Topics in Companion An Med 45 (2021) 100565

Case Report

Restoration of Reproductive Hormone Concentrations in a Male Neutered Dog Improves Health: A Case Study

Linda Brent[a,*], Elaine A. Lissner[a], Michelle A. Kutzler[b]

Keywords:
gonadectomy
canine
neuter
hormone therapy
testosterone
luteinizing hormone

[a]*Parsemus Foundation, San Francisco, CA, USA*
[b]*Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR, USA*

A B S T R A C T

This case study reports on the use of hormone therapy to treat a dog with a range of physical and behavioral signs that began after gonadectomy. A male mixed breed dog neutered at 7 months of age presented at 1 year with health issues impacting quality of life. Reduced mobility, limping, rapid weight gain, and fear of unfamiliar people were treated over the next 3 years with trials of pain medication, joint supplements, thyroxine, antidepressant, and significant diet restrictions. Frequent carprofen administration and daily joint supplements reduced limping, but mobility was still poor. Weight stabilized on a strict diet but fear and anxiety responses to strangers continued to worsen. Hormone restoration therapy was initiated when the dog was almost 4 years of age. Weekly subcutaneous administration of testosterone cypionate (0.5 mg/kg) significantly reduced pain and increased muscle mass, thereby improving mobility. However, supraphysiologic concentrations of luteinizing hormone were not reduced with testosterone therapy so a gonadotropin-releasing hormone agonist was implanted. After hormone restoration, appetite was reduced, and anxiety and fear behaviors became manageable. The testosterone and gonadotropin-releasing hormone agonist treatment was easily administered, had no known side effects, and the owners were pleased with the outcome.

Background

Orchiectomy, ovariohysterectomy, or ovariectomy (referred herein as gonadectomy) are used to sterilize dogs and reduce diseases of the sexual organs, sex-specific behaviors, and pet overpopulation. However, research over the past 2 decades has indicated that the loss of gonadal hormones can result in sequalae such as increased incidence of obesity, urinary incontinence, endocrine disorders, orthopedic conditions, cancer, and behavior and cognitive changes.[1-3] The mechanism underlying these problems may be the lack of negative feedback by gonadal hormones on the pituitary and hypothalamus, resulting in supraphysiologic levels of luteinizing hormone (LH).[2] The impact of gonadectomy varies by breed, size, sex, and age at neuter[4,5] supporting an individualized approach to sterilization of dogs.[6] As a result of these concerns, a growing number of veterinarians are offering hormone-preserving sterilization procedures, including vasectomy and hysterectomy.[7]

For dogs that have already received gonadectomy and suffer from various health conditions that may be related to the loss of gonadal hormones, there is no standard of treatment for hormone replacement except for the treatment of urinary incontinence. Treatment with conjugated estrogens, diethylstilbesterol, estriol, gonadotropin-releasing hormone (GnRH) agonists (e.g., deslorelin), or GnRH immunization have been successful in restoring continence in formerly spay-induced incontinent dogs. These treatments restore urinary continence by reducing LH concentrations in spayed female dogs.[8-10] There has been only 1 study investigating the use of testosterone for the treatment of urinary incontinence in neutered male dogs.[11]

The purpose of this case study was to describe the health improvements in a neutered dog following restoration of reproductive hormone concentrations.

Case History and Presentation

A 1-year-old neutered male American bulldog/Walker coonhound mixed breed dog weighing 27.7 kg (Table 1) presented with a history of various health issues negatively impacting quality of life. He was obtained from an animal shelter at an estimated age of 7 months, at which time he was gonadectomized prior to adoption. The dog reportedly displayed normal behavior at the time of adoption. Within 5 months following adoption, his behavior slowly changed to high levels of fear around strangers (manifested as barking, shaking, tail between legs, wide eyed, and avoidance). In addition, the dog had gained weight rapidly and became lame in the right pelvic limb, localized to the coxofemoral joint (e.g., limping after exercise, inability to jump, reluctance to run or exercise vigorously).

Diagnostics and Response to Treatment

At thirteen months of age, radiographs of both hips revealed bilateral shallow acetabula and a misshapen right femoral head. A daily supplement of concentrated omega-6 and omega-3 fatty acids (1200 mg fish oil, NatureMade, CA, USA) and weight loss (e.g., reduced calorie diet) were recommended. The WSAVA body composition score was 8 (obese).[12] At twenty months of age, no improvements were noted and a chemistry and thyroid panel revealed all parameters were within the normal range (Texas A&M Veterinary Medical Diagnostic Laboratory) (Table 2).Given that some signs apparent in this dog (weight gain, lethargy, and behavior changes) were consistent with hypothyroidism,[13,14] levothyroxine sodium (0.5 mg BID; Soloxine, Virbac, TX, USA) was prescribed at 24 months.

*Corresponding author. Linda Brent.
E-mail address: LindaBrent@ParsemusFoundation.org (L. Brent).
Abbreviations: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone

http://dx.doi.org/10.1016/j.tcam.2021.100565

Table 1
Body Weight History

Age (mo)	Wt (kg)
13.0	27.7
20.9	31.8
25.8	32.4
26.5	29.9
46.6	29.6
48.3	28.4
49.2	28.5
50.1	29.0
51.8	28.8
54.8	29.0
55.0	29.0
57.3	28.8
66.2	32.5

Table 2
Serum Chemistry and Thyroid Results at 20 Months of Age

Test	Test Result	Reference Range
Total Serum Protein	6.4 g/dL	5.6-7.9
Albumin	4.0 g/dL	2-4.5
Calcium	10.7 mg/dL	7.2-12.8
Phosphorus	3.31 mg/dL	2.3-6.5
Glucose	109 mg/dL	60-120
BUN	17 mg/dL	8-30
Creatinine	0.75 mg/dL	0.5-1.4
Total Bilirubin	<0.1 mg/dL	0.1-0.4
ALP	21 U/L	12-122
CK	91 U/L	58-241
AST (SGOT)	21 U/L	13-52
ALT (SGOT)	30 U/L	13-79
Globulins	2.4 g/dL	1.8-4.2
A/G Ratio	1.7	0.8-2.2
GGT	<3 U/L	0-10
Amylase	510 U/L	454-1380
Cholesterol	195 mg/dL	124-335
Sodium	149 mEq/L	141-156
Potassium	4.5 mEq/L	3.8-5.5
Na/K Ratio	33.1	
Chloride	109 mEq/L	109-124
T4 (Chl.)	2.06 ug/dL	1.51-3.11
Free T4 (RIA)	28.93 pmol/L	12-33

All results within normal range.

At 25 months tramadol (50 mg BID) and carprofen (Rimadyl, Zoetis, NJ, USA) (50 mg BID for 5 days) were prescribed to reduce joint pain. The tramadol was discontinued after 14 days but the carprofen was continued on an as-needed basis for pain. Tramadol has been reported as less effective on osteoarthritis pain in dogs than carprofen.[15] Minor weight reduction occurred at 26 months (Table 1). The thyroxine was discontinued after 2 months because follow up testing revealed elevated T4 (3.91 ug/dL) and free T4 (53 pmol/L). A glucosamine-chondroitin joint supplement (Flexadin Plus Chews; Vetoquinol, TX, USA) was given for 2 months and then replaced with a fatty acid joint supplement (Antinol; Vetz Petz, CA, USA). Anxiety and fear of strangers was treated with an antidepressant (Fluoxetine SSRI; 10 mg TID) at 34 months of age with no noticeable change in behavior after 81 days and then discontinued.

At 46 months of age after unsatisfactory response to other treatments, gonadal hormone replacement therapy was initiated. Extra-label use of testosterone cypionate (USP 1000 mg/10 mL, Sun Pharmaceutical Industries, Mumbai, IN) was administered subcutaneously at a monthly dose of 1.7 mg/kg. Serum testosterone was measured on the same day of the week and same time of day with levels of 2.44, 1.06 and 0.32 ng/mL at 1-, 14-, and 28-days post-injection, respectively. Thereafter, weekly testosterone injections (0.5 mg/kg) maintained testosterone levels at 1.2-2.2 ng/mL (normal range for intact dog) (Fig 1). Serum concentrations of testosterone and LH were measured using a double antibody radioimmunoassay at Colorado State University Endocrinology Laboratory. For LH, 200 uL canine serum was incubated in the presence of anti-LH antibody at a dilution of 1:400,000, for 24 hours at 40°C. Following incubation, 125I-LH was added to each tube and further incubated for 24 hours at 40°C. After 24 hours, a secondary antibody was added to precipitate the immune complexes and the reaction was incubated for 72 hours at 40°C. Following incubation, cold pour-off buffer was added, the tubes were centrifuged to pellet the immune complexes and the pellet was counted in a gamma spectrophotometer. For testosterone, prior to analysis serum samples were organically extracted, and reconstituted in assay buffer, to eliminate any matrix effects in the assay. Briefly, 200 uL reconstituted serum was incubated in the presence of 125I-testosterone and anti-testosterone antibody, at a dilution of 1:200,000, for 6 hours at 40°C. After 6 hours, a secondary antibody was added to precipitate the immune complexes and the reaction was further incubated for 72 hours at 40°C. Following incubation, cold pour-off buffer was added, the tubes were centrifuged to pellet the immune complexes and the pellet was counted in a gamma spectrophotometer.

Luteinizing hormone concentrations remained persistently elevated despite testosterone supplementation (34.6-88.2 ng/mL; normal range: 0.8-11.2 ng/mL for intact dogs; 7.2-27 ng/mL for castrated dogs). At 50 months of age (114 days after initiation of testosterone therapy), a GnRH agonist implant (9.4 mg deslorelin, Suprelorin, Virbac, TX, USA) that is labelled for 12 months of contraceptive efficacy in intact male dogs was administered subcutaneously near the umbilicus for ease of monitoring and removal if necessary. LH concentrations dropped to 1.89 ng/mL (normal range for intact dog) following implant administration (Fig 1).

Within 45 days after initiation of hormone therapy, mounting another dog with pelvic thrusting was observed. This behavior subsided without intervention by 95 days after testosterone treatment initiation and was observed infrequently thereafter. In addition, by 95 days after testosterone treatment initiation, the patient's muscle mass had increased notably (especially in the hind legs and hips) and mobility improved markedly (such that he could run and jump like the other dog in the household). Body condition score improved (BCS = 7 heavy). While the dog continued to show fear responses and avoidance of strangers, his behavioral responses were reduced in intensity (Table 3).

Vital signs as measured by a collar monitor (Pet Pace Smart Collar, MA, USA) during the first 4 months of hormone therapy revealed little variability (pulse $\bar{x} = 62.02$ bpm, SD = 2.0; respiration $\bar{x} = 17.59$, SD = 0.5, calories $\bar{x} = 1134.95$ kcal, SD = 96.8; heart rate variability $\bar{x} = 11.54$, SD = 0.05).

Follow-up

Twelve months after administering the GnRH agonist implant, appetite and begging for food noticeably increased and within 2 months (at 66 months of age), weight had also increased. Although testosterone concentrations remained in the normal range for intact males, measurement of LH concentrations revealed elevated values (19 ng/mL) indicating that the implant was no longer releasing hormone and ready for replacement.

Discussion

There is growing evidence linking gonadectomy with lifelong health and behavioral problems in some dogs.[1-3] However, little information exists on the effects of reproductive hormone therapy. This case study reported the physical and behavioral improvements following restoration of reproductive hormone concentrations in a neutered male dog.

In the presented case, the dog began exhibiting behavioral, mobility, and body condition problems within a few months following

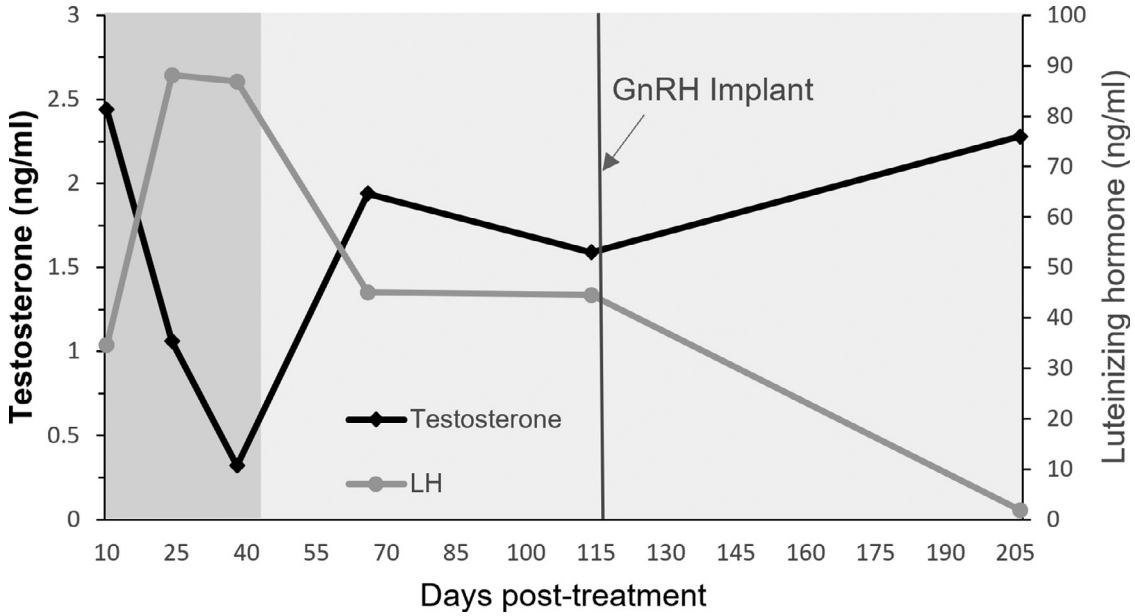

Fig 1. Serum testosterone and luteinizing hormone levels. Testosterone y-axis on left and LH y-axis on right. Dark grey shaded area indicates monthly testosterone injection timepoints and light grey shaded area indicates weekly testosterone injection timepoints. Vertical line indicates when GnRH agonist implant was applied. Note the inverse relationship between testosterone and LH, as well as the 95.8% drop in LH after implantation of the GnRH agonist.

gonadectomy. These conditions worsened over the next 3 years despite treatment with anti-inflammatories, anxiolytics, joint health supplements, and diet restrictions. Treatment aimed at restoring reproductive hormone concentrations resulted in significant improvements in behavior, mobility, and body condition.

Factors other than restoration of reproductive hormone concentrations (e.g., age, desensitization to external stimuli over time) may also have impacted health and behavior. However, the fact that the patient displayed increasingly debilitating orthopedic and behavioral signs for 3 years prior to hormone therapy, and then had notable and lasting improvements shortly afterward, supports a conclusion that restoration of reproductive hormone concentrations was effective in returning this dog to improved physical health. While anxious behaviors decreased, consultation with a veterinary behavior professional in conjunction with anxiolytic medication may result in greater improvement. The initial therapy with thyroid supplementation in this case may not have been indicated given test results. Further assessment of thyroid function – including TSH and thyroid autoantibodies – is warranted since gonadectomized dogs have a higher risk of hypothyroidism.[16]

In this case study, no adverse effects were noted although long-term, in-depth laboratory testing was not conducted. In the only published report of testosterone supplementation in male dogs, no adverse effects were reported but the authors cautioned that owners should watch for clinical signs.[11] Possible side effects of testosterone administration include hepatopathy, dermatopathies, epiphora, perianal adenomas/adenocarcinomas, increased liver enzymes, cervical skin thickening, increased preputial discharge, increased body odor, and increased male sexual behaviors. Androgens are contraindicated in any animal with a poorly functioning liver and in breeds that are predisposed to copper storage diseases such as Bedlington terriers and Labrador retrievers.[17] Liver function testing is advised before and during testosterone administration.

The most challenging aspect of restoring reproductive hormone concentrations was determining the appropriate type and dosage of testosterone because few studies have evaluated testosterone therapy for dogs. Intramuscular testosterone cypionate (1.5 mg/kg/month) was used to treat urinary incontinence in neutered male dogs.[11] Testosterone cypionate at 0.5-3 mg/kg/month for male dogs was also noted as a treatment in a patent application.[18] It is

Table 3
Details of Behavioral and Physical Issues Before and After Hormone Therapy

Issue	Details and Impact Before Hormone Therapy	Change Following Hormone Therapy
Fear of humans	Extreme fear responses to humans outside home; inability to take dog outside home except if necessary (veterinarian)	No change in fear response to veterinary visit; moderate improvement when unfamiliar people at a distance and ability to go for walk in park
	Fear response when familiar and unfamiliar people visit house	Moderate improvement and friendly behavior to routine visitors; no change to unfamiliar people
Hip pain	Unable to run very far or jump, avoidance of activity	Significant improvement in running; jumping on furniture and owners; increase in vigorous activity
	Unable to stretch back legs or sit on haunches	Able to stretch normally; no change in sitting posture
	Inability to go on walks; unable to play rough with other dog	Significant improvement in exercise stamina on long walks (30+ min); significant increase in initiation and participation in play with other dog
	Limping after exercise requiring pain medication (4-8 times/month)	Significant reduction in limping and need for pain medication (twice/year)
Body condition	Overweight, body condition score = 8 (obese)	Lost weight prior to hormone therapy with feeding restrictions. No change in weight during hormone therapy but improved body condition score = 7 (heavy); increase in weight when GnRH implant started to wear off
	Muscle atrophy in rear	Noticeable increase in muscle mass in hip and hind legs (e.g., gluteal and biceps femoris)

important to mention that benign prostatic hyperplasia was induced in beagles treated with testosterone-filled subcutaneous silastic implants delivering a higher dose of 0.25 mg/kg/day.[19] In the current case report, no signs of benign prostatic hyperplasia were observed so the size of the prostate was not measured. Routine monitoring for benign prostatic hyperplasia will be necessary for the subject because it is the most common disease in dogs with normal hormone levels.[20]

The current case report utilized a subcutaneous route of administration for the testosterone cypionate. This route was chosen because its use in humans supports favorable outcomes in terms of less pain, consistent and stable testosterone levels, and ease of home administration when compared to intramuscular injection.[21-22] It is important to note that testosterone is a schedule III non-narcotic due to the potential for human abuse and dependence. In the current case, the weekly dosing frequency of 0.5 mg/kg provided more consistent testosterone concentrations than the monthly testosterone dose. This finding agrees with research in humans reporting deviations above and below the normal range for serum testosterone when dosing is administered at frequencies longer than 1 week.[21]

This case report highlights the importance of considering LH concentrations in addition to testosterone when restoring reproductive hormone concentrations after gonadectomy in male dogs. Early research in castrated dogs reported that a single subcutaneous injection of testosterone had little impact on LH, but levels were reduced after several daily injections of the same dose.[23] In this case study, testosterone supplementation did not return extremely high LH concentrations to normal levels, but they were reduced to the normal range following administration of a GnRH agonist implant. Continuous release of the GnRH agonist from the implant desensitizes pituitary cells to GnRH, resulting in a cessation of LH secretion. The implant has a good safety and efficacy profile and can be reimplanted at 6- or 12-month schedules (depending on dose).[24] In the current case report, when the implant stopped secreting the GnRH agonist 12 months after insertion, LH concentration rose, and the dog's appetite and weight increased despite no change in activity. Within 1 week of administration of a new implant, appetite returned to normal. Stimulation of LH receptors in the gastrointestinal tract and in the hypothalamus reduce satiety and result in hyperphagia.[25-27] The change in appetite may be a noninvasive method to determine when a new implant is required.

It is possible that only reducing LH to below the level of detection ($<$1 ng/mL) with a GnRH agonist may be sufficient to address gonadectomy-related health issues, though this single case report cannot provide a clear answer. This dog showed improvement in muscle mass and mobility 95 days following restoration of testosterone levels and prior to administration of the GnRH agonist implant, with continued health improvements thereafter. Low circulating androgen levels in humans are known to have a negative impact on muscle proliferation and maintenance.[28] Additional research is needed to assess the impact of normalizing the levels of testosterone, LH, or both, and to identify health and behavior issues responsive to hormone restoration following gonadectomy.

Summary

Restoring reproductive hormone concentrations was associated with significant physical health and behavioral improvements in a neutered canine. The owners were pleased with the response to the hormone restoration therapy and have continued this treatment for their dog. Clinicians interested in reproductive hormone restoration should seek informed consent from the client after explaining the possible negative side effects of androgen treatment. Additional research on restoring reproductive hormone concentrations in gonadectomized dogs is needed to evaluate different treatment regimens and long-term effects.

Authors' contributions

Linda Brent: Conceptualization, Methodology, Formal Analysis, Investigation, Writing - Original Draft, Project Administration. Elaine Lissner: Conceptualization, Resources, Writing - Review & Editing. Michelle Kutzler: Conceptualization, Methodology, Resources, Writing - Review & Editing.

Acknowledgment

The authors thank Dr James B. Woodley (Waller Veterinary Clinic, Waller TX) for implementing the treatment plan. The study could not have been completed without the participation of Toby Rogers.

References

1. Houlihan KE. A literature review on the welfare implications of gonadectomy of dogs. *JAVMA* **250:**1155–1166, 2017
2. Kutzler MA. Possible relationship between long-term adverse health effects of gonad-removing surgical sterilization and luteinizing hormone in dogs. *Animals* **10:**599, 2020
3. Urfer SR, Kaeberlein M. Desexing dogs: a review of the current literature. *Animals* **9:**1086, 2019
4. Hart BL, Hart LA, Thigpen AP, Willits NH. Assisting decision-making on age of neutering for mixed breed dogs of five weight categories: associated joint disorders and cancers. *Front Vet Sci* **7:**472, 2020
5. Hart BL, Hart LA, Thigpen AP, Willits NH. Assisting decision-making on age of neutering for 35 breeds of dogs: associated joint disorders, cancers, and urinary incontinence. *Front Vet Sci* **7:**388, 2020
6. The Board of Directors of the Society for Theriogenology and the American College of Theriogenologists, Montgomery AL. Basis for position on mandatory spay-neuter in canine and feline. Available at: https://cdn.ymaws.com/www.theriogenology.org/resource/resmgr/Docs/spay-neuter_basis.pdf (Accessed January 19, 2021).
7. Kutzler MA. Gonad-sparing surgical sterilization in dogs. *Front Vet Sci* **7:**342, 2020
8. Mandigers RJ, Nell T. Treatment of bitches with acquired urinary incontinence with oestriol. *Vet Rec* **149:**764–767, 2001
9. Veronesi M, Rota A, Battocchio M, Faustini M, Mollo A. Spaying-related urinary incontinence and oestrogen therapy in the bitch. *Acta Vet Hung* **57:**171–182, 2009
10. Donovan CE, Weston A, Kutzler MA. Gonadotropin-releasing hormone immunization to treat urethral sphincter mechanism incompetence in a bitch that experienced an adverse reaction to phenylpropanolamine. *J Vet Sci Med Diagn* **2:**3, 2013
11. Palerme J-S, Mazepa A, Hutchins RG, Ziglioli V, Vaden SL. Clinical response and side effects associated with testosterone cypionate for urinary incontinence in male dogs. *Am Anim Hosp Assoc* **53:**285–290, 2017
12. World Small Animal Veterinary Association Body Condition Score. Available at: https://wsava.org/wp-content/uploads/2020/01/Body-Condition-Score-Dog.pdf (Accessed July 22, 2021).
13. Camps T, Amat M, Manteca X. A review of medical conditions and behavioral problems in dogs and cats. *Animals* **9:**1133, 2019
14. Panciera DL. Hypothyroidism in dogs: 66 cases (1987-1992). *J Am Vet Med Assoc* **204:**761–767, 1994
15. Budsberg SC, Torres BT, Kleine SA, Sandberg GS, Berjeski AK. Lack of effectiveness of tramadol hydrochloride for the treatment of pain and joint dysfunction in dogs with chronic osteoarthritis. *J Am Vet Med Assoc* **252:**427–432, 2018
16. Milne KL, Hayes Jr. HM. Epidemiologic features of canine hypothyroidism. *Cornell Vet* **71:**3–14, 1981
17. Johnston SD, Kustritz Root, Olson PS. *Canine and Feline Theriogenology*. 1st ed. Philadelphia: PA:Saunders; 2001
18. Bieber D. *Testosterone Replacement Therapy in Animals, Including Dogs* inventor. United States Patent application US; 2014 0371189 2014 Dec 18
19. Berry SJ, Coffey DS, Strandberg JD, Ewing LL. Effect of age, castration, and testosterone replacement on the development and restoration of canine benign prostatic hyperplasia. *Prostate* **9:**295–302, 1986
20. Cunto M, Mariani E, Anicito Guido E, Ballotta G, Zambelli D. Clinical approach to prostatic diseases in the dog. *Reprod Domest Anim* **54:**815–822, 2019
21. McFarland J, Craig W, Clarke NJ, Spratt DI. Serum testosterone concentrations remain stable between injections in patients receiving subcutaneous testosterone. *J Endocr Soc* **1:**1095–1103, 2017
22. Spratt DI, Stewart II, Savage C, et al. Subcutaneous injection of testosterone is an effective and preferred alternative to intramuscular injection: demonstration in female-to-male transgender patients. *J Clin Endocrinol Metab* **102:**2349–2355, 2017
23. Falvo RE, Vincent DL, Lathrop J, Toenjes A. Effect of testosterone and testosterone propionate administration on luteinizing hormone secretion in the male mongrel dog. *Biol Reprod* **21:**807–812, 1979
24. Driancourt MA, Briggs JR. Gonadotropin-releasing hormone (GnRH) agonist implants for male dog fertility suppression: a review of mode of action, efficacy, safety, and uses. *Front Vet Sci* **7:**483, 2020
25. Rozkowska E, Fonberg E. Salivary reactions after ventromedial hypothalamic lesions in dogs. *Acta Neurobiol Exp* **33:**553–562, 1973

26. Houpt K, Coren B, Hintz HF, Hilderbrant JE. Effect of sex and reproductive status on sucrose pareference, food intake, and body weight of dogs. *J Am Vet Med Assoc* **174:**1083–1085, 1979
27. Daniel J, Foradori C, Whitlock B, Sartin J, Whitlock BK. Hypothalamic integration of nutrient status and reproduction in the sheep. *Reprod Domest Anim* **48:**44–52, 2013
28. Martín AI, Priego T, López-Calderón A. Hormones and muscle atrophy editor. In: Xiao J, editor. *Muscle Atrophy*, *Advances in Experimental Medicine and Biology*, Singapore: Springer Nature; 2018. p. 207–233