Clinical use of deslorelin implants for the long-term contraception in prepubertal bitches: Effects on epiphyseal closure, body development, and time to puberty

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Abstract
Long-acting GnRH agonists have been used both for canine estrus induction and prevention. The objective of the study was to investigate the use of a deslorelin implant as a long-term and reversible contraceptive in prepubertal bitches with special regard to the time of epiphyseal closure. Thirteen healthy, crossbreed, medium-sized prepubertal female dogs were used in this study. An implant containing 9.4 mg (G1, n = 5) and 4.7 mg (G2, n = 4) deslorelin acetate (Suprelorin) or a placebo (sodium chloride 0.9%; G3, n = 4) was inserted subcutaneously in the interscapular region. Estrus was monitored once daily by physical and sexual behavioral changes. Body development, vaginal cytology, and serum progesterone and estradiol 17β concentration were monitored weekly for the first 5 weeks, and then every 3 weeks throughout the treatment period. Radiographic examinations were performed monthly to determine the epiphyseal closure. Half of the deslorelin-treated bitches (G1: n = 2 and G2: n = 2) came into estrus during the 83-week observation period. All animals in the control group showed estrus between the 39th and 64th weeks of observation. Time to puberty averaged 82.7 ± 8.9 and 61.9 ± 9.7 weeks in the deslorelin-treated (G1 and G2) and the control bitches, respectively (P < 0.02). Both deslorelin implants (9.4 and 4.7 mg) can be used efficiently for the long-term prevention of estrus in prepubertal bitches; however, epiphyseal closure is clearly delayed which was without any clinical effect in the present study.

1. Introduction

Gonadotropin-releasing hormone (GnRH) is a decapetide hypothalamic hormone that acts on GnRH receptors in the pituitary. It is secreted in a pulsatile manner into the hypothalamo–hypophysial portal system and has a short half-life of 2 to 5 minutes because of rapid cleavage by proteases. In the pituitary, GnRH stimulates production and secretion of both luteinizing hormone and follicle-stimulating hormone which in turn act on the gonads regulating steroid production, spermatogenesis, ovarian follicular development, and ovulation [1,2].

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Prolonged administration of GnRH agonists leads to desensitization of the pituitary gland and effectively inhibits the pituitary–gonadal axis. In recent years, slow-release depot formulations of GnRH agonists have been developed for long-term fertility control of domestic animals. These products have a broad range of potential applications in domestic animal reproduction [2,3]. One of these products, a deslorelin implant, has been used both for suppression and induction of estrus in bitches [4–7].

A limited number of studies have been conducted on the efficacy of long-term release GnRH agonists to postpone puberty and its physiological effects on ovarian function and body development in female dogs [8–10]. In a recent study, Marino et al. [10] evaluated short- and long-term effects of repeated 4.7-mg deslorelin implant application on the genital tract and body development in prepubertal bitches.

It has been reported that hormonal changes after prepubertal gonadectomy cause delayed closure of growth plates in cats and dogs [11,12]. However, the effects of different dosages of deslorelin on the epiphyseal closure (EC) are not yet fully investigated. We hypothesized that prepubertal application of a GnRH agonist would cause comparable effects.

Therefore, the objective of this study was to investigate the deslorelin implant Suprelorin (9.4 and 4.7 mg) for use as a long-term, safe, and reversible contraceptive in prepubertal medium-sized bitches with special regard to the time of EC.

2. Materials and methods

2.1. Animals and treatments

The study was conducted at the Clinics of the Department of Veterinary Medicine, Kafkas University, Kars, Turkey, from 2011 to 2013. Thirty healthy, crossbreed, medium-sized, prepubertal female dogs (age range, 4–5.1 months; body weight range, 6–15 kg) were used in the study. To provide a random distribution, dogs were distributed into groups according to the order they were brought to the clinic. The dogs were housed in indoor–outdoor runs and had a maximum daylight per day. They were fed with a standard commercial puppy food, and water was available ad libitum. For ease of applications, dogs were also identified with letter numbers (e.g., b1, b2, b3, b4, and henceforth). Only bitches that clearly proved to be prepubertal after gynecologic observation including vaginal cytology, estradiol 17β (E2), and progesterone (P4) measurement were used in the present study. After the first vaginal cytologic and blood sampling, an implant containing 9.4-mg (G1, n = 5) and 4.7 mg (G2, n = 4) deslorelin (Suprelorin; Virbac, France) or a placebo (sodium chloride 0.9%; G3, n = 4) was administered subcutaneously in the interscapular region by using a single use applicator. One of the treated bitches in G2 (b7) died in the 34th week of the treatment period for an unknown reason. Therefore, only the deslorelin concentrations of this dog were used in the statistical calculations. This study was reviewed and approved by the Animal Local Ethics Committee (KAÜ-HADYEK; 2010/30) of the Faculty of Veterinary Medicine, Kafkas University, Turkey.

2.2. Clinical and radiographic observation, vaginal cytology, and blood sampling

After implant insertion, estrus was monitored once daily by physical (vulvar appearance and swelling, serosanguineous vaginal discharge) and sexual behavioral changes, until the occurrence of estrus. Body development (body weight [kg], height at withers [cm], size of vulva [cm], and humeral length [mm]) and vaginal cytology and serum P4 and E2 concentration were monitored weekly for the first 5 weeks, and then every third week throughout the treatment period. The size of vulva was measured with a metal Vernier caliper. For determination of height at withers, an assistant kept the dog in a standing position, and measurement was performed from the withers to the floor using a measuring tape. For standardization, the same person performed all body measurements. After the mean period of suppression (G1, 9.4 mg: up to 48 weeks; G2, 4.7 mg: up to 24 weeks), sampling period was shortened from 3 weeks to 2 weeks in each group. Furthermore, serum concentrations of deslorelin were monitored weekly for the first 4 weeks, and radiographic examinations were performed monthly throughout the treatment period to monitoring EC. A portable X-ray device (Dynamic X-Ray; DRX 3-I, GM/094101, Ankara, Turkey) was used for radiographic examinations. Mediolateral radiographs of the right humerus were taken using a standardized direct radiographic technique (45–55 kV and 2.5 mAs X-Ray radiation doses with related to the size of the dog). Radiographic observation included measurements of humerus length and closure time of epiphyseal plate of the proximal humerus. The disappearance of the radiolucent line between the epiphysis and the metaphysis on the radiographic images was accepted as indicator for completion of the EC.

2.3. Hormone assays

The concentrations of P4 (nmol/L) and E2 (pmol/L) in peripheral blood serum samples were measured using “electrochemiluminescence immunoassay” with the fully automated Cobas Modular E170 Analyzer (Roche Diagnostics, Mannheim, Germany) in a special laboratory (Düzen Laboratories Group, Ankara, Turkey), as published by Agaoglou et al. [13]. Serum concentrations of deslorelin were measured by means of a commercially available Competitive Enzyme Immunoassay Kit (CEK 0100-06; AB Biolabs, Sovereign CT, USA) as recommended by the manufacturer. The assay is 100% specific for deslorelin. No cross-reactivity with physiological GnRH or the GnRH agonists triptorelin and goserelin was detectable. The assay sensitivity is 10 to 100,000 pg/mL. Photometric measurements were done with an ELx808 Absorbance Microplate Reader (BioTek Instruments, Inc., Winooksi, VT, USA). Because of material restrictions, deslorelin resorption could only be analyzed during the first 4 weeks after implant insertion. Only in two dogs, this was possible until Days 49 and 70, respectively.
2.4. Statistical analyses

The commercial software PASW statistics 18.0 software for Windows (SPSS; Chicago, IL, USA) was used for all statistical analyses. The concentrations of P4, E2, and deslorelin during the study period were evaluated individually. Normality and homogeneity of groups were determined by the Shapiro–Wilk test. For normally distributed data, differences between the groups were compared using one-way ANOVA. Changes in the body weight, height at withers, size of vulva, and humeral length of bitches were compared by repeated measures define factor. Body weight, height at withers, EC time, and time to puberty among groups were compared by one-way ANOVA and equal variances assumed post hoc test. Data are expressed as mean ± standard deviation. Values of P < 0.05 were considered statistically significant.

3. Results

Epiphysial closure was completed within 83.5 ± 8.5, 73.4 ± 4.5, and 60.9 ± 9.9 weeks of age in G1, G2, and G3, respectively (G1:G3 = P < 0.001). Half of the deslorelin-treated bitches (G1: n = 2 and G2: n = 2) came into estrus during the 83 weeks of observation. All animals in the control group showed estrus between the weeks 39 and 64 after placebo treatment. Time to puberty averaged 82.7 ± 8.9 and 61.9 ± 9.7 weeks in the deslorelin-treated (G1 and G2) and the control bitches (P < 0.02), respectively. Some bitches (b1, b3, b5, b6) did not show estrus within the observation period, which lasted on average 101.5 weeks. No clinically detectable systemic side effects were observed in any of the treated bitches. However, six bitches in the deslorelin treatment (G1 and G2; 75%) and three in the control group (G3; 75%) developed a mild form of juvenile vaginitis between the weeks 10 and 72 of the observation period, which recovered spontaneously without any specific treatment. In one bitch of G1 (b3), cystic follicular degeneration was determined during routine ovariohysterectomy operation. The follow-up of individual clinical findings is summarized in Table 1.

Serum concentrations of P4 and E2 varied throughout the study period (Fig. 1A-D). An increase in serum P4 (>3.18 nmol/L) concentrations was observed in two animals in G1 (2 of 5) during the first 3 weeks of treatment (20.25 nmol/L and 4.70 nmol/L, Fig. 1A). However, only one of these two animals (b1) showed an increase in serum E2 concentrations up to 135.79 pmol/L (Fig. 1B). In G2, an increase in serum P4 concentrations was found in two animals (2 of 3), at weeks 40 (b6; 8.33 nmol/L) and 48 (b8; 13.89 nmol/L) after the implant insertion (Fig. 1C).

A total of six bitches in G1 (n = 3) and G2 (n = 3) showed an increase in serum E2 concentrations (>73 pmol/L) at 37 to 49 weeks after implant insertion, however without a clinical flare-up (Fig. 1B, D). In the control group, only one animal showed an increase in serum E2 concentrations in week 14 of the observation period (Fig. 1F).

In Figure 2A, B, the course of the serum deslorelin concentration of G1 and G2 bitches is shown. In G1 bitches, the values showed individual courses but always were less than 400 pg/mL. On Day 28, all bitches showed basal values between 20 and 50 pg/mL. Two bitches were followed up until Days 49 and 70, respectively, and deslorelin was still measurable after this time period (56 and 24 pg/mL).

Body development was unaffected by treatment. The changes in body weight, height at withers, size of vulva, and humeral length were not significantly different (P > 0.05) between the treatment and control groups for 40 weeks after the implant insertion (Fig. 3A–D).

4. Discussion

The present study was designed to evaluate the effects of a long-term GnRH agonist deslorelin on the endocrine and body development of medium-sized prepubertal bitches and its effect on the time of EC. To provide a homogenous group and data useful for any comparison between the groups, only medium-sized bitches, all living in a Mediterranean climate with an average of 12 hours of daylight and that clearly were prepubertal, as evaluated clinically and endocrinologically, were used.

Long-term administration of GnRH agonists results in downregulation and desensitization of pituitary GnRH receptors and a complete suppression of gonadal function [7,8,14]. A significant advance for their clinical use was obtained by the development of slow-release agonists that can be easily administered intramuscularly or implanted subcutaneously, delivering continuous doses of GnRH for 3 to 12 months depending on the formulation [4,8,15,16]. Although some GnRH agonists proved to be efficient for contraception in adult bitches for 1 year, there are limited data concerning the efficacy of long-term release GnRH agonists to delay the puberty in female dogs [4,8–10]. The present study shows that both the 4.7- and the 9.4-mg deslorelin implants can be used efficiently for the long-term prevention of estrus.

In dogs, major growth occurs between 3 and 6 months of age, and most growth plates close between 4 and 12 months of age, depending on the anatomic site and breed of dogs. The mean closure time of the growth plates for the proximal humerus of a medium-size dog was
reported to be 10 to 12 months of age [17]. The literature [11] indicates that prepubertal gonadectomy delayed growth plate closure which resulted in extended growth periods in dogs both neutered at 7 weeks and 7 month old, compared with sexually intact dogs. Rubion et al. [8] reported no side effects and neither body weight nor growth appeared to be affected by long-term release GnRH agonist treatment (Gonazon), which is in accordance to our findings. Municchi et al. [18] reported that pubertal delay induced by deslorelin treatment significantly increased predicted adult height in human adolescents. To our knowledge, no data are available concerning the effect of the deslorelin implant on epiphyseal plate closure in bitches. In the present study, we reported that in prepubertal medium-sized dogs, implanted once with either 4.7- or 9.4-mg deslorelin, the EC was significantly delayed until 20 months of age, however without any clinical impact.

It has been described that GnRH agonists decreased gonadal steroids and prevented puberty when administered daily in male and female prepubertal dogs during 23 months of treatment [19]. However, after insertion of a long-acting GnRH agonist implant, we observed individual variable serum E2 and P4 concentrations during the observation period of 8 to 19.5 months in the treatment groups (G1 and G2).

In sexually intact bitches, serum E2 concentration increases from 18 to 55 pmol/L initially to reach a peak of 147 to 440 pmol/L during proestrus [20]. Although some of the investigated animals in our study showed an increase in serum average E2 concentrations from less than 18 to 462 pmol/L throughout the study period, values quickly decreased to less than 18 pmol/L thereafter and remained constantly low in treated animals of both G1 and G2 without estrus signs. Possible reasons for this variability of serum P4 and E2 concentrations could be individual differences in drug absorption or metabolism between the bitches. In contrary to the findings of Marino et al. [10], the bitches in the present study developed normally; it might be supposed that this difference results from an incomplete suppression of hormone secretion after a single implant insertion. This incomplete suppression might also be responsible for the normal height at withers despite

Fig. 1. Changes in individual progesterone (P4; A, C, E) and estradiol 17β (E2; B, D, F) concentrations in groups. (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)

b 1-13 = individual bitches
delayed EC. The very low measurable concentrations of steroid hormones obviously were not enough to induce EC but might have been able to slow down the humerus growth. In their study, Marino et al. [10] inserted three implants, at 4.5, 9, and 13.5 months, respectively, and only a slight increase in E2 serum concentration was assessed on Days 8 and 10 after the first implant. The concentrations remained basal thereafter and until the end of the study. Progesterone values never exceeded 3.5 nmol/L. We suppose that the repeated insertion every 4.5 months better suppressed sexual hormone secretion; therefore, the external genitalia stayed juvenile, which was not the case in the present study. More cases, frequent blood sampling and long-term observations for several years are needed to better understand the different effects of both application schemes.

However, in the present study, we observed that none of the prepubertal bitches that received a deslorelin (4.7 and 9.4 mg) implant, showed a flare-up during the first 4 weeks after implant insertion, as reported previously by us [21]. In prepubertal bitches, insertion of a GnRH agonist device did not induce estrus, when applied at the age of 4 months or earlier, whereas a flare-up may occur in older prepubertal bitches and adult bitches in anestrus within 1 month after implantation [2,7–9,21,22]. In a placebo-controlled study with prepubertal bitches, Trigg et al. [4] showed that the age at the implant insertion is important for the response to the GnRH agonist. Although the age, clearly, is not the only parameter defining puberty, it becomes evident that the probability of a flare-up increases with increasing age. It should be emphasized that a thorough clinical examination including at least vaginal cytology and if possible E2

![Graph A](image1.png)

**Group 1 (Suprelorin-9.4)**

b 1-5 = individual bitches

![Graph B](image2.png)

**Group 2 (Suprelorin-4.7)**

b 6-9 = individual bitches

*Fig. 2. Changes in individual serum deslorelin concentrations in G1, 9.4 mg (A) and G2, 4.7 mg (B) deslorelin-treated bitches. (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)*
measurement should be performed before the implant insertion.

It has been supposed that the individually highly heterogeneous hormone concentrations might be due to individual absorption patterns of deslorelin. We therefore measured the concentration once weekly with a commercially available assay. Unfortunately, we could only analyze the first 4 weeks after the implant insertion, and we observed highly individual concentrations with maximum values after 7 to 21 days that slowly decreased within 28 days and never exceeded 1400 pg/mL; in the 9.4-mg deslorelin group, the maximum concentration never exceeded 400 pg/mL. The lower concentrations in the 9.4-mg group might be incidental and due to low patient numbers; alternatively, the delayed resorption might have been accompanied by reactions of the surrounding tissue slowly decreasing the local perfusion. However, assessed results in this study partly corresponds with the results from Kutzler et al. [5] who found a decrease of concentrations to levels lower than 1100 pg/mL until Day 25 after subcutaneous administration. The authors observed that all bitches reached maximum concentrations 1 to 6 days after insertion, which might be because of a lower deslorelin dosage and another deslorelin preparation. Kutzler et al. [5] used Ovuplant implant originally manufactured for short-term application (ovulation induction in mares) with the intention that deslorelin concentrations would only be in circulation for 48 hours. In the mentioned study, Kutzler et al. [5] reported that, in dogs, deslorelin from Ovuplant could be detected in circulation for days to weeks. In the present study, the authors used a preparation (Suprelorin) that should remain in circulation for several months to suppress fertility. Unfortunately, we could not follow the course of deslorelin during the whole study period. However, we still assessed measurable concentrations 70 days after insertion of a 4.7-mg implant in one dog. In our study, serum concentrations of deslorelin seemed to be independent from the administered dosage. Instead, individual differences were considerable, probably due to differing blood supply at the injection site. Kutzler et al. [5] reported that after vestibular insertion of a deslorelin implant, the deslorelin concentrations quickly decreased from a maximum after 6 days to basal values within 12 days; however, it has to be emphasized that Ovuplant is another preparation of deslorelin, and all comparisons should consider this. At last, we did not found a relation between the serum concentrations of deslorelin at different time points and the measured serum \( E_2 \) or \( P_4 \) concentrations. For more solid conclusions, a follow-up study with more patients and more frequent blood sampling over a prolonged period is required.

There are few data about the side effects of GnRH agonists in veterinary medicine. In recent studies, persistent estrus related or not to ovarian cysts, uterine disorders, urinary incontinence, hair anomalies, and juvenile vaginitis have been reported [2,10,23,24]. In mares, application of a higher dose (11 mg) of deslorelin implant led to suppression of ovarian activity and in some cases to limited follicular activity or inactivity (follicles \(<20\) mm) [25]. In our study, in six bitches from the treatment groups, a mild form of juvenile vaginitis was observed which recovered spontaneously without any specific treatment. This is
similar to the findings of Marino et al. [10]. However, additional cases of vaginitis were observed in the control group; we therefore suppose that these cases are not related to the GnRH treatment. Some cases of vaginitis might be caused by the repeated vaginal smear applications during the study period. Furthermore, cystic follicular degeneration was determined during routine ovariohysterectomy operation in one bitch from G1. This was not found by Marino et al. [10] who only detected cortical primordial and primary follicles, few secondary follicles and no antral follicles, and CL after ovariohysterectomy of the treated bitches at 18 months of life, probably a result of better hormonal suppression. Arit et al. [23] similarly reported the development of follicular cysts in a 7-year-old deslorelin-treated bitch. Ovarian cysts seem to be possible side effects caused by incomplete suppression of follicular development and because deslorelin is not registered for the use in bitches. The owner should be thoroughly informed before the implant insertion. However, more studies are necessary before a recommendation can be made concerning the best age and frequency of implant administration in prepubertal bitches.

4.1. Conclusions

In conclusion, both deslorelin implants (4.7 and 9.4 mg) can be used efficiently for the long-term medical prevention of estrus in proven prepubertal bitches of medium body size, when implanted at the mean age of 4 months. Body weight, height at withers, and development of external genitalia are not significantly affected with one implant; however, the EC is clearly delayed which was without any clinical effect in the present study. More studies are needed for a profound knowledge of the side effects after repeated insertion.

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