Received: 28 July 2022 | Accepted: 19 September 2022

DOI: 10.1111/rda.14266

Reproduction in Domestic Animals WILEY

ORIGINAL ARTICLE

The function of the pituitary-testicular axis in dogs prior to and following surgical or chemical castration with the GnRH-agonist deslorelin

Annemarie Spruijt[1] | Hans Kooistra[1] | Christine Oei[2] | Claudia Vinke[3] | Auke Schaefers-Okkens[1] | Jeffrey De Gier[1]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[2]Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[3]Department of Population Health Sciences, Division Animals in Science & Society, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Correspondence
Annemarie Spruijt, Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, PO Box 80154, NL-3508 TD, Utrecht, The Netherlands.
Email: a.spruijt@uu.nl

Funding information
This study was partially funded by Virbac SA, France.

Abstract

Chemical castration, that is the reduction of circulating testosterone concentrations to castrate levels by administration of a GnRH-agonist implant, is a popular alternative to surgical castration in male dogs. Detailed information concerning the pituitary-testicular axis following administration of a GnRH-agonist implant is still scarce. Therefore, GnRH-stimulation tests were performed in male dogs, prior to and after surgical and chemical castration. This approach also allowed us to determine plasma concentrations of testosterone and oestradiol in intact male dogs for future reference and to directly compare the effects of surgical and chemical castration on the pituitary-testicular axis. In intact male dogs ($n = 42$) of different breeds GnRH administration induced increased plasma LH, FSH, oestradiol and testosterone concentrations. After surgical castration basal and GnRH-induced plasma FSH and LH concentrations increased pronouncedly. Additionally, basal and GnRH-induced plasma oestradiol and testosterone concentrations decreased after surgical castration. After chemical castration, with a slow-release implant containing the GnRH-agonist deslorelin, plasma LH and FSH concentrations were lower than prior to castration and lower compared with the same interval after surgical castration. Consequently, plasma oestradiol and testosterone concentrations were lowered to values similar to those after surgical castration. GnRH administration to the chemically castrated male dogs induced a significant increase in the plasma concentrations of LH, but not of FSH. In conclusion, after administration of the deslorelin implant, the plasma concentrations of oestradiol and testosterone did not differ significantly from the surgically castrated animals. After GnRH-stimulation, none of the dogs went to pre-treatment testosterone levels. However, at the moment of assessment at 4,4 months (mean 133 days ± SEM 4 days), the pituitary gonadotrophs were responsive to GnRH in implanted dogs. The increase of LH, but not of FSH, following GnRH administration indicates a differential regulation of the release of these gonadotrophins, which needs to be considered when GnRH-stimulation tests are performed in implanted dogs.

KEYWORDS
buserelin, FSH, GnRH-stimulation, GNRH-implant, gonadotrophins, LH

1 | INTRODUCTION

Castration of male dogs is a routine procedure in veterinary medicine for a variety of indications. Examples of indications for castration are behavioural problems ascribed to the influence of androgens such as intermale competition, urine marking, roaming and mounting and persistent purulent discharge from the prepuce (Giammanco et al., 2005; Maarschalkerweerd et al., 1997). Besides that, benign prostate hyperplasia and perianal adenoma with their sequelae (Johnston et al., 2001; Niżański et al., 2020) are indications to castrate a male dog.

Surgical castration in male dogs has traditionally been the method of choice to induce infertility and to cease the influence of the gonadally secreted sex hormones, such as testosterone. However, surgical castration is a major invasive procedure and irreversible. Furthermore, behavioural problems, attributed to the influence of androgens, are not always solved by surgical castration (Maarschalkerweerd et al., 1997; Neilson et al., 1997; Roulaux et al., 2020). Therefore, a reversible therapy is considered desirable for these indications. Additionally, androgen-dependent medical problems often arise in middle-aged and older dogs (Davidson, 2020; Lopate, 2010) when the anaesthetic risk can be substantial, rendering surgical castration undesirable. Therefore, reversible alternatives such as chemical castration are currently widely used. Progestogens, such as delmadinone acetate and osaterone acetate, can be used to reversibly suppress the effects of androgens with minor or no inhibition of the pituitary-testicular axis and with minor or no effects on spermatogenesis (Lange et al., 2001; Tsutsui et al., 2001). However, treatment with progestogens may result in side effects such as diabetes mellitus and growth hormone excess (Court et al., 1998; Kooistra et al., 1998). In addition, galactorrhoea may be observed due to increased prolactin secretion after termination of the progestogen treatment (Braun et al., 1984). These potential side effects and the lack of scientific data concerning the effects on behaviour of progestogens negatively influence their potential use as a treatment approach in dogs where castration may be the desired end solution.

An alternative for chemical castration is the use of GnRH-agonists (Goericke-Pesch, 2017; Goericke-Pesch et al., 2012; Junaidi et al., 2007; Romagnoli et al., 2012; Trigg et al., 2006). For this purpose, slow-release formulations have been developed from which a GnRH-agonist, such as deslorelin or azagly-nafarelin, is continuously released into the systemic circulation (Goericke-Pesch et al., 2010; Junaidi et al., 2003; Trigg et al., 2001; Vickery et al., 1984). The resulting continuously high circulating GnRH-agonist concentration, as opposed to the physiological pulsatile secretion pattern of hypothalamic GnRH, results in downregulation of the GnRH receptors and desensitization of the pituitary gonadotrophs (Junaidi et al., 2007; Vickery et al., 1984). Consequently, plasma concentrations of LH, FSH, oestradiol and testosterone decrease, followed by the arrest of spermatogenesis (Goericke-Pesch et al., 2009; Vickery et al., 1984). The effects of these slow-release formulations of GnRH-agonists have been shown to be fully reversible (Goericke-Pesch, 2017; Goericke-Pesch et al., 2009; Junaidi et al., 2003; Junaidi, Williamson, Trigg, et al., 2009; Tremblay & Bélanger, 1984; Trigg et al., 2001).

Despite studies on their clinical effects, information on the effects of surgical or chemical castration on the function of the hypothalamic–pituitary-testicular axis in male dogs is lacking. In most studies on this topic, basal plasma hormone concentrations have been measured (DePalatis et al., 1978; Goericke-Pesch et al., 2010; Günzel-Apel et al., 1990; Inaba et al., 1996; Junaidi et al., 2003; Junaidi, Williamson, Martin, et al., 2009; Lacoste et al., 1988). However, hormone measurements in a single blood sample can be unreliable because of the pulsatile secretion pattern of the gonadotrophins. Stimulation tests, using GnRH, are more appropriate to investigate the secretory potential of the pituitary and pituitary responsiveness in intact and castrated dogs (de Gier et al., 2012; Romagnoli et al., 2017).

In some studies, GnRH- or hCG-stimulation tests have indeed been performed in intact dogs, mostly focussing on the circulating concentrations of LH and testosterone (Günzel-Apel et al., 1994; Knol et al., 1993). In some studies, also FSH was measured (Purswell & Wilcke, 1993; Vickery et al., 1984). Junaidi et al. (2007) studied GnRH-stimulation in GnRH (deslorelin) implanted dogs. Native GnRH was injected on days 15, 25, 40 and 100 after implantation, whereas bovine LH was injected on days 16, 26, 41 and 101. On all occasions, after days 25, 26 post-implantation, exogenous GnRH and LH elicited higher plasma concentrations of LH and testosterone in the four control dogs than in the four deslorelin-treated animals ($p < .05$). It was concluded that, in male dogs, implantation of a GnRH superagonist desensitized the pituitary gonadotrophs to GnRH and also led to a desensitization of the Leydig cells to LH (Junaidi et al., 2007). Vickery et al. (1984) injected male dogs once daily with D-Nal(2)6-LHRH, a potent LHRH agonist, for periods up to 42 days. Blood samples collected at regular intervals were assayed for LH, FSH, and testosterone. The first injection of D-Nal(2)6-LHRH caused an acute elevation in plasma levels of LH, FSH, and testosterone, measured at 2 and 4 h after the injection. This acute response to injection was attenuated with each successive injection, and by 2 weeks, no elevation was seen, suggesting a downregulation of pituitary responsiveness. Levels of LH and testosterone were maximally depressed by 4 days of treatment. These studies were an important starting point for understanding the pituitary-testicular axis but unfortunately mostly based on limited numbers of animals in a restricted number of breeds. To gain more insight into the function of the pituitary-testicular axis and the effects of castration on this axis, it is essential to compare gonadotrophic hormone, testosterone

and oestradiol concentrations pre- and post-GnRH-stimulation in a larger number of male dogs. Therefore, in an earlier study (de Gier et al., 2012), the results of GnRH-stimulation tests in larger numbers of male and female dogs of several breeds, both pre- and post-gonadectomy were evaluated. GnRH-stimulation tests were performed in 14 female and 14 male client-owned dogs of several breeds, pre- and post-gonadectomy. Basal plasma LH and FSH concentrations were increased significantly after gonadectomy in both bitches and male dogs. GnRH administration pre-gonadectomy resulted in an increase in plasma LH and FSH concentrations. GnRH administration post-gonadectomy produced an increase in plasma LH and FSH concentrations in castrated male dogs. GnRH administration pre-gonadectomy resulted in a significant increase in plasma testosterone concentration. Basal plasma oestradiol concentrations were significantly higher in intact males than in castrated males and their ranges did not overlap. In this study, the aim was to further explore the function of the pituitary-testicular axis in male dogs by studying the effects of chemical castration with a GnRH-agonist implant. This approach also allowed us to determine plasma concentrations of testosterone and oestradiol in intact male dogs for future reference.

2 | ANIMALS, MATERIALS AND METHODS

2.1 | Animals

Forty-two client-owned dogs were used in this study. The following inclusion criteria were used: all dogs had to be healthy and to possess two scrotal testicles. In addition, they had to be at least 18 months old, in order to assure a mature hypothalamus-pituitary-testis axis at the start of the study (Günzel-Apel et al., 1990; Mialot et al., 1988). The dogs were assigned to one of two treatment groups, surgical castration (SC) or chemical castration (CC), based on the owners' preference.

Eighteen dogs (six mix breed dogs, one Beagle, one Chesapeake Bay Retriever, two Golden Retrievers, one Maltese, one standard Poodle, one Rottweiler, two miniature Schnauzers, one Shih Tzu, one St. Bernard dog and one Weimaraner) were surgically castrated. Their mean (± SD) age and body weight at the onset of the study were 33 (±12.9) months and 22.4 (±18.7) kg, respectively.

Twenty-four dogs were chemically castrated with implants containing 4.7 mg deslorelin, all belonging to 1 batch (Suprelorin®, Virbac Nederland BV, Barneveld, The Netherlands), injected subcutaneously between the shoulder blades. This group consisted of one mix breed dog, one American Cocker Spaniel, one American Staffordshire Terrier, one Belgian Shepherd Dog, one Bichon Frisé, one Bouvier des Flandres, one Canadian White Shepherd, one miniature Dachshund, one Short-haired Dutch Shepherd Dog, one English Bulldog, three Flatcoated Retrievers, one German Wire-haired Pointing Dog, one Jack Russel terrier, three Labrador Retrievers, one miniature Pincher, one Rottweiler, two Tibetan Terriers, one Västgötaspets and one Welsh Springer Spaniel. Their mean age and body weight at the onset of the study were 41 (±22.6) months and 26.2 (±14.2) kg, respectively.

Testicular length (a) and width (b), including the scrotal skin, were measured with callipers. As a measure of testicular size, the surface of the oval that is defined by the length and width of the testicles was calculated with the following equation: area (cm^2) = $1/2a^* 1/2b^* \pi$. (Goericke-Pesch et al., 2010)

2.2 | Ethics of experimentation

This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Utrecht University. All owners of the dogs included in this study read and signed an informed consent form in which all procedures concerning this study were explained.

2.3 | GnRH-stimulation test, blood sampling and hormone assays

The first GnRH-stimulation tests were performed in the week before castration in all dogs. The second GnRH-stimulation tests were performed 162±8 (mean±SEM) days (5.4 months) after surgical castration and at 133±4 days (4.4 months) after chemical castration. All GnRH-stimulation tests were performed after an overnight fast. Blood samples were collected at −40, 0, 10, 60, and 90 min, at which 8, 8, 4, 8, and 4 ml blood, respectively, were collected. At T = 0, immediately after collecting the second basal blood sample, buserelin (Receptal®, Intervet/Schering-Plough Animal Health, Boxmeer, the Netherlands), a GnRH analogue, was administered intravenously via the cephalic vein in a dose of 0.4 μg/kg B.W. Blood was collected from the jugular vein in chilled heparin-coated tubes and centrifuged at 1500×*g* for 10 min at 4°C. All plasma samples were stored at −25°C until analysis. The plasma samples were used for determination of the hormone concentrations of LH and FSH (at −40, 0, 10, and 60 min), testosterone (at 0, 60, and 90 min) and oestradiol (−40, 0, 60, and 90 min). The samples, per hormone, were analysed in batches. The intervals of sample storage until analysis were 151–552 days for LH, 202–396 days for FSH, 69–533 days for testosterone and 443–858 days for oestradiol.

Plasma LH concentration was measured by a heterologous radioimmunoassay (RIA) as described previously (Nett et al., 1975), with the following modifications. A rabbit antiserum raised against ovine LH (CSU-204, kindly supplied by G.D. Niswender, Colorado State University, CO, USA), radio iodinated bLH-7981 as prepared for the bovine LH assay (Dieleman & Bevers, 1987), and canine pituitary standard LER 1685–1 (a gift from Dr. L.E. Reichert, Albany Medical College, NY, USA) were used in this assay. The intra-assay and inter-assay coefficients of variation (CVs) for values above 0.5 μg/L were 2.3% and 10.5%, respectively. The lower limit of quantitation was 0.3 μg/L.

Plasma FSH concentration was measured by immunoradiometric assay (IRMA) (AHROO4, Biocode SA, Liège, Belgium), according

to the manufacturer's instructions and as described previously by (Beijerink et al., 2007). The intra-assay and interassay CVs were 3.0% and 6.0%, respectively. The lower limit of quantitation was 0.5 μg/L.

Plasma oestradiol-17β concentration was measured by RIA (Coat-A-Count TKE; Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's instructions with modifications as described previously (Dieleman & Bevers, 1987) and validated for the dog (van Haaften et al., 1994). The intra-assay and interassay CVs were 14% and 11.8%, respectively. The lower limit of quantitation was 7 pmol/L.

Plasma testosterone concentration was measured by RIA (Coat-A-Count® Total Testosterone, Diagnostic Product Corporation, Los Angeles, CA) according to the manufacturer's protocol with previously described modifications to increase the sensitivity (Buijtels et al., 2006). The intra-assay and interassay CVs were 5% and 6%, respectively. The lower limit of quantitation was 51 pmol/L.

2.4 | Analysis and presentation of data

Statistical analysis was performed using SPSS® for Windows (SPSS Inc.). Before statistical evaluation, left and right testicular sizes were added, and the resulting cumulative testicular size was used for statistical analysis. Data concerning testicular size, body weight and time interval between castration and the second GnRH-stimulation test were normally distributed. Results are presented as mean ± SD or mean ± SEM and differences were evaluated using the Student's *t*-test.

If plasma concentrations of LH, FSH, oestradiol or testosterone were below the limit of quantitation, the respective lower limit value was assigned to that sample. Basal LH, FSH, and oestradiol concentrations were calculated as the mean of the values in the samples collected at −40 min and immediately before GnRH administration.

Non-parametric statistical tests were used for comparison of LH, FSH, oestradiol and testosterone values because the plasma concentrations were not normally distributed. Differences between treatment groups for one hormone at a given time were compared by the Wilcoxon rank sum test. Within-group differences before and after gonadectomy were compared by the Wilcoxon signed rank test. Within-group changes in hormone concentrations during the GnRH-stimulation test were analysed by an ANOVA for repeated measures with a simple contrast, the basal plasma concentration as reference category, and Bonferroni corrected for repeated measurements. $p < .05$ was considered significant. Results are presented as median and range.

Cut-off values with corresponding sensitivity, specificity and 95% confidence intervals (95% CI) were calculated by receiver operating characteristic (ROC) analysis with after treatment as the positive state. Only cut-off values with 100% sensitivity and 100% specificity are given.

Plots for the graphic presentation of data were created with Sigmaplot (Systat Software Inc.). In the box-and-whisker plots, the box represents the interquartile range from the 25th to the 75th percentile. The horizontal bar through the box indicates the median and the whiskers represent the main body of data between the 5th and 95th percentiles.

TABLE 1 Plasma concentrations of LH, FSH, oestradiol, and testosterone, pre- and post-GnRH administration in 42 healthy and gonadally intact male dogs of different breeds

	Median	Range
Basal LH (μg/L)	2.7	1.0–12.2
LH 10 min after GnRH (μg/L)	16.3	3.2–116.4
LH 60 min after GnRH (μg/L)	16.3	5.6–103.9
Basal FSH (μg/L)	2.4	0.4–14.0
FSH 10 min after GnRH (μg/L)	3.2	0.5–20.4
FSH 60 min after GnRH (μg/L)	4.3	0.5–23.3
Basal oestradiol (pmol/L)	36.9	24.6–73.2
Oestradiol 60 min after GnRH (pmol/L)	41.4	29.7–73.8
Oestradiol 90 min after GnRH (pmol/L)	43.7	23.9–74.9
Basal testosterone (nmol/L)	14.0	1.3–46.8
Testosterone 60 min after GnRH (nmol/L)	24.2	12.8–84.5
Testosterone 90 min after GnRH (nmol/L)	25.3	10.4–67.0

3 | RESULTS

Before treatment, the cumulative mean (± SEM) testicular size (13.3 ± 1.9 cm^2) in the surgically castrated (SC) dogs did not differ significantly from that in the chemically castrated (CC) dogs (15.1 ± 1.2 cm^2). The mean cumulative testicular size decreased ($p < .001$) to 8.5 ± 0.9 cm^2 after chemical castration.

Mean (± SEM) body weight of the SC dogs before castration (22.4 ± 4.4 kg) did not differ significantly from that after castration (22.9 ± 4.4 kg). In the CC dogs, body weight after chemical castration (27.3 ± 3.0 kg) was higher ($p = .003$) than that before treatment (26.2 ± 2.9 kg). At the time of the second GnRH-stimulation test, none of the CC dogs showed signs of returning to the pre-castration stage.

The data collected from all dogs before surgical or chemical castration ($n = 42$) also allowed us to determine plasma concentrations of testosterone and oestradiol in intact male dogs for future reference (Table 1, Figure 1). In the gonadally intact dogs, GnRH administration induced increased plasma LH and FSH concentrations at 10 min and increased plasma oestradiol and testosterone concentrations at 60 min. The plasma FSH and oestradiol concentrations further increased between 10 and 60 min, and 60 and 90 min, respectively. The GnRH-stimulated plasma LH and testosterone concentrations at 10 and 60 min, and 60 and 90 min, respectively, did not differ significantly. The ranges of the GnRH-stimulated plasma LH, FSH, oestradiol and testosterone concentrations overlapped with the ranges of the basal values.

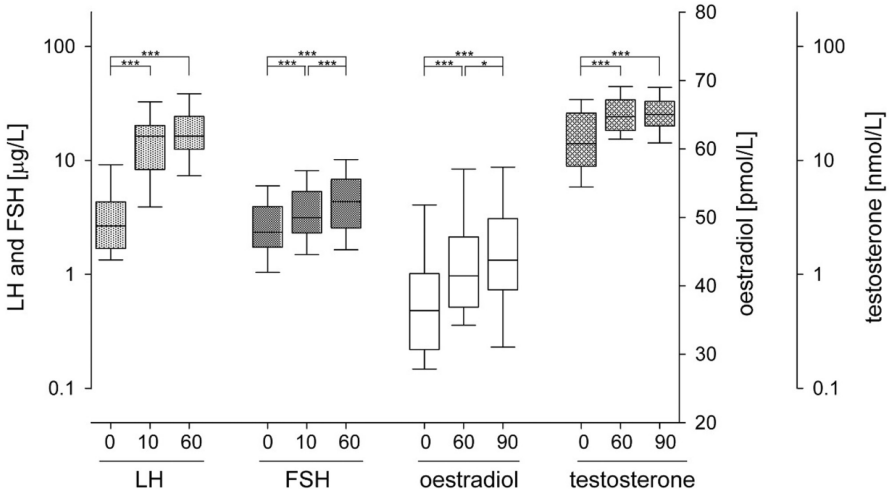

FIGURE 1 Plasma LH and FSH concentrations before and at 10 and 60 min after GnRH administration, and plasma oestradiol and testosterone concentrations before and at 60 and 90 min after GnRH administration in 42 gonadally intact male dogs of different breeds. Asterisks denote a significant difference: *: $p < .05$; ***: $p < .001$. Note the different scales of the y-axis.

The basal (p = .001) and GnRH-stimulated (p = .01) plasma LH concentrations after surgically castration were higher than those before castration. By contrast, after CC, basal and GnRH-stimulated plasma LH concentrations were lower ($p < .001$) than before chemical castration (Table 2 and Figure 2). ROC analysis revealed a cut-off value of 1.1 µg/L for the basal plasma LH concentration in CC dogs. In both SC and CC dogs, before and after castration, plasma LH concentrations were significantly higher compared with the basal value at T = 10 min and T = 60-min post-GnRH administration (Figure 2). In all individual dogs, before and after SC and before CC, the plasma LH concentration was increased at T = 10 min and T = 60-min post-GnRH administration compared with the basal value. After chemical castration, no increase in plasma LH concentration was observed in one dog at T = 10. In this and two more dogs the plasma LH concentration was lower at T = 60 than the basal value.

The basal and GnRH-stimulated plasma FSH concentrations after SC were higher ($p < .001$) than those before castration. By contrast, after CC, basal and GnRH-stimulated plasma FSH concentrations were lower ($p < .001$) than before CC (Table 2 and Figure 3). ROC analysis revealed a cut-off value of 7.7 µg/L for the basal plasma FSH concentration in SC dogs. In all dogs before and in the SC dogs after castration, plasma FSH concentrations were higher at T = 10 and T = 60 min after GnRH administration compared with the basal value (Figure 3). After CC, the plasma FSH concentrations at T = 10 min and T = 60 min after GnRH administration did not differ significantly from the basal value (Table 2 and Figure 3). In all individual dogs, before and after SC and before CC, the plasma FSH concentration was increased at T = 10 min and T = 60 min post-GnRH administration compared with the basal value.

The basal LH:FSH ratios in both groups before castration were similar. In SC dogs, the basal LH:FSH ratio decreased after surgical castration without overlap of the ranges before and after castration (Table 2). ROC analysis revealed a cut-off for the basal LH:FSH ratio of 0.45. In CC dogs, the basal LH:FSH ratio increased after chemical castration with overlap of the ranges before and after castration. After castration, the basal LH:FSH ratio was higher ($p < .001$) in CC than in SC dogs.

The basal and GnRH-stimulated plasma oestradiol concentrations, both before and after castration were similar when the SC and CC dogs were compared, and in both groups lower ($p < .001$) than those before castration (Table 2 and Figure 4). ROC analysis revealed a cut-off value for the basal plasma oestradiol concentration of 22 pmol/L in both SC and CC dogs. Before castration, in both SC (p = .02) and CC ($p < .001$) dogs, the plasma oestradiol concentrations were significantly higher at T = 90 min after GnRH administration and in the CC dogs also at T = 60 ($p < .001$) compared with the basal value. Before surgical castration, the plasma oestradiol concentration only tended to be higher at T = 60 (p = .06) compared with the basal value (Table 2 and Figure 4). After castration, in both SC and CC dogs, the plasma oestradiol concentrations at T = 60 and T = 90 min after GnRH administration did not differ significantly from the basal value.

The basal plasma testosterone concentrations, both before and after castration, and the GnRH-stimulated plasma testosterone concentrations before castration were similar when the SC and CC dogs were compared (Table 2 provides the ranges). In both groups, the basal and GnRH-stimulated plasma testosterone concentrations were higher before ($p < .001$) than those after castration (Table 2 and Figure 5). In both groups, it decreased approximately 200–400-fold after castration. After castration, GnRH-stimulated plasma testosterone concentrations in the CC dogs were higher than those in the SC dogs at T = 60 min (p = .02) and T = 90 min (p = .008). Although the highest measured plasma testosterone concentrations in CC dogs post-stimulation were 8.2 nmol/L (T = 60 min) and 7.7 nmol/L (T = 90 min), the median plasma concentrations at those time points were 0.06 and 0.09 nmol/L. None of the dogs went to pre-treatment testosterone levels. ROC analysis revealed cut-off values for the basal plasma testosterone concentration of 0.7 nmol/L and 2.2 nmol/L in SC and CC dogs, respectively. In both SC and CC dogs, before castration plasma testosterone concentrations were significantly higher at T = 60 min and T = 90 min after GnRH administration

TABLE 2 Plasma concentrations of LH, FSH, oestradiol, and testosterone, the LH:FSH ratio, and the testosterone: Oestradiol (Te:E2) ratio pre- and post-GnRH administration, prior to and following surgical ($n = 18$) or chemical ($n = 24$) castration

	Surgical castration						Chemical castration					
	Before treatment			After treatment			Before treatment			After treatment		
	median	range	N =	median	range	N =	median	range	N =	median	range	N =
Basal LH (μg/L)	3.2	1.0–11.3	14	8.6	2.5–36.0	14	2.6	1.1–12.2	24	0.68	0.4–1.0	23
LH 10 min after GnRH (μg/L)	16.7	3.9–92.6	14	30.2	9.4–65.1	14	15.1	3.2–116.4	24	1.11	0.4–3.8	22
LH 60 min after GnRH (μg/L)	15.5	5.6–103.9	13	27.4	8.6–49.0	13	19.2	6.0–71.4	24	0.80	0.4–2.7	21
Basal FSH (μg/L)	2.0	0.4–6.4	16	28.4	8.9–128.3	15	3.1	0.9–14.0	24	0.5	0.5–3.6	22
FSH 10 min after GnRH (μg/L)	2.7	0.5–8.4	16	32.1	9.8–137.5	15	3.6	1.2–20.4	24	0.5	0.5–3.6	22
FSH 60 min after GnRH (μg/L)	3.2	0.5–11.1	14	32.0	10.9–78.8	14	4.8	1.3–23.3	24	0.5	0.5–3.7	22
Basal LH: FSH	1.59	0.5–3.6	14	0.27	0.1–0.4	13	1.0	0.2–7.2	24	1.3	0.2–1.8	22
Basal oestradiol (pmol/L)	36.5	25.1–60.1	17	12.2	9.2–19.1	16	36.3	24.6–73.2	21	14.58	8.6–19.9	21
Oestradiol 60 min after GnRH (pmol/L)	40.0	30.3–59.0	15	11.3	7.9–18.3	14	42.7	29.7–73.8	21	13.52	8.6–28.0	20
Oestradiol 90 min after GnRH (pmol/L)	44.3	23.9–62.8	17	13.1	9.5–20.5	13	43.2	31.4–74.9	20	14.79	10.8–24.2	17
Basal testosterone (nmol/L)	17.4	1.3–34.8	18	0.05	0.05–0.07	18	11.0	3.8–46.8	24	0.05	0.05–0.7	23
Testosterone 60 min after GnRH (nmol/L)	25.5	15.9–45.3	17	0.05	0.05–0.07	17	23.9	12.8–84.5	24	0.06	0.05–8.2	23
Testosterone 90 min after GnRH (nmol/L)	25.7	17.0–43.9	17	0.05	0.05–0.07	15	24.9	10.4–67.0	23	0.09	0.05–7.7	22
Basal Te:E2 * 1000	562.5	211–1046	17	4.6	2.7–7.1	16	335	122–997	21	4.3	2.9–33.3	21

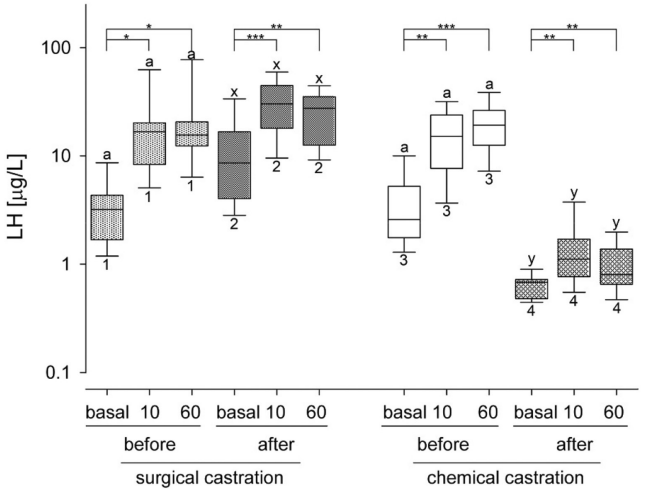

FIGURE 2 Plasma LH concentrations before and at 10 and 60 min after GnRH administration before and after surgical ($n = 18$) or chemical ($n = 24$) castration. Asterisks denote a significant difference between the basal value and the value after GnRH administration: *: $p < .05$; **: $p < .01$; ***: $p < .001$. Different letters denote a significant difference ($p < .001$) between SC and CC dogs for values collected at the same time point of the GnRH-stimulation test. Different numbers denote a significant difference ($p < .001$, except SC T = 10 min: $p = .01$; SC T = 60 min: $p = .01$) between before and after castration within the groups of SC or CC dogs. Note the logarithmic scale of the y-axis.

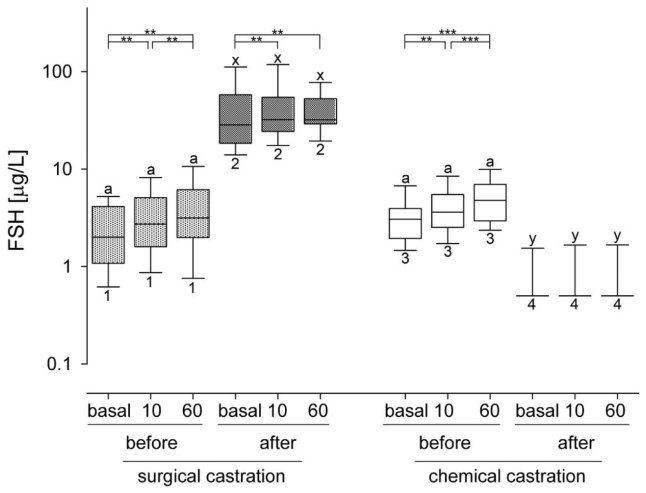

FIGURE 3 Plasma FSH concentrations before and at 10 and 60 min after GnRH administration before and after surgical ($n = 18$) or chemical ($n = 24$) castration. Asterisks denote a significant difference between the basal value and the value after GnRH administration: **: $p < .01$; ***: $p < .001$. Different letters denote a significant difference ($p < .001$) between SC and CC dogs for values collected at the same time point of the GnRH-stimulation test. Different numbers denote a significant difference ($p < .001$) between before and after castration within the groups of SC or CC dogs. Note the logarithmic scale of the y-axis.

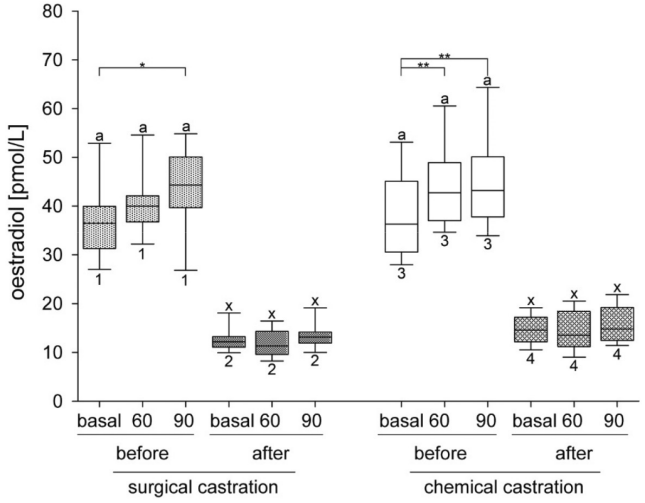

FIGURE 4 Plasma oestradiol concentrations before and at 60 and 90 min after GnRH administration before and after surgical ($n = 18$) or chemical ($n = 24$) castration. Asterisks denote a significant difference between the basal value and the value after GnRH administration: *: $p < .05$; **: $p < .01$. Different letters denote a significant difference ($p < .001$) between SC and CC dogs for values collected at the same time point of the GnRH-stimulation test. Different numbers denote a significant difference ($p < .001$) between before and after castration within the groups of SC or CC dogs.

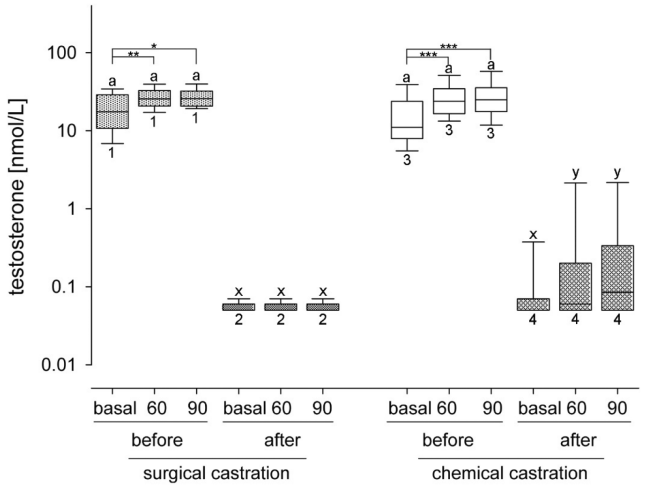

FIGURE 5 Plasma testosterone concentrations before and at 60 and 90 min after GnRH administration before and after surgical ($n = 18$) or chemical ($n = 24$) castration. Asterisks denote a significant difference between the basal value and the value after GnRH administration: *: $p < .05$; **: $p < .01$; ***: $p < .001$. Different letters denote a significant difference ($p < .001$) between SC and CC dogs for values collected at the same time point of the GnRH-stimulation test. Different numbers denote a significant difference ($p < .001$) between before and after castration within the groups of SC or CC dogs. Note the logarithmic scale of the y-axis.

compared with the basal value with no difference between both time points (Figure 5). After surgical or chemical castration, the plasma testosterone concentrations after GnRH administration, both at T = 60 min and T = 90 min, did not differ significantly from the basal value.

The basal testosterone: oestradiol ratios were similar, both before and after castration, when the SC and CC dogs were compared (Table 2). In both groups, a significant and approximately 100-fold decrease ($p < .001$) was observed after castration without overlap of the ranges of the testosterone: oestradiol ratio before and after castration. ROC analysis revealed cut-off testosterone: oestradiol ratios of 109 and 78 for SC and CC dogs, respectively.

4 | DISCUSSION

In this study, more insight into the function of the pituitary-testicular axis was obtained by performing GnRH-stimulation tests and comparing hormone concentrations in male dogs prior to and following surgical or chemical castration. Besides that, this approach also allowed us to determine plasma concentrations of testosterone and oestradiol in 42 intact male dogs of several breeds that can be used for future reference. Based on our study, the expected increase in oestradiol 60 and 90 min after buserelin stimulation is, respectively, 112% and 118% and for testosterone, respectively, 173% and 180% in healthy intact male dogs. This study was, however, not specifically designed to establish reference values. Additional procedural steps that follow the ASVCP guidelines for de novo determination of reference intervals (Friedrichs et al., 2012) are needed to validate these values.

GnRH (buserelin) administration induced a significant increase in the plasma concentrations of LH, FSH, oestradiol and testosterone in the intact male dogs. Results were largely similar to previously reported data (Falvo et al., 1982; Günzel-Apel et al., 1994; Jones et al., 1976; Junaidi et al., 2007; Knol et al., 1993; Purswell & Wilcke, 1993), but instead of a short-lived increase in the plasma LH concentration after GnRH administration that was observed in most other studies, plasma LH was still increased at T = 60 min in the present study, probably caused by the longer half-life of buserelin relative to native GnRH (Padula, 2005). LH is the main regulator of testosterone secretion, and the interval between increase of plasma LH and plasma testosterone has been shown to be 30–60 min in dogs (DePalatis et al., 1978; Günzel-Apel et al., 1994; Knol et al., 1993). These factors might explain why also the plasma testosterone concentration did not significantly further increase between T = 60 min and T = 90 min.

Chemical castration with deslorelin implants results, after an initial temporary stimulation of gonadotrophin secretion, in secondary hypogonadism (Junaidi et al., 2007; Ludwig et al., 2009; Trigg et al., 2001). Consequently, testis size had decreased significantly in the CC dogs at 4.4 months after implantation, which can be an indication of the arrest of spermatogenesis in these dogs at the level of the spermatogonia/primary spermatocytes as has been shown before (Cavitte et al., 1988; Goericke-Pesch et al., 2009; Junaidi, Williamson, Trigg, et al., 2009; Ludwig et al., 2009; Tremblay & Bélanger, 1984; Vickery et al., 1984). We did not evaluate spermatogenesis by sperm analysis or by histological examination of the testes to confirm that statement.

In agreement with previous findings, basal plasma concentrations of both FSH and LH were significantly higher after surgical castration than before treatment (de Gier et al., 2012; DePalatis et al., 1978; Olson et al., 1992). Additionally, basal plasma concentrations of both FSH and LH were significantly lower after administration of the deslorelin implant compared to before treatment (Cavitte et al., 1988; Goericke-Pesch et al., 2009; Junaidi et al., 2007). After surgical castration, the negative feedback of the gonadally secreted steroid and protein hormones on GnRH secretion ceases abruptly. This is probably mediated via the KiSS-1/gpr-54 system, as the Kisspeptin neurons, that have been shown to induce GnRH secretion by the GnRH neurons, express the sex steroids receptors that the GnRH neurons lack (Albers-Wolthers et al., 2014; Oakley et al., 2009; Roseweir & Millar, 2009; Tsutsui et al., 2010). The removal of the negative feedback is followed by an increased secretion of LH and FSH (DePalatis et al., 1978; Reichler et al., 2004; Winter et al., 1982; Winter et al., 1983). In contrast to SC, plasma LH and FSH concentrations decreased after CC, which is in line with the proposed mechanism of action, namely downregulation of the GnRH receptors and desensitization of the pituitary gonadotrophs as a result of the continuously elevated plasma GnRH concentration (Jones et al., 1976; Junaidi et al., 2007; Vickery et al., 1984). After SC, GnRH secretion is also elevated, but probably still pulsatile as suggested by the increased, but pulsatile plasma LH and FSH concentrations after ovariectomy in bitches (Concannon, 1993). Due to the pulsatile GnRH secretion, there is no desensitization of the gonadotrophs. This emphasizes the importance of the pulsatile nature of GnRH secretion in the regulation of gonadotrophin secretion.

The increase in basal and GnRH-stimulated plasma FSH concentrations after surgical castration was more pronounced than that of LH, which has been shown before in male dogs and bitches (Olson et al., 1992; Reichler et al., 2004). Because the increase in basal plasma FSH concentration after surgical castration was so high that there was no overlap in the ranges between before and after castration, in contrast to the levels of LH, it was possible to calculate a cut-off value with a 100% sensitivity and specificity. This can thus discriminate gonadally intact dogs from dogs after SC. However, we need to keep in mind that these values are based on this group of dogs only. Research has shown that, although LH and FSH are secreted by the same gonadotrophic cells with GnRH as the stimulating hormone, LH and FSH are differentially regulated in the bitch (de Gier et al., 2006). In vitro studies have shown that LH and FSH are stored in different granules within the same cell type (Ascoli & Narayan, 2014; Moyle & Campbell, 1996). Furthermore, the magnitude of FSH secretion in response to secretagogues is smaller than that of LH (Chowdhury & Steinberger, 1975; Muyan et al., 1994). Additionally, LH release is highly responsive to increased GnRH pulse frequency (Marshall et al., 1991), whereas plasma FSH concentration increases in response to decreased GnRH

pulse frequency (Gross et al., 1987). Differential feedback effects of the gonadally secreted steroid and protein hormones, probably via the KiSS-1/gpr-54 system, constitute part of the differentially regulated pituitary gonadotrophin secretion (Roseweir & Millar, 2009; Shupnik, 1996). For example, the negative feedback by oestradiol and inhibin on the gonadotrophs mainly targets FSH secretion (Hayes et al., 2001; O'Connor & De Kretser, 2004; Roseweir & Millar, 2009; Shupnik, 1996). This suggests that the negative feedback effect by gonadally secreted hormones on FSH secretion is more important than on LH secretion, which explains the more pronounced increase of plasma FSH than LH after SC. The difference in effect of gonadectomy on basal plasma LH and FSH concentrations is also reflected by the decreased basal LH:FSH ratio following castration compared to prior to castration.

The decrease in the basal plasma LH concentration following castration by long-term GnRH administration has been shown in several studies in dogs (Cavitte et al., 1988; Goericke-Pesch et al., 2009; Inaba et al., 1996; Junaidi et al., 2003; Ludwig et al., 2009; Paramo et al., 1993; Vickery et al., 1984) and was to be expected. Interestingly, in our study, plasma LH concentration increased significantly post-GnRH administration in the implant group, indicating that the pituitary gonadotrophs were responsive to GnRH in this group at this time point (4.4 months). In a recent study, testicular LHR gene expression in five adult male dogs implanted with deslorelin was lower 4 months after implantation than in untreated adults (Balogh et al., 2021). Junaidi et al. (2007) performed GnRH-stimulation tests at different intervals after administration of an implant containing deslorelin. In that study, no increase of plasma LH concentration after intravenous injection of gonadorelin was detected at 100 days after start of treatment. This difference might be explained by the fact that these authors performed the GnRH-stimulation test with native GnRH, whereas we used the more potent buserelin (Padula, 2005) in another dosage. In addition, the dogs in the study of Junaidi et al. (2007) were smaller than the dogs used in our study (15–22 kg and 26.2 ± 14.2 kg, respectively), indicating a higher deslorelin dose per kg body weight. Furthermore, the longest interval from castration to GnRH-stimulation test was 100 days in that study versus 133 days (4.4 months) in our study. It is possible that some dogs in our study were completely downregulated at an earlier time but had started to regain pituitary responsiveness to GnRH. Partial reversal of the effect of deslorelin would fit in with the suggestion of Junaidi, Williamson, Trigg, et al. (2009), who stated that the dose–response relationship with deslorelin is expressed with respect to the maximum duration of suppression and not the degree of suppression (Junaidi, Williamson, Martin, et al., 2009).

Few data on the basal plasma FSH concentrations before and after chronic treatment with GnRH-agonists in dogs have been published. Goericke-Pesch et al. (2009) demonstrated a similar and significant effect of the GnRH-agonist azagly-nafarelin on basal FSH compared with our study, whereas the data of Cavitte et al. (1988) were highly suggestive of such an effect by the GnRH-agonist D-Trp6-LH-RH. This is in line with the expected downregulation of pituitary gonadotrophin secretion. Interestingly, the plasma FSH concentration, in contrast to the plasma LH concentration, did not increase after GnRH administration in CC dogs. The difference in response to GnRH-stimulation of FSH compared with that of LH after chemical castration may also be ascribed to differential regulation of pituitary gonadotrophin secretion as described above. GnRH induces both LH and FSH secretion, but it preferentially stimulates LH secretion (Urban et al., 1988).

Basal and GnRH-stimulated plasma oestradiol concentrations decreased to similar levels after surgical and chemical castration in SC and CC dogs. The approximately 50% decrease in basal plasma oestradiol after castration is similar to data in other studies (Goericke-Pesch et al., 2010; Ludwig et al., 2009). Plasma oestradiol was still above the detection limit of the assay after SC, which is an indication for extragonadal production of oestradiol. Although peripheral aromatization of androgens to oestradiol contributes to the total plasma oestradiol concentration, the adrenals probably are the main source of plasma oestradiol after castration (Santen et al., 1980).

Basal and GnRH-stimulated plasma testosterone concentrations decreased approximately 200–400-fold after surgical and chemical castration. GnRH administration after chemical castration did not induce a significant increase in the plasma testosterone concentration, as was also shown by Junaidi et al. (2007). Despite the absence of a GnRH-induced increase of testosterone in CC, the GnRH-stimulated plasma testosterone concentrations were higher in CC than in SC dogs. This indicates that, at the time of performing the GnRH-stimulation test, the pituitary gonadotrophs were responsive, as was also suggested by the increase of LH after GnRH administration in CC dogs. Although the highest measured plasma testosterone concentrations in CC dogs post stimulation were 8.2 and 7.7 nmol/L, at T = 60 and T = 90, respectively, the median plasma concentrations at those time points were 0.06 and 0.09 nmol/L, indicating that most dogs were still downregulated.

The basal testosterone: oestradiol ratio was similar in both groups pre- and post-treatment. It decreased approximately 50-fold after treatment, reflecting the more pronounced effect of SC and CC on the basal plasma testosterone concentration than on the plasma oestradiol concentration. Mischke et al. (2002), in a study on endocrinological findings in male dogs with testicular pathology, suggested that the testosterone: oestradiol ratio more accurately predicts the clinical effects of the gonadally secreted steroids in dogs with testicular tumours than plasma concentrations of testosterone or oestradiol alone (Mischke et al., 2002).

The interval between the castration and second GnRH-stimulation tests was 5.4 months for SC and 4.4 months for CC. The reasons for the timing of the second GnRH-stimulation test in the SC and CC was based on multiple factors. We wanted the groups to be as biologically comparable as possible. The timing of the second GnRH-stimulation test for SC dogs was based on the study of Reichler et al. (2004) in which stabilization of gonadotropins concentration after castration in female dogs was researched. Concentrations of both gonadotropins increased steadily and stabilized at week 29. In the CC dogs, we wanted to be sure that the chemical castration would have had enough time to ascertain complete downregulation

and, on the contrary, would not already be regaining pituitary responsiveness to GnRH again. So, to make the intervals as comparable as possible, we performed the stimulation tests in the SC dogs earlier that the 29 weeks. Additionally, it were client-owned dogs so we needed to consider the logistical argument as well. Despite the strong design of this study, it needs to be considered that the range of the hormone values at 10 min and 60 min after GnRH-stimulation are rather high and that an age and breed effect cannot be excluded. By providing the ranges of the hormone concentrations, we think this study is a good basis for future research.

5 | CONCLUSIONS

GnRH administration in intact male dogs induced increased secretion of the pituitary gonadotrophins, LH and FSH, and the sex steroids oestradiol and testosterone. Surgical castration resulted in increased plasma concentrations of LH and FSH. Chemical castration with a continuous release of the GnRH-agonist deslorelin induced decreased plasma concentrations of LH and FSH and consequently lowered plasma oestradiol and testosterone concentrations to values that did not differ significantly to those after surgical castration. However, at the moment of assessment at 4,4 months (mean 133 days ± SEM 4 days), the pituitary gonadotrophs were responsive to GnRH in implanted dogs. The increase in LH, but not of FSH, following GnRH administration indicates a differential regulation of the release of these gonadotrophins.

AUTHOR CONTRIBUTIONS

JDG, CV, AS and HK were involved in the conceptualization and design of the study. Data acquisition, analysis and validation were performed by JDG and CO. JDG did the funding acquisition. All authors were involved in drafting the article and revising it critically. All authors approved on the final version of the article.

ACKNOWLEDGEMENTS

We thank Miss D.H.G. Morsink, DVM, Miss R.M. Gudde, MSc, Miss S.C. van Wijk, DVM, Miss drs. E.R. Steur and Miss L.A. Kramer for assistance in performing the experiments and Dr. B.E. Belshaw for editing.

FUNDING INFORMATION

This study was partially funded by Virbac SA, France.

CONFLICT OF INTEREST

This study was partially funded by Virbac SA, France. The funding source had no role in the study design, collection, analysis and interpretation of data or in writing of the report and decision to submit the article for publication.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Annemarie Spruijt https://orcid.org/0000-0002-0995-5503

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How to cite this article: Spruijt, A., Kooistra, H., Oei, C., Vinke, C., Schaefers-Okkens, A., & De Gier, J. (2023). The function of the pituitary-testicular axis in dogs prior to and following surgical or chemical castration with the GnRH-agonist deslorelin. *Reproduction in Domestic Animals*, *58*, 97–108. https://doi.org/10.1111/rda.14266