

# **STEROID PROFILES IN THE DIAGNOSIS OF CANINE ADRENAL DISORDERS**

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## **INTRODUCTION**

Diagnosis of adrenal disease in domestic animals usually is dependent on the manipulation of the hypothalamo-pituitary-adrenal axis (HPA) and the measurement of cortisol (i.e., ACTH stim test; low dose dexamethasone suppression (LDDS) test; urine cortisol/creatinine ratio test; or the combined dexamethasone suppression/ACTH stimulation test). More recently, other steroid measurements have been utilized to evaluate the HPA, including steroid hormone profiles<sup>1,2</sup>, and 17-hydroxyprogesterone,<sup>3-5</sup> which have revealed that suspected adrenal disease conditions may be caused by steroids other than cortisol (or in addition to cortisol).<sup>3</sup> Determination of pituitary-dependent hyperadrenocorticism (PDH), or adrenal-dependent hyperadrenocorticism (ADH), is now usually made by evaluation of the 4-hour timepoint of the LDDS test<sup>6</sup>, by endogenous ACTH measurement,<sup>7</sup> or by ultrasound visualization of the adrenal glands.<sup>7,8</sup> Hyperadrenocorticism (HAC) is defined as an overproduction of steroid hormones by the adrenal cortex.<sup>4</sup> Cushing's syndrome refers to all causes of hyperadrenocorticism with excess production of cortisol,<sup>6</sup> while atypical Cushing's disease refers to hyperadrenocorticism caused by increased levels of intermediate adrenal steroids that frequently are referred to as "sex steroids".<sup>9</sup>

## **STEROID HORMONE PROFILES/GENERAL**

Steroid hormone profiling in veterinary medicine was begun at The University of Tennessee Clinical Endocrinology Service, with the premise being that multiple steroid hormone analyses would increase the diagnostic accuracy of adrenal function tests.<sup>1</sup> Measurement of multiple steroids in Pomeranians<sup>2</sup> led to the recognition of a syndrome called "Alopecia-X"<sup>11</sup> by dermatologists. Others have reported on adrenal syndromes in dogs called "atypical Cushing's disease",<sup>3,9</sup> or "adrenal hyperplasia-like syndrome",<sup>9,10-13</sup> that used steroid profiling. Cortisol is known to have negative control effect on the HPA axis, but it's now also understood that other steroids can have this effect as well.<sup>9,14,15</sup> Steroid profiling in dogs and cats has led to the realization that HAC can be due to primary adrenal tumors that secrete other steroids besides cortisol.<sup>16-21</sup> Steroid profiling in ferrets led to the realization that HAC in this species is primarily due to increased levels in blood of estradiol, 17-hydroxyprogesterone and/or androstenedione,<sup>9,22</sup> and measurement of these steroids has helped define medical control of ferret adrenal disease.<sup>23-27</sup> Steroid profiles have also helped to better understand the condition of SARDS in dogs, where steroids other than cortisol frequently are involved.<sup>28</sup> Steroid profiling is also helping to understand drug effects on adrenal secretory activity (mitotane, trilostane, melatonin).<sup>29-31</sup>

## **STEROID HORMONE PROFILES/SPECIFIC**

Steroid hormone profiles are indicated when other routine tests of adrenal function are negative (ACTH stim; LDDS; combined dexamethasone suppression/ACTH stim) and the dog still exhibits signs of Cushing's syndrome, indicating the likelihood of atypical Cushing's disease being present.<sup>3,9</sup> The issue of non-adrenal illness has been raised as a possible consideration in atypical Cushing's disease cases.<sup>21</sup> Results of studies in dogs with chronic illness, but without clinical evidence of HAC, have shown that 17-hydroxyprogesterone (17OHP) concentration may be increased.<sup>21</sup> However, results of other studies of adrenal function testing in dogs with non-adrenal illness have demonstrated only minor effects on test results.<sup>32,33</sup> Also, in studies that have measured only 17OHP as a means of detecting HAC, the sensitivity and specificity of using post-ACTH 17OHP concentration as a diagnostic test for HAC were low, and post-ACTH 17OHP analysis was not recommended as a screening test for HAC.<sup>4</sup> These studies provide evidence that measurement of a singular adrenal intermediate steroid (such as 17OHP) may give equivocal results, but when profiles of steroid intermediates are used, the sensitivity and specificity of the test procedure is much improved.<sup>29</sup> It has been emphasized that adrenal function testing should be performed in dogs with clinical and/or biochemical evidence of HAC, and not in dogs with non-adrenal related disease.<sup>6</sup>

Steroids that may be involved with atypical Cushing's disease are androstenedione, estradiol, 17-hydroxyprogesterone, progesterone and aldosterone.<sup>9</sup> Other steroids that aren't commonly measured may be involved as well, such as corticosterone<sup>21</sup> and deoxycortisone.<sup>16</sup> Estradiol is unique because treatment of excess estradiol can be difficult, the hormone can be secreted by tissues other than the gonads or adrenals<sup>35-38</sup> and because secretion is independent of ACTH stimulation or dexamethasone suppression testing, as currently done. For dogs with atypical Cushing's disease (PDH etiology), expect hepatomegaly, hepatopathy and bilateral adrenomegaly to be present along with increased endogenous ACTH level and the usual clinical signs, bloodwork and often haircoat problems. For dogs with atypical Cushing's disease (ADH etiology), expect hepatomegaly, hepatopathy and unilateral adrenomegaly to be present (and maybe atrophy of contra-lateral gland) along with decreased endogenous ACTH level and the usual clinical signs, bloodwork and often haircoat problems. For primary hyperaldosteronism conditions, due to primary adrenal tumor or bilateral adrenal hyperplasia, expect hypertension in association with hypernatremia and muscular weakness (cervical ventroflexion, hindlimb weakness) due to hypokalemia.<sup>39</sup> Retinal hemorrhage and blindness<sup>40</sup> and renal disease<sup>41</sup> can occur in cats. For hyperadrenocorticoid cases that also have low aldosterone levels, this pattern can be indicative of a primary adrenal tumor, and ultrasound is indicated to confirm a tumor's presence or absence.<sup>29</sup>

## **Treatment Implications**

Primary adrenal tumors. Adrenal steroid profiles reveal that adrenal tumors in dogs, cats and ferrets have a variety of secretory patterns, with serum cortisol levels often being normal.<sup>19,17,18-22,29,39</sup> Similar findings have been reported in humans with adrenoadenomas.<sup>42</sup> In ferrets, mice, rats, guinea pigs and hamsters, sex steroid-producing adrenocortical tumors occur following gonadectomy, in association with the significant increase in serum gonadotropin levels that develop.<sup>43-47</sup> The elevated luteinizing hormone (LH) level that occurs following gonadectomy leads to neoplastic transformation and expression of LH hormone receptors on sex steroid-producing adrenocortical cells in ferrets<sup>46,47</sup> and rodents.<sup>48</sup> Also, in humans, there is evidence of stimulatory effects of LH on adrenocortical cell growth and function,<sup>49</sup> and LH receptor protein has been identified in the zona reticularis layer of the adrenal gland by immunohistochemical staining.<sup>50</sup> In spayed female dogs, plasma gonadotropin levels post-gonadectomy rise to levels ten times what they were pre-gonadectomy, providing evidence of the strong and continuous LH stimulus that possibly plays a role in adrenocortical tumor development.<sup>51,52</sup> Evidence is accumulating in human studies that some cortisol and other steroid-producing adrenal tumors or hyperplasias are under the control of ectopic or aberrant hormone receptors (e.g., gastrointestinal peptide, beta-adrenergic, vasopressin, serotonin and angiotensin II), and that these receptors may provide alternative mechanisms for pharmacologic control of adrenal tumors.<sup>53,54</sup> Control of the secretory activity of adrenal tumors with beta-adrenergic and LH receptors has now been demonstrated by use of beta-receptor antagonists (propranolol)<sup>53</sup> and gonadotropin antagonists such as leuprolide and deslorelin.<sup>23,24</sup> Surgical removal of adrenal tumors is usually indicated, but age and health considerations impact this decision. If surgery is not an option, then mitotane is usually the next consideration. Adrenal profiles are indicated to determine the functionality of adrenal tumors in light of the multiple hormone secretion patterns that are seen.

Mitotane. Adrenal hormone profiles reveal that most intermediate hormones are decreased by mitotane the same as for cortisol, but that estradiol may remain unaffected. In cases that continue to have elevated estradiol levels, varying clinical signs of Cushing's disease will be present.<sup>29</sup>

Trilostane. Enzyme inhibition by trilostane occurs for 3-beta hydroxysteroid dehydrogenase, but also for 11-beta hydroxylase.<sup>30</sup> Thus, 11-deoxycortisol levels build-up in dogs treated with trilostane. It is also apparent that other intermediate steroid levels increase (androstenedione, 17-hydroxyprogesterone, estradiol and progesterone) in dogs treated with trilostane,<sup>29</sup> which could be due to the 11-beta hydroxylase inhibition, and possibly 21-hydroxylase enzyme inhibition.<sup>29</sup> The reason why only 11-deoxycortisol levels were increased in the above study<sup>30</sup> may be due to the length of trilostane exposure (3-7 weeks), compared to dogs that are exposed to trilostane for extended periods. Trilostane reportedly offers effective control of Cushing's syndrome,<sup>30</sup> but the long-term effects of the elevated intermediate steroids remain ill-defined. Some dogs do have return of clinical signs of Cushing's syndrome while on trilostane.<sup>29</sup> Because trilostane seems to pre-

dispose dogs to increased adrenal toxicity with mitotane, an acute switch from trilostane to mitotane treatment should not be done.<sup>29</sup>

Aromatase enzyme inhibitors (anastrozole, exemestane, melatonin). The aromatase enzyme occurs in gonadal and adrenal tissues (and other tissues such as fat and skin cells), and converts androstenedione to testosterone or estrone, both of which are then converted to estradiol. Neither estrone nor testosterone have been observed to be increased in dogs with adrenal disease, but estradiol frequently is increased, and causes many of the clinical signs associated with Cushing's disease.<sup>29</sup> Aromatase enzyme-inhibiting drugs will decrease estradiol levels, but currently are infrequently used (except melatonin) in animals due to cost considerations.

Anti-gonadotropin drugs (melatonin, leuprolide acetate, deslorelin acetate, androgens). Adrenal tissues in different species (e.g., ferrets, rodents, humans) are known to have luteinizing hormone (LH) receptors present.<sup>44-50</sup> In ferret studies, anti-gonadotropin drugs are effective in lowering sex steroid levels.<sup>23-24,27</sup> Sex steroid levels are also decreased in dogs with adrenal disease that are treated with melatonin,<sup>31</sup> but it is not known if LH receptors are present in canine adrenal tissues. Androgenic drugs have anti-gonadotropin effects via negative feedback effects on the hypothalamo-pituitary tissues.

Melatonin. Results of *in vitro* cell culture (human H295R adrenocortical carcinoma cells) studies in our lab<sup>55</sup> revealed that both 21-hydroxylase and aromatase enzymes were inhibited by melatonin. Also, in dogs with adrenal disease that are treated with melatonin, and repeat adrenal steroid panels are done, cortisol levels are consistently reduced, and estradiol levels are variably reduced.<sup>29</sup> Inhibition of the 21-hydroxylase enzyme would lower cortisol levels, and inhibition of the aromatase enzyme would lower estradiol levels. Estradiol levels were decreased in a prior study of dogs treated with melatonin.<sup>31</sup> Results of *in vitro* studies with human MCF-7 breast cancer cells also revealed that melatonin inhibited aromatase enzyme, which resulted in reduced estradiol levels.<sup>56</sup> Melatonin treatment for cases of mild adrenal disease in dogs may be effective, and particularly in cases where sex steroids are increased.

Melatonin plus phytoestrogens. Melatonin has the above listed effects, and phytoestrogens (isoflavones, lignans, genistein) are known to inhibit 3-beta hydroxysteroid dehydrogenase.<sup>57,58</sup> Lignans and genistein are also known to decrease the activity of aromatase enzyme in MCF-7 cells *in vitro*.<sup>58</sup> So, combinations of melatonin and phytoestrogens may have efficacy in treating hyperestrinism conditions.

## **Hyperestrinism in Dogs**

Hyperestrinism in dogs may be a new and emerging disease entity. In sample submissions to the Clinical Endocrinology Service (2005) at The University of Tennessee, 40% of adrenal panels had elevated estradiol levels present (>70 pg/ml).<sup>29</sup> In hyperestrinism cases, estradiol is the estrogen that is increased, ACTH stim and LDDS tests are usually normal for cortisol, thyroid function is normal or controlled, liver problems are frequent and typical (very elevated alkaline phosphatase, hepatomegaly, steroid hepatopathy, hyperechoic liver by ultrasound), PU/PD is frequent, panting may be present, haircoat problems often are present, skin biopsy results suggest an endocrinopathy, there is no change in estradiol level in response to ACTH stim or LDDS tests as currently conducted, resistance to mitotane may occur and increase often occurs in response to trilostane. Effective treatment options for hyperestrinism in dogs is limited at the present time, and drugs that could be expected to be efficacious (aromatase inhibitors – excluding melatonin) often are limiting due to cost. Melatonin and phytoestrogen treatment may be effective for the above listed reasons. Mitotane will likely be effective if the source of estradiol is the adrenal tissues. Trilostane treatment frequently results in increased estradiol levels,<sup>29</sup> and this may be a reason why less than effective treatment with the drug sometimes occurs.

## **CONCLUSIONS**

Steroid hormone profiles in dogs are indicated when hyperadrenocorticism is suspected due to typical clinical and/or biochemical signs of disease, but the usual tests of adrenal function have been normal. The profiles are effective in ruling out presence of atypical Cushing's disease, cases of hyperestrinism and for

delineating the secretory profile of adrenal tumors (functionality), which often are associated with elevated levels of steroids other than cortisol.

## KEY WORDS

Hormone, Tumor, Endocrine, Atypical, Hyperadrenocorticism

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