Steroid hormone concentration profiles in healthy intact and neutered dogs before and after cosyntropin administration

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Abstract

The purpose of this study was to determine steroid hormone concentration profiles in healthy intact and neutered male and female dogs. Seventeen intact female dogs, 20 intact male dogs, 30 spayed female dogs, and 30 castrated male dogs were used in this study. Serum samples were collected before and 1 h after cosyntropin administration, and serum concentrations were determined for cortisol, progesterone, 17-OH progesterone (17-OHP), dehydroepiandrosterone sulfate (DHEAS), androstenedione, testosterone, and estradiol. Intact male dogs had greater concentrations of DHEAS, androstenedione, and testosterone. Intact female dogs had greater concentrations of progesterone. There was no significant difference in estradiol concentration among the four groups. Intact male dogs had lower concentrations of cortisol post-stimulation. DHEAS and testosterone did not increase in response to ACTH in intact males, and estradiol concentrations did not increase in response to ACTH in any group. Results from this study will enhance interpretation of suspected adrenal and/or gonadal disorders of dogs. Because estradiol concentrations were similar in all groups of dogs, measuring estradiol may not be a useful diagnostic test. Cortisol concentrations for intact male dogs with hyperadrenocorticism may be lower than those of female or neutered dogs.
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1. Introduction

Steroid hormones are commonly measured in dogs to detect a number of abnormal conditions. The most common of these are determining if a female dog has been spayed or if ovarian tissue remains after a spay procedure and if a retained testicle or Sertoli cell tumor exists in a male dog [1–4]. When determining the presence of ovarian tissue, some advocate measuring estradiol either as a baseline or dynamic test [4,5] while others recommend measuring progesterone concentrations [1,6]. To determine if a dog is bilaterally cryptorchid (versus castrated), testosterone is frequently measured [3,7].

In addition to gonadal tissue, the adrenal glands produce steroid intermediates and sex hormones. Thus, measuring steroid intermediates and sex hormones also reflect adrenocortical activity [8–10]. One potential application for measuring steroid intermediates in veterinary medicine is as a screening test for dogs with "Atypical" Cushing's syndrome, whereby dogs present with clinical signs of Cushing's syndrome but do not have hypercortisolemia [28]. It is theorized that these dogs may have increases in steroid intermediates which may be responsible for the clinical signs. A recent study [11] reported two normal-cortisolemic dogs with adrenocortical neoplasia that had substantial increases in 17-OH progesterone (17-OHP) post-ACTH stimulation, thus supporting this theory.

Preliminary studies that were designed to investigate the role of adrenal hormones in alopecia of Pomeranians established normal baseline and post-ACTH stimulation ranges for steroid hormone intermediates using 9 intact female and 10 intact male dogs [12]. Estimates of reference intervals for sex hormones in dogs at UTCVM endocrinology laboratory are based on relatively small sample sizes (10 intact male dogs, 5 intact female dogs, 9 neutered male dogs, 8 neutered female dogs) [13]. Establishment of accurate reference intervals, by increasing sample size, will increase our ability to classify dogs as normal or abnormal and to recognize associations between steroid hormone concentrations and disease. The purpose of this study was to determine normal steroid hormone concentration profiles for intact and neutered dogs of both sexes prior to and after stimulation with ACTH.

2. Materials and methods

2.1. Dogs

Thirty spayed female (SF) dogs, 30 castrated male (CM) dogs, 20 intact female (IF) dogs 3 months after their last heat (when in anestrus), and 20 intact male (IM) dogs were used for this study. Dogs were entered into the study over a period of 6 months between June and November of 2000. All were client-owned dogs except for one intact female and five intact males which were housed at a research facility. The research dogs were housed and maintained according to the university animal care and use committee guidelines. All client-owned dogs were enrolled with the informed consent of their owners. The study was approved by the University's Office of Lab Animal Care. All dogs were normal on physical examination and had no recent history of illness or steroid administration. Three intact females were withdrawn, due to progesterone concentrations greater than 1 ng/ml, indicative of diestrus [1].

2.2. Experimental protocol

The ACTH stimulation test was performed in the morning following an overnight fast. Two blood samples (before and 1-h post-ACTH) were obtained from the jugular vein of each dog. A 1-h sampling time was chosen because cortisol is maximally stimulated at this time and is, therefore, the protocol followed by convention in veterinary medicine [12–15,28]. This protocol parallels that used in people in which maximum stimulation of adrenal intermediates post-ACTH stimulation was achieved at 30 min and maintained through the 60-min sampling time [8]. Blood was allowed to clot at room temperature prior to centrifugation. Serum was collected and stored at $-70\,^{\circ}$C until assays were performed. Serum samples from all dogs were evaluated for progesterone, 17-OH progesterone (17-OHP), dehydroepiandrosterone sulfate (DHEAS), androstenedione, testosterone, estradiol, and cortisol. Synthetic ACTH (cosyntropin[1]) was administered intravenously via the cephalic vein at a dose of 5 μg/kg. This dose has been shown to maximally stimulate cortisol production of the adrenal glands [14].

Serum steroid hormone concentrations were determined in our Clinical Endocrinology Service[2] by radioimmunoassay procedures.[3] Sensitivity for cortisol, progesterone, 17-OHP, DHEAS, androstenedione, testosterone, and estradiol RIA was 0.2 μg/dl, 0.02, 0.1, 0.5, 0.1, and 0.04 ng/ml, and 10 pg/ml, respectively. Intra- and inter-assay coefficient of variation for the RIA were: cortisol, 5.4 and 5.9%; progesterone, 7.3 and 13.0%; 17-OHP, 10.7 and 9.8%; DHEAS, 3.0 and 10.9%; androstenedione, 7.7 and 7.1%; testosterone, 9.3 and 7.9%; and estradiol, 7.3 and 7.3%. During the course of the study, the commercial assay for measuring DHEAS was discontinued by the manufacturer. Therefore, DHEAS concentrations were not determined for two castrated and four intact male dogs.

2.3. Statistical analysis

Data was analyzed using a statistical software package.[4] Hormone values within sex were tested for normal distribution. Data were summarized using the median and range. A non-parametric Kruskal–Wallis test was used to detect differences in baseline and post-stimulation hormone values among categories of sex. Values were considered significant if $P < 0.05$. When significance was detected, pairwise comparisons were made and subjected to the Bonferroni adjustment to distinguish differences in hormone levels between sex groups. The sign test for paired data was used to determine difference between baseline and post-stimulation values within sex.

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[1] Cortrosyn, Organon Inc., West Orange, NJ.
[2] Clinical Endocrinology Service, College of Veterinary Medicine, University of Tennessee, JW Oliver, Director; EM Bailey, Analytical Support.
[3] Estradiol 17-beta [125I] RIA, ICN Pharmaceuticals, Inc., Costa Mesa, CA. Progesterone [125I] RIA, Diagnostic Products Corp., Los Angeles, CA. Testosterone [125I] TIA, Diagnostic Products Corp., Los Angeles, CA. DHEAS [125I] RIA, ICN Pharmaceuticals, Inc., Costa Mesa, CA. 17-OHP [125I] RIA, ICN Pharmaceuticals, Inc., Costa Mesa, CA. Androstenedione [125I] RIA, ICN Pharmaceuticals, Costa Mesa, CA. Cortisol [125I] RIA, Diagnostic Products Corp., Los Angeles, CA.
[4] SAS, version 8e, SAS Institute, Inc., Cary, NC.

3. Results

The mean age for SF and CM dogs was 4.5 years (range 2–8 years); for IF it was 2.8 years (range 1.5–5 years); and for IM dogs it was 3.4 years (range 1.5–5 years). Breeds in the SF group included 2 beagles, 2 Boston terriers, 2 boxers, 2 hound dogs, 2 rottweilers, a German shepherd dog, and 20 mixed breed dogs. Breeds in the CM group included 3 rottweilers, 2 dachshunds, 2 hounds, an Australian cattle dog, golden retriever, German shepherd dog, Labrador retriever, Pekinese, schnauzer, Shetland sheep dog, springer spaniel, and 15 mixed breed dogs. Breeds in the IF group included 5 doberman pinschers, 2 Pekinese, 3 Tibetan spaniels, 2 basset hounds, an Australian shepherd, German shepherd dog, hound dog, Pharough hound, and a mixed breed dog. Breeds in the IM group included 9 hound dogs, 3 Australian shepherds, 2 American Staffordshire terriers, a basset hound, boxer, English bulldog, Great Dane, Welch Corgie, and a mixed breed dog.

The results of tests on the distribution of data, for each of the hormones by sex of the dog, indicated that more than half of the observations were non-normally distributed. Therefore, to maintain consistency, all data are presented as the median, 5th and 95th percentiles to more accurately reflect the distribution of values. Intact male dogs had significantly greater concentrations of androstenedione, DHEAS, and testosterone than the other three groups (Figs. 1–3, Tables 1 and 2). There was no statistical difference among SF, IF, or CM dogs for any of these three hormones. In SF, IF, and CM dogs, both testosterone and DHEAS had slight but significant increases in response to ACTH stimulation; however, in IM dogs, neither testosterone nor DHEAS increased in response to ACTH (Tables 1 and 2).

Intact female dogs had greater median concentrations of progesterone (both baseline and post-stimulation) than the other three groups although the baseline concentration was not significantly different from IM dogs (Tables 1 and 2, Fig. 4). There was no significant difference between baseline or post-stimulation progesterone concentrations for SF and CM dogs. Intact dogs (both male and female) had significantly greater concentrations of both baseline and post-stimulation 17-OHP than neutered dogs (Fig. 5). There was no statistical difference

Table 1

Median (5th–95th percentile) sex hormone (ng/ml) and cortisol concentrations (µg/dl) pre- and post- stimulation with ACTH in normal male dogs

Hormone	Group			
	Intact male ($n = 20$)		Neutered male ($n = 30$)	
	Pre	Post	Pre	Post
Androstenedione	24.5 (2.7–48.8)	34.2[a] (6.8–85.7)	2.2 (0.7–10.4)	14.1[a] (5.9–72.2)
DHEAS[b]	10.2 (5.2–30.5)	11.5 (6.6–39)	4.6 (3.5–15.3)	7.5[a] (2.3–56.8)
Progesterone	0.1 (0.02–0.5)	1.0[a] (0.7–1.8)	0.04 (0–0.3)	1.0[a] (0.3–1.9)
17-OHP	0.3 (0.06–0.8)	1.6[a] (0.8–2.9)	0.08 (0.03–0.3)	1.1[a] (0.3–2.8)
Estradiol (pg/ml)	50.3 (33.6–66.6)	48.7 (30.0–63.5)	47.4 (27.7–65.1)	45.4 (26.8–69.4)
Testosterone	2.8 (0.01–41.5)	2.8 (0.2–22.1)	0.04 (0.01–0.1)	0.05[a] (0.02–0.7)
Cortisol	1.54 (0.4–4.53)	8.51[a] (7.09–10.68)	1.13 (0.22–4.32)	10.14[a] (7.33–14.91)

[a] Indicates significant increase post-stimulation ($P < 0.05$).

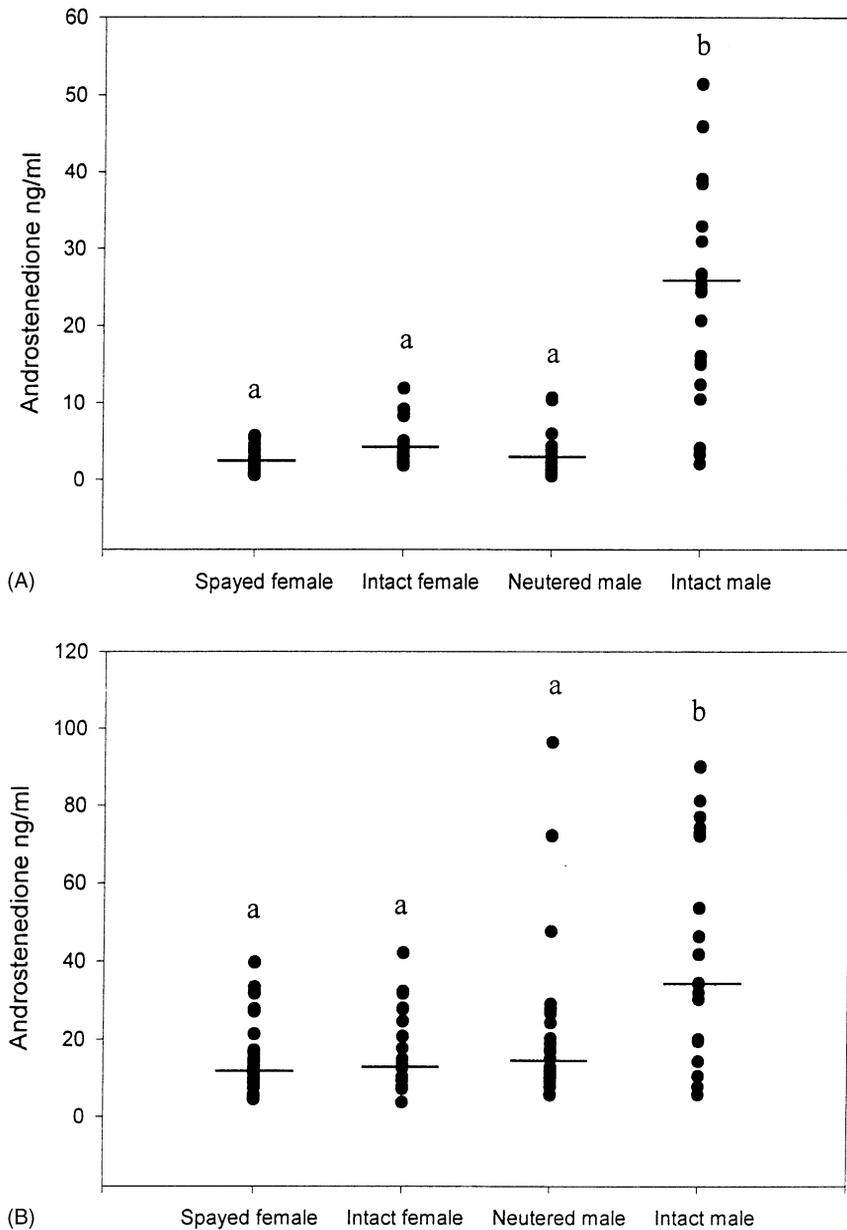[b] $n = 16$ intact males; $n = 28$ neutered males.

Fig. 1. Point plots of baseline (A) and post-ACTH stimulation (B) serum androstenedione concentrations in 30 spayed female, 17 intact female, 30 neutered male, and 20 intact male dogs. The horizontal bar is the median. Different letters indicate significant difference ($P < 0.05$).
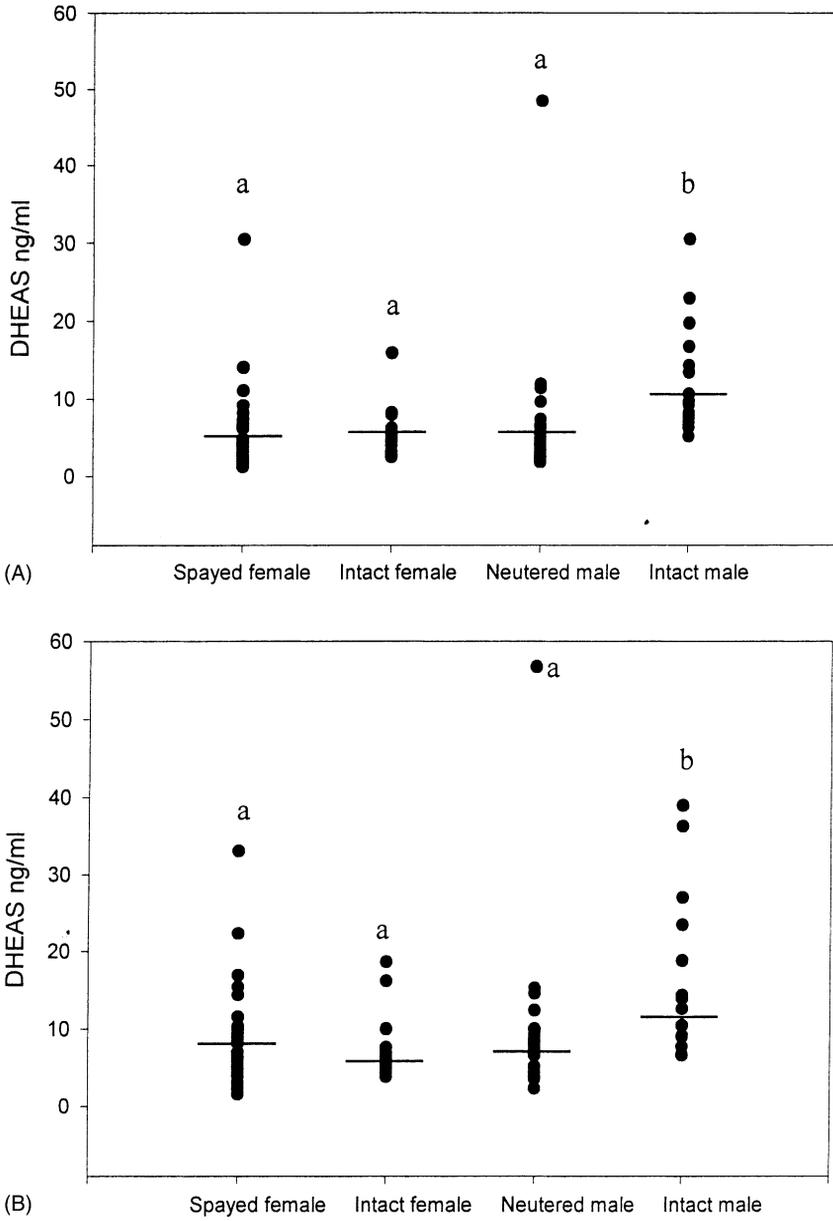
Fig. 2. Point plots of baseline (A) and post-ACTH stimulation (B) serum DHEAS concentrations. See Fig. 1 for explanation.
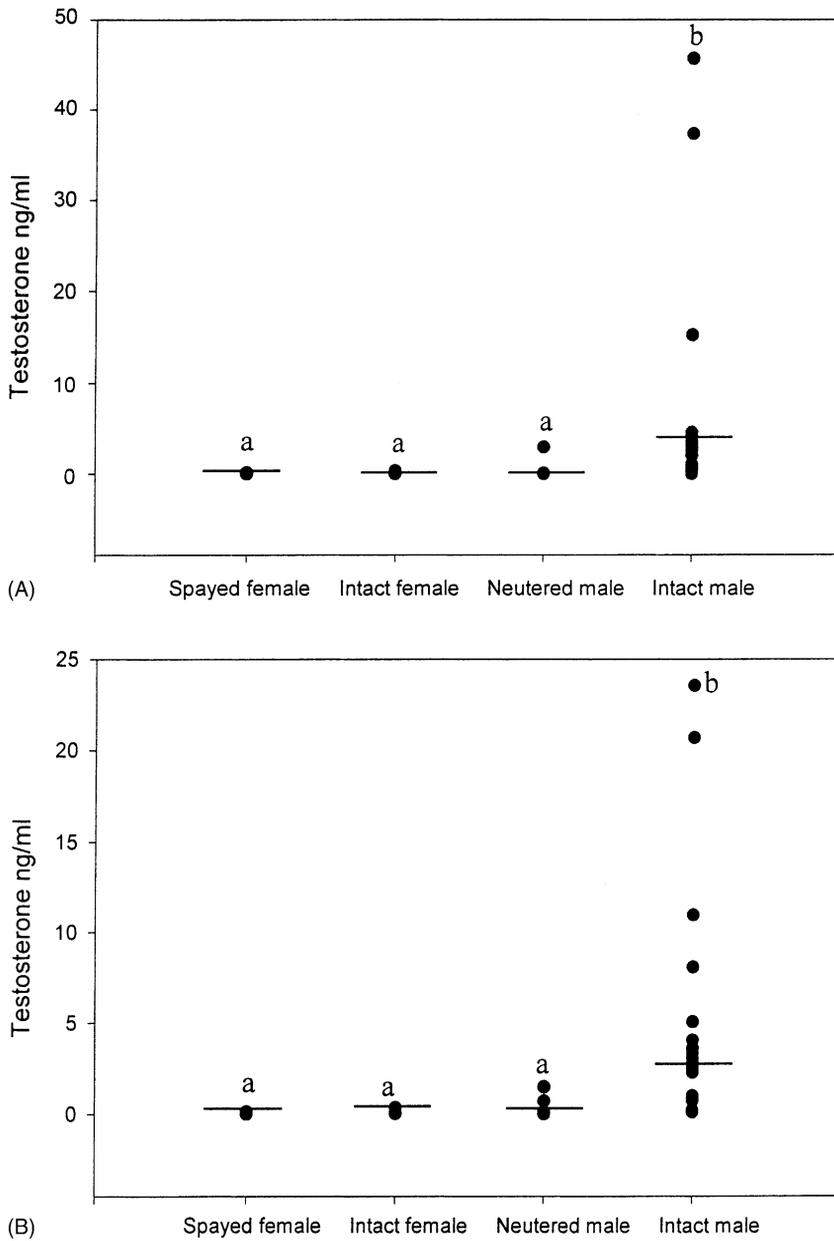
Fig. 3. Point plots of baseline (A) and post-ACTH stimulation (B) serum testosterone concentrations. See Fig. 1 for explanation.
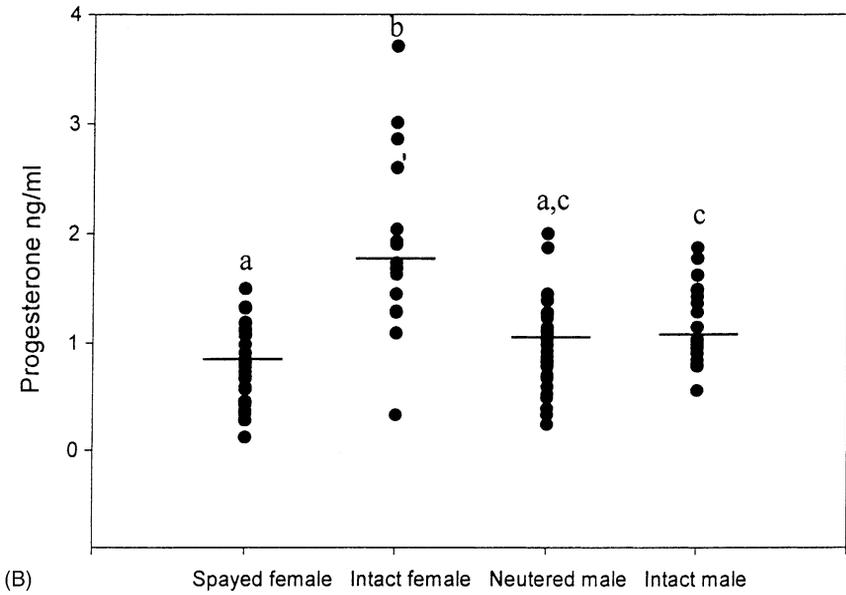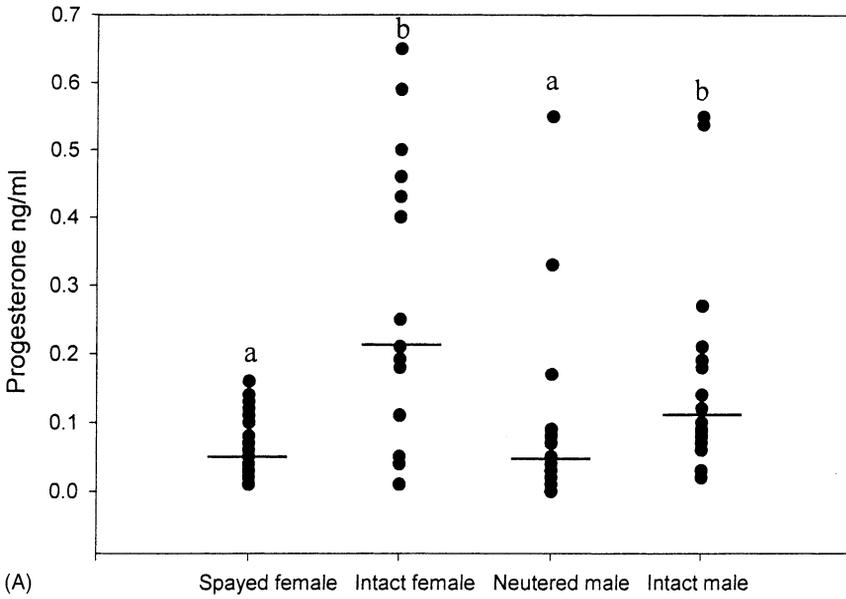
Fig. 4. Point plots of baseline (A) and post-ACTH stimulation (B) serum progesterone concentrations. See Fig. 1 for explanation.
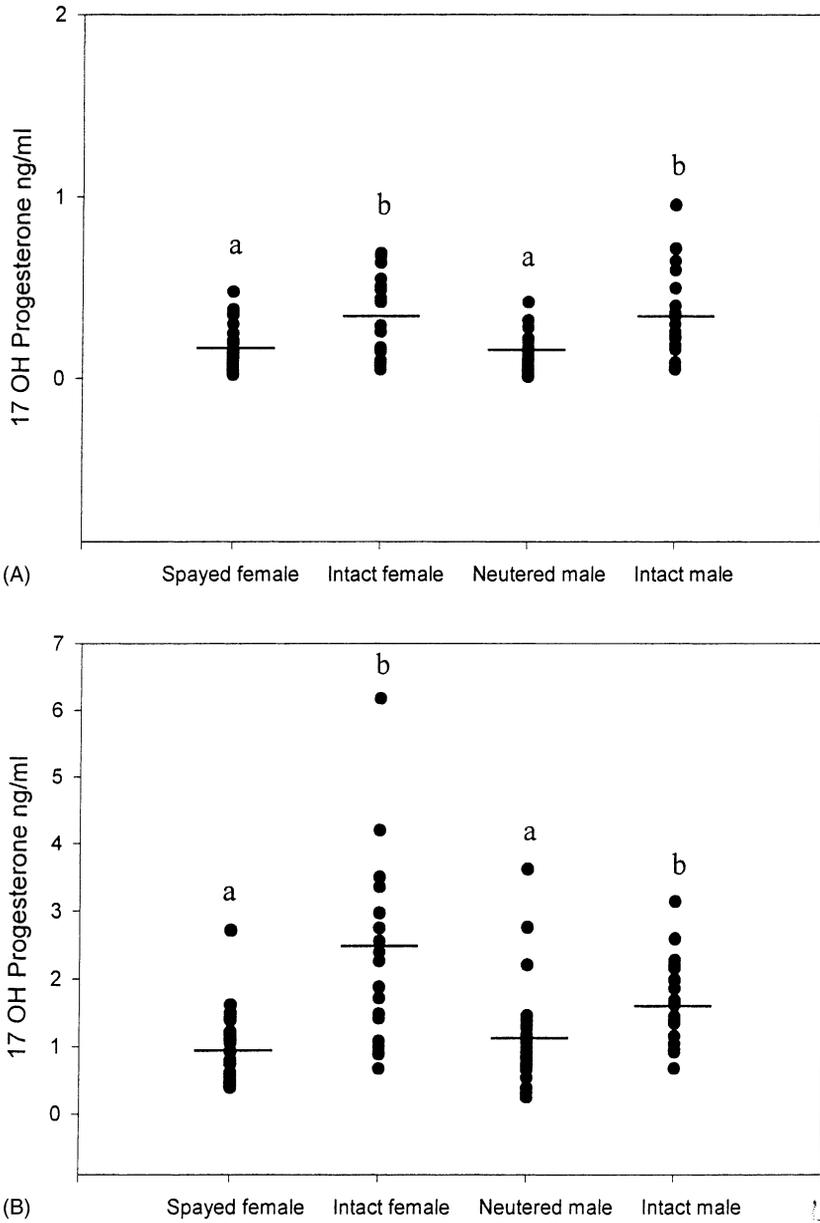
Fig. 5. Point plots of baseline (A) and post-ACTH stimulation (B) serum 17-OH progesterone concentrations. See Fig. 1 for explanation.

Table 2
Median (5th–95th percentile) sex hormone (ng/ml) and cortisol concentrations (μg/dl) pre- and post- stimulation with ACTH in normal female dogs

Hormone	Group			
	Intact female ($n = 17$)		Spayed female ($n = 30$)	
	Pre	Post	Pre	Post
Androstenedione	3.2 (1.9–11.9)	14.2[a] (3.8–42.1)	2.45 (0.7–5.3)	12.4[a] (5.5–33.4)
DHEAS	4.7 (2.5–15.9)	5.7[a] (3.8–18.6)	4.5 (1.8–14.1)	8.3[a] (2.3–22.4)
Progesterone	0.21 (0.01–0.65)	1.7[a] (0.3–3.7)	0.04 (0.01–0.14)	0.8[a] (0.3–1.3)
17-OHP	0.3 (0.05–0.6)	2.3[a] (0.7–4.2)	0.1 (0.03–0.4)	0.9[a] (0.5–1.6)
Estradiol (pg/ml)	47.6 (31.5–69.0)	48.0 (31.8–63.9)	45.5 (32.9–68.7)	45.4 (27.9–67.1)
Testosterone	0.04 (0.01–0.3)	0.06[a] (0.03–0.4)	0.05 (0.01–0.07)	0.05[a] (0.02–0.1)
Cortisol	1.03 (0.4–5.99)	9.78[a] (6.67–17.48)	1.55 (0.3–4.16)	9.96[a] (6.5–14.55)

[a] Indicates significant increase post-stimulation ($P < 0.05$).

between neutered males and females or intact males and females. For all groups, progesterone concentration significantly increased in response to ACTH stimulation (Tables 1 and 2).

Baseline estradiol concentrations were similar among the four groups and did not increase significantly after administration of ACTH (Fig. 6, Tables 1 and 2). Individual outliers existed in each group of dogs.

Baseline cortisol concentrations were similar among the four groups (Fig. 7A, Tables 1 and 2). Post-stimulation cortisol was significantly lower in the IM dogs ($P < 0.05$) (Fig. 7B). For all groups, cortisol concentration significantly increased in response to ACTH stimulation (Tables 1 and 2).

4. Discussion

Steroid hormones were determined for intact and neutered dogs of both sexes, before and after stimulation with ACTH. Determination of normality using statistical tests and observations of histograms is problematic [16]. Since the distribution of values for the majority of hormones were considered to be non-normally distributed, we chose to take a conservative approach by using non-parametric tests for statistical comparisons and summarizing our data with the median and percentiles. As expected, IM dogs had higher baseline concentrations of androstenedione, testosterone, and DHEAS than all other groups. Neither DHEAS nor testosterone concentrations increased significantly above baseline following ACTH stimulation. This differs from people in which DHEAS is secreted in response to ACTH [17]. One possible explanation is that DHEAS is secreted into a large plasma pool and has a long circulating half-life [17]; therefore, significant increases may be hard to detect. It is also possible that DHEAS secretion post-stimulation occurs either earlier or later than our 1-h sampling time [18]. Also, as expected, baseline and post-stimulation progesterone concentrations were highest in IF dogs, even while in anestrus, although baseline concentration was similar to that of IM dogs.
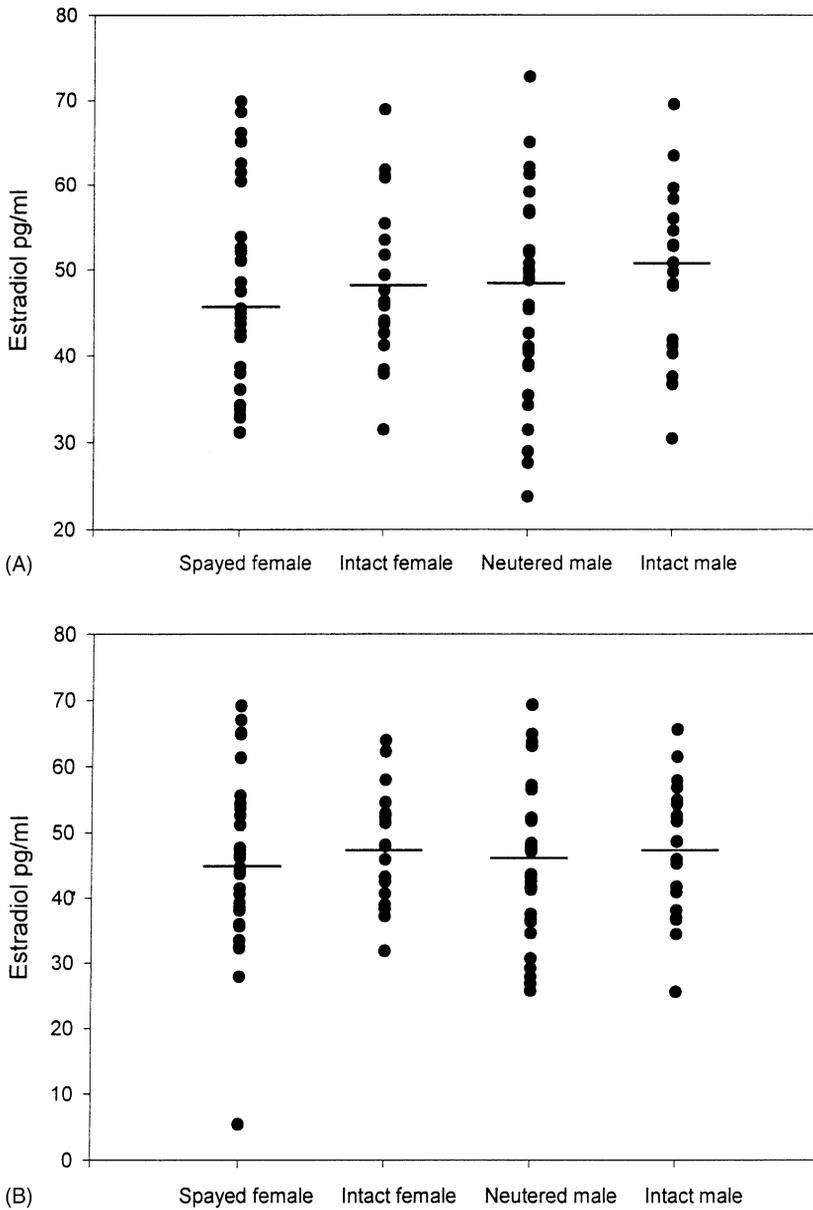
Fig. 6. Point plots of baseline (A) and post-ACTH stimulation (B) serum estradiol concentrations. See Fig. 1 for explanation.
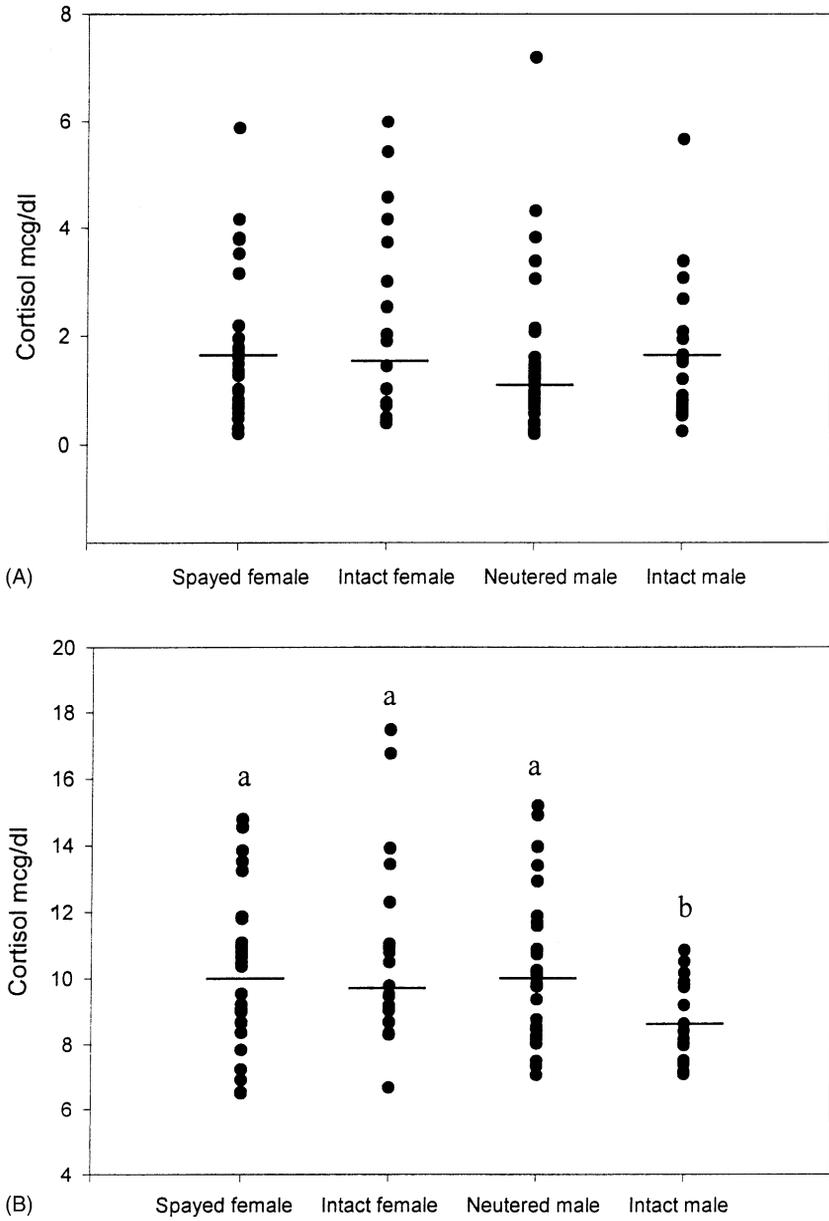
Fig. 7. Point plots of baseline (A) and post-ACTH stimulation (B) serum cortisol concentrations. See Fig. 1 for explanation.

Most laboratories establish their own normal reference ranges without publishing the data for peer review. With this in mind, there are conflicting reports in the literature as to the normal estradiol concentrations in dogs. The hormone concentrations published in one text book are those established by UTCVM endocrinology service [13]. Estradiol concentrations were greater in CM ($n = 9$) and SF ($n = 8$) dogs as compared to IM ($n = 10$) and IF ($n = 5$) dogs. In another published report, IF dogs ($n = 5$) had higher concentrations of estradiol as compared to IM dogs ($n = 5$); however, statistical comparison between the groups was not done [15]. One study of 7 IM and 6 CM beagles found IM to have a slightly lower concentration of estradiol than CM dogs; however, when multiple samples were taken from individual dogs over a 6-h period and 1 month later, a wide range of concentrations were found, ranging from 15 to 115 pg/ml in one dog [29]. Investigators in another study evaluated steroid hormones in 9 CM and SF control dogs and 12 dogs with recurrent flank alopecia during different photoperiods [19]. They demonstrated an increase in estradiol concentration during maximum photoperiod for both groups; however, no statistical evaluations were performed comparing the two photoperiods. These differences in the literature emphasize the difficulty in interpreting estradiol concentrations in the dog.

In the present study, estradiol concentrations were similar among intact and neutered male and female dogs. The source of estradiol is similar, regardless of neuter status, provided intact females are in anestrus. In these individuals estradiol is primarily produced from aromatization of androstenedione and testosterone within peripheral tissues [20,21]. Our results add to the variability of estradiol concentrations described in the literature. Based on our findings and those previously discussed, estradiol concentrations should not be used to classify a dog as to its sex or neuter status.

Only estradiol concentrations failed to increase in response to intravenous ACTH for all groups, and the increases seen for testosterone and DHEAS, while significant, were not substantial. This is not unexpected since both testosterone and estradiol can be produced in the adrenal glands by conversion of adrenal intermediates (e.g. androstenedione) via aromatase enzyme action or, more importantly, they can be produced from aromatization in extra-adrenal tissues such as fat, hair follicles, or liver [20,22]. In addition, testosterone can be converted from DHEAS which has a long circulating half-life [17].

One unexpected finding was the significantly lower concentration of cortisol post-ACTH stimulation in IM dogs. Previous studies determining cortisol concentrations in dogs do not differentiate between intact and neutered animals [23–26]. However, a recent study comparing the adrenocortical responses of bulls and steers found bulls to produce significantly less cortisol in response to ACTH than steers [27]. They theorized that this could be a result of direct modulation of steroidogenesis by androgens, altered negative feedback on the pituitary or hypothalamus, or an effect from higher centers. These results suggest that different normal values may be needed to diagnose hyperadrenocorticism in intact male dogs.

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