# The trials and tribulations of *endocrine testing*

**Jenni Donald**, of Gribbles Veterinary Hamilton, outlines some useful approaches to the tricky business of reaching a diagnosis with endocrine testing.

**THIS ARTICLE IS** not intended to be a complete review of all endocrine testing, but picks out a few useful facts on things I have come across over the years. None of the endocrine tests that we have available has sensitivity and specificity of 100%, and this always needs to be considered when undertaking this testing so there are no false expectations. Test results must be interpreted with the history, clinical signs and physical examination findings. Also we may be using assays developed for humans, which further complicates interpretation because full validation is not always possible.

## HORSES

A case earlier in the year highlighted the difficulties we sometimes have reaching a diagnosis with endocrine testing. In this case, a Thoroughbred mare presented with symptoms suggestive of a granulosa cell tumour (GCT), and ultrasound findings supported the diagnosis. Serum was sent to the laboratory to test for anti-müellerian hormone (AMH), which is considered the most reliable test for identifying the presence of a GCT. AMH is produced by granulosa cells in the ovary, and levels are similar throughout the oestrus cycle and during pregnancy. When there is proliferation of granulosa cells with a GCT there is generally a marked increase in AMH in serum. In a study of 44 mares with histologically confirmed GCT, the sensitivity of AMH for the detection of GCTs was 98%.

Given this, we were surprised when the result came back from the referral laboratory within normal limits. The result was 3.90pmol/L, with a reference interval of 1.57–21.0pmol/L for normal mares, while levels typically are >99pmol/L in mares with GCT reported in the literature.

A sample was then sent to Monash University in Melbourne, to test for inhibin. The sensitivity of inhibin to detect GCT has been reported to be 80%. The inhibin result was 1.01ng/ml, which is also within normal limits but towards the upper end (reference interval is 0.34–1.66ng/ml).

Surgery was carried out to remove the mass and histology confirmed a GCT. So although we have tests reported to have high sensitivity, in this case they did not support the diagnosis. Mention was made on the histology report that theca cells predominated in the mass, which may explain why the AMH and inhibin levels were low, as these are produced by the granulosa cells.



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The following are some other assays used for horses who have specific requirements or features that need to be considered to achieve the greatest diagnostic accuracy.

### Endogenous adrenocorticotropic hormone (ACTH) for the diagnosis of pituitary pars intermedia dysfunction (PPID).

An excellent review of this topic has been prepared by Lisa Hulme-Moir of Gribbles Veterinary. Here are some key points to remember:

- ACTH is possibly more stable than we originally thought, but care with sample handling is still recommended, and it is best to stick with the original guidelines.
- Endogenous ACTH can be falsely increased with pain and severe illness, and possibly with exercise.
- Testing in autumn is recommended to take advantage of the exaggerated response seen at this time of the year in PPID cases.
- The thyrotropin-releasing hormone (TRH) stimulation test is reported to have improved diagnostic precision, but there are difficulties with obtaining the TRH. This may change in the future.

Work is still progressing on establishing reference intervals for horses and donkeys in New Zealand, and there is an ongoing search for markers overseas, particularly for early detection of PPID.

### Insulin for equine metabolic syndrome (EMS)

Insulin dysregulation in EMS can result from insulin resistance or excessive postprandial insulin release. Different tests are needed to differentiate these. The insulin assay available in New Zealand has been validated in horses, and this can be combined with glucose and challenge studies to try to establish the diagnosis.

### Combination PPID-EMS cases

Many of the tests for insulin dysregulation can be abnormal in both PPID and EMS. At Liphook Equine Hospital there has been some preliminary work measuring adiponectin, and this test may be useful in PPID cases to establish if there is also EMS.

## DOGS

Other than progesterone for breeding, the most common endocrine assays we run in dogs are for the diagnosis of hyperadrenocorticism (Cushing's disease), hypoadrenocorticism (Addison's disease) and hypothyroidism.

### Hyperadrenocorticism

For the diagnosis of hyperadrenocorticism, cortisol is measured either in serum with the low-dose dexamethasone suppression test (LDDST) or ACTH stimulation test, or in urine in the urine cortisol:creatinine ratio (UCCR). Cortisol is quite stable and results tend not to be badly affected by sample artefact such as haemolysis or lipaemia, but, as always, it is best to look after the samples.

The LDDST is regarded as the test of choice for hyperadrenocorticism.

- Most normal dogs will show suppression of the cortisol level at eight hours to <40nmol/L.
- About 90% of dogs with hyperadrenocorticism will have eight-hour level >40nmol/L and six to eight percent have borderline levels (25–36nmol/L).
- Combining results from many studies the reported sensitivity and specificity of the LDDST range from 85–100% and from 44–73% respectively.
- There will be false positives in dogs who are systemically ill, so testing should be avoided during this time.
- LDDST has the added advantage in distinguishing pituitary-dependent disease from adrenal disease in about 50% of cases.
- Occasionally dogs who are on phenobarbital will not show suppression mimicking hyperadrenocorticism, so results need to be interpreted with caution.

The ACTH stimulation test assesses adrenocortical reserve and is the test of choice for iatrogenic hyperadrenocorticism and hypoadrenocorticism. Because of low sensitivity it is inferior to the LDDST for the diagnosis of spontaneous hyperadrenocorticism in dogs.

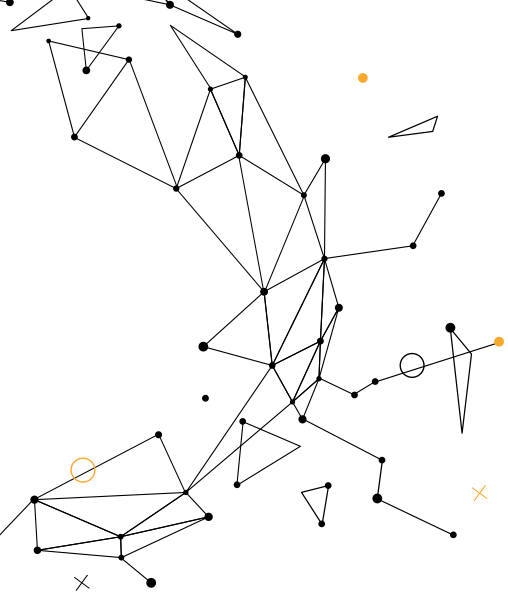
- For adrenal tumours, sensitivity of the ACTH stimulation test is reported to be 57–63%.
- For dogs with pituitary disease the sensitivity is 80–83%.
- Specificity ranges from 59% to 93%.
- It has been found that progestogens, ketoconazole and glucocorticoids will decrease the response to ACTH. Phenobarbital does not appear to affect the results.

The urine test UCCR is a simple and inexpensive test. The reported sensitivity and specificity for the UCCR when a single random urine sample is collected from dogs in veterinary hospitals was 75–100% and 20–25% respectively. If two urine samples were collected at home by the owners on two separate days at least two days after the visit to the veterinarian, sensitivity increased to 99% and specificity to 77%.

There are rare cases that are not ruled out by this test. If the clinical signs are convincing, the more specific tests are needed.

The poor specificity of the UCCR means that high levels of cortisol in the urine can be due to other stressful disease or hyperadrenocorticism. Interpreting the results alongside all the other findings is imperative.

Glucocorticoids and other drugs that suppress cortisol secretion can decrease the UCCR. Phenobarbital treatment does not affect the UCCR.





FT4 MEASURED BY EQUILIBRIUM DIALYSIS WAS FOUND TO BE ELEVATED IN MORE THAN 98% OF CASES. **HOWEVER, TEST SPECIFICITY IS POOR, WITH UP TO 20% OF SICK EUTHYROID CATS HAVING A FALSE INCREASE IN fT4.**

The high-dose dexamethasone suppression test (HDDST) can be used to aid differentiation of pituitary-dependent disease from disease due to adrenal tumours. About 75% of pituitary-dependent disease will show suppression with the high dose, whereas adrenal tumours will not.

### Hypoadrenocorticism

Resting/basal cortisol concentrations are not useful in dogs, other than to rule out Addison's disease. If the resting cortisol is >55nmol/L, Addison's disease is very unlikely. Conversely, a resting cortisol <55nmol/L supports a diagnosis of Addison's disease. An ACTH stimulation test in this case is warranted.

Two studies looking at baseline cortisol in dogs with Addison's disease demonstrated a sensitivity of 85.7–100% and specificity of 91.8–98.2% for basal cortisol concentrations <28nmol/L. For basal cortisol concentrations <55nmol/L, the sensitivity was 100% in both studies, but specificity was 63.3–78.2%.

### Hypothyroidism

Checking total T4 levels for hypothyroidism in dogs is a common request at laboratories. The ancillary tests of free T4 (fT4) and thyroglobulin stimulating hormone (TSH) are often also part of the assessment. Thyroid function tests should only be done when there is clinical suspicion of thyroid disease – lethargy, weight gain despite an indifferent appetite, coat changes, and ideally with hypercholesterolemia or mild anaemia.

In healthy patients a low T4 is most likely to be a spurious result that can occur from normal fluctuation of the T4 level. Older dogs and very fit dogs can also have lower levels. I am always reminded of a family of Rhodesian Ridgebacks that ran miles every day, and all had T4 levels in the 10–14nmol/L range.

There are also breed variations, with the sight hounds (Greyhounds, Whippets, Salukis, etc) having T4 and fT4 below the general canine reference range. Basenjis have also been found to have thyroid profiles similar to sight hounds.

In sick dogs, low T4 is often the result of sick euthyroidism. The rare exception would be hypothyroid myxedema, which can be life threatening. If this is suspected, then checking fT4 and TSH would be indicated, as would T4 supplementation started while awaiting results.

There are also drugs that will lower T4 levels. This has been documented for:

- phenobarbital
- trimethoprim-sulfonamide
- zonisamide
- clomipramine
- aspirin
- glucocorticoids (including topicals on eyes, ears and skin).

Although fT4 is less affected by factors that can falsely lower T4, it can still occur with sick euthyroidism and with some drugs.

An elevated TSH is a fairly specific test for true hypothyroidism when combined with a low T4 and fT4. About 10% of normal dogs could have a high TSH but should have a normal T4 and fT4. TSH is not very sensitive, however, with about 25% of dogs with hypothyroidism having a TSH level within normal limits. Phenobarbital and trimethoprim-sulfa have been shown to cause TSH elevations.

A test that is part of the screening pre-breeding programme in the US is thyroglobulin autoantibodies (TGAA). It is generally considered that if T4 and fT4 are normal, then the animal should be considered euthyroid, even if TGAA are present. In a study where 234 dogs with normal T4 and TSH levels and elevated TGAA were followed for a year, 19% became hypothyroid, 15% became TGAA-negative and the remainder were still TGAA-positive, with normal T4 and fT4 and considered euthyroid.

## CATS

The most common test we do in cats is testing for hyperthyroidism. Most cases are straightforward, with high T4 levels in 90% to support the clinical picture.

There can be differences in assays used by the different analysers, whether in the reference laboratory or in-clinic. Any of the assays can occasionally give a falsely low or high result. The T4 assay is generally robust, with minimal effects from haemolysis or lipaemia.

Difficulties generally arise in three situations:

- The cat is hyperthyroid, but T4 is within normal limits.
- There is a palpable thyroid nodule, but the T4 is normal.
- When a cat with no clinical signs is

misdiagnosed as hyperthyroid based on falsely increased T4 levels.

About 10% of all hyperthyroid cats (and 40% of cats with early or mild disease) will have T4 within normal limits. Often the T4 level will be in the mid- to high-end of the range. This typically occurs from one of two causes:

- Fluctuations in the T4 level. The T4 level will generally increase with time allowing diagnosis if they are truly hyperthyroid.
- Other non-thyroidal illness is suppressing T4 at the time of testing.

Options are to retest or check fT4.

fT4 measured by equilibrium dialysis was found to be elevated in more than 98% of cases. However, test specificity is poor, with up to 20% of sick euthyroid cats having a false increase in fT4. For this reason, fT4 can never be regarded as the gold standard for diagnosing hyperthyroidism.

Measuring TSH is not recommended in cats as the canine assay is not particularly good at detecting feline TSH, and we would be looking for decreased amounts, which would be difficult to differentiate from normal.

## OTHER ASSAYS

There is a human assay available in New Zealand for aldosterone that can be used to test for hyperaldosteronism in cats. This had not been validated, but may be useful as an adjunct to other diagnostics. Unfortunately, the insulin assay that works for horses does not work for dogs, so no insulin assay is available in New Zealand for this species. We need to send samples overseas to get insulin measured in dogs.

The same is true for parathyroid hormone. This hormone is very unstable, however, which makes it more difficult to send overseas.

There is a human assay for the catecholamines, metanephrine and normetaneprine, which can be used to support the diagnosis of phaeochromocytoma. These hormones are measured in urine.

With endocrine testing, I am always reminded of quote from a specialist when a student commented, "You seem to keep testing until you get the result you want". It's true that it often does seem that way, but that is because the clinical picture is so important in these diseases. VS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