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Fertility Restoration in Progestogen-Treated Hungarian Wirehaired Vizslas: Effects of GnRH Agonist on Reproductive Activity

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ABSTRACT

Deslorelin implants are increasingly utilized in veterinary reproductive management as a reversible alternative to surgical sterilization in dogs. The present study evaluated the biphasic response to deslorelin (Suprelorin®, 4.7 mg; Virbac®, France) in Hungarian Wirehaired Vizsla bitches after long-term progestogen-induced estrus suppression and assessed its efficacy for estrus induction and hormonal response sterilization. Thirty healthy bitches, aged 2-3 years and weighing from 19.8 to 23.7 kg, were enrolled in the study, which was divided into three groups, including a control group (C1) and two experimental groups (E1 and E2), each comprising 10 bitches. All of these dogs received subcutaneous deslorelin implants during anestrus. In E1, bitches previously treated with medroxyprogesterone acetate for 18 to 24 months received the implant for estrus induction, which was surgically removed on day 7. In E2, the implant was used for prolonged estrus suppression. Cytology and hormone analysis samples (progesterone, luteinizing hormone) were collected from the cephalic vein at specific time points; during anestrus (day 160), before implantation in controls, proestrus (day 3), estrus (days 1-3), the flare-up phase (72 hours post-implantation), and post-removal (day 7, group E1). Diestrus was monitored on days 7, 9, 11, 14, 15, 30, and 60 for the E2 group under suppression. Cytological assessment was performed using Diff-Quik™ staining, and hormone levels were measured via chemiluminescent immunoassay and ELISA. Ultrasonography and physical examination were used to monitor reproductive tract status. The present study confirmed a biphasic response to deslorelin, characterized by an initial flare-up phase, during which luteinizing hormone (LH) levels and estrous behavior were elevated, peaking on days 3 or 4, followed by effective suppression from day 14 through day 60. Implant removal in group E1 successfully induced fertile estrus, demonstrating Suprelorin's utility for fertility restoration in progestogen-treated bitches. In group E2, the implant provided sustained reproductive suppression for up to 12 months without adverse effects. These results highlighted the dual role of deslorelin in inducing or suppressing estrus, providing a valuable, evidence-based pharmacological option for managing canine reproduction.

Keywords: Deslorelin, Estrus induction, Hormonal sterilization, Luteinizing hormone, Progesterone, Reproductive management

INTRODUCTION

Modern veterinary reproductive medicine is advancing toward individualized, evidence-based strategies that emphasize reversible and minimally invasive methods for controlling fertility. Deslorelin, a potent gonadotropin-releasing hormone (GnRH) agonist administered via Suprelorin® implants, has emerged as a promising pharmacological tool for managing reproductive function in female dogs (Hinderer et al., 2021; Schäfer-Somi et al., 2022; Moxon et al., 2024). By modulating the hypothalamic-pituitary-gonadal axis, deslorelin induces a controlled anestrus, enabling precise reproductive planning without the need for permanent sterilization (Hollinshead and Hanlon, 2019; Brändli et al., 2021; Gontier et al., 2022). While the short-term contraceptive effects of deslorelin are well documented, data on its application after prolonged use of synthetic progestogens remain limited, especially in underrepresented breeds such as the Hungarian Vizsla (Lo et al., 2022; Kowalewski, 2023).

Traditional progestogen-based contraception carries known risks, including suppression of cyclicity, ovarian pathology, and delayed return to estrus (Ferré-Dolcet et al., 2022; Karadağ et al., 2024), which complicates future reproductive management. Recent investigations have expanded our understanding of deslorelin's clinical utility in restoring reproductive function following hormonal suppression, demonstrating both its safety and therapeutic potential (Gontier et al., 2022; Holumbiiovska, 2023; Baldan et al., 2025). These findings support the integration of deslorelin-based protocols into routine veterinary reproductive care, particularly in cases that require breed-specific considerations. As interest increases in alternatives to surgical sterilization, hormone-based interventions provide a scientifically

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grounded, humane solution that aligns with contemporary standards of animal welfare and reproductive health management (Romagnoli et al., 2019; Amaral et al., 2023; Barnes and Vansandt, 2025).

The application of long-acting hormonal implants in canine reproductive management enables controlled and reversible modulation of fertility, providing a clinically validated, non-surgical alternative to traditional gonadectomy (Demattio et al., 2024; Baldan et al., 2025). This strategy enhances safety and preserves reproductive potential, making it particularly advantageous for both companion animals and breeding stock (Barnes and Vansandt, 2025). By utilizing reversible hormonal treatments, such as Suprelorin, reproductive cycles can be managed with greater flexibility, enabling owners to make informed decisions based on the specific needs of their pets (Cowl et al., 2018; Ferré-Dolcet et al., 2022; Karadağ et al., 2024). This method of implanting hormonal medication is particularly beneficial for breeding animals because it preserves future reproductive potential while reducing the risks associated with permanent sterilization (Romagnoli et al., 2019; Spruijt et al., 2023; Schaper et al., 2025). Moreover, accumulating evidence from recent studies supports the clinical reliability and safety profile of this non-invasive reproductive control method, reflecting its increasing integration into routine veterinary reproductive protocols (Ferré-Dolcet et al., 2022; Amaral et al., 2023; Moxon et al., 2024). As advancements in reproductive medicine continue to evolve, non-invasive fertility regulation strategies provide a humane and scientifically supported alternative to conventional spaying procedures.

The application of Suprelorin in female dogs after fertility suppression with progestogens represents a significant advancement in modern veterinary reproductive medicine. Suprelorin, which contains deslorelin as its active ingredient, is widely recognized for its effectiveness in temporarily regulating animal reproductive functions (Schäfer-Somi et al., 2018; Oliveira et al., 2021; Wang et al., 2023). This GnRH agonist functions by downregulating the hypothalamic-pituitary-gonadal axis, resulting in reversible suppression of estrous cycles (Gontier et al., 2022). The application of a progestogen-based hormonal implant for fertility control represents a promising approach to managing reproductive health, while also reducing the risks linked to prolonged hormonal therapies (da Silva et al., 2021; Kowalewski, 2023; Demattio et al., 2024). Recent studies by Furthner et al. (2020), Schäfer-Somi et al. (2022), and Karadağ et al. (2023) have highlighted the benefits of this approach, demonstrating its effectiveness in maintaining reproductive control without the long-term side effects commonly observed with other hormonal therapies. As veterinary medicine continues to evolve, the integration of Suprelorin into reproductive management protocols offers a safer and more controlled alternative for long-term fertility regulation in female dogs (Palerme et al., 2021).

The administration of progestogens in female dogs requires careful consideration due to their potential long-term effects on reproductive health. These hormonal treatments can induce significant and prolonged alterations in the estrous cycle, potentially disrupting normal hormonal balance and fertility (Ferré-Dolcet et al., 2022; Cermakova et al., 2023; Moxon et al., 2024). The studies have demonstrated that exogenous progestogens may suppress natural ovarian function, leading to extended anestrus or irregular estrous cycles (Karadağ et al., 2024). Studies by Brändli et al. (2021), Gontier et al. (2022), and Moxon et al. (2024) have highlighted these concerns, emphasized the necessity of veterinary supervision when utilizing such treatments. Therefore, veterinarians and pet owners should weigh the benefits and risks of progestogen therapy, considering alternative approaches whenever possible to maintain optimal reproductive health in female dogs (Driancourt and Briggs, 2020; Holumbiiovska et al., 2021; Kowalewski, 2023).

Suprelorin exerts its effects by acting on the hypothalamus, stimulating the secretion of gonadotropin-releasing hormone (GnRH), which in turn triggers the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland (Zhang et al., 2023; Demattio et al., 2024; Baldan et al., 2025). These essential gonadotropic hormones are integral to the regulation of ovarian physiology, as they stimulate folliculogenesis and facilitate the re-establishment of regular estrous cycling in bitches (Lo et al., 2022; Kowalewski, 2023; Moxon et al., 2024). This mechanism makes Suprelorin particularly valuable in cases where progestogens have induced reproductive suppression, as it facilitates a controlled and gradual return to fertility (Lindh et al., 2023). The studies conducted by Ferré-Dolcet et al. (2022) and Schaper et al. (2025) have demonstrated the efficacy of this approach, highlighting its ability to regulate reproductive function without the need for invasive procedures. As veterinary reproductive medicine continues to evolve, Suprelorin presents a scientifically supported and reversible solution for fertility management, offering both veterinarians and pet owners a safer and more flexible alternative to permanent sterilization (Moxon et al., 2023a; Zhelavskyi et al., 2024).

It has been demonstrated that the implantation of Suprelorin induces a significant increase in follicular growth in female dogs, confirming its effectiveness in restoring reproductive function following hormonal suppression (Glocova et al., 2020; Gontier et al., 2022; Conley et al., 2023). By stimulating the hypothalamic-pituitary-gonadal axis, Suprelorin promotes the release of key reproductive hormones, facilitating the reactivation of ovarian activity and the progression of normal follicular development (Limmanont et al., 2024). This process is essential for re-establishing regular estrous cycles, making Suprelorin a valuable tool in veterinary reproductive management. Studies by Gontier et al. (2022), Amaral et al. (2023), and Romagnoli et al. (2024) have provided strong evidence supporting its efficacy, demonstrating its ability to enhance fertility recovery in previously hormonally suppressed females. As non-invasive reproductive control methods continue to gain traction in veterinary medicine, Suprelorin offers a promising alternative to conventional fertility management strategies, ensuring safe and reversible reproductive regulation in female dogs (Stempel and Goericke-Pesch, 2020).

This study expands current knowledge by evaluating the long-term endocrine and clinical effects of deslorelin in bitches previously subjected to prolonged progestogen-induced suppression, with a focus on the reactivation of folliculogenesis and the hypothalamic-pituitary-gonadal axis. Unlike prior studies that emphasized short-term outcomes in reproductively intact dogs, this study focused on post-suppression recovery and highlighted breed-specific responses, using the Hungarian Vizsla as a model. The findings support evidence-based, reversible fertility management protocols tailored to individual reproductive histories and breed characteristics, thereby improving clinical practice and promoting animal welfare (Romagnoli et al., 2019; Amaral et al., 2023). The restoration of normal ovarian function after progestogen treatment remains unpredictable, creating challenges in reproductive planning. The present study addressed a critical gap by evaluating if Suprelorin can support the recovery of estrous cycling in bitches previously treated with progestogens, thereby providing a more predictable and controlled alternative for managing fertility.

The present study aimed to evaluate the efficacy of deslorelin (Suprelorin, 4.7 mg implant; Virbac®, France) for inducing estrus in Hungarian Vizsla dogs after prolonged fertility suppression with progestogens, as well as to assess its impact on long-term hormonal sterilization. This study uniquely examined the long-term effects of Suprelorin® following progestogen use in Hungarian Vizslas, addressing a lack of breed-specific data on hormonal fertility control.

MATERIALS AND METHODS

Ethical approval

The clinical investigations were conducted in accordance with the Law of Ukraine "On Protection of Animals from Cruel Treatment" (February 21, 2006, No. 3447-IV) and adhered to the European Commission's guidelines on the treatment of vertebrates, ensuring protection from thirst, hunger, malnutrition, discomfort, fear, pain, and suffering. All studies adhered to bioethics standards, with written owner consent, local animal protection guidelines, and national legislation. All procedures and manipulations were performed in compliance with aseptic and antiseptic protocols. Written consent for the study was obtained from the owners before conducting any procedures. All animal research procedures were ethically conducted in full compliance with international welfare guidelines and institutional animal care protocols.

Study design and animal selection

The study was conducted on Hungarian Wirehaired Vizslas (also known as Hungarian Vizslas). An experimental protocol was implemented to assess the effect on reproductive function. A total of 30 healthy bitches, aged 2-3 years and weighing from 19.8 to 23.7 kg. The control group (C1) and two experimental groups, E1 and E2, which received a subcutaneous deslorelin implant (Suprelorin, 4.7 mg implant, Virbac®, France), were formed, each group consisting of 10 dogs. Standard clinical parameters, including rectal temperature, heart rate, and respiratory rate, were routinely assessed throughout the study period. Group allocation was based on findings from cytological evaluations and results from hormonal profiles.

Group formation was considered based on the animals' reproductive history, alongside initial concentrations of key hormones such as progesterone and luteinizing hormone. Reproductive status was evaluated using vaginal cytology and laboratory hormone assays for serum progesterone (P4) and LH. The study was conducted from 2022 to 2025 at a specialized breeding facility and in the veterinary clinic (Doctor VET, Kamyanets-Podilsky, Ukraine).

During 2022-2023, hormonal suppression of estrus in the first experimental group (E1) was achieved using medroxyprogesterone acetate (50 mg/mL, Depo-Promone, Zoetis, USA) at a dose of 1.0 mL, administered subcutaneously in the neck region during anestrus (Romagnoli et al., 2024). Estrus was suppressed for 18-24 months (duration of progestogen action). For estrus induction, the deslorelin implant (Suprelorin, 4.7 mg implant, Virbac®, France) was administered subcutaneously (Romagnoli et al., 2024). The implant was surgically removed on day 7 after the onset of estrus. In the second experimental group (E2), deslorelin was used for long-term (up to 12 months) suppression of estrus and inhibition of reproductive function.

Samples collection

Samples for cytological analysis and hormone testing (progesterone and luteinizing hormone) were obtained from the cephalic vein (4.0 ml) at 09:00 AM to evaluate the estrous cycle phase during anestrus, before deslorelin implant placement in the control group (day 160), proestrus (day three), estrus (days 1-3), at the flare-up effect time point (72 hours post-implantation), after implant removal (estrus induction was noted on day 7 post-implantation in E1, confirming the flare-up effect of the deslorelin implant), and during diestrus (days 7, 9, 11, 14, 15, 30, and 60) under suppressed sexual activity in E2.

Cytological examination

The vaginal samples in dogs were collected by inserting sterile cotton swabs or cytological brushes into the vagina to a depth of 3-5 cm, using rotational movements to collect cellular material. The samples were collected using sterile cytology brushes (Nu-Life Medical and Surgical Supplies Inc., Canada) and transferred onto glass slides, followed by air fixation. The slides were dried for 3 minutes at room temperature (18 °C). Staining was performed using the Diff-Quik™ method (SIEMENS RAL DIFF-QUICK Stain, Germany). The Diff-Quik™ staining method was used, which is a modified Romanowsky technique. It involved fixation in methanol, followed by sequential staining with an eosinophilic dye for cytoplasmic elements and a basophilic dye for nuclear material, enabling clear evaluation of cellular morphology (Oliveira et al., 2021; Zhelavskyi, 2024).

The procedure included sequential immersion of the dried smears in a fixing solution (70% methyl alcohol, BASF SE, Germany) for 10 to 15 seconds, staining solution 1 (eosin) for 5 to 10 seconds, and staining solution 2 (methylene blue) for 5-10 seconds, followed by rinsing with distilled water, drying (Oliveira et al., 2021; Zhelavskyi, 2024), and microscopy (Axioscope 5, Zeiss, Germany) at ×1000 magnification with immersion oil.

Blood collection and analyses

The concentrations of progesterone (P4 nmol/L) were evaluated using the Catalyst Dx Chemistry Analyzer (IDEXX Laboratories, Inc., USA), a precise and reliable platform for hormone assays, to monitor reproductive status and hormonal dynamics following sample collection, calibration, and chemiluminescent immunoassay-based quantification (Conley et al., 2023; Tiberiu et al., 2024). The determinations of LH pmol/L in dogs were conducted using an enzyme-linked immunosorbent assay (ELISA). For this purpose, plates coated with antibodies that bound LH, enzyme-conjugated antibodies, and a substrate that changes color in the presence of the enzyme were used. Optical densities were measured using spectrophotometers (Thermo Fisher Scientific, USA; Bio-Rad, USA). The assay employed specific antibodies targeting LH, sourced from commercial suppliers (Abcam, UK; Sigma-Aldrich, USA). Secondary antibodies conjugated with detection enzymes were sourced from leading manufacturers (Thermo Fisher Scientific, USA; RandD Systems, USA). For signal development, ELISA substrates were acquired from established diagnostic and biotechnology companies (Thermo Fisher Scientific, USA; Bio-Rad, USA).

Suprelorin implant administration

Before administration, clinical examinations were conducted, and vaginal and blood samples were collected for hormonal analysis (progesterone, LH). Suprelorin implants (Virbac, France), each containing 4.7 mg of deslorelin, were administered subcutaneously (at a depth of 0.5 cm) using specialized implanters (Figure 1). The injection sites were located in the abdominal area, paramedian (1.5 cm) to the midline, and cranial (2.0 cm) to the level of the umbilicus. Before implant insertion, the injection sites were shaved and disinfected with 70% ethyl alcohol (Decon Laboratories, Inc., USA) using sterile cotton swabs. After the implants were administered, their locations were confirmed by palpation. The implants were removed on day three of the flare-up effect (on day seven following subcutaneous administration in E2). Following general and local clinical examinations, regional anesthesia was administered using 2% lidocaine hydrochloride (Lidocaine, Mylan Pharmaceuticals, Italy). Local anesthesia with lidocaine was administered during the implant retrieval procedure. The implant sites were disinfected with sterile cotton swabs soaked in 70% ethyl alcohol (Emsure®, Sigma-Aldrich, Germany). Skin and subcutaneous tissue incisions (0.7 cm in length) were made using scalpels (B. Braun blade 22, B. Braun Medical Inc., USA). The implants were then carefully extracted using surgical (Figure 2) tweezers (Adson-Brown, 12 cm, J-16-130, B. Braun Medical Inc., USA). The wound surfaces were treated with swabs soaked in 70% ethyl alcohol (Emsure®, Sigma-Aldrich, Germany), and spray adhesive (Sigvaris Fix Special Glue 00000011100, Switzerland) was applied. A 10-day postoperative monitoring protocol was implemented after implant removal to evaluate potential adverse effects. No complications such as bleeding, hematomas, swelling, inflammation, or other adverse effects were observed. No clinically significant complications were documented at administration sites, with particular attention given to monitoring for hematoma formation, local inflammatory responses, or other pathological manifestations (Romagnoli et al., 2024).

Statistical analysis

The statistical evaluation was conducted using Statistica® 12.6 software (StatSoft Inc., USA), with a significance threshold established at $p < 0.05$. Data distribution was assessed for normality, and subsequent analyses were carried out using parametric methods, including Student's t-test.

RESULTS

Cytological characteristics at different stages of the canine estrous cycle in Hungarian Vizsla

During anestrus, the vaginal cytology demonstrated a predominance of parabasal cells (92.14 ± 0.12%) and occasional small intermediate cells (7.12 ± 0.11%). Neutrophils were observed only in trace amounts (1.02 ± 0.03%; Figure 3). Biochemical evaluation of blood plasma confirmed basal progesterone levels (1.59 nmol/L; Table 1). Clinically, the animals exhibited no signs of sexual receptivity and displayed no interest in males. As the cycle progressed, a gradual increase in clear, odorless vulvar discharge was noted.

By day three, the cytological profile indicated an increase in superficial cells (72.12 ± 0.73%), along with the presence of erythrocytes (12%) and large intermediate cells (10.07 ± 0.05%). By the sixth day, against a backdrop of rising progesterone levels (Table 1), the typical cytological features of estrus were evident: a dominance of superficial cells (92.12 ± 0.57%), a minor presence of large intermediate cells (5.12 ± 0.04%), and a small number of erythrocytes (2.12 ± 0.04%).

Table 1. Plasma luteinizing hormone concentrations (ng/ml) during the estrous cycle in 2-3-year-old Hungarian Vizsla bitches (n = 10)

Stages	Concentrations LH (ng/ml)
Anestrus	0.34 ± 0.09
Proestrus	3.35 ± 0.07*
Estrus	18.56 ± 0.82*
Diestrus	1.17 ± 0.05*

Mean ± Standard deviation; LH: Luteinizing hormone; Significance*, $p < 0.05$ relative to the anestrus stage

Hormonal dynamics during the natural estrous cycle in Hungarian Vizsla

Luteinizing hormone and progesterone concentrations exhibited marked fluctuations throughout the reproductive cycle. During anestrus, LH levels remained low (0.34 ± 0.09 ng/mL), indicative of functional quiescence within the reproductive axis. In proestrus, LH began to rise (3.35 ± 0.07 ng/mL; $p < 0.05$), stimulating follicular maturation and estrogen secretion.

A sharp LH surge was documented during estrus (18.56 ± 0.82 ng/mL; $p < 0.05$), acting as the physiological trigger for ovulation, which typically followed within 48 hours. The onset of diestrus coincided with a significant decline in LH (1.17 ± 0.05 ng/mL; $p < 0.05$) and the formation of the corpus luteum. Progesterone levels rose sequentially from baseline values in anestrus 1.42 ± 0.16 nmol/L to 6.07 ± 0.47 nmol/L in proestrus ($p < 0.05$; Table 2), peaking during estrus at 24.27 ± 1.67 nmol/L ($p < 0.05$), and reaching their maximum during diestrus at 42.02 ± 0.62 nmol/L ($p < 0.05$), creating optimal conditions for potential implantation or the manifestation of pseudopregnancy.

Hormonal response to Suprelorin 4.7 mg implantation

Before the implantation of Suprelorin 4.7 mg, dogs exhibited baseline hormone levels characteristic of anestrus: LH (0.34 ± 0.09 ng/mL) and progesterone (1.42 ± 0.16 nmol/L). Within 72 hours post-implantation, a pharmacologically induced "flare effect" was observed, characterized by a rapid LH increase peaking at 48 hours (23.31 ± 0.32 ng/mL), accompanied by a gradual rise in progesterone to 9.60 ± 0.31 nmol/L (Figures 4 and 5). This profile mimicked a natural pre-ovulatory LH surge, although it was exogenously triggered (Group E1).

A similar initial response was observed in Group E2, with LH peaking at 48 hours (22.12 ± 0.21 ng/mL) and progesterone levels reaching 16.71 ± 0.27 nmol/L by day five. However, by days 14-15, suppression of the hypothalamic-pituitary-gonadal axis became evident. By days 30 and 60 post-implantation, LH concentrations in both groups (E1 and E2) remained persistently low (0.14 ± 0.04 ng/mL), while serum progesterone levels declined to baseline values (1.07 ± 0.08 nmol/L) with no further fluctuations. Clinically, there were no signs of estrus, behavioral changes, or vulvar discharge. Cytological evaluation of vaginal smears consistently indicated anestrus, as evidenced by the dominance of parabasal cells and the absence of erythrocytes and superficial epithelial cells.

Table 2. Plasma progesterone concentrations (nmol/L) during the estrous cycle in 2-3-year-old Hungarian Vizsla bitches (n = 10)

Stages	**Concentrations P4 (nmol/L)**
Anestrus	1.42 ± 0.16
Proestrus	6.07 ± 0.47*
Estrus	24.27 ± 1.67*
Diestrus	42.02 ± 0.62*

Mean ± Standard deviation; P_4: Progesterone; Significance*, $p < 0.05$ relative to the anestrus stage

Figure 1. Administration of a 4.7 mg deslorelin (Suprelorin®) implant in 3-year-old Hungarian Vizsla bitches

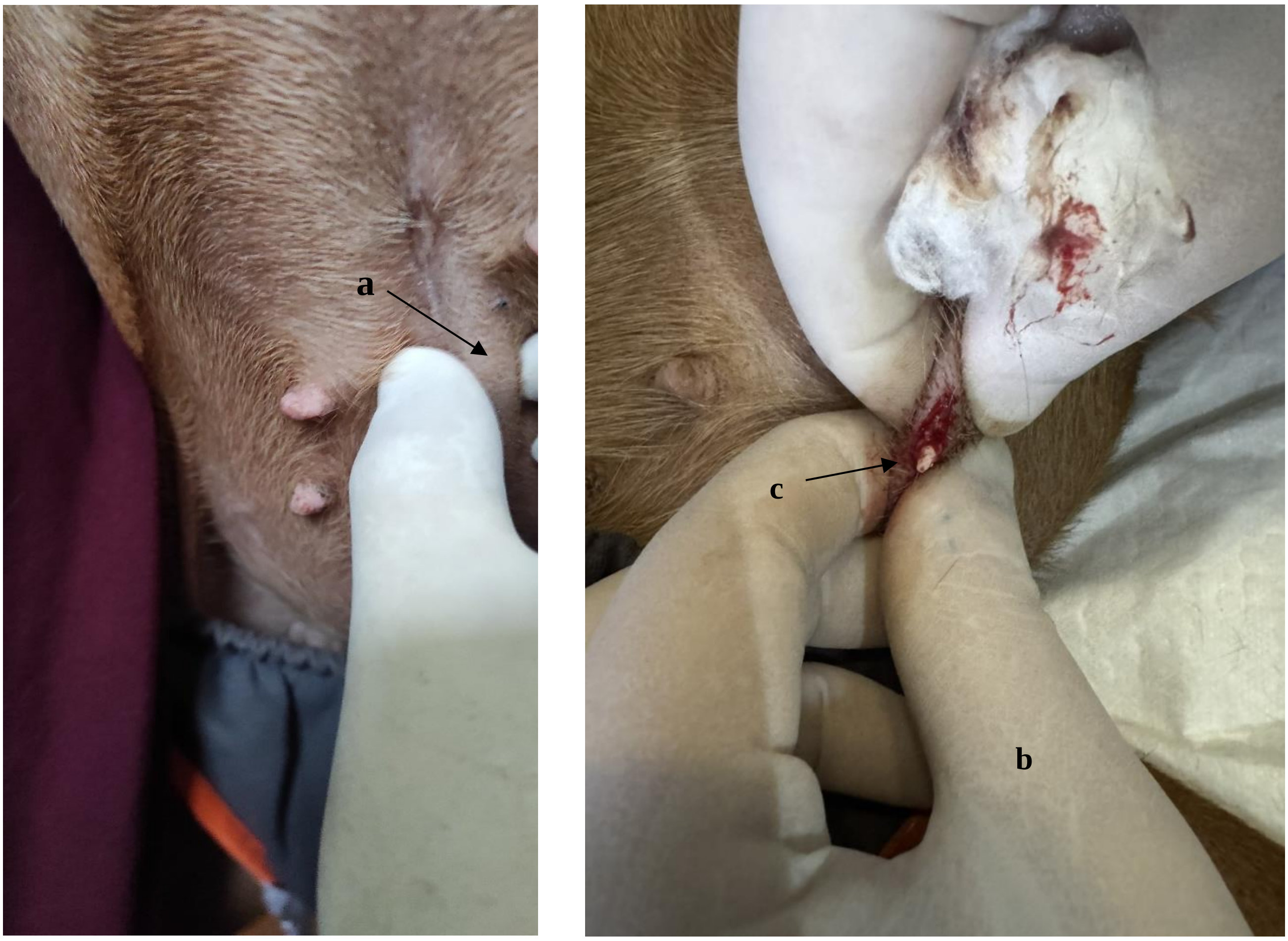

Figure 2. Surgical removal of the Suprelorin® (4.7 mg deslorelin) implant in 3-year-old Hungarian Vizsla bitches. **a**: Localization of the implant, **b**: Extraction from the incision site, **c**: Macroscopic appearance of the removed implant.

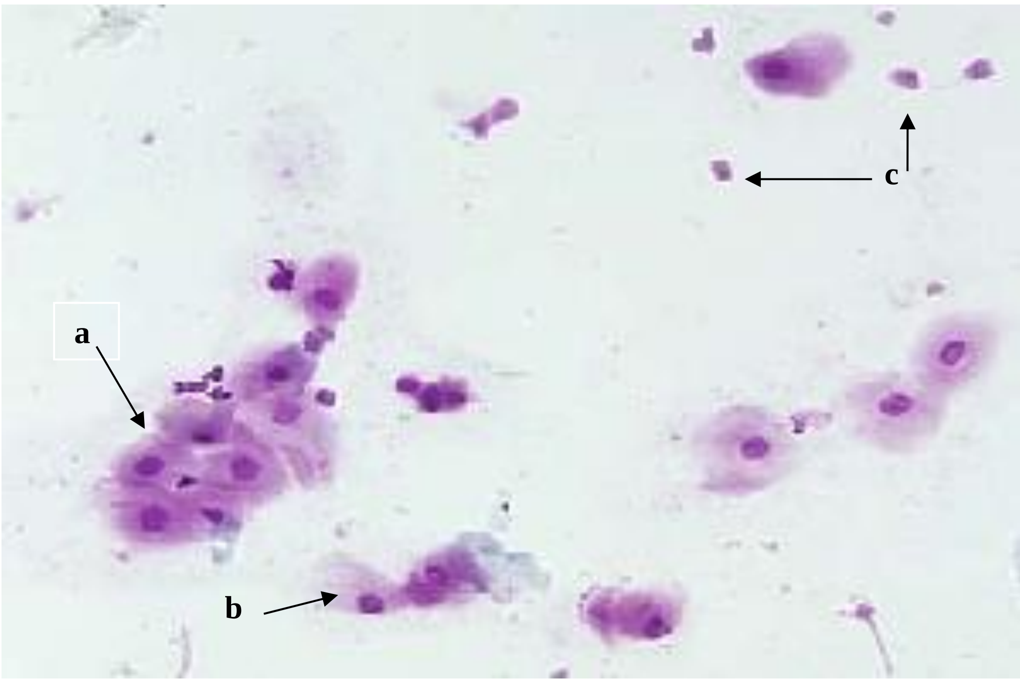

Figure 3. Cytology of a vaginal sample during the anestrus stage (day 160) in 3-year-old Hungarian Vizsla bitches before Suprelorin implantation. The slides show parabasal cells (**a**), small intermediate cells (**b**), and erythrocytes (**c**), Magnification 100×, Diff-Quik™ staining.

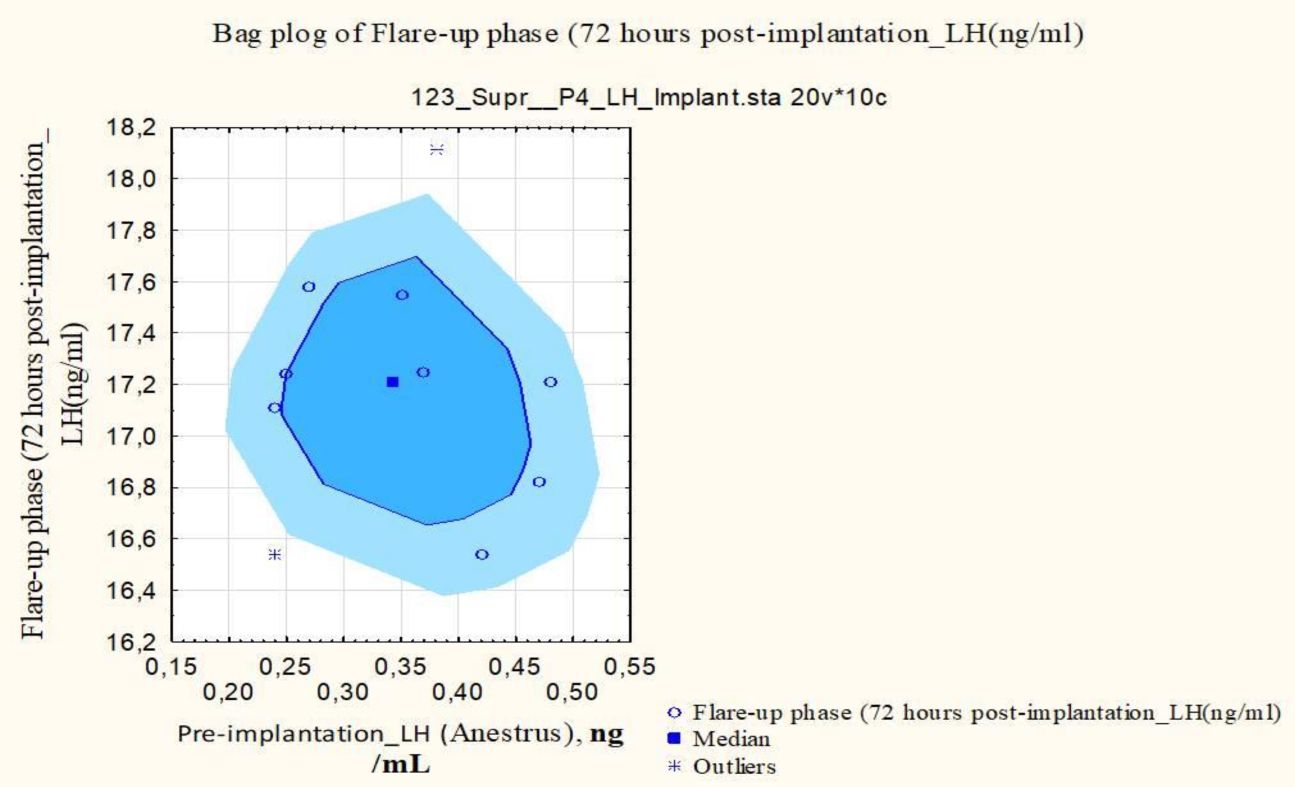

Figure 4. Bag plot illustrating changes in luteinizing hormone (LH) concentrations in the blood serum during the flare-up phase (72 hours post-implantation) compared to baseline LH levels during anestrus (pre-implantation). The dark blue region represents the central 50% of the data (bag), while the light blue area encompasses 95% of the observations, excluding outliers. Blue crosses indicate outliers, and the dark blue square marks the median. The graph demonstrates a pronounced increase in LH levels following implantation, consistent with the flare-up effect.

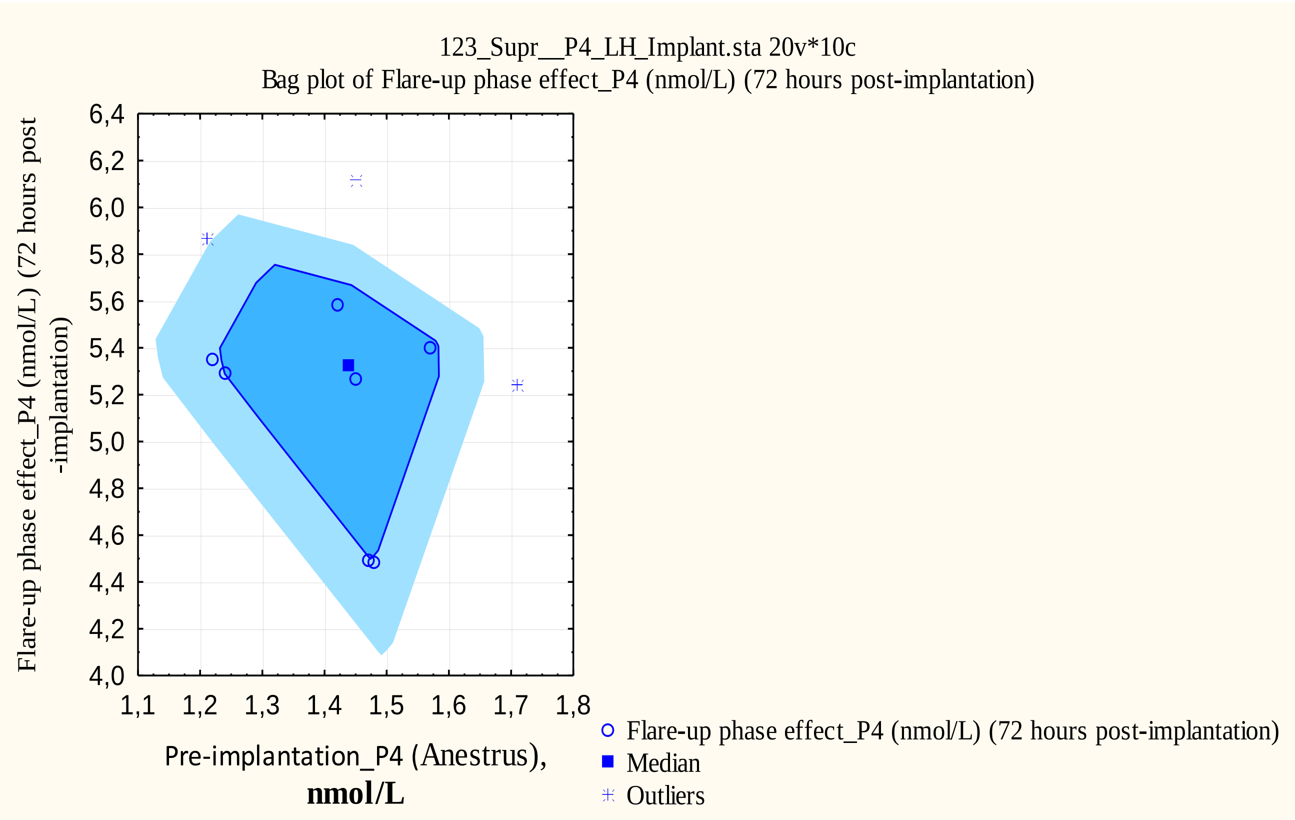

Figure 5. Bag plot of serum progesterone (P4) concentrations in the "flare-up" phase (72 hours after hormonal implantation) compared to pre-implantation levels during anestrus. The inner contour (bag) represents 50% of the central data, while the outer contour (fence) captures 95% of the observations, excluding outliers. The median value is indicated by the square marker.

DISCUSSION

Suprelorin, which contains the active ingredient deslorelin, is a GnRH agonist commonly used to induce temporary infertility in sexually mature male dogs (Driancourt and Briggs, 2020). The application of Suprelorin in veterinary practice has become a crucial tool for regulating reproductive function, especially in cases where traditional castration is not desirable, as it effectively downregulates the hypothalamic-pituitary-gonadal axis (Brändli et al., 2021; Gontier et al., 2022; Spruijt et al., 2023).

Clinical studies confirmed that Suprelorin (deslorelin acetate) demonstrates potent gonadotropin-inhibiting effects, achieving over 90% suppression of LH and FSH secretion within 4 to 6 weeks after implantation (Stempel et al., 2022). This downregulation of the hypothalamic-pituitary-gonadal axis results in a transient but significant reduction in serum testosterone levels (typically to < 0.5 ng/mL; Spruijt et al., 2023) and complete cessation of spermatogenesis lasting 6 to 12 months, depending on the formulation (Romagnoli et al., 2024). This feature makes it a valuable tool for veterinarians and pet owners seeking reversible fertility control while avoiding the permanent physiological and behavioral changes associated with surgical intervention, castration. As research continues to expand on the efficacy and long-term effects of deslorelin-based treatments, Suprelorin remains an essential option for managing reproductive health in male dogs. Suprelorin (deslorelin 4.7 mg implant) is an implant based on deslorelin, a synthetic analog of GnRH. Initially, it stimulates the secretion of LH and FSH through the activation of GnRH receptors in the pituitary gland (a phase known as the flare effect), which may temporarily increase sex hormone levels, testosterone in males (Glocova et al., 2020), or estradiol in females. However, prolonged use leads to receptor desensitization (Falceto et al., 2024), resulting in reduced LH and FSH secretion and, consequently, suppression of sexual activity (Bonacina et al., 2020; Romagnoli et al., 2022; Demattio et al., 2024; Greiner et al., 2025). The pharmacokinetics of deslorelin are characterized by the slow release of the active substance from the implant, ensuring its prolonged effect, which lasts up to 6 to 12 months (Cowl et al., 2018). The metabolism of the drug primarily occurs in the liver, while excretion takes place through the kidneys (Joonè and Cavalieri, 2023).

In Hungarian Vizslas, the biphasic endocrine response induced by deslorelin, featuring a 3-4-day flare phase characterized by simultaneous surges in LH and progesterone, followed by prolonged hormonal suppression, was consistent with the pharmacokinetic profile of GnRH agonists described by Schäfer-Somi et al. (2018). The observed delay in the onset of the flare phase, however, may reflect breed-specific pharmacokinetic or pharmacodynamic variability, as proposed by Spruijt et al. (2023). The emergence of behavioral estrus in 83% of individuals during this early stimulatory window supported the transient fertility concerns raised by Schaper et al. (2025), while the rapid luteinization, evidenced by the temporal synchrony of LH and progesterone elevation, reinforced endocrine patterns previously demonstrated in multi-breed cohorts (Hinderer et al., 2021; Holumbiiovska et al., 2025).

These findings have expanded Hollinshead and Hanlon (2019) and Romagnoli et al. (2022) contraceptive efficacy data to hunting breeds while underscoring the necessity for breed-specific protocols as cautioned in the World Small Animal Veterinary Association (WSAVA) reproduction control guidelines (Romagnoli et al., 2024).

Suprelorin is also used for controlling reproductive function in cats (to suppress sexual activity and prevent unwanted pregnancies (Baldan et al., 2025), ferrets (for managing hyperadrenocorticism associated with excessive sex hormone secretion; Romagnoli et al., 2024), as well as in ornamental birds (Borsdorf and Petritz, 2025) and rodents (for regulating reproductive behavior and preventing hormone-dependent diseases; Glocova et al., 2020; Viudes-de-Castro et al., 2024). Additionally, Suprelorin has been applied in chameleons (Cermakova et al., 2023) and grey mouse lemur (Noiret et al., 2024). Deslorelin exerts its clinical efficacy through the downregulation of LH and FSH secretion, thereby providing a reliable pharmacological strategy for regulating reproductive activity and managing associated behavioral and physiological disorders (Cowl et al., 2018; Socha et al., 2021; Lo et al., 2022).

The use of GnRH agonist implants enables a reversible and temporary suppression of both endocrine and germ cell function in the testes of male dogs (Stempel and Goericke-Pesch, 2020; Greiner et al., 2025). Although not always approved for all uses, off-label applications in male dogs include managing hormone-related anxiety behaviors, benign prostatic hyperplasia, small hepatoid gland adenomas, and alopecia X (Zhelavskyi et al., 2025). Deslorelin implants have also demonstrated success in suppressing estrus in juvenile animals and treating hyperadrenocorticism in ferrets (Romagnoli et al., 2024). Hormonal castration can be similarly accomplished in both male and female cats (Romagnoli et al., 2019; Furthner et al., 2020). However, the onset and duration of effects can vary significantly among individuals, posing challenges for breeders (Sasidharan et al., 2021; Moxon et al., 2023b). In male rabbits and guinea pigs, the treatment has not demonstrated sufficient contraceptive efficacy (Glocova et al., 2020). Nonetheless, it effectively suppressed the estrous cycle in females of these species and reduced reproductive activity in both male and female rats (Viudes-de-Castro, 2024). In avian and reptilian species, the physiological response to deslorelin implants is notably species-specific, exhibiting substantial variability in efficacy, onset of action, and duration of hormonal suppression. In birds, complete inhibition of oviposition has been successfully achieved in species such as chickens, Japanese quails, and psittacines. Significant reproductive differences arise in avian species, with female pigeons exhibiting only partial (40-60%) suppression of oviposition compared to the complete cessation observed in other bird species (Borsdorf and Petritz, 2025). The implants have also been reported to assist in managing undesirable reproductive behaviors such as feather-picking and aggression. In some male birds, including Danio rerio and Japanese quails, deslorelin effectively reduces testosterone levels (Troisi et al., 2020; Stempel et al., 2022; Spruijt et al., 2023).

The administration of progestagens for estrus suppression in bitches raises significant clinical concerns, such as metabolic disturbances (increased body weight and fat deposition; Romagnoli et al., 2024), reproductive pathologies (endometrial hyperplasia progressing to pyometra in 15-30% of cases; Hagman, 2024), and immunocompromised states (reduced lymphocyte function; Zhelavskyi et al., 2020). These effects arise from the prolonged suppression of the hypothalamic-pituitary-gonadal axis (Spruijt et al., 2023), with the risk of mammary neoplasia increasing 2-to 4-fold after extended use (Moon et al., 2022). Notably, 20-30% of treated bitches develop behavioral changes (lethargy or aggression) due to neuroendocrine effects (Gontier et al., 2022; Tiberiu et al., 2024), while alterations in hepatic enzymes (ALT/AST elevation) and insulin resistance (Romagnoli et al., 2024) further complicate long-term use. Contemporary studies demonstrated the superior safety of GnRH agonists, achieving over 90% efficacy without metabolic side effects (Brändli et al., 2021), which supported their preferential use in breeding populations (Romagnoli et al., 2024; WSAVA Guidelines, 2024).

The observed LH dynamics indicate a statistically significant increase in hormone levels during the flare-up phase compared to the baseline during anestrus (Lo et al., 2022; Kowalewski, 2023). This trend is consistent with findings from other studies, which described an early endocrine response to hormonal stimulation. Such a response is characteristic of the physiological reaction to gonadotropin-releasing hormone (GnRH) or its analogs, which are commonly employed to induce ovulation or synchronize estrus in reproductive protocols (Zhang et al., 2023).

The reduction in LH levels during the suppression phase indicated that the majority of values were concentrated below the initial anestrus level, which demonstrated the efficacy of the hormonal preparation in suppressing pituitary LH secretion over the extended period post-implantation. Such a profile is indicative of negative feedback regulation typically mediated by progestagens, which inhibit GnRH-induced LH release (Karadağ al., 2023). This mechanism is aligned with the known physiological effects of long-acting contraceptive implants or suppressive hormone therapies used in reproductive management (Moxon et al., 2023a; Zhang et al., 2023).

Despite the promising findings, the current study presented several limitations that should be acknowledged. Firstly, the sample size (n = 30) was relatively small, which may affect the statistical power and generalizability of the results. Additionally, no blinding procedures were implemented, introducing the risk of observer bias, particularly during behavioral and cytological assessments. The prior long-term use of progestogens in E1 group bitches might also serve as a confounding factor influencing the endocrine response to deslorelin, as individual variations in hormonal sensitivity were not controlled. Furthermore, although the bitches belonged to the same breed and had similar age and weight, other intrinsic factors, such as genetic variability and prior reproductive history, could have influenced the outcomes. Future studies with larger, randomized, and blinded cohorts are needed to confirm these results and further evaluate the long-term safety and efficacy of deslorelin in various clinical contexts.

The increase in serum P4 concentrations during the flare-up phase was noticeable, with a median value noticeably higher than the pre-implantation levels. This elevation reflected the rapid endocrine response triggered by the implanted hormone, likely mediated through stimulation of luteal tissue activity or mimicking of luteotropic signals. The pattern aligned with previous findings indicating that progestin-based implants can induce an acute increase in circulating progesterone concentrations shortly after administration (Schäfer-Somi et al., 2018; Pereira et al., 2024). Such an early flare-up effect is commonly associated with enhanced hypothalamic-pituitary-ovarian axis stimulation during the initial phase of hormonal synchronization protocols (Stempel et al., 2022; Moxon et al., 2023b).

The increase in P4 levels during the early post-implantation period may reflect a temporary stimulatory effect of the implant, possibly linked to an initial hormonal surge before the onset of suppression. Similar temporary endocrine responses have been reported in prior studies examining the early-phase effects of GnRH agonist implants (Gontier et al., 2022; Karadağ et al., 2023).

The obtained results suggested that the prolonged effect of the implant led to a decrease or stabilization of P4 levels in the late post-implantation period. This may indicate the effective suppression of endogenous progesterone production as a result of the hormonal implant's action. A similar trend has been reported by Lo et al. (2022), Romagnoli et al. (2024), and Holumbiiovska et al. (2025), confirming the suppressive impact of prolonged progestogen exposure on ovarian steroidogenesis. This pattern aligned with results reported in previous research, which highlighted the prompt activation of the endocrine system following hormonal stimulation. Such early hormonal responses suggest a rapid physiological adaptation to exogenous endocrine cues (Stempel et al., 2022; Romagnoli et al., 2024; Viudes-de-Castro et al., 2024).

Immunocastration offers a humane and effective alternative to traditional sterilization methods, reducing pain, stress, and recovery time while controlling population growth, improving meat quality, and addressing hormone-related disorders; yet, its long-term effects and ethical considerations remain topics of discussion (Wang et al, 2023). Traditionally, animal sterilization has been performed using surgical and mechanical castration, but these approaches are associated with high levels of pain, stress, extended recovery periods, and postoperative infections (Joonè and Cavalieri, 2023). Immunocastration is a new, safe, and painless castration technique used to prevent undesirable sexual behavior, reduce aggressive behavior, prevent unwanted pregnancies, control wild and stray animal populations, enhance growth performance, improve meat quality, and treat various hormone-dependent disorders (Karadağ et al., 2023; Moxon et al., 2024). The mechanism of immunocastration involves the immunological blockade of the hypothalamic-pituitary-gonadal (HPG) axis, which suppresses gonadotropin secretion, causes atrophy of gonadal tissue, and inhibits gametogenesis, leading to infertility in both female and male mammals (Picone et al., 2025). By the mid-1990s, various immunocastration vaccines had been tested on different animal models to achieve successful castration effects (Ahmed et al., 2022). Recently, genetic immunocastration, particularly DNA vaccines, has garnered increasing attention due to its safety, animal friendliness, and ease of use (Ahmed et al., 2022).

Suprelorin offers a natural approach to regulating reproductive functions by directly affecting the HPG axis. It imitates the body's natural hormonal control mechanisms to enable reversible fertility suppression, minimizing the physiological stress and risks typically associated with surgical sterilization (Bonacina et al., 2020; Demattio et al., 2024; Pereira et al., 2024; Greiner et al., 2025). Moreover, its extended effects provide a practical and less invasive option for managing reproduction in both domestic and wild animals, which includes applications in population control, behavioral modification, and addressing hormone-dependent disorders (Driancourt and Briggs, 2020; Conley et al., 2023; Falceto et al., 2024).

CONCLUSION

The present study confirmed a biphasic response to deslorelin in Hungarian Vizslas, comprising an initial hormonal stimulation phase that peaked on days 3 to 4, followed by effective suppression of reproductive function. While the animals exhibited estrous behavior during the flare-up phase, a complete hormonal suppression, characterized by a notable decline in LH levels, was observed from day 14 onward. Notably, the peak LH levels did not compromise overall contraceptive effectiveness. Suprelorin has demonstrated potential as a therapeutic option for re-establishing fertility in bitches that have undergone prior progestagen-based reproductive suppression. Suprelorin's ability to stimulate normal gonadotropin secretion makes it a valuable asset in veterinary reproductive management. Based on the study results, Suprelorin may represent a future method for addressing reproductive disorders in bitches that have undergone prolonged progestagen therapy. Future research efforts can focus on clarify the long-term metabolic consequences of contraceptive interventions, especially in breeds with high physical activity levels, while refining dosage regimens to reduce flare-phase effects without compromising the overall efficacy of hypothalamic-pituitary-gonadal axis suppression.

DECLARATIONS

Availability of data and materials

The data supporting the findings of the present study are available upon reasonable request from the corresponding author.

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Authors' contributions

Mykola Zhelavskyi carried out the study conception and design. Mykola Zhelavskyi and Serhii Kernychnyi performed data acquisition. Mykola Zhelavskyi, Valerii Tsvilikhovskyi, Volodymyr Mizyk, Tamara Betlinska, and

Maksym Luchka conducted laboratory investigations. Mykola Zhelavskyi played the lead role in composing the manuscript and conducting its critical review. All authors reviewed and approved the final version of the submitted article for publication in this journal.

Competing interests

The authors certify that there are no actual or perceived financial, academic, or personal conflicts of interest that could have influenced the design, execution, or reporting of this research. The study was conducted with complete scientific independence, and no external influences affected the design, execution, or reporting of the findings.

Ethical considerations

The research protocol was developed in strict adherence to international standards for ethical scientific conduct, including principles of data integrity, originality, and proper attribution of sources.

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