relationship of neoplasm with breed, age, and body weight. *J. Vet. Intern. Med.* **2013**, *27*, 1143–1152. https://doi.org/10.1111/jvim.12136.
22. Sanders, K.; Galac, S.; Meij, B.P. Pituitary tumour types in dogs and cats. *Vet. J.* **2021**, *270*, 105623 https://doi.org/10.1016/j.tvjl.2021.105623.
23. Miller, A.D.; Miller, C.R.; Rossmeisl, J.H. Canine Primary Intracranial Cancer: A Clinicopathologic and Comparative Review of Glioma, Meningioma, and Choroid Plexus Tumors. *Front. Oncol.* **2019**, *9*, 1151. https://doi.org/10.3389/fonc.2019.01151.
24. Maarschalkerweerd, R.J.; Endenburg, N.; Kirpensteijn, J.; Knol, B.W. Influence of orchiectomy on canine behaviour. *Vet. Rec.* **1997**, *140*, 617–619. https://doi.org/10.1136/vr.140.24.617.
25. Hart, B.L.; Eckstein, R.A. The role of gonadal hormones in the occurrence of objectionable behaviours in dogs and cats. *Appl. Anim. Behav. Sci.* **1997**, *52*, 331–344. https://doi.org/10.1016/S0168-1591(96)01133-1.

26. Knol, B.W.; Egberink-Alink, S.T. Treatment of problem behaviour in dogs and cats by castration and progestagen administration: A review. *Vet. Q.* **1989**, *11*, 102–107. https://doi.org/10.1080/01652176.1989.9694206.
27. Kutzler, M. Prevention of breeding in the male. In *BSAVA Manual of Canine and Feline Reproduction and neonatology*, 2nd ed; BSAVA: Gloucester, UK, 2010; pp. 34–43.
28. Stempel, S.; Körber, H.; Reifarth, L.; Schuler, G.; Goericke-Pesch, S. What Happens in Male Dogs after Treatment with a 4.7 mg Deslorelin Implant? II. Recovery of Testicular Function after Implant Removal. *Animals* **2022**, *12*, 2545. https://doi.org/10.3390/ani12192545.
29. Goericke-Pesch, S. Long-term effects of GnRH agonists on fertility and behaviour. *Reprod. Domest. Anim.* **2017**, *52*, 336–347. https://doi.org/10.1111/rda.12898.
30. Higuchi, T.; Burcham, G.N.; Childress, M.O.; Rohleder, J.J.; Bonney, P.L.; Ramos-Vara, J.A.; Knapp, D.W. Characterization and treatment of transitional cell carcinoma of the abdominal wall in dogs: 24 cases (1985–2010). *J. Am. Vet. Med. Assoc.* **2013**, *242*, 499–506. https://doi.org/10.2460/javma.242.4.499.
31. de Brot, S.; Robinson, B.D.; Scase, T.; Grau-Roma, L.; Wilkinson, E.; Boorjian, S.A.; Gardner, D.; Mongan, N.P. The dog as an animal model for bladder and urethral urothelial carcinoma: Comparative epidemiology and histology. *Oncol. Lett.* **2018**, *16*, 1641–1649. https://doi.org/10.3892/ol.2018.8837.
32. Knapp, D.W.; Glickman, N.W.; DeNicola, D.B.; Bonney, P.L.; Lin, T.L.; Glickman, L.T. Naturally-occurring canine transitional cell carcinoma of the urinary bladder A relevant model of human invasive bladder cancer. *Urol. Oncol.* **2000**, *5*, 47–59. https://doi.org/10.1016/s1078-1439(99)00006-x.
33. Paltiel, H.J.; Diamond, D.A.; Di Canzio, J.; Zurakowski, D.; Borer, J.G.; Atala, A. Testicular volume: Comparison of orchidometer and US measurements in dogs. *Radiology* **2002**, *222*, 114–119. https://doi.org/10.1148/radiol.2221001385.
34. Arlt, S.P.; Spankowsky, S.; Heuwieser, W. Follicular cysts and prolonged oestrus in a female dog after administration of a deslorelin implant. *New Zealand Vet. J.* **2011**, *59*, 87–91. https://doi.org/10.1080/00480169.2011.552858.
35. Palm, J.; Reichler, I.M. The use of Suprelorin in companion animal medicine. *Schweiz. Arch. Tierheilkd.* **2012**, *154*, 7–12. https://doi.org/10.1024/0036-7281/a000286.
36. Brandli, S.P.; Palm, J.; Kowalewski, M.P.; Reichler, I.M. Long-term effect of repeated deslorelin acetate treatment in bitches for reproduction control. *Theriogenology* **2021**, *173*, 73–82. https://doi.org/10.1016/j.theriogenology.2021.07.015.
37. Fontaine, E.; Fontbonne, A. Clinical use of GnRH agonists in canine and feline species. *Reprod. Domest. Anim.* **2011**, *46*, 344–353. https://doi.org/10.1111/j.1439-0531.2010.01705.x.
38. Reichler, I.M.; Welle, M.; Sattler, U.; Jochle, W.; Roos, M.; Hubler, M. Comparative quantitative assessment of GnRH- and LH-receptor mRNA expression in the urinary tract of sexually intact and spayed female dogs. *Theriogenology* **2007**, *67*, 1134–1142. https://doi.org/10.1016/j.theriogenology.2007.01.001.
39. Reichler, I.M.; Barth, A.; Piché, C.A.; Jöchle, W.; Roos, M.; Hubler, M.; Arnold, S. Urodynamic parameters and plasma LH/FSH in spayed Beagle bitches before and 8 weeks after GnRH depot analogue treatment. *Theriogenology* **2006**, *66*, 2127–2136 https://doi.org/10.1016/j.theriogenology.2006.06.009.
40. Norris, A.M.; Laing, E.J.; Valli, V.E.O.; Withrow, S.J.; Macy, D.W.; Ogilvie, G.K.; Tomlinson, J.; McCaw, D.; Pidgeon, G.; Jacobs, R.M. Canine Bladder and Urethral Tumors: A Retrospective Study of 115 Cases (1980-1985). *J. Vet. Intern. Med.* **1992**, *6*, 145–153. https://doi.org/10.1111/j.1939-1676.1992.tb00330.x.
41. Houlihan, K.E. A literature review on the welfare implications of gonadectomy of dogs. *J. Am. Vet. Med. Assoc. 2017, 250, 1155–1166*. https://doi.org/10.2460/javma.250.10.1155.
42. Teske, E.; Naan, E.C.; van Dijk, E.M.; Van Garderen, E.; Schalken, J.A. Canine prostate carcinoma: Epidemiological evidence of an increased risk in castrated dogs. *Mol. Cell. Endocrinol.* **2002**, *197*, 251–255. https://doi.org/10.1016/s0303-7207(02)00261-7.
43. Schrank, M.; Romagnoli, S. Prostatic neoplasia in the intact and castrated dog: How dangerous is castration? *Animals* **2020**, *10*, 85. https://doi.org/10.3390/ani10010085.
44. Sundburg, C.R.; Belanger, J.M.; Bannasch, D.L.; Famula, T.R.; Oberbauer, A.M. Gonadectomy effects on the risk of immune disorders in the dog: A retrospective study. *BMC Vet. Res.* **2016**, *12*, 278. https://doi.org/10.1186/s12917-016-0911-5.

3.2 Follow up

3.2.1 Frida – Spayed Rhodesian Ridgeback

Since the last follow up and the article's publication, Frida's owner has moved abroad and left her with his parents. Consequently, accurate monitoring of the bitch's clinical condition and signs is not currently possible. With regard to the date of the most recent implant, we know that she was reimplanted with a new 9.4 mg deslorelin implant in summer of 2022 and that the current caretakers intend to repeat the treatment again after a year in summer 2023. The follow-up call in June 2023 also reported that Frida had been doing well and no signs of urinary leakage had been noticed at home.

3.2.2 Jack – American Staffordshire Male Dog

This patient was last implanted with a 9.7 mg implant in September 2021, but since then his family moved to Malta. For the following 17 months the owners reported that Jack was well-controlled by the treatment. In September 2022, the owners decided not to administer a new implant. This decision was due to two reasons: the use of Suprelorin™ 9.4 mg is currently not authorized in local Maltese veterinary practices (only the 4.7 mg implant is authorized, but it is used rarely) and they hoped the dog's age (10 years old) might have reduced his testosterone levels. However, in March 2023, they started to notice his testes enlarging and, in the following 2 months, they witnessed a gradual increase of the dog's excitement and sexual arousal. He became very agitated and while on walks he would obsessively smell every scent and lick all the urine marks on their path. He started to mount both owners again, especially after meals, when he needed to be isolated in a room until he calmed down.

In April 2023, Jack fell off the couch and started limping on his right hind leg. He was examined by the local vet who prescribed meloxicam (Loxicom™, Norbrook, Newry, Northern Ireland) to treat the pain. After a week the treatment was suspended as Jack developed diarrhea, which was treated with probiotics. He then resumed the gabapentin treatment he had been prescribed in 2019 to manage his chronic back pain (suspected cervical hernia), with 300 mg daily administration. To manage the dog's high energy and support his mobility recovery, the owners started to take him swimming every day, which has given excellent results.

In May the owner noticed some preputial green liquid, which seemed to ooze out rather than be ejaculated and contacted the reproduction team in the VTH of UNIPD. At this time no further signs of prostatitis were present, but it is to be noted that the patient was experiencing diarrhea due

to the aforementioned NSAID treatment. Given a past similar occurrence resulting to be prostatitis and the dog's intense discomfort, we advised to treat the current condition before proceeding with an ultrasonographic check-up and then, hopefully, a new 4.7 mg deslorelin implant at a willing local clinic. After email consultation to understand the available drugs in Malta and find an alternative to the unattainability of osaterone acetate (Ypozane™ , Virbac, Carros, France), in the beginning of June the condition was treated as a recurrence of prostatitis with cyproterone acetate (Androcur®, Bayer, Leverkusen, Germany) 50 mg BID for a week then 25 mg BID for 10-15 days and marbofloxacin (Marbocyl™, Vetoquinol, Magny-Vernois, France) 60 mg SID for 20 days. After the first few days Jack seemed to already experience an improvement of his condition, but at the end of the treatment the owners reported no effect of the antiandrogenic in reducing his hypersexuality and agitatedness.

The dog was brought to a local veterinarian to further investigate the prostatic condition and treatment efficacy, monitor hepatic function and evaluate the general condition for deslorelin insertion. The physical examination was normal, but the implant was not administered at this time since upon US exam of the prostate the clinician noticed it was enlarged, with irregular volume and a neoformation.

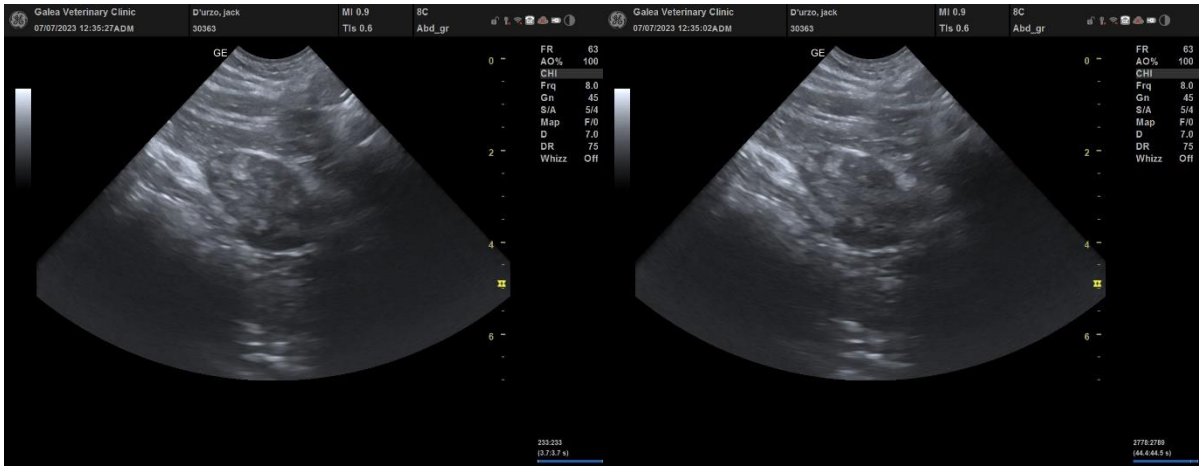

Figure 4.1 Abdominal ultrasound of an American Staffordshire dog treated with marbofloxacin and cyproterone acetate for a suspected prostatitis recurrence. The scan shows the ultrasonographic appearance of the prostate.

Serum biochemistry evaluation showed low amylase (377 U/L), slightly increased globulin (46 g/L) and normal hepatic values except for a slightly increased ALT (137 U/L). This exam did not include AST assay. However, it should be underlined that this dog has shown high hepatic enzymes since his first blood chemistry analyses as a puppy. This alteration had been further investigated with US of the liver, but no other alterations were observed. The liver function was

not investigated further, but every prostate ultrasound was paired with liver imaging too, when possible, always showing normal size, margins and parenchyma of the organ.

On the 21st of July a new deslorelin implant was administered, albeit 4.7 mg due to the local availability. We recommended an antiandrogenic to avoid flare up effect from exacerbating further the condition but Ypozane™ remains unavailable and, due to the inefficacy of the latest treatment and liver values, the owners are against the use of Androcur™ (Bayer, Leverkusen, Germany). Over the months they have learned to manage Jack's high libido and intended to manage the flare up with swimming and playing to release his excessive energy and reduce his agitation.

3.2.3 Baloo – Mixed-Breed Male Dog

Baloo has received a total of 3 implants, the first two were 4.7 mg and the third was 9.4 mg. A follow-up call in July 2023 for updates revealed that the third implant started losing effect after around 6 months (March 2022). Gradually the dog started presenting normal erections and sperm production, progressively returning to his original complaints. The owner is a veterinarian and suspects a progressive reduction of clinical effect of deslorelin with repeated administrations. No further implants have been applied.

In October 2022, the dog was brought to the owner's clinic for investigations on a hemorrhagic diarrhea, although there is no recorded image, the owner reported that on ultrasound they identified an enlarged prostate with cysts.

Around the same time, the owner adopted a female dog and our patient was consequently castrated. After the surgery he occasionally mounts, but not as obsessively as before the implants. In March 2023 the prostate had returned to normal size with no visible cysts.

4. Discussion

4.1 Duration of treatment

The 2 deslorelin formulations commercially available have an efficacy of 6 months for 4.7 mg implants and 12 months for 9.4 mg implants according to the manufacturer's instructions. However, duration of the implant's effect is unpredictable in dogs, as efficacy seems to be dose related (Trigg et al., 2001) and reports show significant individual variability even within the same dose (Maenhoudt et al., 2014)

Information about duration of effects in females is more limited. Long-term suppression of the reproductive cycle and restoration of normal fertility after the end of efficacy or deslorelin implant removal have been reported (Borges et al., 2015; Lucas, 2014). Overall, the male time frame seems to be widely fitting in bitches too.

Our case series is overall consistent with the claimed duration of implants, although serial reimplantation does not allow to completely evaluate the duration when reimplantation occurs before the effect of the previous implant has extinguished.

Figure 4.1 summarizes relevant information on all the treatments in our case series, with reference to the average duration of the 4.7 and 9.4 mg implant effect for each dog. Some of these cases' duration should however be further discussed.

Specifically, in regard to Ben, where the implants were intended to treat BPH, it's difficult to verify whether or not duration was shorter than 6 months as prostatic growth is a slow process. Once the prostate has become almost atrophic at the end of a deslorelin treatment it may take weeks or a few months before it starts giving problems due to BPH, making a shorter (e.g. 4 or 5 months) than normal duration of action difficult to detect unless serial ultrasonographic exams are performed.

Furthermore, the mean time of observed 9.4 implant duration of effect is difficult to measure in Jack as implants generally maintained efficacy until reimplantation about 11 months later, except in two cases. Our original explanation for the two shorter durations with the implants of 2019 and 2020 was that they were due to use of an implant which expired during its use. This was unexpected as the expiration date of deslorelin had always been interpreted as related to the time of initial use (i.e. the implant will last for its expected duration as long as it is administered prior to the expiration date). However, Virbac has denied the possibility of the implants in question

Table 4.1 Study of 6 dogs ordered by reason for chronic treatment with deslorelin implant; dog name, age at first treatment, number of 4.7 mg or 9.4 mg implants, mean time interval between implants for 4.7 mg or 9.4 mg of deslorelin, time of observed duration of effect of 4.7 or 9.4 mg implants; major side effects (requiring treatment to maintain animal welfare or life-threatening) and minor side effects (transient and self-limiting changes that did not require any treatment or mild persistent side effects not affecting the dog's health) are shown.

Dog	**Ben**	**Letizia**	**Frida**	**Matley**	**Jack**	**Baloo**
Breed	German shepherd	Boxer	Rhodesian ridgeback	Maremma shepherd	American Staffordshire	Mixed
Sex	Male, intact	Female, spayed	Female, spayed	Male, intact	Male, intact	Male, intact
Reason for treatment	BPH	UI	UI	Contraception	Control of reproductive behavior	Control of reproductive behavior
Age at first deslorelin implant	8 years old	7 years old	4.5 years old	2 years old	3 years old	3 years old
Number of 4.7 mg implants	3	4	1	13	5	2
Mean time interval in between 4.7 implants	5.5-6 months	6-6.5 months	/	5.5 months	5 months (prior to the delay due to unavailability in Malta)	5.5 months
Mean time of observed 4.7 implant duration of effect	Until new implant	6 months, except only 1 month with 4th implant	5 months	Until new implant, even 8 months	Until new implant	*Discussed below*
Number of 9.4 mg implants	1	1	3	2	6	1
Mean time interval in between 9.4 implants			10 months	13.5 months	11 months	
Mean time of observed 9.4 implant duration of effect	At least 9 months		Until new implant	Until new implant	Until new implant, except only 7 months twice	6 months
Overall duration of treatment	> 2.5 years	3.5 years	3 years	10 years	9.5 years	1.5 years
Minor side effects	None reported	None reported	None reported	Owners reported reduced liveliness and tendency to gain weight with 9.4 mg implant	None reported	None reported
Major side effects	None reported	Pituitary carcinoma?	None reported	Bladder carcinoma?	None reported	None reported

expiring during the period of expected duration and confirmed that implants do not require to be administered 6 or 12 months prior to their expiration date for the 4.7 or 9.4 mg implants, respectively (personal communication). According to the manufacturing company any occurrence of duration of action shorter than the indicated period is to be considered as abnormal and should be suspected of lack of efficacy due to issues with the technique of administration of the implant, impaired quality of the implant or to the dog's metabolism (personal communication). Nonetheless, we feel that this finding should be taken into account, both for future investigation

and in clinical practice, until further data is available ruling out this possible cause of dysfunction of the implants. The fact that Jack's last two implants' duration of effect respected the expected period, even after the 2 occurrences of reduced duration of the implants, shows that he remained sensitive to deslorelin and the previous abnormal occurrence was not due to the development of resistance or immunization.

Lastly, the duration of implant effect in Baloo appeared to decrease with each new implant, as the first implant remained effective for 5.5 months, while the second one only lasted 5 months. Even more surprising was the owner reporting that with the third implant, the only 9.4 mg implant administered, signs of hypersexuality began resurfacing after 6 months.

The finding that repeated implants may reduce duration of efficacy is inconsistent with our other cases and with literature documenting chronic use of deslorelin, however the lack of information on multiple deslorelin implant administration in dogs makes further reasoning on this issue difficult and perhaps inconclusive. Documentation of case series such as ours may be useful in bringing awareness to unexpected and/or unknown features of deslorelin use.

4.2 Efficacy of off label use of Deslorelin

The findings of this investigation suggest that the chronic use of deslorelin exhibits efficacy in treating conditions such as BPH, disorders related to reproductive behavior and UI.

The treatment was considered successful when the condition was controlled and no major side effects occurred in the observation period. Major side effects were defined as all conditions that required medical or surgical treatment to maintain animal welfare and/or were life-threatening. In contrast, minor side effects included transient and self-limiting changes that did not require any treatment or persistent side effects not affecting the dog's health, such as mild changes in behavior.

It's relevant to note that in addition to contraception, deslorelin also causes significant shrinkage of the prostate gland (Junaidi et al., 2009), which is a clinical advantage for patients with BPH. Studies using deslorelin implants in dogs have shown a significant progressive reduction in prostate volume (Jurczak et al., 2010; Ponglowhapan and Lohachit, 2010; Ström et al., 2010), even more rapid in BPH compared to normal healthy dogs with a resolution of clinical signs within two weeks after implantation (Ponglowhapan and Lohachit, 2010). Based on these studies, it may be concluded that GnRH agonist releasing implants are an effective method to treat BPH. However, it is important to underline that initially, due to the 'flare-up' effect of GnRH agonists, it is possible that the clinical condition due to BPH may temporarily worsen. The ideal patient for this treatment has been defined as to be the asymptomatic dog with BPH, in which the progression of the prostatic disorder is prevented (Lucas, 2014). However, a clinically evident BPH may still be treated with deslorelin implants provided that the flare up effect is monitored and treated when present.

Another indication for deslorelin use is to control certain objectionable behaviors linked to the action of testosterone in male dogs. Studies have demonstrated that surgical and chemical castration with deslorelin implants induce similar effects with regard to decreased testosterone values and sexually dimorphic male behaviors, including aggression, fear (Strom et al. 2010), sexual behavior and libido, hypersexuality (comprised of excessive mounting, whining and stray behavior), intermale conflict and excessive territorial urine marking in dogs (Goericke-Pesch, 2017). Clinically, the implants can be used to reliably produce the behavioral changes that would result from surgical castration. It seems that deslorelin implants are an alternative for owners seeking change in testosterone-mediated behaviors in male dogs, without undergoing castration, or as a trial to what might be achieved by a potential surgical sterilization (Driancourt and Briggs, 2020).

Lastly, although the precise pathway of deslorelin's effect on urinary incontinence is yet to be confirmed, there is already relevant literature supporting the presence of GnRH receptors not only in reproductive organs but also in the urinary tract, skin, intestine, and bone marrow (Cheung and Wong, 2008; Reichler et al., 2007, Coit et al., 2009). The expression of these receptors in the urinary tract may explain the effect of GnRH agonists on UI and urinary function. A potential role of deslorelin in directly affecting tumor formation in these organs has also been proposed (Brandli et al., 2021), although the currently available information on this aspect is inconclusive.

We could not establish any obvious correlation between the insurgence of a bladder carcinoma and the chronic use of GnRH agonists in Ben (case n.1), especially given his advanced age, nonetheless this finding should be taken into account and cross-referenced with future findings.

Overall, the use of deslorelin implants in the treatment of post-spaying USMI in female dogs has been reported to be effective in many studies (Lucas, 2014; Reichler et al., 2006) and is further supported by our own experience and publication.

4.3 Gonadectomy vs. chronic deslorelin use

There are a number of interrelated variables that have been shown to influence individual pet owners' choices in regard to surgical gonadectomy of their dogs. These factors include the repercussions of the procedure on the reproductive capacity, health, welfare and behavior, as well as personal beliefs regarding the procedure's perceived pain or necessity. These factors collectively generate arguments either favoring or opposing the procedure. Moreover, considerations also include the procedure's cost and accessibility, veterinary counseling, as well as basic knowledge and awareness of castration as a viable option (Driancourt and Briggs, 2020). It appears likely that if these factors influence decision-making regarding surgical castration, they might equally influence decision-making regarding non-surgical contraceptive alternatives, as well.

In the broader context, there is scarcity of scientific information concerning human attitude toward surgical compared to non-surgical fertility control methods for pet dogs, and fewer still pertaining to attitudes and conduct concerning the utilization of the commercialized deslorelin implants (Adams et al., 2016). These investigations have thus far been confined to the veterinary community, which, while not being the ultimate decision-makers in the adoption of a product or procedure, holds a pivotal role in shaping the choices of dog owners and caretakers pertaining to the management of canine fertility.

An effort should be made by the veterinarian to inform on non-surgical neutering techniques especially when surgery is a risk due to age or other factors increasing the anesthesia risk. This is also a viable option when owners ask for reversible fertility suppression of breeding males or are reluctant to castrate or as a "test-run" before deciding to proceed with surgical castration, offering the opportunity to examine the effect of the hormonal deprivation on the dog's behavior and health. It may be useful when male and female dogs are living in the same household for a temporary period and has shown promising results in the management of inappropriate reproductive behavior (Ström et al., 2010). Lastly, veterinarians may advise the off-label use of deslorelin to treat BPH, UI, alopecia X and perianal gland tumors (Driancourt and Briggs, 2020).

Reluctancy of owners occurs even when the dog is not intended for breeding and their hesitation to consider traditional surgical sterilization might be due to evidence of long-term health problems associated with removal of the gonads: obesity, UI, endocrine disorders (i.e. hypothyroidism, diabetes), orthopedic disorders (i.e. cranial cruciate ligament rupture, hip dysplasia), behavioral disorders (i.e. aggressiveness, fear) and neoplasia (i.e. osteosarcoma, hemangiosarcoma, mast cell tumor, lymphoma) (Kutzler, 2018[a]). As already mentioned, there are numerous nonsurgical methods for fertility management in male and female dogs which are safe, reliable, and reversible.

Deslorelin is marketed for temporary infertility and has proven to be very effective for this purpose. In males, effects on spermatogenesis, steroidogenesis and behavior have been demonstrated to be fully reversible (Stempel et al., 2022). Treatment produces detrimental effects on all aspects of semen quality, with azoospermia and aspermia reported as a consequence (Goericke-Pesch, 2017). In females, all effects have been postulated to be fully reversible which means that after the end of efficacy, bitches come spontaneously into estrus and ovulate.

Very few adverse effects were recorded during studies done with deslorelin implants in male dogs, with the exception of moderate swelling at the implant site (Driancourt and Briggs, 2020) and the short term flare up phase already described. Other side effects during the implants release period were very uncommon and limited to coat changes or owners reporting temporary reduction of activity after treatment (EMA, 2008).

In female dogs a wider range of side effects have been recorded after treatment with deslorelin: persistent estrus, endometrial proliferation and secretion, proliferation of mammary parenchyma during the first 2-4 weeks after treatment, cervical closure, possible atrophy of mammary parenchyma, induced lactation and/or lactational arrest, pyometra, weight gain, coat and behavioral changes (Fontaine and Fontbonne, 2010; Arlt et al., 2011; Palm and Reichler, 2011). In addition to these, after prolonged GnRH treatment in intact females some studies report hip dysplasia, false pregnancy, urinary incontinence, endometrial hyperplasia, and ovarian tumors (Brandli et al., 2021). One of the few case studies documenting the effects of repeated deslorelin administration in young bitches has shown major side effects in a fifth of the patients, including ovarian cancer, uterine diseases, UI and mammary tumors (Brandli et al., 2021), however data should not be overinterpreted given the study's reduced sample size and absence of a control group.

In addition to the primary consequence of inducing sterility in canines, surgical neutering has been demonstrated to exert a dual effect on the occurrence of various health conditions, resulting in both diminished and augmented incidence rates when compared to intact dogs. In theory, deslorelin implants possess the capacity to modulate the probability of specific adverse conditions, such as obesity and cancer, while still offering contraceptive benefits.

Among side effects of neutering, gonadectomy is associated with higher incidence of obesity in dogs in several studies (Kustritz et al., 2017). There is very limited information documenting whether factors contributing to obesity following surgical sterilization also exist following fertility control using deslorelin implants as clinical studies with deslorelin are often not designed to evaluate this outcome and they mostly involve small sample sizes with limited statistical power (Driancourt and Briggs, 2020). A recent clinical study with use of repeated deslorelin implants in

young female dogs for reproduction control showed an increase in body weight in 15 bitches after treatment (Brandli et al., 2021), however no controlled trial was performed and only 32 bitches were treated repeatedly with deslorelin implants, hence correlation with spayed female dogs of comparable age and breed is not possible. Data on male dogs and deslorelin specifically are limited and should be further researched as, if confirmed, less likelihood of weight gain might be an attractive feature of the implant rather than traditional neutering. Further investigation is necessary to explore the interaction between age of administration and the effect of treatment on body weight in dogs, and to directly address a comparison between neutering vs. deslorelin implant treatment on body weight.

In regards to neoplasia, as previously mentioned LH is suspected to be involved in the etiology of cancers with increased incidence after gonadectomy, given the expression of LH receptors in the tissues most commonly manifesting neoplastic evolution. Treatment with deslorelin implants decreases serum LH, in contrast to the overproduction of LH seen in surgically castrated dogs. Therefore, some authors hypothesize that if the LH receptors expressed on lymphocytes or bones are somehow related to the development of lymphoma or osteosarcoma, respectively, then the use of deslorelin, instead of or following surgical castration, may have a protective effect on the risk of lymphoma and osteosarcoma incidence or their progression rate (Driancourt and Briggs, 2020). However, once again, further investigation is needed to explore this hypothesis.

5. Conclusions

The results of this work indicate that the long-term use of deslorelin acetate implants may be effective in conditions such as benign prostate hyperplasia, urinary incontinence or reproductive behavior disorders. In addition, according to the results from these case studies, deslorelin is a safe drug to use for chronic treatment, where the dog undergoes multiple serial implant administration spanning several years in the canine lifespan, as few to no side effects have been reported. However, our sample size doesn't allow to consider ample individual variations and further investigations with wider difference in patient's reason for treatment, breed and age should be considered. Further studies are needed to explore the possibility of a correlation between the long-term use of deslorelin and the development of neoplasia (e.g. pituitary carcinomas and/or bladder carcinoma). Moreover, as surgical neutering has been associated with the occurrence of urinary tract and prostatic neoplasia, the possibility exists that side effects of the prolonged absence of gonadal hormones may also occur during a chronic deslorelin treatment.

Hence, the use of deslorelin could be useful for long-term treatment or contraception in cases where anesthesia is considered unsafe, but, especially in female dogs, further investigations are necessary to assess the development of major side effects.

6. Bibliography

1. Adams V., Walker S. and Taylor C. (2016). Attitudes to and opinions of neutering in dogs: results of a canine reproduction survey of veterinary surgeons. In *BSAVA Congress Proceedings 2016* (pp. 475-476). BSAVA Library.
2. Albanese F., Malerba E., Abramo F., Miragliotta V. and Fracassi F. (2014). Deslorelin for the treatment of hair cycle arrest in intact male dogs. *Veterinary dermatology*, *25*(6), 519-588.
3. Alliance for Contraception in Cats and Dogs. About ACC&D page. Available at: http://acc-d.org/About. Accessed March 8th, 2021
4. Amaral J., Briantais P., Fontaine C. and Rigaut D. (2023). Efficacy and Safety of 4.7 mg Deslorelin Acetate Implants as a Neutering Option in Male Cats: A Large-Scale Multicentre Randomised Controlled Study. *Animals*, *13*(3), 379.
5. Anderson R.K., Gilmore C.E. and Schnelle G.B. (1965). Utero-ovarian disorders associated with use of medroxyprogesterone in dogs. *Journal of the American Veterinary Medical Association*, *146*, 1311-1316.
6. Arlt S.P., Spankowsky S. and Heuwieser W. (2011). Follicular cysts and prolonged oestrus in a female dog after administration of a deslorelin implant. *New Zealand Veterinary Journal*, *59*(2), 87-91.
7. Arnold S., Hubler M. and Reichler I.M. (2009). Urinary incontinence in spayed bitches: new insights into the pathophysiology and options for medical treatment. *Reproduction in domestic animals - Zuchthygiene*, 44(Suppl), 190-192.
8. Arnold, S., Arnold, P., Hubler, M., Casal, M., & Rüsch, P. (1989). Incontinentia urinae bei der Kastrierten Hündin: Häufigkeit und Rassedisposition [Urinary incontinence in spayed female dogs: frequency and breed disposition]. *Schweizer Archiv fur Tierheilkunde*, 131(5), 259–263.
9. Asa C.S. (2018). Contraception in dogs and cats. *Veterinary Clinics: Small Animal Practice*, *48*(4), 733-742.
10. Augsburger H.R. and Cruz-Orive L.M. (1998). Influence of ovariectomy on the canine striated external urethral sphincter (M. urethralis): a stereological analysis of slow and fast twitch fibres. Urological research, 26, 417-422.
11. Bamberg-Thalén B. and Linde-Forsberg C. (1992). The effects of medroxyprogesterone acetate and ethinylestradiol on hemogram, prostate, testes, and semen quality in normal dogs. *Journal of Veterinary Medicine Series A*, *39*(1-10), 264-270.
12. Barsanti J.A and Finco D.R. (1995). Medical management of canine prostatic hyperplasia. In *Current Veterinary Therapy, XII*; ed. Bonagura and Kirk. Philadelphia, USA: Saunders, 1033-1034.
13. Beijerink N.J., Bhatti S.F.M., Okkens A.C., Dieleman S.J., Mol J.A., Duchateau L. and Kooistra H.02S. (2007). Adenohypophyseal function in bitches treated with medroxyprogesterone acetate. *Domestic Animal Endocrinology*, *32*(2), 63-78.
14. Bell A.G., Jones B.R. and Scott M.F. (1993). Growth hormone responsive dermatosis in three dogs. *New Zealand Veterinary Journal*, *41*(4), 195-199.
15. Bell F.W., Klausner J.S., Hayden D.W., Lund E.M., Liebenstein B.B., Feeney D.A. and Isaacs W.B. (1995). Evaluation of serum and seminal plasma markers in the diagnosis of canine prostatic disorders. *Journal of Veterinary Internal Medicine*, *9*(3), 149-153.

16. Bittman E.L., Dempsey R.J. and Karsch F.J. (1983). Pineal Melatonin Secretion Drives the Reproductive Response to Daylength in the Ewe. *Endocrinology*, 113(6), 2276–2283.
17. Borges P., Fontaine E., Maenhoudt C., Payan-Carreira R., Santos N., Leblond E. and Fontbonne A. (2015). Fertility in adult bitches previously treated with a 4.7 mg subcutaneous deslorelin implant. *Reproduction in Domestic Animals*, *50*(6), 965-971.
18. Bradecamp E.A. (2007). Estrous Synchronization. In *Current Therapy in Equine Reproduction*, ed. Samper, Pycock, McKinnon. Philadelphia, USA: W.B. Saunders, 22-25.
19. Brändli S.P., Palm J., Kowalewski M.P. and Reichler I.M. (2021). Long-term effect of repeated deslorelin acetate treatment in bitches for reproduction control. *Theriogenology*, *173*, 73-82.
20. Brass W., Ficus H.J. and Jochle W. (1971). Antiandrogen treatment of prostate enlargement in male dogs. *Kleintierpraxis*, *16*, 95-99.
21. Brodey R.S. (1966). Clinical and pathological findings in bitches treated with progestional compounds. *J. Am. Vet. Med. Assoc.*, *149*, 1406-1414.
22. Broqua P., Riviere P.J.M., Conn P.M., Rivier J.E., Aubert M.L. and Junien J.L. (2002). Pharmacological profile of a new, potent, and long-acting gonadotropin-releasing hormone antagonist: degarelix. *Journal of Pharmacology and Experimental Therapeutics*, *301*(1), 95-102.
23. Bryan J.N., Keeler M.R., Henry C.J., Bryan M.E., Hahn A.W. and Caldwell C.W. (2007). A population study of neutering status as a risk factor for canine prostate cancer. *The prostate*, *67*(11), 1174-1181.
24. Burke T.J. (1982). Pharmacologic control of estrus in bitch and queen. *The Veterinary Clinics of North America. Small Animal Practice*, *12*(1), 79-84.
25. Burke T.J. and Reynolds Jr H. A. (1975). Megestrol acetate for estrus postponement in the bitch. *Journal of the American Veterinary Medical Association*, *167*(4), 285-287.
26. Burke T.J., Reynolds H.A. and Sokolowski J.H. (1977). A 280-day tolerance-efficacy study with mibolerone for suppression of estrus in the cat. *American Journal of Veterinary Research*, *38*(4), 469-477.
27. Burrow R., Batchelor D. and Cripps P. (2005). Complications observed during and after ovariohysterectomy of 142 bitches at a veterinary teaching hospital. *Veterinary Record*, *157*(26), 829-833.
28. Byron J.K., Taylor K.H., Phillips G.S. and Stahl M.S. (2017). Urethral sphincter mechanism incompetence in 163 neutered female dogs: diagnosis, treatment, and relationship of weight and age at neuter to development of disease. *Journal of Veterinary Internal Medicine*, *31*(2), 442-448.
29. Cheung L.W. and Wong A.S. (2008). Gonadotropin-releasing hormone: GnRH receptor signaling in extrapituitary tissues. *The FEBS journal*, *275*(22), 5479-5495.
30. Christensen B. W. (2018). Canine prostate disease. *Veterinary Clinics: Small Animal Practice*, *48*(4), 701-719.
31. Christie D.W. and Bell E.T. (1971). Some observations on the seasonal incidence and frequency of oestrus in breeding bitches in Britain. *Journal of Small Animal Practice*, *12*(3), 159-167.
32. Cohen S.M., Werrmann J.G., Rasmusson G.H., Tanaka W.K., Malatesta P.F., Harris G. and Nett T.M. (1995). Comparison of the effects of new specific azasteroid inhibitors of steroid 5α-reductase on canine hyperplastic prostate: Suppression of prostatic DHT correlated with prostate regression. *The prostate*, *26*(2), 55-71.
33. Coit V.A., Dowell F.J. and Evans N. P. (2009). Neutering affects mRNA expression levels for the LH-and GnRH-receptors in the canine urinary bladder. *Theriogenology*, *71*(2), 239-247.

34. Concannon P.W. (2011). Reproductive cycles of the domestic bitch. *Animal reproduction science*, *124* (3-4), 200-210.
35. Cooley D.M., Beranek B.C., Schlittler D.L., Glickman N.W., Glickman L.T. and Waters D.J. (2002). Endogenous gonadal hormone exposure and bone sarcoma risk. *Cancer Epidemiology Biomarkers & Prevention*, *11*(11), 1434-1440.
36. de la Riva G.T., Hart B.L., Farver T.B., Oberbauer A.M., Messam L.L.M., Willits N. and Hart L.A. (2013). Neutering dogs: effects on joint disorders and cancers in golden retrievers. *PloS one*, *8*(2).
37. D'Occhio M.J. and Aspden W.J. (1996). Characteristics of luteinizing hormone (LH) and testosterone secretion, pituitary responses to LH-releasing hormone (LHRH), and reproductive function in young bulls receiving the LHRH agonist deslorelin: effect of castration on LH responses to LHRH. *Biology of reproduction*, *54*(1), 45-52.
38. Dorn C.R., Taylor D.O.N. and Schneider R. (1968). Survey of animal neoplasms in Alameda and Contra Costa counties, California. In *Cancer morbidity in dogs and cats from Alameda County*. Journal of National Cancer Institute, 40, 307-318.
39. Driancourt M.A. and Rhodes L. (2018). Male contraception in animal species. In *Encyclopedia of Reproduction* (2nd ed.), ed. Skinner. San Diego, USA: Academic Press, 553-558
40. Driancourt M.A. and Briggs J.R. (2020). Gonadotropin-releasing hormone (GnRH) agonist implants for male dog fertility suppression: A review of mode of action, efficacy, safety, and uses. *Frontiers in Veterinary Science*, *7*, 483.
41. Duygu K.A.Y.A. and Serhan Serhat S.S. (2017). Effect of Deslorelin on the Genital Tract in the Bitch: Mechanism of Action and Contraceptive Efficacy. *Turkiye Klinikleri J Vet Sci Obstet Gynecol - Special Topics*, *3*(1), 17-23.
42. Eigenmann J.E., Eigenmann R.Y., Rijnberk A., van der Gaag, I., Zapf J. and Froesch E.R. (1983). Progesterone-controlled growth hormone overproduction and naturally occurring canine diabetes and acromegaly. *European Journal of Endocrinology*, *104*(2), 167-176.
43. England G.C. (1997). Effect of progestogens and androgens upon spermatogenesis and steroidogenesis in dogs. *Journal of Reproduction and fertility. Supplement*, *51*, 123-138.
44. England G.C. (2010a). Physiology and endocrinology of the female. In *BSAVA manual of canine and feline reproduction and neonatology* (2nd ed.). Gloucester, UK: British Small Animal Veterinary Association, 1-12
45. England G.C. (2010b). Physiology and endocrinology of the male. In *BSAVA manual of canine and feline reproduction and neonatology* (2nd ed.). Gloucester, UK: British Small Animal Veterinary Association, 13-22
46. European Medicines Agency (EMA). Suprelorin 4.7mg European Public Assessment Report – Product Information; Annex 1, Summary of Product Characteristics. (2008). Available online at: https://www.ema.europa.eu/en/documents/product-information/suprelorin-epar-product-information_en.pdf
47. European Medicines Agency (EMA). Summary of Opinion (for Suprelorin 9.4mg Implant). (2010). Available online at: https://www.ema.europa.eu/en/documents/smop/cvmp-post-authorisation-summary-positive-opinion-suprelorin_en.pdf
48. Farquhar V.J., McCue P.M., Carnevale E.M., Nett T.M. and Squires E.L. (2002). Deslorelin acetate (Ovuplant) therapy in cycling mares: effect of implant removal on FSH secretion and ovarian function. *Equine veterinary journal*, *34*(4), 417-420.
49. Fekete G. and Szeberenyi S.Z. (1965). Data on the mechanism of adrenal suppression by medroxyprogesterone. *Steroids*, *6*(2), 159-166.

50. Ferré-Dolcetsìand Sussan B. (2021). Acute colonic impaction and faecaloma due to canine benign prostatic hyperplasia–Case report. *Acta Veterinaria Hungarica*, *69*(1), 9-13.
51. Fieni F., Merot J., Tainturier D., Bruyas JF and DATTU. (1998). Medical termination of pregnancy in the female dog. *Compendium of Veterinary Medicine*, *174* (7-8), 141-150.
52. Fontaine E. and Fontbonne A. (2010). Use of deslorelin to control fertility in the bitch. In *Proc 7th EVSSAR Congress.* Louvain la Neuve, Belgium. 15-17.
53. Fontaine E. and Fontbonne A. (2011). Clinical use of GnRH agonists in canine and feline species. *Reproduction in domestic animals*, *46*(2), 344-353.
54. Fontaine E., Mir F., Vannier F., Gérardin A., Albouy M., Navarro C. and Fontbonne A. (2011). Induction of fertile oestrus in the bitch using Deslorelin, a GnRH agonist. *Theriogenology*, *76*(8), 1561-1566.
55. Fontbonne, A. (2010). Clinical approach to unwanted mating and pregnancy termination. In *BSAVA manual of canine and feline reproduction and neonatology* (2nd ed.). Gloucester, UK: British Small Animal Veterinary Association, 106-115
56. Forsee K.M., Davis G.J., Mouat E.E., Salmeri K.R. and Bastian R.P. (2013). Evaluation of the prevalence of urinary incontinence in spayed female dogs: 566 cases (2003–2008). *Journal of the American Veterinary Medical Association*, *242*(7), 959-962.
57. Fossati P. (2022). Spay/neuter laws as a debated approach to stabilizing the populations of dogs and cats: An overview of the European legal framework and remarks. *Journal of Applied Animal Welfare Science: JAAWS*, 1–13. doi:10.1080/10888705.2022.2081807
58. Furman B.L. (2007). Deslorelin. *Biomedical Sciences.*
59. García Romero G., Mattioli G., Rosa D., Díaz J.D., Abeya M. and Gobello C. (2012). A single administration of the GnRH antagonist acyline inhibits basal and GnRH-stimulated serum testosterone concentrations in male dogs. *Reproduction in domestic animals*, *47*(3), 32-35.
60. Gobello C. (2006). Dopamine agonists, anti-progestins, anti-androgens, long-term-release GnRH agonists and anti-estrogens in canine reproduction: A review. Theriogenology, 66, 1560-1567.
61. Gobello C. (2012). Effects of GnRH Antagonists vs Agonists in Domestic Carnivores, a Review. *Reproduction in Domestic Animals*, *47*, 373-376.
62. Goericke-Pesch S., Spang A., Schulz M., Özalp G., Bergmann M., Ludwig C. and Hoffmann B. (2009). Recrudescence of spermatogenesis in the dog following downregulation using a slow release GnRH agonist implant. *Reproduction in domestic animals*, *44*, 302-308.
63. Goericke-Pesch S. (2017). Long-term effects of GnRH agonists on fertility and behaviour. *Reproduction in Domestic Animals*, *52*, 336-347.
64. Grubaugh W., Sharp D.C., Berglund L.A., McDowell K.J., Kilmer D.M., Peck L.S. and Seamans K.W (1982). Effects of pinealectomy in Pony mares. *Journal of Reproduction and fertility*. Supplement,32:293–295. PMID: 6962863.
65. Grüntzig K., Graf R., Boo G., Guscetti F., Hässig M., Axhausen K.W. and Pospischil A. (2016). Swiss canine cancer registry 1955–2008: occurrence of the most common tumour diagnoses and influence of age, breed, body size, sex and neutering status on tumour development. *Journal of comparative pathology*, *155*(2-3), 156-170.
66. Hardman JG. and Limbird LE. (2001). Gonadotropin-releasing hormone and gonadotropic hormones. In Parker K.L. and Schimmer B.P. Pituitary Hormones and Their Hypothalamic Releasing Factors. *The Goodman & Gilman's The Pharmacological Basis of Therapeutics* (10th ed.). New York, USA: McGraw-Hill, 1552-1558.
67. Hart B.L. and Eckstein R.A. (1997). The role of gonadal hormones in the occurrence of objectionable behaviours in dogs and cats. *Applied Animal Behaviour Science*, *52*(3-4), 331-344.

68. Hart B.L., Hart L.A., Thigpen A.P. and Willits N.H. (2014). Long-term health effects of neutering dogs: comparison of Labrador retrievers with golden retrievers. *PloS one*, *9*(7), 102-241.
69. Hart B.L., Hart L.A., Thigpen A.P. and Willits N.H. (2016). Neutering of German Shepherd Dogs: associated joint disorders, cancers and urinary incontinence. *Veterinary Medicine and Science*, *2*(3), 191-199.
70. Heber D., Dodson R., Swerdloff R.S., Channabasavaiah K. and Stewart J.M. (1982). Pituitary receptor site blockade by a gonadotropin-releasing hormone antagonist in vivo: mechanism of action. *Science*, *216*(4544), 420-421.
71. Herbert C.A. and Trigg T.E. (2005). Applications of GnRH in the control and management of fertility in female animals. *Animal Reproduction Science*, *88*(1-2), 141-153.
72. Hoffmann B., Höveler R., Hasan S.H., and Failing K. (1992). Ovarian and pituitary function in dogs after hysterectomy. *Reproduction*, *96*(2), 837-845.
73. Hopkins S.G., Schubert T.A. and Hart B. L. (1976). Castration of adult male dogs: effects on roaming, aggression, urine marking, and mounting. *Journal of the American Veterinary Medical Association*, *168*(12), 1108-1110.
74. Höveler R., Evers P. and Hoffmann B. (1987). Investigations on endocrine responses in the bitch following hysterectomy. *European Journal of Endocrinology*, *116*(3_Supplement), S110-S111.
75. Iguer-Ouada M. and Verstegen J. P. (1997). Effect of finasteride (Proscar MSD) on seminal composition, prostate function and fertility in male dogs. Journal of reproduction and fertility. Supplement, 51, 139-149.
76. Inaba T., Umehara T., Mori J., Torii R., Tamada H. and Sawada T. (1996). Reversible suppression of pituitary-testicular function by a sustained-release formulation of a GnRH agonist (leuprolide acetate) in dogs. *Theriogenology*, *46*(4), 671-677.
77. Jackson E.K.M. (1984). Contraception in the dog and cat. *British Veterinary Journal*, *140*(2), 132-137.
78. Jiang G., Stalewski J., Galyean R., Dykert J., Schteingart C., Broqua P. and Rivier J.E. (2001). GnRH antagonists: a new generation of long acting analogues incorporating p-ureido-phenylalanines at positions 5 and 6. *Journal of medicinal chemistry*, *44*(3), 453-467.
79. Jochle W. (1991). Pet population control in Europe. *Journal of the American Veterinary Medical Association*, *198*, 1225–1230
80. Jordan A. (1994). Toxicology of depot medroxyprogesterone acetate. *Contraception*, *49*(3), 189-201.
81. Junaidi A., Williamson P.E., Trigg T.E., Cummins J.M. and Martin, G.B. (2009). Morphological study of the effects of the GnRH superagonist deslorelin on the canine testis and prostate gland. *Reproduction in domestic animals*, *44*(5), 757-763.
82. Jurczak A., Domoslawska A., Janowski T. and Zdunczyk S. (2010). Treatment of benign prostatic hyperplasia (BPH) in dogs using GnRH agonist implant Suprelorin-preliminary results. In *Proc 7th EVSSAR congress.* Louvain la Neuve, Belgium.
83. Kaszak I., Kanafa S., Ruszczak A., Parada R., Śmiech M., Poznański P. and Jurka P. (2016). Different mammary tumors, ovarian cysts, uterocervical stump inflammation in hysterectomized bitch. *Animal Science Papers and Reports*, *34*(4), 399-403.
84. Kim K.S. and Kim O. (2005). Cystic endometrial hyperplasia and endometritis in a dog following prolonged treatment of medroxyprogesterone acetate. *Journal of veterinary science*, *6*(1), 81-82.

85. Kloosterboer H.J., Vonk-Noordegraaf C.A. and Turpijn E W. (1988). Selectivity in progesterone and androgen receptor binding of progestagens used in oral contraceptives. Contraception, 38(3), 325-332.
86. Knol B.W. and Egberink-Alink S.T. (1989). Treatment of problem behaviour in dogs and cats by castration and progestagen administration: A review. *Veterinary Quarterly*, *11*(2), 102-107.
87. Körber H., Wehrend A., Hoffmann B. and Goericke-Pesch S. (2013). The use of Suprelorin® for estrus suppression in the bitch. *Reproductive Biology*, (13), 30-31.
88. Kustritz M.V.R., Slater M.R., Weedon G.R. and Bushby P.A. (2017). Determining optimal age for gonadectomy in the dog: a critical review of the literature to guide decision making. *Med Acces Dec*, *9*, 167-11.
89. Kustritz M.V.R. (2018). Population control in small animals. *Veterinary Clinics: Small Animal Practice*, *48*(4), 721-732.
90. Kutzler M.A., Wheeler R., Lamb S. and Volkmann D.H. (2002). Deslorelin implant administration beneath the vulvar mucosa for the induction of synchronous estrus in bitches. In *Proceedings of Third EVSSAR European Congress*.
91. Kutzler M.A. (2010). Prevention of breeding in the male. In *BSAVA manual of canine and feline reproduction and neonatology* (2nd ed.). Gloucester, UK: British Small Animal Veterinary Association, 34-43.
92. Kutzler M.A. (2018[a]). Estrus suppression in dogs. *Veterinary Clinics: Small Animal Practice*, *48*(4), 595-603.
93. Kutzler M.A. (2018[b]). Estrous cycle manipulation in dogs. *Veterinary Clinics: Small Animal Practice*, *48*(4), 581-594.
94. Kutzler M.A. (2020). Possible relationship between long-term adverse health effects of gonad-removing surgical sterilization and luteinizing hormone in dogs. *Animals*, *10*(4), 599.
95. Lucas X. (2014). Clinical use of deslorelin (GnRH agonist) in companion animals: a review. Reproduction in domestic animals, 49, 64-71.
96. Maarschalkerweerd R.J., Endenburg N., Kirpensteijn J. and Knol B.W. (1997). Influence of orchiectomy on canine behaviour. Veterinary Record, 140(24), 617-619.
97. Maenhoudt C., Santos N.R. and Fontbonne A. (2014). Suppression of fertility in adult dogs. *Reproduction in Domestic Animals*, *49*, 58-63.
98. Maute A.M., Koch D.A. and Montavon P.M. (2001). Perineale Hernie beim Hund–Colopexie, Vasopexie, Cystopexie und Kastration als Therapie der Wahl bei 32 Hunden. *Schweiz Arch Tierheilkd*, *143*(7), 360-367.
99. Maarschalkerweerd R.J., Endenburg N., Kirpensteijn J. and Knol B.W. (1997). Influence of orchiectomy on canine behaviour. *Veterinary Record*, *140*(24), 617-619.
100. Max A., Jurka P., Dobrzynski A. and Rijsselaere T. (2014). Non-surgical contraception in female dogs and cats. *Acta Scientiarum Polonorum. Zootechnica*, *13*(1).
101. McCann J.P., Altszuler N., Hampshire J. and Concannon P.W. (1987). Growth hormone, insulin, glucose, cortisol, luteinizing hormone, and diabetes in beagle bitches treated with medroxyprogesterone acetate. *European Journal of Endocrinology*, *116*(1), 73-80.
102. McGreevy P.D., Thomson P.C., Pride C., Fawcett A., Grassi T., Jones B. (2005). Prevalence of obesity in dogs examined by Australian veterinary practices and the risk factors involved. *Veterinary Record*,156, 695-702. https://doi.org/10.1136/
103. Misdorp W. (1988). Canine mammary tumours: protective effect of late ovariectomy and stimulating effect of progestins. *Veterinary Quarterly*, *10*(1), 26-33.

104. Misdorp W. (1991). Progestagens and mammary tumours in dogs and cats. *Acta endocrinologica*, *125*, 27-31.
105. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 25077495, Deslorelin. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Deslorelin. Accessed July 22nd, 2023
106. Nelson L.W. and Kelly W.A. (1976). Progestogen-related gross and microscopic changes in female Beagles. *Veterinary pathology*, *13*(2), 143-156.
107. Neilson J.C., Eckstein R.A. and Hart B.L. (1997). Effects of castration on problem behaviors in male dogs with reference to age and duration of behavior. *Journal of the American Veterinary Medical Association*, *211*(2), 180-182.
108. Neri, R. (1989). Pharmacology and pharmacokinetics of flutamide. *Urology*, *34*(4), 19-21.
109. Niżański, W., Levy, X., Ochota, M., & Pasikowska, J. (2014). Pharmacological treatment for common prostatic conditions in dogs–benign prostatic hyperplasia and prostatitis: an update. *Reproduction in domestic animals*, *49*, 8-15.
110. Noakes D.E., Parkinson T.J., England G.C.W. and Arthur G.H. (2001a). Endogenous and exogenous control of ovarian cyclicity. In *Arthur's veterinary reproduction and obstetrics* (8th ed.). Philadelphia, USA: W.B. Saunders, 3-53
111. Noakes D.E., Parkinson T.J., England G.C.W. and Arthur G.H. (2001b). Normal reproduction in male animals. In *Arthur's veterinary reproduction and obstetrics* (8th ed.). Philadelphia, USA: W.B. Saunders, 673-694
112. Noakes D.E., Parkinson T.J. and England G.C.W. (2019). Pharmacological Control of Reproduction in the Dog and Cat. In *Veterinary Reproduction & Obstetrics* (10th ed.), ed. England. London, UK: W.B. Saunders, 613-617.
113. Oglesby, R. N. (2005). Ovuplant & Deslorelin. Available at: https://horseadvice.com/horse-equine/medications/reproductive-drugs/ovuplant-deslorelin/ Accessed April 2nd, 2021
114. Okafor C.C., Pearl D.L., Lefebvre S.L., Wang M., Yang M., Blois S.L. and Dewey C.E. (2013). Risk factors associated with struvite urolithiasis in dogs evaluated at general care veterinary hospitals in the United States. *Journal of the American Veterinary Medical Association*, *243*(12), 1737-1745.
115. Okkens A.C. and Kooistra H.S. (2006). Anoestrus in the Dog: a Fascinating Story. *Reproduction in Domestic Animals*, 41: 291-296. https://doi.org/10.1111/j.1439-0531.2006.00702.x
116. Okkens A.C., van de Gaag I., Biewanga W.J., Rothuizen J. and Voorhout G. (1981) Urological complications following ovariohysterectomy in dogs. *Tijdschrift voor Diergeneeskunde* 106, 1189-1198
117. Palm J. (2011). Wirksamkeit von Deslorelinazetat zur Läufigkeitsunterdrückung der Hündin: eine retrospektive Analyse von 102 Fällen (Doctoral dissertation, University of Zurich).
118. Palm J. and Reichler I.M. (2011). The use of Deslorelin acetate for the suppression of heat in the bitch: A retrospective study of 102 cases. In *Reproduction in domestic animals.* Malden, USA: Wiley-Blackwell, *46*, 33-34.
119. Pawson A.J. and McNeilly A.S. (2005). The pituitary effects of GnRH. *Animal reproduction science*, 88(1-2), 75-94.
120. Pearson H. (1973). The complications of ovariohysterectomy in the bitch. *Journal of Small Animal Practice* 14, 257- 266.

121. Peptech Animal Health. (1998). Freedom of information (FOI) summary: Ovuplant. From FDA website: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/581. Accessed January 1st, 2023
122. Pérez-Marín C.C., López R., Domínguez J.M. and Zafra R. (2006). Clinical and pathological findings in testis, epididymis, deferens duct and prostate following vasectomy in a dog. *Reproduction in Domestic Animals*, *41*(2), 169-174.
123. Ponglowhapan, S. and Lohachit C. (2010). Clinical use of GnRH agonist Deslorelin in benign prostatic hyperplasia in dogs. In *Proc 7th EVSSAR congress.* Louvain la Neuve, Belgium.
124. Priester W.A. (1979). Occurrence of mammary neoplasms in bitches in relation to breed, age, tumour type, and geographical region from which reported. *Journal of Small Animal Practice*, 20, 1-11.
125. Prymak C., McKee L.J., Goldschmidt M.H. and Glickman L.T. (1988). Epidemiologic, clinical, pathologic, and prognostic characteristics of splenic hemangiosarcoma and splenic hematoma in dogs: 217 cases. *Journal of the American Veterinary Medical Association*, *193*(6), 706-712.
126. Reichler I.M., Hubler M., Jöchle W., Trigg T.E., Piché C.A. and Arnold S. (2003). The effect of GnRH analogs on urinary incontinence after ablation of the ovaries in dogs. *Theriogenology*, *60*(7), 1207-1216.
127. Reichler I.M., Jöchle W., Piché C.A., Roos M. and Arnold S. (2006). Effect of a long acting GnRH analogue or placebo on plasma LH/FSH, urethral pressure profiles and clinical signs of urinary incontinence due to Sphincter mechanism incompetence in bitches. *Theriogenology*, *66*(5), 1227-1236.
128. Reichler I.M., Welle M., Sattler U., Jöchle W., Roos M., Hubler M. and Arnold S. (2007). Comparative quantitative assessment of GnRH-and LH-receptor mRNA expression in the urinary tract of sexually intact and spayed female dogs. *Theriogenology*, *67*(6), 1134-1142.
129. Reichler I.M. (2009). Gonadectomy in cats and dogs: a review of risks and benefits. *Reproduction in Domestic Animals*, *44*, 29-35.
130. Rhodes L. (1996). The role of dihydrotestosterone in prostate physiology: comparison among rats, dogs and primates. In *Proceedings of the Annual Meeting of the Society of Theriogenology*, 124-135.
131. Romagnoli S. (1992). Il ciclo estrale nella cagna: comportamento sessuale, citologia vaginale e profilo endocrino. *Praxis Veterinaria*, 13 (1), 12-18.
132. Romagnoli S. and Concannon, P.W. (2003). Clinical use of progestins in bitches and queens: a review. *Recent Advances in Small Animal Reproduction. International Veterinary Information Service (www. ivis. org), Ithaca, New York, USA*.
133. Romagnoli S. (2006). Deslorelin in small animal andrology. In *Proceedings of 5th EVSSAR biannual congress.* Budapest, Hungary. 204-207.
134. Romagnoli S. and Sontas H. (2010). Prevention of breeding in the female. In *BSAVA manual of canine and feline reproduction and neonatology* (2nd ed.). Gloucester, UK: British Small Animal Veterinary Association, 23-33.
135. Romagnoli S., Stelletta C., Milani C., Gelli D., Falomo M.E. and Mollo A. (2009). Clinical use of deslorelin for the control of reproduction in the bitch. *Reproduction in domestic animals*, *44*, 36-39.
136. Romagnoli S. (2009). Non-surgical contraception in dogs and cats. In *Proceeding of 34th Congress of the World Small Animal Veterinary Association*.

137. Romagnoli S., Siminica A., Sontas B.H., Milani C., Mollo A. and Stelletta C. (2012). Semen quality and onset of sterility following administration of a 4.7-mg deslorelin implant in adult male dogs. *Reproduction in Domestic Animals*, *47*, 389-392.
138. Ru G., Terracini B. and Glickman L.T. (1998). Host related risk factors for canine osteosarcoma. *The Veterinary Journal*, *156*(1), 31-39.
139. Rubion S., Desmoulins P.O., Rivière-Godet E., Kinziger M., Salavert F., Rutten F. and Driancourt M.A. (2006). Treatment with a subcutaneous GnRH agonist containing controlled release device reversibly prevents puberty in bitches. *Theriogenology*, *66*(6-7), 1651-1654.
140. Sawada T., Tamada H., Inaba T. and Mori J. (1992). Prevention of estrus in the bitch with chlormadinone acetate administered orally. *Journal of Veterinary Medical Science*, *54*(3), 595-596.
141. Scott D.W. and Concannon P.W. (1983). Gross and microscopic changes in the skin of dogs with progestagen-induced acromegaly and elevated growth hormone levels. *The Journal of the American Animal Hospital Association (USA)*.
142. Scott D.W. (1990). Seasonal flank alopecia in ovariohysterectomized dogs. *The Cornell Veterinarian*, *80*(2), 187-195.
143. Selman P.J., Mol J.A., Rutteman G.R. and Rijnberk A. (1991). Progestins and growth hormone excess in the dog. *Acta endocrinologica*, *125*, 42-47.
144. Selman P.J., Wolfswinkel J. and Mol J.A. (1996). Binding specificity of medroxyprogesterone acetate and proligestone for the progesterone and glucocorticoid receptor in the dog. *Steroids*, *61*(3), 133-137.
145. Selman P.J., Mol J.A., Rutteman G.R., Van Garderen E., Van den Ingh T.S. and Rijnberk, A. (1997). Effects of progestin administration on the hypothalamic-pituitary-adrenal axis and glucose homeostasis in dogs. *Journal of Reproduction and fertility. Supplement*, *51*, 345-354.
146. Sirivaidyapong S., Mehl N.S. and Trigg, T. E. (2012). Delay of Puberty and Reproductive Performance in Male Dogs Following the Implantation of 4.7 and 9.4 mg G n RH-Agonist Deslorelin at an Early Pre-pubertal Age. *Reproduction in Domestic Animals*, *47*, 400-402.
147. Sjaastad O. V., Sand O. and Hove K. (2013). La riproduzione. In *Fisiologia degli animali domestici*, ed. Mattioli. Milan, Italy: CEA - Casa Editrice Astoria, 698-708
148. Sirinarumitr K., Johnston S.D., Kustritz M.V.R., Johnston G.R., Sarkar D.K. and Memon M.A. (2001). Effects of finasteride on size of the prostate gland and semen quality in dogs with benign prostatic hypertrophy. *Journal of the American Veterinary Medical Association*, *218*(8), 1275-1280.
149. Slauterbeck J.R., Pankratz K., Xu K.T., Bozeman S.C. and Hardy D.M. (2004). Canine ovariohysterectomy and orchiectomy increases the prevalence of ACL injury. *Clinical Orthopaedics and Related Research*, *429*, 301-305.
150. Sloan J.M. and Oliver I.M. (1975). Progestogen-induced diabetes in the dog. *Diabetes*, *24*(4), 337-344.
151. Smith D., Enever R., Dey M., Latta D. and Weierstall R. (1993). Pharmacokinetics and bioavailability of medroxyprogesterone acetate in the dog and the rat. *Biopharmaceutics & drug disposition*, *14*(4), 341-355.
152. Spain C.V., Scarlett J.M. and Houpt K.A. (2004). Long-term risks and benefits of early-age gonadectomy in dogs. *J of the American Veterinary Medical Association*, *224*(3), 380-387.
153. Spain C.V. (2006). Non-Reproductive Effects of Spaying and Neutering: Effects on Growth, Hip Dysplasia, Immunology and Tumors. In *Third International Symposium on Non-Surgical Contraceptive Methods for Pet Population Control*, Alexandria.

154. Squires E.L. and Simon B.W. (2011). Evaluation of a new sustained-release deslorelin acetate for induction of ovulation in mares. In *AAEP Proceedings*, *57*, 53-54.
155. Stempel S., Körber H., Reifarth L., Schuler G. and Goericke-Pesch S. (2022). What Happens in Male Dogs after Treatment with a 4.7 mg Deslorelin Implant? II. Recovery of Testicular Function after Implant Removal. *Animals*, *12*(19), 2545.
156. Ström H.B., Jigler E., Hagman R. and Frössling J. (2010). Deslorelin treatment of male dogs. In: *Proc 7th EVSSAR congress*. Louvain la Neuve, Belgium.
157. Sundburg, C. R., Belanger, J. M., Bannasch, D. L., Famula, T. R., & Oberbauer, A. M. (2016). Gonadectomy effects on the risk of immune disorders in the dog: a retrospective study. *BMC veterinary research*, *12*(1), 1-10.
158. Thrusfield M.V., Holt P.E. and Muirhead R.H. (1998). Acquired urinary incontinence in bitches: its incidence and relationship to neutering practices. *Journal of small animal practice*, *39*(12), 559-566.
159. Trevejo R., Yang M. and Lund E.M. (2011). Epidemiology of surgical castration of dogs and cats in the United States. *J of the American Veterinary Medical Association*, *238*(7), 898-904.
160. Trigg T.E., Wright P.J., Armour A.F., Williamson P.E., Junaidi A., Martin G.B. and Walsh J. (2001). Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function in male and female domestic dogs. *Journal of reproduction and fertility. Supplement*, *57*, 255-261.
161. Trigg T.E., Doyle A.G. and Walsh J.D. (2004). Advances in the use of the GnRH agonist deslorelin in control of reproduction. In *5th International Symposium on Canine and Feline reproduction. Sao Paolo, Brazil*, 49-51.
162. Trigg T.E., Doyle A.G., Walsh J.D. and Swangchan-Uthai T. (2006). A review of advances in the use of the GnRH agonist deslorelin in control of reproduction. *Theriogenology*, *66*(6-7), 1507-1512.
163. Turcotte J.G., Haines R.F., Brody G.L., Meyer T.J. and Schwartz S.A. (1968). Immunosuppression with medroxyprogesterone acetate. *Transplantation*, *6*(2), 248-260.
164. Van Goethem B., Schaefers-Okkens A.U.K.E. and Kirpensteijn J. (2006). Making a rational choice between ovariectomy and ovariohysterectomy in the dog: a discussion of the benefits of either technique. *Veterinary Surgery*, *35*(2), 136-143.
165. Verstegen-Onclin K. and Verstegen J. (2006). Non-reproductive effects of spaying and neutering: effects on the urogenital system. In *Proceedings of the Third International Symposium on Non-Surgical Contraceptive Methods for Pet Population Control* *http://www.acc-d.org/2006%20Symposium%20Docs/Session%20I.pdf*
166. Vickery B.H., McRae G.I., Goodpasture J.C. and Sanders L.M. (1989). Use of potent LHRH analogues for chronic contraception and pregnancy termination in dogs. *Journal of reproduction and fertility. Supplement*, *39*, 175-187.
167. Ware W.A. and Hopper D.L. (1999). Cardiac tumors in dogs: 1982–1995. *Journal of Veterinary Internal Medicine*, *13*(2), 95-103.
168. Weems C.W., Weems Y.S. and Randel R.D. (2006). Prostaglandins and reproduction in female farm animals. *The Veterinary Journal*, *171*(2), 206-228.
169. White C.R., Hohenhaus A.E., Kelsey J. and Procter-Gray E. (2011). Cutaneous MCTs: associations with spay/neuter status, breed, body size, and phylogenetic cluster. *Journal of the American Animal Hospital Association*, *47*(3), 210-216.
170. Wiebe V.J. and Howard J.P. (2009). Pharmacologic advances in canine and feline reproduction. *Topics in companion animal medicine*, *24*(2), 71-99.

171. Wilkins L. (1960). Masculinization of female fetus due to use of orally given progestins. *Journal of the American Medical Association*, *172*(10), 1028-1032.
172. Wilson G.P. and Hayes Jr H.M. (1979). Castration for treatment of perianal gland neoplasms in the dog. *Journal of the American Veterinary Medical Association*, *174*(12), 1301-1303.
173. Wisener L.V., Pearl D.L., Houston D.M., Reid-Smith R.J. and Moore A.E. (2010). Risk factors for the incidence of calcium oxalate uroliths or magnesium ammonium phosphate uroliths for dogs in Ontario, Canada, from 1998 to 2006. *American J of Vet Res*, *71*(9), 1045-1054.
174. Withers A.R. and Whitney J.G. (1967). The response of the bitch to treatment with medroxyprogesterone acetate. *Journal of Small Animal Practice*, *8*(5), 265-271.
175. Wright P.J., Verstegen J.P., Onclin K., Jöchle W., Armour A.F., Martin G.B. and Trigg T.E. (2001). Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin. *Journal of Reproduction and fertility. Supplement*, *57*, 263-268.
176. Zhang Y., Wang X., Chen Z. and Huang X. (2012). Long-term reproductive consequences of no-scalpel vasectomy in beagles. *Journal of Huazhong University of Science and Technology [Medical Sciences]*, *32*, 899-905.
177. Zink M.C., Farhoody P., Elser S.E., Ruffini L.D., Gibbons T.A. and Rieger R.H. (2014). Evaluation of the risk and age of onset of cancer and behavioral disorders in gonadectomized Vizslas. *Journal of the American Veterinary Medical Association*, *244*(3), 309-319.