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RESEARCH ARTICLE

Clinical and Endocrine Short-Term Effects of GnRH Analogue Deslorelin in Prepubertal Bitches: Does A "Flare-up" Occur? [1]

Duygu KAYA * Selim ASLAN ** Semra KAYA *
Mushap KURU * Cihan KAÇAR * Sabine SCHÄFER-SOMI ***

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* Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Kafkas University, TR-36040 Kars - TURKEY
** Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Ankara University, TR-06110 Ankara - TURKEY
*** Centre for Artificial Insemination and Embryo Transfer, University of Veterinary Medicine, A-1210, Vienna, AUSTRIA

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Summary

Long-acting GnRH agonists have been used both in canine estrus induction and prevention. The objective of the study was to investigate the clinical and endocrine short-term effects of a deslorelin implant (4.7 and 9.4 mg) in terms of initial "*flare-up*" in prepubertal bitches. Fourteen healthy, cross-breed prepubertal female dogs, aged 4 months, were used in the study. An implant containing 9.4 mg (G1, n=5) and 4.7 mg (G2, n=4) deslorelin (Suprelorin®) or a placebo (sodium chloride 0.9%, G3, n=5) was inserted subcutaneously. Estrus was monitored once daily by physical and sexual behavioral changes. Vaginal cytology, serum progesterone (P4) and estradiol 17β (E2) concentrations were monitored weekly for the first five weeks throughout the treatment. None of the bitches in the treatment (G1 and G2) and control (G3) group showed any signs of estrous throughout the study period. No local reactions were observed at the implantation site. Two bitches showed an increase in serum P4 concentrations (>1.0 ng/ml) in G1 (2/5; 40%), however, only one of these two animals (P4=6.37 ng/ml) showed an increase in serum E2 concentrations up to 37 pg/ml. No marked increase in serum P4 and E2 concentrations were observed during the first 13 weeks of treatment in G2. These data demonstrate that in prepubertal bitches, insertion of a deslorelin implant does not cause a "*flare-up effect*" which commonly occurs in anestrus adult bitches during the first month after implantation. However, further studies are needed to demonstrate that the "*flare-up*" effect of deslorelin implant in bitches especially during later prepubertal stages.

Keywords: *Prepubertal bitches, Suprelorin®, Flare-up effect*

Prepubertal Köpeklerde GnRH Analoğu Deslorelinin Kısa Dönem Klinik ve Endokrinolojik Etkileri: "Flare-up" Gözlenir mi?

Özet

Uzun etkili GnRH agonistleri köpeklerde hem östrusun uyarılmasında hem de baskılanmasında kullanılmaktadırlar. Bu çalışmada, pubertas öncesi dönemdeki dişi köpeklerde deslorelin implant (4.7 ve 9.4 mg) kullanımının "*flare-up*" dönemi olarak değerlendirilebilecek klinik ve endokrinolojik kısa dönem etkilerinin varlığı araştırılmıştır. Bu amaçla pubertas öncesi dönemde olduğu belirlenen ortalama 4 aylık yaşta on dört adet sağlıklı melez dişi kopek kullanıldı. Köpeklere deri altı yolla 9.4 mg (G1, n=5) ve 4.7 mg (G2, n=4) deslorelin (Suprelorin®) etken maddesi içeren implantlar ya da plasebo (sodyum klorür %0.9; G3; n=5) uygulandı. Östrus belirtileri günlük olarak fiziksel değişiklikler ve davranış değişiklikleri yönünden takip edildi. Ayrıca çalışma süresince vaginal sitoloji, serum progesteron (P4) ve östradiol 17β (E2) konsantrasyonları haftalık olarak izlendi. Tedavi (G1 ve G2) ve kontrol grubundaki (G3) hayvanların hiçbirinde östrus bulgusu belirlenmedi. İmplantın yerleştirildiği bölgede herhangi bir lokal yan etki gözlenmedi. Grup 1'de iki köpekte (2/5; %40) serum P4 düzeyinde bir artış gözlenirken, bu köpeklerden yalnızca birinde (P4=6,37 ng/ml) serum E2 düzeyinin 37 pg/ml'ye kadar yükseldiği belirlendi. Grup 2'de 13 haftalık tedavi süresince serum P4 ve E2 düzeylerinde önemli bir değişiklik gözlenmedi. Bu çalışmadan elde edilen bulgular, pubertas öncesi köpeklerde deslorelin implantın, yetişkin köpeklerde implante edildikten sonraki ilk bir ay içerisinde sıklıkla gözlenen "*flare-up etkisine*" neden olmadığını göstermiştir. Bununla birlikte deslorelin implant uygulamalarının özellikle pubertasa yakın dönemlerde oluşturabileceği "*flare-up*" etkisine dair yeni çalışmalara ihtiyaç vardır.

Anahtar sözcükler: *Prepubertal köpek, Suprelorin®, Flare-up etkisi*

İletişim (Correspondence)
+90 474 2426836/5236
dygkaya@gmail.com

INTRODUCTION

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

Long-acting GnRH agonists can be used for indications such as preventing puberty in both sexes; controlling of estrus cycle and treating post-spaying urinary incontinence [3-6] and controlling the risk of recurrence of mammary neoplasia, prevention of mammary tumour metastatic disease and the termination of pregnancy in bitches [7-9].

GnRH agonists have been used both in canine estrus induction and prevention [3,5,6,10]. Chronic administrations of GnRH agonists result in downregulation and desensitation of pituitary GnRH receptors and, therefore, in a complete suppression of gonadal function [5,6,11]. 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 thus delivering constant doses of GnRH for 3-12 months depending on the formulation [5,12-14].

In dogs, GnRH agonist administration is an effective, safe and reversible method to prevent estrus cycles for a long period exceeding one year [2,11]. Bitches exhibit different responses to administration of deslorelin implants depending on the stage of the estrus cycle. A sharp, initial stimulatory gonadotropin response to GnRH agonist treatment known as '*flare-up*' occurs after implant insertion which results in an increased release of FSH and LH [15,16]. In dogs, estrus can be induced within 3 to 10 days after treatment [2,6,17]. Trigg et al.[12] noted the same phenomenon for animals aged seven months or more. In these animals, estrus systematically induced in the 1-2 months following implantation. Likewise, Inaba et al.[17] reported induction of estrus after use of a sustained-release formulation of a GnRH agonist (leuprolide acetate) in one year old prepubertal bitches; however, estrus induction has not been observed after implant insertion in prepubertal bitches with an average age of 4 months [5,12].

The objective of this study was to investigate the short-term effects of the deslorelin implant Suprelorin® (4.7 and 9.4 mg; Virbac®, Vienna, Austria) on vaginal cytological and hormonal changes and as a safe and reversible contraceptive in prepubertal bitches with special regard to the occurrence a flare-up effect.

MATERIAL and METHODS

Animals and Treatments

Fourteen healthy, crossbred prepubertal female dogs (mean age: 4.25±0.32 months; body weight range: 6-15 kg) were used in the study. The dogs were housed in indoor-outdoor runs and were fed with a standard commercial dog food. Water was available *ad libitum*. After the first vaginal cytological and blood sampling, an implant containing 9.4 mg (G1, n=5) and 4.7 mg (G2, n=4) deslorelin (Suprelorin®; Virbac, Vienna, Austria) or a placebo (2 ml sodium chloride 0.9%; G3, n=5) was inserted subcutaneously in the inter-scapular region by using a single use applicator. This study was approved by Kafkas University, Animal Local Ethics Committee (KAÜ-HADYEK; 2010/30).

Clinical Observation, Vaginal Cytology and Blood Sampling

Following implant insertion, animals were examined for presence local or systemic reactions (e.g. edema, suppuration at the implantation site or anaphylactoid reactions) and estrus signs were monitored once daily by physical (vulvar appearance, the length of the vulva and swelling, sero-sanguineous vaginal discharge) and sexual behavioral changes. Furthermore, vaginal cytology and serum progesterone (P4) and estradiol 17β (E2) concentration were monitored weekly for the first five weeks, and then every three weeks throughout the treatment period. The vaginal smears were obtained using a cotton swab and were stained with Papanicolaou staining technique [18] and examined microscopically to identify the cycle stage.

Hormone Assays

The concentrations of P4 and E2 in peripheral blood plasma were measured using "Electrochemiluminescence Immunoassay" (ECLIA) with the fully automated Cobas Modular E170 Analyzer® (Roche Diagnostics, Mannheim, Germany) in a special laboratory (Düzen Laboratories Group, Ankara, Turkey; internationally certified: TURKAK, TS EN ISO/IEC 17025:2005 laboratory of analysis).

Statistical Analyses

Changes in serum P4 and E2 concentrations were analyzed using Repeated Measures Define Factors with the General Linear Model procedure of PASW Statistics 18. All data presented are means±S.D. Values of $P<0.05$ were considered statistically significant.

RESULTS

Local/Systemic Side Effects

No clinically detectable local or systemic side effects associated with the treatment were observed in any of the treated (0/9) or control (0/4) bitches.

Behavioral and Physical Changes

Sexual behavioral changes were not observed in treatment and control animals during the daily examinations for the study period. The length of the vulva was unaffected by treatment. The changes in vulva size *(Fig. 1)* were not significantly different between treatment and control groups for 13 weeks after start of treatment (P>0.05).

Hormonal and Vaginal Cytological Changes

Serum concentrations of P4 *(Fig. 2-A,B)* and E2 *(Fig. 3-A, B)* varied throughout the study period. An increase in serum P4 (>1.0 ng/ml) concentrations was observed in two animals in G1 (2/5; 40%) during the first three weeks of treatment (6.37 ng/ml and 1.48 ng/ml, *Fig. 2, A*). However, only one of these two animals (1/5; 20%; P4=6.37 ng/ml) showed an increase in serum E2 concentrations up to 37 pg/ml *(Fig 3-A)*. Although one animal (1/4; 25%) from G2 (b8) showed an increase in E2 concentrations up to 41 pg/ml in the 10th week of treatment *(Fig. 3-B)*, similarly to G1, there was no significant increase in serum P4 concentrations during 13 weeks of treatment in this animal *(Fig. 2-B)*.

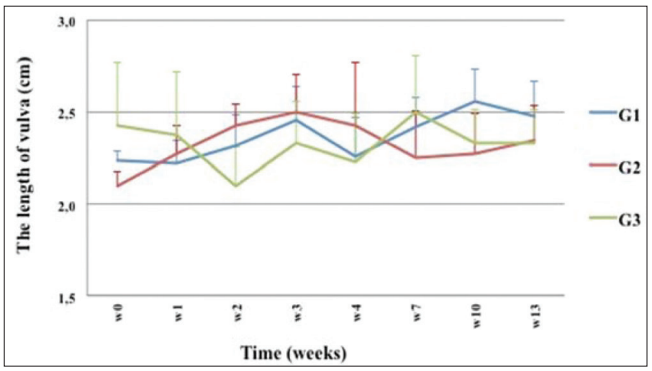

Fig 1. Changes in the length of the vulva (cm) in treatment and control groups (G1:G2:G3=P>0.05)

Şekil 1. Uygulama ve kontrol gruplarında vulva büyüklüklerindeki (cm) değişiklikler (G1:G2:G3=P>0.05)

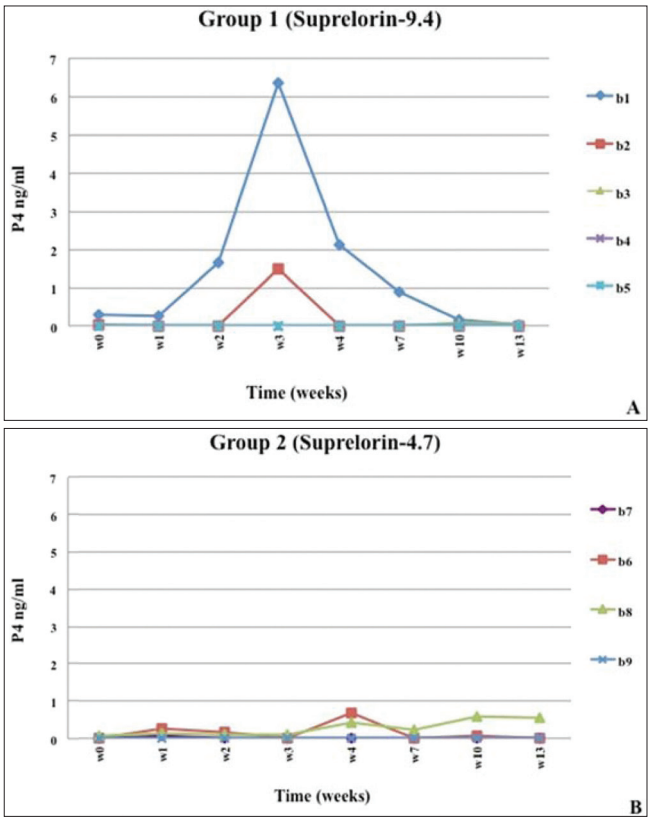

Fig 2. (A, B) Changes in individual P4 levels of treatment groups (G1 and G2)

Şekil 2. (A, B) Uygulama gruplarında (G1 ve G2) P4 düzeylerindeki bireysel değişiklikler

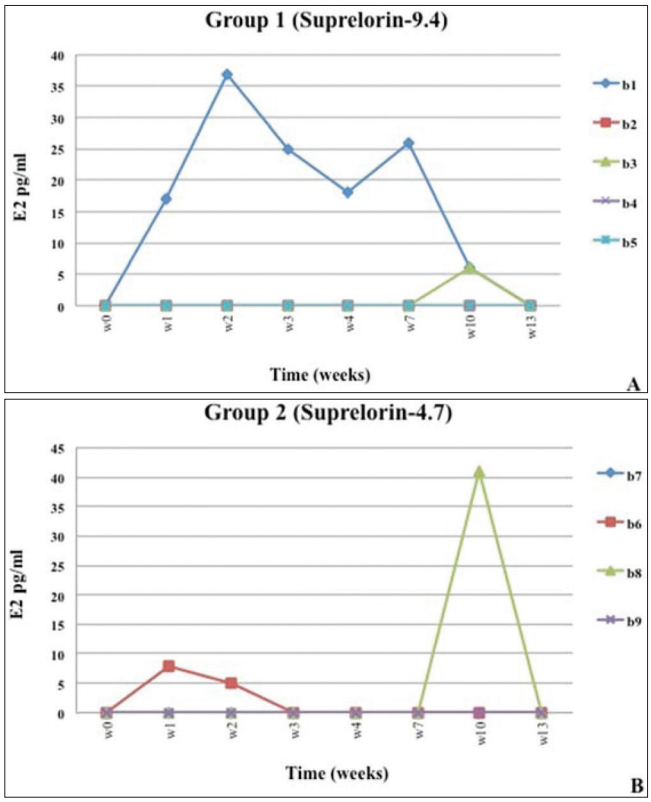

Fig 3. (A, B) Changes in individual E2 levels of treatment groups (G1 and G2)

Şekil 3. (A, B) Uygulama gruplarında (G1 ve G2) E2 düzeylerindeki bireysel değişiklikler

Two out of five (2/5; 40%) dogs in G1 and half of the dogs (2/4; 50%) in G2 showed an increase in superficial cell index (SCI, 15-20%) accompanied by the increase in serum E2 concentrations (6-41 pg/ml) during the first eight weeks after placement of the implant *(Fig. 4)*. Dogs in the control group (G3) showed neither increased SCI nor P4 and E2 concentrations during this period (0/5; 0%). Furthermore, there was no increase in SCI after implant insertion in the control group *(Table 1)*.

DISCUSSION

The present study was designed to evaluate the short-term effects of deslorelin implant Suprelorin® (4.7 and 9.4 mg) on vaginal cytological and hormonal changes and its

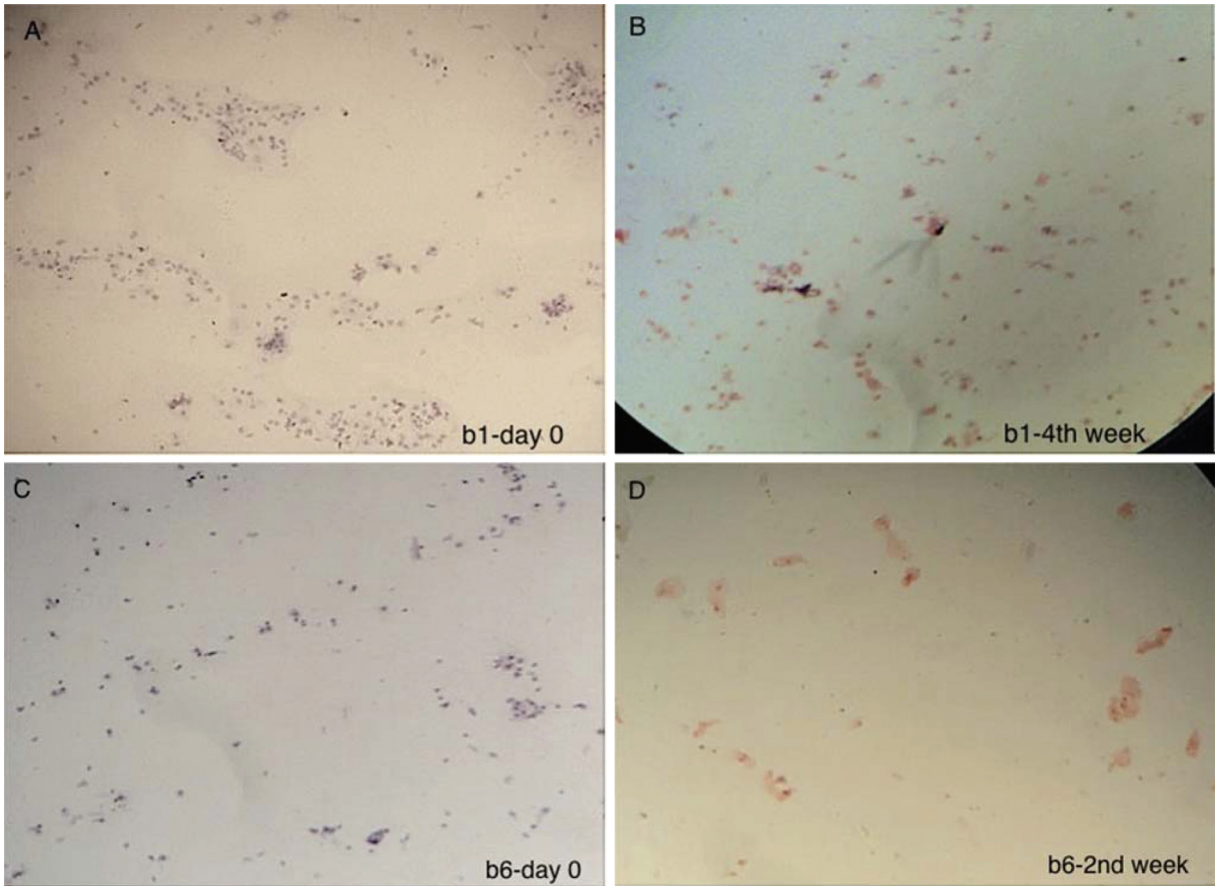

Fig 4. (A-D) Individual changes in superficial cell index of treatment groups (A, B: b1 from G1 and C, D: b6 from G2)
Şekil 4. (A-D) Uygulama gruplarında süperfisyel hücre indeksindeki bireysel değişimler (A, B: G1'den b1 ve C, D: G2'den b6 numaralı hayvan)

Table 1. *Follow-up of individual parameters from treatment and control dogs*
Tablo 1. *Uygulama ve kontrol gruplarındaki köpeklerde takip edilen bireysel parametreler*

Number of the Dog	Groups	Local/Systemic Side Effects	SCI within 13 wk After the Start of Treatment (%)	Clinical Signs of Estrus
b1	G1-9.4 mg	none	5	none
b2	G1-9.4 mg	none	not observed	none
b3	G1-9.4 mg	none	10	none
b4	G1-9.4 mg	none	20	none
b5	G1-9.4 mg	none	20	none
b6	G2-4.7 mg	none	15	none
b7	G2-4.7 mg	none	10	none
b8	G2-4.7 mg	none	20	none
b9	G2-4.7 mg	none	10	none
b10	G3-control	none	not observed	none
b11	G3-control	none	not observed	none
b12	G3-control	none	not observed	none
b13	G3-control	none	<5	none
b14	G3-control	none	not observed	none

effect on the occurrence of a flare-up effect, and as a safe and reversible contraceptive in prepubertal bitches.

GnRH agonists have been shown to be an effective and reversible alternative to surgical castration in dogs. Prolonged administration of GnRH agonists leads to desensitization of the pituitary gland and effectively inhibited the pituitary-gonadal axis [2,16]. However, treatment with GnRH agonists initially induces a large increase in the concentrations of LH and FSH *(flare-up effect)* that is sometimes associated with estrus induction, with or without ovulation [6,12]. This agonist-induced estrus is generally regarded as an inherent disadvantage when the implant was used to postpone puberty or for suppression of estrus.

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 prepubertal female dogs [5,19,20]. Although insertion of a GnRH agonist device did not induce estrus in prepubertal bitches [5,19,20], which commonly occurs in anestrus adult bitches (flare-up) within one month following implantation [2,6], it was also reported that the age at implantation is important for the response of animals to GnRH agonist [20]. According to the mentioned studies bitches implanted at four months of age showed no sign of estrus, however, all dogs implanted at seven months of age exhibited estrus within 1-2 weeks after implant insertion. In the present study, we observed the same findings for bitches aged four months inserted with deslorelin (4.7 and 9.4 mg) implant (Suprelorin®, Virbac, Austria). None of the bitches in the treatment groups showed any flare-up sign during the first 13 weeks after implant insertion.

Cytological evaluation of the vaginal mucosa and determination of increasing percentage of superficial cells as a bioassay for estrogen influence is even more reliable than a single plasma E2 determination [21]. It was confirmed that two-thirds (4/6) of prepubertal bitches implanted at seven months of age also showed an increase in SCI and increased P4 concentrations. However, an increase in SCI and P4 concentrations was not reported for bitches implanted at four months of age [20,22]. Similarly, Swangchan-Uthai et al.[19] suggested that the majority of vaginal cells were basal and parabasal for four weeks after deslorelin implant insertion in prepubertal bitches. In disagreement with these results, in the present study, we observed an increase in the average rate of 15-20% of SCI accompanied by a moderate increase in serum E2 concentrations in four of the nine treated bitches during the first eight weeks after the implant placement. Similar to our findings, Lanna et al.[23] reported an increase in the proportion of superficial cells, reaching a high average of 44±15% without clinical signs of estrus in adult bitches received a single intramuscular injection of 2 mg deslorelin. In our study, dogs in the control group (G3) did not show an increase in SCI or P4 and E2 concentrations which comparable to anestrus during the observation period.

It has already been described that GnRH agonist implants can be easily implanted and removed without local or systemic side effects [5,6,15]. In agreement with these results, in the present study, we did not observe any local inflammatory reactions and pain symptom or systemic side effects after implant insertion.

In sexually intact bitches, serum E2 concentration increases from 5-15 pg/ml initially to reach a peak of 40-120 pg/ml during proestrus [24]. Although it was shown that administration of GnRH implants led to induction of estrus in adult animals [6,15], lower P4 concentrations were determined after estrus induction with deslorelin implants than during spontaneous estrus in bitches [25]. In the present study, two of the treated prepubertal animals showed an increase in serum E2 concentrations from <5 to 37-41 pg/ml throughout the study period. However, values were decreased to <5 pg/ml thereafter in treated animals of both G1 and G2 without estrus signs.

In conclusion, our results clearly demonstrate that deslorelin treatment (4.7 and 9.4 mg) did not cause a "flare-up" and can be used safely without any clinical short-term side effect in prepubertal bitches when inserted at a mean age of four months. Although GnRH agonists appear to be a promising alternative for the reversible control of ovarian activity in domestic carnivores, further studies are needed to understand the hormonal changings and the long term effects on the development and health of bitches especially during later prepubertal stages.

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