Effect of a subcutaneous implant of deslorelin acetate on serum testosterone concentrations in male Hermann's (*Testudo hermanni* sp.) and Greek (*Testudo graeca* sp.) tortoises

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Abstract

Deslorelin acetate is a gonadotropin-releasing hormone agonist formulated in a controlled-release subcutaneous implant and designed for reversible suppression of testosterone production in dogs. It has also been demonstrated to be effective in other animal species, but no data on its effectiveness in male land tortoises are available. This study aimed to evaluate the effect of a 4.7-mg deslorelin acetate implant on serum testosterone concentrations in male Hermann's (*Testudo hermanni* sp.) and Greek (*Testudo graeca* sp.) tortoises. Twenty adult male tortoises housed under the same environmental conditions were enrolled for the study and randomly assigned to a treatment (D, n = 10) or a control (C, n = 10) group. Starting in May, males from the D group were implanted with a 4.7-mg deslorelin acetate device, whereas males from the C group (n=10) did not receive any treatment. Blood samples were collected once immediately before implant

application (S0-May) and at 15 days (S1-June), 2 (S2-July), and 5 (S3-October) months after application. Serum testosterone at each sampling time was measured through a solid-phase, enzyme-labeled, competitive chemiluminescent immunoassay. Median serum testosterone concentrations were not significantly different between the two groups in all sampling times, and no interaction between treatment and sampling time was observed. The present study, therefore, suggests that a single treatment with a 4.7-mg deslorelin acetate implant has no effect on testosterone circulation in male Hermann's and Greek tortoises during the following 5 months.

Keywords: deslorelin acetate; testosterone; male; *Testudo hermanni* sp; *Testudo graeca* sp.

Introduction

The influence of testosterone (T) on sexual behavior has been investigated in behavioral endocrinology in male and female mammals, birds and reptiles (Dorner 1976; Nelson 2005). Notably, it has been shown that an elevation of circulating T in reptiles stimulates mate-searching effort, territoriality, and aggressiveness, promoting various sexual behaviors and activities (Paries et al. 2014). Male chelonians are regularly presented with increased sexual behavior due to high T levels (Garstka et al. 1991), resulting in competition and aggression amongst each other and leading to high stress and traumatic mating conditions in females because of the frequent chasing, bites, and sexual acts (Paries et al. 2014).

Surgical removal of testes can solve aggression and mating problems in European tortoises if separation of the animals is impossible. Orchiectomy via shell osteotomy has been successfully applied, but it is time-consuming and associated with prolonged healing times and frequent complications (Brannian 1984; Bennet 2000; Hernandez-Divers 2006; Innis et al. 2013). Recently, laparoscopic orchiectomy on male tortoises has been implemented (Kinney et al. 2011; Paries et al. 2014), but testicular anatomy is quite variable among the 300 different species (Kuchling 1999; Innis and Boyer 2002). The role of marked annual variations of T levels observed in many chelonians remains obscure, especially regarding seasonal changes and sexual activity in general (Sereau et al. 2010). Many chelonians often have a temporal disconnection between gametogenesis, fecundation, sexual behaviors, and hormone levels (Kuchling 1999). A biphasic annual pattern of T circulation

has been previously reported in the Greek tortoise (Licht et al. 1985; Shelby et al. 2000; Sereau et al. 2010) and in the *Testudo hermanni* (Kuchling 1981; Huot-Daubremont et al. 2003), while a study on the subtropical *Chrysemys dorbigni* revealed a single seasonal androgen peak coincident with maximal testicular growth (Silva et al. 1984).

Deslorelin acetate is a gonadotropin-releasing hormone (GnRH) agonist formulated in a controlled-release subcutaneous implant for reversible suppression of T production. It is commercially available as 4.7- and 9.4-mg implants for male dogs and ferrets (Suprelorin®, Virbac, Carros, France), but its use has also been described in other species (Rowland 2011; Petritz et al. 2013; Schoemaker 2018; Harley et al. 2019). Deslorelin implant was demonstrated to be effective in dogs (Junaidi et al. 2003, 2009), cats (Novotny et al. 2012), and boars (Kopera et al. 2008, 2009), and only partially effective in bulls (Aspden et al. 1997, 1998; D'Occhio et al. 2000) and stallions (Gautier et al. 2018). Studies on the Japanese quail (Petritz et al. 2013) and on the Bearded dragon (Rowland 2011) indicated that deslorelin may be effective also in birds and reptiles, while research on chelonians could not demonstrate deslorelin implants' effects on female and male adult pond sliders (Potier et al. 2017; Bardi et al. 2021). To the best of the authors' knowledge, no data regarding the effectiveness of deslorelin in male Hermann's and Greek tortoises are available. Therefore, the purpose of the present study was to evaluate the effect of a 4.7-mg deslorelin acetate implant on T serum concentrations in intact adult Hermann's (*Testudo hermanni* sp.) and Greek (*Testudo graeca* sp.) male tortoises.

Materials and methods

Twenty healthy, intact male Hermann's (*Testudo hermanni* sp., n=12) and Greek (*Testudo graeca* sp., n=8) adult tortoises belonging to the Wildlife Recovery Centre (CRAS, Bernezzo, Italy) were enrolled. The study was performed during the active period amidst the hibernation periods, indicated as from April to October by Mazzotti et al (2002). Normal morphology of carapace, absence of current/previous pathologies, and negativity to parasitosis were adopted as selection criteria. Age ranged from 10 to 25 years. First, each subject was weighted, marked on the carapace, and randomly assigned to a treatment (D, n = 10) or control (C, n = 10) group. The random assignment to group D or C was performed separately for each species (Hermann's and Greek) so that in the end, both D and C groups consisted of 6 male Hermann's and 4 male Greek tortoises. The

78 males were randomly housed in two large outdoor enclosures, with free access to a sheltered and sunny area;
79 the areas were placed side by side and far from female tortoises.

80 Starting from May (before the beginning of the breeding season) and immediately before inserting the
81 device, a blood sample (S0-May) was taken from the jugular vein into 3,5 mL plain tubes (Vacuette® Greiner
82 Bio-One) from all 20 subjects for T analysis. A 4.7 mg deslorelin acetate implant (Suprelorin® Virbac, Carros,
83 France) was then introduced in the femoral muscle of the tortoises randomly assigned to the D group (n = 10).
84 Throughout the procedure, the animals were restrained in a calm environment without stress sources. Each
85 tortoise was weighed, and blood was collected at 15 days and two and five months after the device's application
86 (S1-June, S2-July, and S3-October, respectively). All the above-described procedure, except for device
87 application, was also performed in subjects of the C group (n = 10). Blood samples were immediately
88 centrifuged (1000g for 20 minutes), and the obtained sera were stored at -20°C until analysis.

89 Serum testosterone was measured at the MYLAV laboratory in Milan (Italy) through a solid-phase,
90 enzyme-labeled, competitive chemiluminescent immunoassay (IMMULITE®2000 Total Testosterone,
91 Siemens Medical Solutions – Diagnostics - USA). The functional sensitivity for the T assay on this system
92 was 0.15 ng/mL, and the intra- and inter-assay CV were 5.1% and 7.2%, respectively. Serum T concentrations
93 are expressed as ng/mL.

94 Statistical analysis was performed using the Analyze-it software (Analyze-it software ltd, Leeds, UK). A
95 T-test for independent samples was used to compare body weight between the two groups (C vs. D). The
96 median T values of the two groups were analyzed by a two-way ANOVA with interaction. The model was
97 testosteroneijk= treatmenti + timej +treatment x timeij + errork. A $p<0.05$ was considered statistically
98 significant.

99

100 **Results**

101 No differences were detected between the median body weight of C (0.487 kg) and the D group (0.441 kg).
102 Median concentrations of serum T ranged between a minimum of 5.3 ng/mL at S0 (May) to a maximum of 30
103 ng/mL at S2 (July), both registered in the C group. The D group's minimum and maximum median values were

9.3 ng/mL and 21.1 ng/mL, respectively. Neither time nor treatment or their interaction affected T levels in all sampling times. Median T levels in the two groups in the different sampling times are reported in Figure 1.

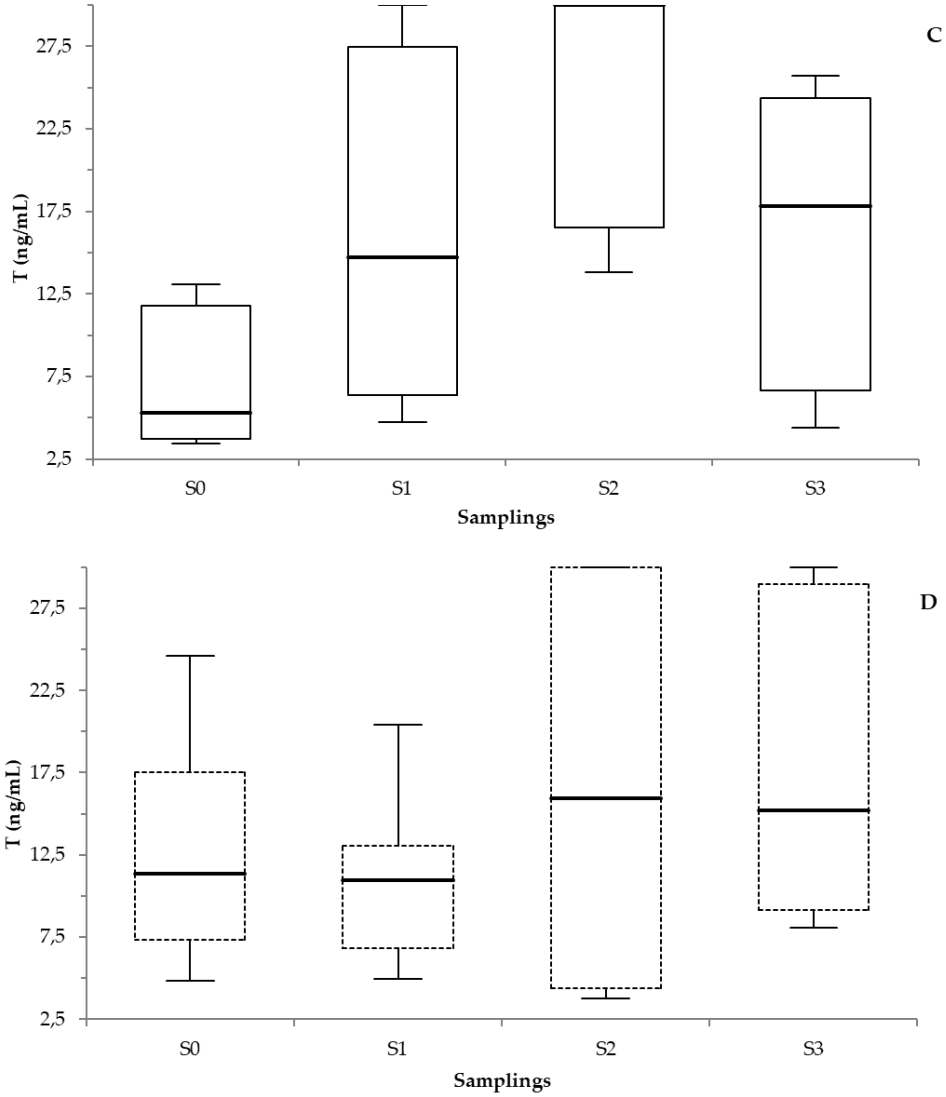

Fig. 1 Variation of serum testosterone concentrations in adult males *Testudo hermanni* and *Testudo graeca* in control (C) and treatment (D) groups. The boxes indicate the I-III interquartile range (IQR), the horizontal line the median value, and the whiskers extend to further observation within quartile I minus 1.5 x IQR or to further observation within quartile III plus 1.5 x IQR

Discussion

The pattern of T serum concentrations variations registered in the C group was similar to those previously reported in *Testudo greca* and *T. hermanni* (Kuchling 1981; Huot-Daubremont et al. 2003; Sereau et al. 2010;

Bonnet et al. 2016), with lower levels at the emergence from hibernation and increases during summer associated with the main mating period. Nevertheless, the increasing trend of serum T in the C group of the present study was not significant, maybe due to inter-individual variability and the small sample size. In the present study, median T concentrations in the C group at S0 and S1 (5.8 ng/mL and 14.7 ng/mL, respectively) appeared consistent with those reported by Huot-Daubremont et al. (2003) in April/June (10 ng/mL), while the peak registered in S2-July (30 ng/mL) was lower than the one reported by the same authors (50 ng/mL). Regarding the D group, the median T concentrations at S1 were superimposable to those at S0 (9.9 ng/mL and 9.3 ng/mL, respectively).

According to these results, an effect of a synthetic GnRH agonist on circulating T concentrations in males of two tortoise species could not be demonstrated. These findings showed no stimulatory effect on T concentrations soon after implantation, and no suppressive effect throughout the study, consistently with other researches on reptiles. In green iguanas, no effects on testosteronaemia were found in males treated with GnRH agonist (Kirchgessner et al. 2009; Grundmann et al. 2013,), although GnRH implants were successful to stop the ovarian activity in a group of captive female green iguanas (Knotek et al. 2009). In *Trachemis scripta,* subcutaneous deslorelin implant produced no differences in serum hormonal concentration or reproductive activity between case and control groups, both in male and female (Grundmann et al. 2018), and the same was found for female leopard geckos (Cermakova et al. 2019). Several individual green sea turtles showed a rise in immunoreactive LH following an injection of mammalian GnRH (Kuchling 1999), but without changes in sexual steroids. Another study (Licht et al. 1984) tested the ability of mammalian and chicken GnRH and their agonistic analogs in stimulating gonadotropin release in the cobra and musked turtle; neither of the GnRH preparations or their agonists produced significant changes in circulating hormones in either of the reptiles (Licht et al. 1984).

Some authors investigated the molecular features of GnRH receptors (GnRHR) in a leopard gecko (Ikemoto et al. 2004), revealing that it has a distinct genomic organization compared with all the other GnRHR genes. The low-expression level in the pituitary gland indicates the possibility that multiple types of GnRHR are expressed in this reptile and, possibly, also in other reptiles. One hypothesis is, therefore, that the unresponsiveness to a GnRH agonist in reptiles may be due to a species specificity of the reptilian GnRHR. Nevertheless, discrepancies in T secretion response to a controlled release deslorelin implant were also found

among mammalian species, like in the bovine (Aspden et al. 1998; D'Occhio et al. 2000); those authors speculated that the pulsatile release of LH in bulls is not required to stimulate the synthesis of steroidogenic enzymes that sustain elevated T secretion (D'Occhio et al. 2000). A similar mechanism may also occur in other species, thus explaining the lack of efficacy of deslorelin implant.

GnRH agonists have two distinguishing features compared with the natural GnRH: a higher affinity for GnRH receptors and a longer half-life in circulation (Karten and Rivier 1986). While the mammalian response to long-term GnRH agonists has been widely described, the release rate and pharmacokinetics of these implants in reptiles are unknown. Regarding the present study, it is possible that a flare-up effect on T levels may have preceded sampling S1, as Potier et al. (2017) showed a transient flare-up effect on T concentrations in yellow-bellied sliders around 12 days after implant. Also, a suppressive effect on T concentrations may have occurred after the last blood sample (S3) performed 5 months after device application. A deeper knowledge of pharmacokinetic response in these two tortoise species would allow better scheduling of blood sampling times.

The unresponsiveness to treatment may also be related to the dosage employed. Potier et al. (2017) registered only a transient stimulatory effect of a single 4.7-mg deslorelin implant on the anterior pituitary in yellow-bellied sliders without negative feedback on T production. These authors suggested further studies with the employment of a higher deslorelin dosage to support the obtained results. Still, recently Bardi et al. (2021) demonstrated that neither single nor double deslorelin implant successfully suppresses gonadal activity and prevents reproduction during a one-season follow-up in adult female pond sliders. The same authors stated that since a partial effect was noted in the double-implant group, failure to suppress gonadal activity in these animals is unlikely due to differences in the hormonal regulation of the reproductive cycle (Bardi et al. 2021). Gonadotropin response in reptiles is also highly temperature dependent (Pang and Schreibman 1991), and even prolonged treatment may have no measurable effect under certain conditions (Licht et al. 1985). Seasonal fluctuations in body temperature complicate the interpretation of seasonal hormonal patterns, especially in terrestrial tortoises, where temperature variations are more marked than in sea chelonians, as it largely depends on air temperature fluctuations.

In conclusion, the present study suggests that a 4.7-mg deslorelin acetate implant does not affect circulating T concentrations in male Hermann's and Greek tortoises during the following 5 months. Interpretation of these

data is hindered by scarce information on the physiological pattern of T secretion in this species, pharmacokinetic response, and the degree of species specificity of the reptilian GnRH receptors, which may have limited treatment effectiveness.

References

Aspden WJ, Rao A, Rose K, Scott PT, Clarke IJ, Trigg TE, Walsh J, D'Occhio MJ (1997) Differential responses in anterior pituitary luteinizing hormone (LH) content and LHβ- and α-subunit mRNA, and plasma concentrations of LH and testosterone, in bulls treated with the LH-releasing hormone agonist deslorelin. Domest Anim Endocrinol 14:429-437. https://doi.org/10.1016/s0739-7240(97)00048-9

Aspden WJ, Rodgers RJ, Stocco DM, Scott PT, Wreford N, Trigg TE, Walsh J, D'Occhio MJ (1998) Changes in testicular steroidogenic acute regulatory (STAR) protein, steroidogenic enzymes and testicular morphology associated with increased testosterone secretion in bulls receiving the luteinizing hormone releasing hormone agonist deslorelin. Domest Anim Endocrinol 15:227-238. https://doi.org/10.1016/s0739-7240(98)00013-7

Bardi E, Manfredi M, Capitelli R, Lubian E, Vetere A, Montani A, Bertoni T, Talon E, Ratti G, Romussi S (2021) Determination of Efficacy of Single and Double 4.7 mg Deslorelin Acetate Implant on the Reproductive Activity of Female Pond Sliders (Trachemys scripta). Animals 11:660. https://doi.org/10.3390/ani11030660

Bennet RA (2000) Nonreproductive surgery in reptiles. Vet Clin North Am Exot Anim Pract 3:715–732. https://doi.org/10.1016/s1094-9194(17)30071-3

Bonnet X, Golubović A, Arsovski D, Đorđević S, Ballouard JM, Sterijovski B, Ajtić R, Barbraud C, Tomović L (2016) A prison effect in a wild population: a scarcity of females induces homosexual behaviors in males, Behav Ecol 27:1206–1215. https://doi.org/10.1093/beheco/arw023

Brannian R (1984) A soft tissue laparotomy technique in turtles. J Am Vet Med Assoc 185:1416. PMID: 6511609.

Čermakova E, Oliveri M, Knotkova Z, Knotek Z (2019) Effect of GnRH agonist (deslorelin) on ovarian activity in leopard gecko (Eublepharis macularius). Veterinary Medicine (Prague) 64:228-230 https://doi.org/10.17221/167/2018-VETMED

D'Occhio MJ, Fordyce G, Whyte TR, Aspden WJ, Trigg TE (2000) Reproductive responses of cattle to GnRH agonists. Anim Reprod Sci 60:433-442. https://doi.org/10.1016/S0378-4320(00)00078-6

Dorner G (1976) Hormones and brain differentiation. Elsevier Scientific Publishing Company, Amsterdam.

Garstka W, Cooper Jr WE, Wasmund KW, Lovich JE (1991) Male sex steroids and hormonal control of male courtship behavior in the yellow-bellied slider turtle, Trachemys scripta. Comp Biochem Physiol Part A: Physiol 98:271–280. https://doi.org/10.1016/0300-9629(91)90532-H

Gautier C, Schmidt K, Aurich J, Aurich C (2018) Effects of implants containing the GnRH agonist deslorelin on testosterone re-lease and semen characteristics in Shetland stallions. Anim Reprod Sci 195:230-241. https://doi.org/10.1016/j.anireprosci.2018.05.027

Grundmann M, Möstl E, Knotkova Z, Knotek Z (2013) The use of synthetical GnRH agonist implants (deslorelin) for the suppression of reptile endocrine reproductive activity. Proc 1st Int Congr Avian, Rept Exot Mamm, Wiesbaden (Germany), April 20th-26th, p. 248.

Grundmann M, Möstl E, Knotkova Z, Knotek Z (2013) GnRH implants in captive male green iguanas. Proc Abst 31st World Veterinary Congress, Prague, 17-20 September 2013, p.15

Harley JJ, Power A, Stack JD (2019) Investigation of the efficacy of the GnRH agonist deslorelin in mitigating intraspecific aggression in captive male Amur leopards (Panthera pardus orientalis). Zoo Biol 38:214-219. https://doi.org/10.1002/zoo.21475

Hernandez-Divers SJ (2006) Reptile diagnostic coelioscopy: a means to definite diagnosis. North Am Vet Conf - Exotics Reptiles and Amphibians 20:1622.

Huot-Daubremont C, Bradshaw D, Bradshaw F, Kuchling G, Grenot C (2003) Variation of plasma sex steroid concentrations in wild and captive populations of Hermann's tortoise (Testudo hermanni hermanni) in Southern France. Gen Comp Endocrinol 130:299-307. https://doi.org/10.1016/S0016-6480(02)00623-8

Ikemoto T, Enomoto M, Park MK (2004) Identification and characterization of a reptilian GnRH receptor from the leopard gecko. Mol Cell Endocrinol 214:137-147. https://doi.org/10.1016/s0378-1119(03)00758-3

Innis CJ, Boyer TH (2002) Chelonian reproductive disorders. Vet Clin North Am: Exot Anim Pract 5:555–578. https://doi.org/10.1016/s1094-9194(02)00013-0

Innis CJ, Feinsod R, Hanlon J, Stahl S, Oguni J, Boone S, Schnellbacher R, Cavin J, Divers SJ (2013) Coelioscopic orchiectomy can be effectively and safely accomplished in chelonians. Vet Rec 172:526. https://doi.org/10.1136/vr.101475

Junaidi A, Williamson PE, Cummins JM, Martin GB, Blackberry MA, Trigg TE (2003) Use of a new drug delivery formulation of the gonadotrophin-releasing hormone analogue deslorelin for reversible long-term contraception in male dogs. Reprod Fertil Dev 15:317-322. https://doi.org/10.1071/RD03039

Junaidi A, Williamson P, Martin G, Blackberry M, Cummins J, Trigg T (2009) Dose-response studies for pituitary and testicular function in male dogs treated with the GnRH superagonist, deslorelin. Reprod Domest Anim 44:725-734. https://doi.org/10.1111/j.1439-0531.2008.01060.x

Karten MJ, Rivier JE (1986) Gonadotropin-releasing hormone analog design. Structure-function studies towards the development of agonists and antagonists: rationale and perspectives. Endocr Rev 7:44-66. https://doi.org/10.1210/edrv-7-1-44

Kinney ME, Johnson SM, Sladky KK (2011) Behavioral evaluation of red-eared slider turtles (Trachemys scripta elegans) ad-ministered either morphine or butorphanol following unilateral gonadectomy. J Herpetol Med Surg 21:54–62. https://doi.org/10.5818/1529-9651-21.2.54

Kirchgessner M, Mitchell M, Domenzain L, Walden M, Dickens MJ, Romero LM (2009) Evaluating the Effect of Leuprolide Acetate on Testosterone Levels in Captive Male Green Iguanas (Iguana iguana). J Herpetol Med Surg 19(4):128-131. https://doi.org/10.5818/1529-9651-19.4.128

Knotek Z, Knotkova Z, Kley N, Mostl E, Kneidinger N (2009) Is Ovariectomy in Female Lizards Necessary? Clinical Study with GnRH Analogue Implants in Green Iguanas. Proc Abstr BVZS Autumn Meeting, York 7–8 november 2009, p.76

Kopera I, Tuz R, Kotula-Balak M, Schwarz T, Koczanowski J, Bilinska B (2008) Morphofunctional alterations in testicular cells of deslorelin-treated boars: an immunohistochemical study. J Exp Zool Part A 309:117-126. https://doi.org/10.1002/jez.437

Kopera I, Tuz R, Hejmej A, Schwarz T, Koczanowski J, Bilińska B (2009) Immunolocalization of androgen receptor in the boar epididymis: the effect of GnRH agonist deslorelin. Reprod Domest Anim 44:266-272. https://doi.org/10.1111/j.1439-0531.2007.01054.x

Kuchling G (1981) Le cycle sexuel male de la tortue Testudo hermanni hermanni Gmelin dans une population naturelle et en cap-tivite. Bull Soc Herpetol Fr 19:29–35.

Kuchling G (1999) The Reproductive Biology of the Chelonia. Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-642-80414-4

Licht P, McCreery BR, Barnes R, Pang R (1983) Seasonal and stress related changes in plasma gonadotropins, sex steroids and corticosterone in the bullfrog, Rana catesbeinana. Gen Comp Endocrinol 50:124-145. https://doi.org/10.1016/0016-6480(83)90249-6

Licht P, Millar R, King JA, McCreery BR, Mendonca MT, Bona-Gallo A, Lofts B (1984) Effects of chicken and mammalian gonadotropin-releasing hormones (GnRH) on in vivo pituitary gonadotropin release in amphibians and reptiles. Gen Comp Endocrinol 54:89-96. https://doi.org/10.1016/0016-6480(84)90203-x

Licht P, Breitenbach GL, Congdon JD (1985) Seasonal cycles in testicular activity, gonadotropin and thyroxine in the painted turtles, Chrysemis picta, under natural conditions. Gen Comp Endocrinol 59:130–139. https://doi.org/10.1016/0016-6480(85)90427-7

Mazzotti S, Pisapia A, Fasola M (2002) Activity and home range of Testudo hermanni in Northern Italy. Amphibia-Reptilia 23, 305-312. https://doi.org/10.1163/15685380260449180

Nelson RJ (2005) An Introduction to Behavioural Endocrinology. 3rd ed, Sinauer Associates, Inc. Massachusetts.

Novotny R, Cizek P, Vitasek R, Bartoskova A, Prinosilova P, Janosovska M (2012) Reversible suppression of sexual activity in tomcats with deslorelin implant. Theriogenology 78:848-857. https://doi.org/10.1016/j.theriogenology.2012.03.035

Pang PKT, Schreibman MP (1991) Vertebrate Endocrinology: Fundamentals and Biomedical Implication, Volume 4, Part B: Reproduction. Academic Press INC. San Diego, California.

Paries S, Funcke S, Ziegler F, Lierz M, Wüst E (2014) Endoscopic assisted orchiectomy in Herman's tortoises (Testudo hermanni sp.) Tierarztl Prax Ausg K 42:383-389. https://doi.org/10.15654/TPK-131089

Petritz OA, Sanchez-Migallon Guzman D, Paul-Murphy J, Fecteau K, Mete A, Kass PH, Hawkins MG (2013) Evaluation of the efficacy and safety of single administration of 4.7-mg deslorelin acetate implants of egg production and plasma sex hormones in Japanese quail (Coturnix coturnix japonica). Am J Vet Res 74:316-323. https://doi.org/10.2460/ajvr.74.2.316

Potier R, Monge E, Loucachevsky T, Hermes R, Göritz F, Rochel D, Risi E (2017) Effects of deslorelin acetate on plasma testosterone concentrations in captive yellow-bellied sliders (Trachemys scripta sp.). Acta Vet Hung 65(3):440-445. https://doi.org/10.1556/004.2017.041

Rowland MN (2011) Use of a deslorelin implant to control aggression in a male bearded dragon (Pogona vitticeps). Vet Rec 169:127. https://doi.org/10.1136/vr.d2007

Sereau BM, Lagarde F, Bonnet X, ElMouden EH, Slimani T, Dubroca L, Trouvé C, Dano S, Lacroix A (2010) Does testosterone influence activity budget in the male Greek tortoise (Testudo graeca graeca)? Gen Comp Endocrinol 167:181–189. https://doi.org/10.1016/j.ygcen.2010.03.002

Shelby JA, Mendonça MT, Horne BD, Seigel RA (2000) Seasonal variation in reproductive steroids of male and female yellow-blotched map turtles, Graptemys flavimaculata. Gen Comp Endocrinol 119:43–51. https://doi.org/10.1006/gcen.2000.7490

Schoemaker NJ (2018) Gonadotrophin-Releasing Hormone Agonists and Other Contraceptive Medications in Exotic Companion Animals. Vet Clin North Am Exot Anim Pract 21:443-464. https://doi.org/10.1016/j.cvex.2018.01.011

Silva AMR, Moraes GS, Wasserman GF (1984) Seasonal variations of testicular morphology and plasma levels of testosterone in the turtle Chrysemys dorbigni. Comp Biochem Physiol 78:153-157. https://doi.org/10.1016/0300-9629(84)90108-7

Declarations

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Author Contributions

All authors contributed to the study's conception and design. Material preparation, animal handling, and data collection were performed by M.C. Pisu, A. Andolfatto, A. Ferro, and S. Esposito. M. Probo and MC Veronesi performed data analysis and statistical analysis. M. Probo wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

All the international, national, and/or institutional guidelines for the care of the animals involved were applied. The study was approved by the Ethical Committee of the University of Milan (OPBA_120_2021). A treatment consensus was signed by both the owner and the veterinarian of the CRAS.

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