Testing and monitoring for equine pituitary Pars Intermedia Dysfunction (PPID)

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As geriatric medicine becomes an increasingly important aspect of equine practice, researchers throughout the world are continuing to determine the best methods for endocrine testing for particular situations. Several recent equine endocrinology conferences and special interest groups have put together sets of guidelines to help equine practitioners formulate plans to diagnose and monitor PPID.

There is currently no perfect commercially available ante mortem diagnostic test for PPID, but age and clinical signs can help to determine the best test for each individual horse. As PPID is a progressive neurodegenerative condition there is a continuum between normal and abnormal with a "grey- zone" in the middle. In the early stages, results of basic tests are often equivocal, and more sensitive tests are required to determine if abnormal pituitary function is contributing to vague clinical signs such as decreased athletic performance, loss of muscle mass, lethargy, weight loss or elevated liver enzymes.

For older equids with at least one characteristic clinical sign of PPID; including hirsutism/hypertrichosis (retained long hair coat), loss of muscle mass, laminitis, lethargy, polyuria/polydipsia, recurrent infections, infertility, and either abnormal fat distribution in the earlier stages and weight loss in the later stages, then the endogenous ACTH concentration has been shown to be equally sensitive to the dexamethasone suppression test, but simpler and safer to perform. However, based on post mortem studies with histological examination of the pars intermedia, both of these tests may miss early or subclinical cases of PPID.[1]

Endogenous ACTH concentrations

Collection of a single blood sample for measurement of ACTH concentration requires a single trip to the farm, carries no risk of laminitis, but can be relatively expensive depending on the laboratory. Using human laboratories and reference ranges from textbooks can be problematic due to differences in assays that are used between laboratories. For example, ELISAs, immunoantibody assays and radioimmunoassays may give different results for the same animal, and even kits made by different manufacturers can give slightly different values, thus resulting in assay dependent reference ranges.[2] One assay may underestimate values at the lower end of the range, while overestimating values at the higher end of a range.[2] The differences in some cases may be small and not of clinical consequence unless values are being compared over time in the same animal. Ideally a veterinary laboratory that has a test validated for equine samples and can provide a laboratory specific reference range for horses should be used. For monitoring purposes, it is warranted to reuse the same laboratory, so that results can be compared over time.

Contrary to previous advice, it is actually beneficial to test in the autumn months. Although normal horses will have slightly elevated ACTH concentrations in the autumn, PPID horses will have a markedly elevated ACTH concentration, thus making it easier to diagnose a horse in the early stages of disease in the autumn.3 Seasonal reference ranges should therefore be used, and care should be made when comparing subsequent test results performed at different times of the year.[4,5] Seasonal reference ranges have recently been published for Perth and Townsville.[6] Diurnal variations are not enough to interfere with a diagnosis of PPID, there sampling can be performed at any time of the day.[7] In contrast to previous advice, there is no advantage of testing more than one sample from a single day.[7] Testing should not be performed immediately after exercise, in acutely ill or severely painful animals.

Single resting ACTH collection technique

1. A single blood sample can be collected at any time of the day.
2. Collect sample into an EDTA (purple) tube (glass or plastic)
3. Chill the sample within 3 hours of collections. Transport in a cooler bag, but do not allow the sample to freeze.
4. Chilled whole blood can be sent directly to the laboratory but ideally the plasma should be separated from the red blood cells using a centrifuge or, if unavailable, after gravity separation.* Place sample in a plastic container (cryovial, empty yellow lid vial, or red top tube with no additive). If the blood sample has been chilled, then the timing of separation is not important – ie the next morning is fine.
5. Chill the sample during delivery to the laboratory in cooler packs, but do not allow the sample to freeze unless separated by centrifugation.
6. Plasma can be kept frozen at -20^{O} C and sent for analysis after several weeks.

Notes:

Exposure to warm temperatures will result in degradation of ACTH, and potential false negative results.

*Separation by centrifuge is ideal, and samples can then be frozen if delivery to the laboratory will be delayed.

Whole blood or plasma separated by gravity should never be frozen as the red cell fragments will cause an artificial increase in the ACTH concentration and potentially result in a false positive result.

Dexamethasone suppression test

The overnight low-dose dexamethasone suppression test is equal in accuracy to the endogenous ACTH test,[1] but requires 2 visits by the veterinarian to the farm and there is a small risk of inducing laminitis. As equids with PPID are already at risk of laminitis it can be impossible to determine if a bout of laminitis immediately after testing was due to the disease itself or the testing procedure. As the dosage of dexamethasone used is small, the risk is considered extremely low, with many experienced veterinarians having performed hundreds of tests over many years with no problems. Dexamethasone 40 µg/kg (0.04 mg/kg IM) is injected and a serum sample is collected approximately 19 hours later. In a normal individual, dexamethasone will cause a subsequent drop in plasma cortisol concentrations (<30 nmol/L, <10 ng/ml or 1 µg/dl) which is maintained at this low concentration for at least 24 hrs. Cortisol concentration will fail to decline (or remain low) in a PPID horse because the tumour in the pituitary gland is not under negative feedback control by glucocorticoids.

Thyroid-releasing hormone (TRH) stimulation test

There are TRH receptors located in both the pars distalis and pars intermedia in horses. After TRH

administration normal horses have a slight increase in ACTH concentration; subclinical PPID horses (ie pituitary hyperplasia at necropsy, but no clinical signs) have a moderate increase in ACTH; while clinical PPID horses have a dramatic increase in ACTH concentrations after TRH administration.[8] Seasonal reference ranges have been published.[9]

T= 0 collect blood for baseline ACTH in an EDTA tube (ideally centrifuge and transfer plasma to a plastic tube then ship on ice or freeze, though whole blood in EDTA can be shipped and tested within 12 hrs). Give 1 mg TRH IV (0.5 mg for ponies). T = 30 min collect plasma for ACTH concentration. This is the most sensitive test available and is ideal for screening older horses for subclinical PPID or when results of less sensitive tests are normal or borderline.[8] TRH can be obtained in a chemical form from Sigma-Aldrich, but must be prepared in a sterile manner and stored at -80^{o}C. As this is an off-label test using a non-sterile, chemical grade product, with the requirement of -80^{o}C storage, referral to a specialist may be desirable. At this time, seasonal reference values are not available in Australian conditions, and interpretation of results in the autumn months may be difficult. Because of the marked autumnal rise in ACTH in PPID horses, a baseline sample may be adequate to identify subclinical cases in the autumn and TRH testing may not be required at this time.

Monitoring after commencement of pergolide therapy

If there are obvious clinical signs of PPID, treatment may be considered without laboratory testing. However, as the dose of pergolide required to control abnormal pituitary hormone production is variable between horses, then a baseline result is useful tocompare to subsequent tests to determine if the dose of pergolide is adequate. It is recommended that testing be repeated 1-2 months after commencement of pergolide treatment. If there have not been substantial improvements in clinical signs and test results, then the dose of pergolide should be increased. As pergolide only reduces the production of abnormal pituitary hormones and does not decrease the size of the pituitary adenoma, the adenoma will continue to grow, and incremental increases in pergolide may be required over subsequent years. Testing should be performed annually as part of a geriatric examination to determine if dose adjustments are required. If clinical signs deteriorate then retesting is also recommended. The dose of pergolide should be increased until clinical signs have vastly improved and test results have normalized. If the horse still looks Cushingoid then the dose is inadequate.

Testing for insulin resistance

All equids with PPID should also be tested for insulin resistance, as insulin resistant animals are more likely to develop laminitis. It is best that horses are kept off feed for 6-8 hrs before testing. A single fasting serum insulin concentration (red top tube) can be used as a screening test. A better test is the oral sugar test, where the owner can administer 1.0 g/kg of glucose or dextrose powder in a small feed of chaff after the 6-8 hr fast. The veterinarian can then collect a serum sample 2 hours after the sugar load to measure insulin concentration. A post-sugar load insulin concentration > 87μU/mL is considered indicative of insulin dysregulation.[10] Many, but not all PPID horses have insulin resistance, and once identified, feeding a low glycaemic diet is warranted.

Implications

As 21% of Australian equids over 15 years of age have PPID,[10] obtaining an accurate diagnosis and instituting an ongoing treatment and monitoring plan can make a vast improvement to the welfare and athletic usefulness of many aging horses and ponies.

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