

## DIAGNOSTIC LABORATORY INSIGHT WITH REGARD TO ADRENAL DISEASE

Dr. Jack W. Oliver, D.V.M., Ph.D. Knoxville, TN  
(20<sup>th</sup> ACVIM Forum Proceedings, Dallas, TX. Pp. 541-543, 2002)

### INTRODUCTION

In recent years, adrenal-associated endocrinopathies in domestic animals have received much attention. For the typical case of hyperadrenocorticism, with increased plasma cortisol levels, diagnosis and treatment of the disease is routine.<sup>1,2</sup> In most dogs and cats with hyperadrenocorticism, bilateral adrenocortical hyperplasia exists in response to excessive production of ACTH from the pituitary gland. Excessive cortisol production in dogs and cats due to a functional adrenal neoplasm will approximate 15-20% and 20-25% of cases, respectively.<sup>1</sup> Although relatively uncommon in cats, hyperadrenocorticism due to adrenal tumors has been well described.<sup>1,5-7</sup> For ferrets, adrenal disease is mainly an adrenal-dependent disorder,<sup>6</sup> and high cortisol secretion is uncommon; rather increased levels of estradiol, androstenedione and 17-hydroxyprogesterone are present, alone or more commonly in combination.<sup>7</sup> Recently, concurrent presence of both pituitary and adrenal tumors in dogs with hyperadrenocorticism has been described.<sup>10,11</sup>

The purpose of this report is to describe diagnostic findings in dogs, cats and ferrets with adrenal disease where adrenal steroid profiling has been performed. These tests were conducted in dogs and cats suspected of having Cushing's syndrome, but where aberrant forms of the disease were present (i.e., the usual adrenal function tests were negative in individuals with variable clinical signs suggestive of Cushing's syndrome). Steroid profiles for ferrets determine suspected adrenal tumor presence.

### REVIEW OF LITERATURE.

Aberrant adrenocortical disease (ACD) in dogs, cats and ferrets is similar to congenital adrenal hyperplasia disorders in humans, where large amounts of adrenal intermediates are secreted but cortisol usually remains normal or is subnormal. Congenital adrenal hyperplasia syndromes in man typically are caused by inherited defects in adrenal steroidogenesis, with decreased negative feedback inhibition of cortisol or pituitary adrenocorticotrophin (ACTH) secretion.<sup>10</sup> With deficient cortisol synthesis and excessive ACTH stimulus, excessive adrenal precursor steroids accumulate, which are converted to adrenal androgens.<sup>10,11</sup> Deficiency of 21-hydroxylase enzyme activity accounts for the majority of cases in humans, with a lesser percentage due to a deficiency of 11 $\beta$ -hydroxylase. In a recent case report of 2 dogs with hyperadrenocorticism caused by adrenal tumors, plasma cortisol concentrations were below normal reference range before and following ACTH stimulation, while levels of progesterone and 17-hydroxyprogesterone (17-OHP) were increased.<sup>12</sup> Low endogenous levels of plasma ACTH were also present.<sup>12</sup> Progestins have been shown to have intrinsic glucocorticoid activity in dogs,<sup>13</sup> which results in suppression of the hypothalamic-pituitary-adrenal (HPA) axis;<sup>14</sup> a situation that appeared to occur in the case report above.<sup>12</sup> Typically, the progestin intermediates of the adrenal glands are converted to other steroids and do not gain access to the circulation. However, in adrenal tumors of dogs,<sup>12</sup> cats<sup>3,4</sup> and ferrets,<sup>7</sup> one or both of these steroids can be elevated in the plasma. The progestins have also been shown to be elevated in pituitary-dependent hypercortisolemia cases.<sup>15,16</sup> In the latter situation, the cortisol levels presumably remain elevated in spite of the high cortisol and progestin levels due to an autonomous, functional tumor of the hypothalamic or pituitary tissues. High serum level of progesterone in cats with adrenal tumors have been associated with suppression of the HPA and a subnormal cortisol response to ACTH stimulation.<sup>3,4</sup> Suppression of the HPA is also known to occur with estradiol.<sup>17</sup> Clinical signs are similar in cats with high progesterone levels to those with hypercortisolemia, including polyuria/polydypsia, diabetes mellitus, temperament changes, cutaneous atrophy and alopecia.<sup>3,4</sup> In ferrets, where adrenal tumors are associated with high plasma levels of the adrenal intermediates androstenedione and 17-hydroxyprogesterone (17-OHP), as well as estradiol, clinical signs include the occurrence of alopecia, return to male sexual behavior as well as swollen vulva or prostatic hyperplasia.<sup>6,18</sup> Adrenal disease in ferrets is also thought to be associated with a higher incidence of diabetes mellitus, similar to that with cats.

The high prevalence of ACD in pet ferrets in the U.S.A. is associated with the practice of spaying and neutering at an early age.<sup>7,19</sup> Early gonadectomy is known to be associated with increased gonadotrophin levels (i.e., luteinizing hormone {LH} and follicle stimulating hormone {FSH}) in ferrets and other species,<sup>20-22</sup> with possible stimulus to gonadal rest cells embedded in the adrenal glands of ferrets.<sup>7,20</sup> In ovario-hysterectomized ferrets, plasma LH and FSH levels are increased,<sup>20,23</sup> and serum LH and FSH concentrations in spayed and neutered dogs are 10-fold above those of intact animals.<sup>24</sup> Interestingly, the reports of cats with elevated intermediate steroid hormone levels, associated with adrenal tumors, were both neutered males.<sup>3,4</sup> However, the adrenal hyperplasia-like syndrome (Alopecia-X) in dogs,<sup>25-28</sup> where serum adrenal steroid intermediates are elevated, certainly occurs in both intact and neutered individuals.<sup>25,28</sup> But, the majority of dogs submitted to our Service (University of Tennessee, Clinical Endocrinology) have been spayed or neutered to eliminate difficulty with interpretation of results due to presence of similar secretory tissues (adrenals/gonads). Luteinizing hormone receptors are known to be present in adrenal tumor tissues from ferrets,<sup>21</sup> and hypersecretion of pituitary gonadotrophins leads to estrogen-secreting adrenal tumors in neonatal-gonadectomized (but not adult-gonadectomized) rodents.<sup>20</sup> In humans, isolated adrenal tumor cells can secrete high levels of estrogen,<sup>29</sup> and testing of adrenal vein effluent from a patient with an adrenal tumor (ACTH responsive) revealed high levels of 17 $\beta$ -estradiol.<sup>30</sup> In dogs, cannulation studies revealed that both androgens and estrogens were secreted by the adrenal.<sup>31</sup> Follicle stimulating hormone stimulates aromatase enzyme activity in gonadal cells, leading to conversion of testosterone and steroid intermediates (i.e., androstenedione) into estrogen.<sup>11,32,33</sup> Luteinizing hormone stimulates enzymatic activity in gonadal tissues,<sup>33</sup> with resultant increase in androstenedione, and similar effect may occur in ACD of ferrets, cats and dogs. Anti-gonadotrophic drug treatment (leuprolide) is effective in reversing clinical signs of ACD in ferrets,<sup>21</sup> which implicates LH and FSH as causative factors in the disease. Currently, interest is developing for the use of anti-gonadotrophic agents like leuprolide to treat adrenal disease in animals,<sup>21,27,34</sup> with efficacy probably limited to adrenal tumors (or

combined pituitary-dependent/adrenal-dependent cases). Another agent that is currently used in the treatment of Alopecia-X with moderate success is melatonin.<sup>35</sup> Lower levels of melatonin are present during long photoperiods, and less melatonin is associated with increased gonadotrophin release. In the cat, long photoperiods result in ovarian follicle development and estrogen production, while lengthening the period of darkness causes increased melatonin release and ovarian inactivity.<sup>36</sup> Because of the association of melatonin with seasonal reproductive activity in ferrets and cats, with increased levels able to inhibit steroidogenesis,<sup>36,37</sup> the hormone probably will have efficacy for ACD in ferrets as experience with its use develops.<sup>38</sup> Similarly, melatonin may have efficacy for aberrant forms of ACD in dogs and cats with appropriate clinical study of effect. Certain other drugs may be useful for the aberrant forms of ACD, such as the aromatase enzyme inhibiting drug anastrozole. Indeed, the limitation with all of the aforementioned drugs for possible use in ACD of ferrets, and aberrant forms of ACD in dogs and cats, is appropriate clinical research studies (with determination of pharmacokinetic parameters) for guidance in appropriate dosage regimens.

## DISCUSSION

Aberrant forms of ACD occur in dogs and cats (i.e., normal or subnormal cortisol response to the usual adrenal function tests), and ACD in ferrets typically involves elevation of three steroids (androstenedione, 17-OHP, estradiol), with cortisol being normal. The response varies somewhat between the three species, with the ferret adrenal steroid profile being the most well defined. For ferrets, abnormally elevated steroid hormone levels in serum samples from ferrets suspected of having ACD (530 cases) were as follows: estradiol, 73%; androstenedione, 66%; 17-OHP, 51%; dihydroepiandrosterone sulfate (DHEAS), 18%; and cortisol, 8%. Basal serum samples are all that's needed for ferret assays. For dogs, the testing results are more variable, but it is common to have elevation of progesterone and 17-OHP. Increase in androstenedione and estradiol occur frequently, but less so than for progesterone and 17-OHP. DHEAS elevation occurs less frequently than the above four hormones, similar to what is seen in ferrets. Testosterone is increased only rarely. For dogs, it is most meaningful to perform an ACTH stimulation test so that comparisons of basal vs. stimulated hormone concentrations can be made. Usually the stimulated values give the most information. There is frequent evidence that feedback relationships occur similar to the study of Syme et al.<sup>12</sup>, where elevated progestins or estradiol can result in subnormal cortisol response. There also is frequent evidence that high levels of estradiol suppress other steroid levels, presumably by negative feedback effects on gonadotrophin and/or ACTH release. For cats, infrequent samples are received for testing, but two recent case reports<sup>3,4</sup> implicate progesterone as being increased along with adrenal androgens and testosterone. Regarding treatments for ferrets, adrenal tumors are commonly removed by surgery, with perhaps 15-20% requiring follow-up surgery within several months for involvement of contra-lateral gland.<sup>18</sup> For medical treatment, good success has been achieved with the anti-gonadotrophin drug leuprolide acetate (Lupron™), although pharmacokinetic studies have not been done and dosage is empirical. Limited success has been achieved with melatonin treatment of ferrets.<sup>38</sup> For dogs, the aberrant forms of ACD can also be treated surgically (depending on risk) when ultrasound studies reveal a unilaterally enlarged gland. For medical treatment, melatonin is frequently used initially because it is cheap and relatively innocuous to dogs. Dogs with aberrant forms of ACD usually respond to mitotane, with high estradiol being the most difficult to control. Increased estradiol levels are sometimes quite resistant to mitotane treatment. For cats, the aberrant forms of ACD are probably best treated surgically where possible, or with mitotane.

In summary, ACD in ferrets may be related to early spay/neuter, chronic stimulation of adrenal tissues by gonadotrophins, resultant development of adrenal neoplasia and abnormal secretion of three specific hormones. For dogs and cats, the aberrant forms of ACD are less well defined. Defects in enzymatic function in dogs, similar to that in humans, may be a reason (e.g., 21-hydroxylase dysfunction). Not enough cases have occurred in cats to provide much detail of etiology. For dogs and cats, where clinical signs and clinical chemistry changes are consistent with Cushing's syndrome, but results of adrenal function tests (cortisol levels) are normal, consideration should be given to evaluating the status of adrenal steroid intermediates before and after ACTH stimulus. Assessment of additional medical treatments besides mitotane are also needed, such as melatonin, the anti-gonadotrophic drugs like leuprolide and the newer drugs that are being pursued for treatment of cancer conditions of reproductive structures in humans.

## REFERENCES.

1. Feldman, E.C. and R.W. Nelson (1996). "Hyperadrenocorticism (Cushing's Syndrome)." In: Canine and Feline Endocrinology and Reproduction, 2<sup>nd</sup> Ed. W. B. Saunders Co., Phil., PA. Pp 187-265.
2. Kempainen, R. and E. Behrend (2000). "CVT Update: Interpretation of endocrine diagnostic test results for adrenal and thyroid disease." In: Kirk's Current Veterinary Therapy XIII, SA Practice. W.B. Saunders Co., Phil., PA. Pp. 321-324.
3. Boord, M. and C. Griffin (1999). "Progesterone secreting adrenal mass in a cat with clinical signs of hyperadrenocorticism." *JAVMA*, 214:666-669.
4. Rossmelsl, J., Jr., J. Scott-Moncrieff, J. Siems, P. Snyder, A. Wells, L. Anothayanontha and J. Oliver (2000). "Hyperadrenocorticism and hyperprogesteronemia in a cat with an adrenocortical adenocarcinoma". *JAAHA*, 36:512-517.
5. Schaer, M. and P.E. Ginn (1999). "Iatrogenic Cushing's syndrome and steroid hepatopathy in a cat". *JAAHA*, 35:48-51.
6. Rosenthal, K.L. and M.E. Peterson (2000). "Hyperadrenocorticism in the Ferret." In: Kirk's Current Veterinary Therapy XIII, Small Animal Practice. W.B. Saunders Co., Philadelphia, PA. Pp. 372-374.
7. Rosenthal, K.L. and M.E. Peterson (1996). "Evaluation of plasma androgen and estrogen concentrations in ferrets with hyperadrenocorticism." *JAVMA*, 209:1097-1102.
8. Peterson, M.E. (2001). "Concurrent pituitary and adrenal tumors in dogs with Cushing's syndrome." Proc. 19<sup>th</sup> ACVIM Meeting, Denver, CO. #781.
9. Greco, D.S., M.E. Peterson, A.P. Davidson, E.C. Feldman, and K. Komurek (1999). "Concurrent pituitary and adrenal tumors in dogs with hyperadrenocorticism: 17 cases (1978-1995)." *JAVMA*, 214:1349-1353.

10. Orth, D.N. and W.J. Kovacs. (1995). "The adrenal cortex." In: Williams Textbook of Endocrinology, 9<sup>th</sup> Edition. Ed. By J.D. Wilson, D.W. Foster, H.M. Kronenberg and P.R. Larsen. W.B. Saunders Co., Philadelphia, PA. Pp. 517-664.
11. Sperling, L.C. and W.L. Heimer (1993). "Androgen biology as a basis for the diagnosis and treatment of adrenogenic disorders in women, I and II." *J. Am. Acad. Dermatol.*, 28:669-683 and 901-916.
12. Syme, H., J. Scott-Moncrieff, N. Treadwell, M. Thompson, P. Snyder, M. White and J. Oliver (2001). "Hyperadrenocorticism associated with excessive sex hormone production by an adrenocortical tumor in two dogs." *JAVMA*, 219:1725-1728.
13. Selman, P.J., J. Wolfswinkel and J.A. Mol (1996). "Binding specificity of medroxyprogesterone acetate and proligestone for the progesterone and glucocorticoid receptor in the dog." *Steroids*, 61:133-137.
14. Selman, P.J., J.A. Mol, G.R. Rutterman, E. vanGarderen, T.S.G.A.M. van den Ingh and A. Rijnberk (1997). "Effects of progestin administration on the hypothalamic-pituitary-adrenal axis and glucose homeostasis in dogs." *J. Reprod. Fertil. Suppl.*, 51:345-354.
15. Frank, L.A., L.P. Schmeitzel and J.W. Oliver (2001). "Steroidogenic response of adrenal tissues after administration of ACTH to dogs with hypercortisolemia." *JAVMA*, 218:214-216.
16. Ristic, J.M.E., H. Evans and M.E. Herrtage (2001). "Plasma 17-hydroxyprogesterone concentrations in the diagnosis of canine hyperadrenocorticism". *Proc. 19<sup>th</sup> ACVIM Meeting, Denver, CO.* #107.
17. Tiligada, E. and J.F. Wilson (1989). "Tonic, neuronal and endocrine influences on the proopiomelanocortin system of the hypothalamus." *Life Sci.*, 46:81-90.
18. Weiss, C.A. and M.V. Scott (1997). "Clinical aspects and surgical treatment of hyperadrenocorticism in the domestic ferret: 94 cases." *JAAHA*, 33:487-493.
19. Shoemaker, N.J., M. Schuurmans, H. Moorman and J.T. Lumeij (2000). "Correlation between age at neutering and age at onset of hyperadrenocorticism in ferrets." *JAVMA*, 216:195-197.
20. Lipman, N.S., R.P. Marini, J.C. Murphy, Z. Zhibo and J.G. Fox (1993). "Estradiol-17 beta-secreting adrenocortical tumor in a ferret." *JAVMA*, 203:1552-1555.
21. Wagner, R.A., E.M. Bailey, J.F. Schneider and J.W. Oliver (2001). "Leuprolide acetate treatment of adrenocortical disease in ferrets." *JAVMA*, 218:1272-1274.
22. Emanuele, N.V., J. Jurgens, N. La Paglia, (1996). "The effect of castration on steady state levels of luteinizing hormone-releasing hormone (LHRH) mRNA and transcription/polymerase chain reaction." *J. Endocrinol.*, 148:509-515.
23. Donovan, B.T. and M.B. TerHaar (1977). "Effects of luteinizing hormone releasing hormone on plasma follicle-stimulating hormone and luteinizing hormone levels in the ferret. *J. Endocrinol.*, 73:37-52.
24. Olson, P.N., J.A. Mulnix and T.M. Nett (1992). "Concentrations of luteinizing hormone and follicle-stimulating hormone in the serum of sexually intact and neutered dogs." *Am. J. Vet. Res.*, 53:762-766.
25. Frank, L.A. and J.W. Oliver (2001). "Adrenal sex hormone imbalance and Alopecia-X; answers or more questions?" *Proc. 17<sup>th</sup> ACVIM Mtg., Denver, Co.* #778.
26. Kintzer, P. (1999). "Adrenal hyperplasia syndrome." *Proc. 17<sup>th</sup> ACVIM Mtg, Chicago, IL.* #435.
27. Schmeitzel, L.P. (1999). "Alopecia X of Nordic breeds." *Proc. 15<sup>th</sup> AAVD/ACVD, Maui, Hawaii.* Pp. 131-138.
28. Schmeitzel, L.P., C.D. Lothrop, Jr and W.S. Rosenkrantz (1995). "Congenital adrenal hyperplasia-like syndrome." In: Kirk's Current Veterinary Therapy XII, Small Animal Practice. Ed. by J.D. Bonagura and R.W. Kirk. W.B. Saunders, Co., Philadelphia, PA. Pp. 600-604.
29. McKenna, T.J., Y. O'Connell, S. Cunningham, M. McCabe and M. Culliton (1990). "Steroidogenesis in an estrogen-producing adrenal tumor in a young woman: Comparison with steroid profiles associated with cortisol- and androgen-producing tumors." *J. Clin. Endocrinol. Metab.*, 70:28-34.
30. Mersen, J.H., L. Ceballos, P. Levin and S. Busky (1988). "Estrogen-Secreting adrenal tumor responsive to ACTH: Localization by adrenal venous sampling." *Southern Med. J.*, 81:275-278.
31. Santen, R.J., E. Samojlik, L. Demers and E. Badder (1980). "Adrenal of male dog secretes androgens and estrogens." *Am. J. Physiol.*, 239:E109-E112.
32. Erickson, G.F. and A.J.W. Hsueh (1978). "Stimulation of aromatase activity by follicle stimulating hormone in rat granulosa cells *in vivo* and *in vitro*." *Endocrinol.*, 102:1275-1282.
33. Carr, B.R. "Disorders of the ovaries and female reproductive tract." In: Williams Textbook of Endocrinology, 9<sup>th</sup> Ed. Ed. by J.D. Wilson, D.W. Foster, H.M. Kronenberg and P.R. Larson. W.B. Saunders, Co., Philadelphia, PA. Pp. 751-817.
34. Johnson-Delaney, C.A. (1998). "Ferret adrenal disease: A fresh perspective." In: Small Mammal Endocrinology. *Proc. Small Mammal and Reptile Program, Ann. Conf. & Expo, AAV. St. Paul, MN.* Pp. 99-111.
35. Paradis, M. (2000). "Melatonin therapy for canine alopecia." In: Kirk's Current Veterinary Therapy XIII, Small Animal Practice. Ed. by J.D. Bonagura. W.B. Saunders, Co., Philadelphia, PA. Pp. 546-549.
36. Leyva, H., L. Addiego and G. Stabenfeldt (1984). "The effect of different photoperiods on plasma concentrations of melatonin, prolactin and cortisol in the domestic cat." *Endocrinol.*, 115:1729-1736.
37. Donovan, B.T., C. Matson and M.J. Kilpatrick (1983). "Effect of exposure to long days on the secretion of oestradiol, oestrone, progesterone, testosterone, androstenedione, cortisol and follicle-stimulating hormone in intact and spayed ferrets." *J. Endocrinol.*, 99:361-368.
38. Paul-Murphy, J. and J. Ramer (2001). "Melatonin use in ferret adrenal gland disease." *Proc. N. Am. Vet. Conf., Vol. 15,* pg . 897.

