Serum $17\alpha$-hydroxyprogesterone concentrations during the reproductive cycle in healthy dogs and dogs with hyperadrenocorticism

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Objective—To determine concentrations of $17\alpha$-hydroxyprogesterone (17OHP) in serum of healthy bitches during various stages of the reproductive cycle and in bitches with hyperadrenocorticism and to compare the dynamics of 17OHP with those of progesterone.

Design—Prospective evaluation study.

Animals—15 healthy sexually intact bitches and 28 spayed bitches with hyperadrenocorticism.

Procedures—11 healthy bitches were evaluated during estrus, nonpregnant diestrus, and anestrus (group 1); 4 other healthy bitches were evaluated during pregnancy and after ovariohysterectomy (group 2). Cycle stages were determined via physical examination, vaginal cytologic evaluation, and serum progesterone concentration. Bitches with hyperadrenocorticism were evaluated at the time of diagnosis (group 3). Serum hormone concentrations were determined with immunoassays.

Results—In group 1, the serum 17OHP concentration was significantly higher in diestrus (median, 1.8 ng/mL) than in estrus (median, 1.1 ng/mL) and anestrus (median, 0.2 ng/mL) and higher in estrus than in anestrus. Changes in serum progesterone concentrations accounted for 22% (estrus) or 23% (diestrus) of the variation in serum 17OHP concentrations. In group 2, 17OHP and progesterone concentrations were significantly higher during pregnancy than after ovariohysterectomy. The serum 17OHP concentration in group 3 was significantly lower (median, 0.2 ng/mL) than in group 1 in estrus and diestrus and in group 2 during pregnancy (median, 0.7 ng/mL) but was not different from 17OHP concentrations in anestrus after ovariohysterectomy (median, 0.2 ng/mL).

Conclusions and Clinical Relevance—Serum 17OHP concentrations in healthy bitches increased during estrus, diestrus, and pregnancy and at those times were higher than in spayed bitches with hyperadrenocorticism. (J Am Vet Med Assoc 2010;236:1208–1214)

Naturally occurring hyperadrenocorticism is a recognized endocrine disorder in dogs characterized by a chronic excess in circulating cortisol concentration. Approximately 85% of affected dogs have hyperadrenocorticism caused by a pituitary gland tumor secreting excessive amounts of ACTH that, in turn, causes excess cortisol synthesis and secretion. Approximately 15% of dogs have an autonomously functioning adrenocortical tumor. Diagnosis of hyperadrenocorticism is based on typical clinical signs and clinicopathologic abnormalities and results of function tests of the hypothalamic–pituitary–adrenal axis and diagnostic imaging. Function tests of the hypothalamic–pituitary–adrenal axis (eg, ACTH stimulation test or LDDS test) evaluate serum cortisol concentrations. However, dogs can have clinical signs, physical examination findings, and clinicopathologic abnormalities suggestive of hyperadrenocorticism and still have serum cortisol concentrations within reference intervals after ACTH stimulation and after administration of a low dose of dexamethasone (atypical hyperadrenocorticism). One precursor of cortisol in the corticosteroid biosynthesis pathway is 17OHP. This steroid hormone is synthesized in the adrenal cortex and gonads. In dogs, serum or plasma 17OHP concentrations before or after ACTH stimulation may increase to exceed the upper reference limits in both typical and so-called atypical hyperadrenocorticism. Such measurements may be useful for the diagnosis of atypical hyperadrenocorti-
cism when cortisol-based test results are not diagnostic.\textsuperscript{11}–\textsuperscript{20} It has been hypothesized that some dogs with naturally occurring hyperadrenocorticism may have abnormalities solely in serum 17OHP concentrations, but it is not known whether 17OHP also contributes to the clinical signs of the disease.\textsuperscript{11}

17α-hydroxyprogesterone can be secreted by the ovaries\textsuperscript{11} and the adrenal glands in healthy female dogs.\textsuperscript{12} Serum progesterone concentrations in sexually intact female dogs undergo cyclic changes, increasing throughout estrus and during the first few weeks of diestrus before reaching a plateau for a few weeks and then decreasing. Progestosterone concentrations remain increased throughout diestrus, and increased concentrations are necessary to maintain pregnancy.\textsuperscript{13–16} These temporal changes are documented, predictable, and repeatable.\textsuperscript{14} Serum progesterone concentrations in bitches of various breeds and age groups are fairly uniform in proestrus, estrus, diestrus, and anestrus.\textsuperscript{13} Serum or plasma progesterone concentrations correlate with 17OHP concentrations during the luteal phase in women.\textsuperscript{17–19} and serum 17OHP concentrations in dogs may undergo similar changes. However, bitches in estrus and diestrus lack the typical clinical signs of hyperadrenocorticism (eg, polyuria, polydipsia,\textsuperscript{20} polyphagia, and hair loss), which would not support an appreciable glucocorticoid effect of 17OHP.

Serum concentrations of 17OHP in healthy sexually intact and neutered dogs before (baseline) and after ACTH stimulation have been determined with various radioimmunoassays.\textsuperscript{18–21} One research group specifically reported 17OHP concentrations at baseline in healthy anestrous bitches (median, 0.3 ng/mL; 5th to 95th percentile, 0.05 to 0.6 ng/mL),\textsuperscript{23} but the dynamics of 17OHP concentrations during the reproductive cycle in dogs have not been evaluated. We hypothesized that 17OHP concentrations in serum increase concurrently with the increase in progesterone concentrations in estrus and diestrus in healthy bitches. The objectives of the study reported here were to evaluate serum 17OHP concentrations during the reproductive cycle in healthy bitches and in bitches with hyperadrenocorticism to assess whether increases in baseline 17OHP concentrations exist during the cycle and how concentrations compare with those in bitches with hyperadrenocorticism. In addition, we sought to compare the dynamics of serum 17OHP concentrations during the female reproductive cycle in dogs with those of serum progesterone concentrations.

**Materials and Methods**

Dogs—The study included 15 healthy bitches (groups 1 and 2) and 28 bitches with naturally occurring hyperadrenocorticism (group 3) that were evaluated at the Veterinary Medical Teaching Hospital of the University of California-Davis from 2004 through 2007. All dogs were privately owned and were enrolled with the informed consent of their owners. The study was performed in compliance with institutional guidelines for research on animals.

All dogs in groups 1 and 2 were sexually intact adult females when enrolled. They were considered healthy on the basis of lack of clinical signs of disease (including clinical signs of hyperadrenocorticism) and unremarkable findings of physical examination. Results of serum biochemical analyses,\textsuperscript{22} CBCs,\textsuperscript{9} urinalyses, and urine cortisol-to-creatinine\textsuperscript{12} ratios were within reference intervals. The only treatments being administered were flea and heartworm preventives. None of the dogs had received glucocorticoids or other medications that could have affected reproductive or adrenal gland function within 6 months before beginning this study.

Dogs with naturally occurring hyperadrenocorticism (group 3) included 17 spayed bitches with PDH and 11 spayed bitches with ATH (9 with adrenocortical carcinomas and 2 with adrenocortical adenomas). None of the dogs in group 3 had been treated for hyperadrenocorticism prior to enrollment into the study. All had clinical signs, physical examination findings, and clinicopathologic abnormalities consistent with hyperadrenocorticism and abnormal results of at least 2 of 3 endocrine screening tests (ACTH stimulation test, LDDS test, or urine cortisol-to-creatinine ratio determination).\textsuperscript{12} Dogs with only 1 positive result of an LDDS or high-dose dexamethasone suppression test consistent with PDH, a high serum cortisol concentration after ACTH stimulation, or both. All also had 2 approximately equal-sized adrenal glands as identified via abdominal ultrasonography. Dogs with ATH had low or undetectable plasma concentrations of endogenous ACTH, ultrasonographic evidence of an adrenal gland mass, and histologic confirmation of an adrenocortical tumor following adrenalectomy.

**Study protocol**—Bitches in group 1 were evaluated during anestrus, estrus, and diestrus of typical nonbreed cycles. The stage of the reproductive cycle was determined from history and physical examination findings, vaginal cytologic findings, and serum progesterone concentrations. Anestrus was defined as a serum progesterone concentration < 1 ng/mL and absence of clinical signs of proestrus or estrus. Estrus was defined by physical examination findings and behavior consistent with estrus (eg, willing to breed), a serum progesterone concentration > 801 ng/mL, and > 80% vaginal superficial cells identified during cytologic evaluation. Diestrus was defined as the period of approximately 2 to 2.5 months after estrus, a serum progesterone concentration > 1 ng/mL, unwillingness to breed, and 80% to 100% vaginal parabasal and intermediate cells identified during cytologic evaluation. Blood samples for determination of 17OHP and progesterone concentrations were obtained in anestrus, estrus, and diestrus (between 2 weeks and 7.5 weeks after estrus had ceased).

Dogs in group 2 were evaluated twice: once during pregnancy and once after ovariohysterectomy. Blood samples for determination of 17OHP and progesterone concentrations from these 4 dogs were collected on days 31, 51, 58, and 59 of pregnancy (day 0 = day of the initial increase in serum progesterone concentration) and at 1, 3, 4, 5, and 10 weeks after ovariohysterectomy. Pregnancy was confirmed in each dog via ultrasonography.

In group 3, blood samples for determination of 17OHP, progesterone, and cortisol concentrations were obtained at baseline (before ACTH stimulation) at the time of diagnosis of hyperadrenocorticism.

**Hormone assays**—Serum was obtained for hormone assays within 60 minutes after blood collection and stored at...
–70°C (–94°F) until assayed. Standard LDDS tests,2 ACTH stimulation tests,24 or both were performed for measurement of serum cortisol concentrations for diagnostic purposes in dogs of group 3. Cortisol and 17OHP concentrations were determined in serum with radioimmunoassays22 validated for use in dogs.24 The sensitivity of the 17OHP assay in the present study was 0.02 ng/mL, determined at 95% binding of the standard curve. Serum progesterone concentration was determined by use of an enzyme immunoassay validated for use in dogs.25 All samples were analyzed in duplicate for each of the 3 hormones.

Intra-assay coefficients of variation of 4 progesterone assays performed for the study were 5.3%, 9.1%, 10.1%, and 11.7%. The interassay coefficient of variation was 9.2%. Although cross-reactivity of progesterone in the 17OHP radioimmunoassay was reportedly low (0.58% in human serum),4 independent assessments were made to verify cross-reactivity by addition of 5 known concentrations of progesterone to aliquots of serum from a castrated male dog (progesterone concentration, 0.2 ng/mL). After addition of progesterone, these 5 aliquots were assayed for 17OHP and revealed negligible effects of progesterone on measured 17OHP concentrations. Cross-reactivity of cortisol in the 17OHP assay was 0.024%.

Statistical analysis—Statistical software was used for all analyses.1 Data distributions were assessed with the Anderson-Darling normality test. Data in group 3 were not normally distributed; therefore, nonparametric tests were used and data are presented as medians and ranges. Friedman tests and Wilcoxon signed-rank tests were used for comparisons of hormone concentrations among multiple groups, the Kruskal-Wallis test was used for comparisons between 2 groups. For comparisons within groups, and Mann-Whitney tests were used for comparisons of hormone concentrations. Data in group 3 were not normally distributed; therefore, nonparametric tests were used and data are presented as medians and ranges. Friedman tests and Wilcoxon signed-rank tests were used and, when differences were significant (ie, \( P < 0.05 \)), horizontal bars represent median values.3 Different superscript letters indicate significant \( (P < 0.05) \) differences in 17OHP concentrations.

Results

Dogs—Group 1 included 11 healthy bitches of the following breeds: German Shepherd Dog (n = 4), Bernese Mountain Dog (2), Labrador Retriever (2), Golden Retriever (2), and Norwich Terrier (1). Median age of these dogs at the time of first blood sample collection was 4.0 years (range, 2.2 to 8.1 years). Four other healthy bitches were included in group 2, consisting of 2 Bernese Mountain Dogs, 1 Flat-Coated Retriever, and 1 Labrador Retriever ranging in age from 2.4 to 6.7 years. Breeds of group 3 dogs included mixed-breed dog (n = 16), Beagle (3), Dalmatian (2), and 1 dog from each of the following breeds: Labrador Retriever, Chow Chow, Shiba Inu, English Springer Spaniel, American Cocker Spaniel, Australian Cattle Dog, and Miniature Schnauzer. Median age of these dogs was 10.2 years (range, 4.1 to 14.1 years).

Hormone assays—In the healthy bitches in group 1, median serum 17OHP concentrations increased 5.5-fold from anestrus to estrus and 9-fold from anestrus to diestrus (Figure 1). Similarly, serum progesterone concentrations increased significantly from anestrus to estrus and diestrus and from estrus to diestrus (Table 1). The associations between serum 17OHP and progesterone concentrations in dogs of group 1 were analyzed separately for each cycle stage because data were not independent. During estrus and diestrus, there were positive albeit nonsignificant relationships between 17OHP and progesterone concentrations (\( r = 0.47 \) \( P = 0.14 \) and \( r = 0.48 \) \( P = 0.14 \), respectively; Figure 2). Simple regression analysis revealed that 22% of the variation in 17OHP concentrations in estrus and 23% of the variation in 17OHP concentrations in diestrus could be explained by serum progesterone concentrations. During anestrus, there was no association between serum 17OHP and progesterone concentrations (\( r = -0.03 \) \( P = 0.94 \)).

In the healthy bitches in group 2, serum 17OHP and progesterone concentrations were significantly higher

![Figure 1—Serum 17OHP concentrations in 11 healthy sexually intact female dogs during anestrus, estrus, and diestrus, and in 28 dogs with hyperadrenocorticism at baseline (HAC). Horizontal bars represent median values. *Different superscript letters indicate significant \( (P < 0.05) \) differences in 17OHP concentrations.](image-url)
during pregnancy than after ovariohysterectomy (Table 1). In these same dogs, a strong positive correlation \((r = 0.99 [P = 0.009])\) of the 4 data pairs of 17OHP and progesterone concentrations in dogs in estrus (A) and diestrus (B). Data for both hormones during anestrus are included for comparison. In estrus and diestrus, there were positive albeit nonsignificant relationships between serum progesterone concentrations and 17OHP concentrations as determined via linear regression \((r = 0.47 [P = 0.14])\) and \(r = 0.48 [P = 0.14]\), respectively. For the anestrus stage, the regression line was too short to be displayed \((r = -0.03; [P = 0.84])\).

![Figure 2](image)

**Discussion**

Results of the study reported here suggested that serum 17OHP concentrations in healthy sexually intact bitches are dependent on the stage of the reproductive cycle. In diestrus and estrus, 17OHP concentrations were greater than in anestrus and greater than in spayed bitches with hyperadrenocorticism. The data from individual dogs (Figure 1) indicated that there was little overlap in 17OHP concentrations of bitches with hyperadrenocorticism and bitches in diestrus or estrus. Only 2 of 28 (7%) bitches with hyperadrenocorticism had 17OHP concentrations that were higher than the lowest 17OHP concentration of dogs in diestrus, and 5 of 28 (18%) bitches with hyperadrenocorticism had 17OHP concentrations higher than the lowest 17OHP concentration of dogs in estrus or pregnancy.

In another study\(^1\) conducted by our research group in which the same 17OHP assay was used, the median serum 17OHP concentration at baseline in neutered dogs of both sexes and sexually intact male dogs with hyperadrenocorticism was 0.2 ng/mL (range, 0.04 to 3.9 ng/mL), which is similar to the median 17OHP concentration of the spayed female dogs with hyperadrenocorticism in the present study. In reports from other research groups using the same assay, median serum 17OHP concentrations in dogs with hyperadrenocorticism at baseline were 0.46 ng/mL (range, <0.33 to 2.34 ng/mL),\(^2\) 0.33 ng/mL (range, 0.03 to 8.8 ng/mL),\(^3\) and 1.1 ng/mL (range, 0.33 to 2.34 ng/mL).\(^4\) Those findings and the results of the present study indicate that median serum 17OHP concentration in estrus or nonpregnant diestrus in healthy bitches is higher than the median 17OHP concentration of most dogs with hyperadrenocorticism at baseline. In other words, far more than half of dogs with hyperadrenocorticism\(^5\) have a lower 17OHP concentration at baseline than the physiologic serum 17OHP concentration in healthy bitches during estrus or diestrus. The present study included a proportionally larger number of dogs with ATH than the typical approximately 10% to 15% of the general population of dogs with hyperadrenocorticism. Serum 17OHP concentrations in almost all dogs with hyperadrenocorticism were greater than in anestrus and greater than in spayed dogs with hyperadrenocorticism and bitches in diestrus or estrus. Despite the increased serum 17OHP concentrations in estrus and diestrus, none of the dogs in groups 1 or

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The key enzyme 3β-HSD has been localized in ovarian follicles and corpora lutea of dogs. Plasma or serum 17OHP concentrations in healthy dogs increase during sexual maturation and are higher in sexually intact than in neutered adults (sexually intact females evaluated in anestrus). Given these findings, we expected even larger differences in serum 17OHP concentrations between pregnant and ovariohysterectomized dogs in group 2. It was our objective to compare hormone concentrations in that group at 1 point in time during pregnancy with 1 point in time after each dog underwent ovariohysterectomy. Each dog served as its own control animal, and data were compared between pregnancy and after ovariohysterectomy, not over time or with other dogs in the group. Dogs were evaluated 1 to 10 weeks after ovariohysterectomy. It may have been ideal to have obtained postovariohysterectomy blood samples from each dog on the same day after ovariohysterectomy. However, once reproductive organs are removed, gonadal hormones should not be detectable in the circulation, independent of the time of sample collection (after a short washout period).

In another study involving pregnant bitches, serum progesterone concentration was > 15 ng/mL. Ovariectomy on gestation day 28 or 35 resulted in a decrease of serum progesterone concentration to undetectable values within a few days. In a different study, progesterone washout was examined in anestrous bitches that received exogenous progesterone up to a peak serum concentration comparable to that in pregnancy or diestrus (mean, 34 ng/mL). By 72 hours after administration, the mean serum progesterone concentration was 0.9 ng/mL, which was comparable to progesterone concentrations prior to injection. The mean half-life of elimination was 12.1 hours (range, 9.5 to 13.8 hours). Interestingly, serum progesterone concentrations in the present study were greater in sexually intact healthy bitches in anestrus than in spayed healthy bitches, although this difference was not significant. Furthermore, serum progesterone concentrations were significantly greater in healthy bitches in anestrus than in spayed bitches with hyperadrenocorticism. These data may have indicated that the presence of ovaries contributes to circulating progesterone concentrations even in periods of reproductive inactivity.

The ovaries are an established source of progesterone and 17OHP in serum. However, in dogs, there are no known seasonal or environmental effects on serum progesterone or 17OHP concentrations. Blood samples in the present study were collected throughout the year, usually in the early afternoon in each of the 12 months. Time of sample collection in healthy dogs was determined by the bitches’ cycle stage, regardless of season. The relationship of serum 17OHP with progesterone concentrations in the present study further supported a common ovarian origin. In healthy bitches, ovarian progesterone secretion increases after the LH surge in late proestrus and estrus. Plasma progesterone concentration reaches a maximum on day 25 after the LH peak and decreases after day 30. In pregnant cycles, it declines to < 1 ng/mL at parturition, whereas the decrease is more variable (31 to 82 days after the LH peak) in nonpregnant cycles. This may explain the greater median progesterone and...
Serum or plasma 17OHP concentrations higher than the upper reference limit at baseline and after ACTH stimulation have been documented in multiple reports of dogs with hyperadrenocorticism. Results of the present study established that the serum 17OHP concentration of healthy bitches is dependent on the stage of the reproductive cycle and is significantly higher in estrus and diestrus than in anestrus. These physiologic increases were far greater than those in bitches with hyperadrenocorticism at baseline and were not associated with clinical signs of hyperadrenocorticism.

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From this month’s AJVR

Evaluation of the relationship between lesions in the gastroduodenal region and cyclooxygenase expression in clinically normal dogs

Jenna G. Wooten et al

Objective—To determine whether clinically normal dogs have lesions in the pylorus and duodenum and to examine the expression of cyclooxygenase (COX) isoforms in the pylorus and duodenum of these dogs.

Animals—27 clinically normal dogs.

Procedures—Physical examination was performed on clinically normal dogs from animal shelters and research projects; the dogs were then euthanized. After the dogs were euthanized, the pylorus and duodenum were photographed and scored for gross appearance of lesions. Samples were obtained for histologic evaluation and determination of COX expression via western blot analyses. Tissues from the pylorus and duodenum were categorized as normal, inflamed, or eroded on the basis of histologic analysis. Each histologic category of tissue was then evaluated to determine the correlation with gross appearance and COX expression.

Results—Of the 27 dogs, 5 had unremarkable histologic findings in the pylorus and duodenum. Inflammation was found in the pylorus of 10 dogs and in the duodenum of 5 dogs. Epithelial erosion was detected in the pylorus of 1 dog and in the duodenum of 3 dogs. Gross appearance was not significantly correlated with histologic appearance. Expression of COX-1 was not upregulated by inflammation, whereas COX-2 expression was increased by inflammation or erosion.

Conclusions and Clinical Relevance—Dogs that appear to be clinically normal may have underlying gastroduodenal lesions associated with upregulation of COX-2. Because of the inability to determine this during routine physical examination, practitioners should be aware of this potential situation when prescribing COX inhibitors. (Am J Vet Res 2010;71:630–635)