Diagnosing Equine Pituitary Pars Intermedia Dysfunction in Ambulatory Practice

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Diagnosis of pituitary pars intermedia dysfunction can be achieved in the field using the dexamethasone suppression test or plasma adrenocorticotropic hormone (ACTH). Equine ACTH is considerably more stable than previously suggested, which makes it useful for field-collected samples. Fasting insulin and circadian cortisol concentration are not discriminating tests. Additionally, season may affect interpretation of diagnostic test results. Author’s address: Department of Physiological Sciences, 264 McElroy, Oklahoma State University, Stillwater, OK 74078; e-mail: diannem@okstate.edu. © 2008 AAEP.

1. Introduction
Equine pituitary pars intermedia dysfunction (PPID, equine Cushing’s disease) is a common endocrinopathy of aged horses and ponies that manifests with a variety of clinical signs, including hirsutism, laminitis, polydipsia/polyuria, and muscle loss. New information has revealed that the diagnosis of PPID is not nearly as straightforward as previously believed. The purpose of this review is to provide concise and current information on how the diagnostic tests for PPID that are used in equine ambulatory practice perform and provide new information on appropriate sample-handling techniques.

2. Clinical Signs
Hirsutism in an aged horse is considered essentially diagnostic for PPID, and specific endocrine testing rarely improves the level of confidence in the diagnosis. In horses with early PPID, clinical signs may overlap with the phenotypic changes that accompany “normal” aging. In addition, the horse with PPID may look similar to horses with other endocrine diseases, in particular equine metabolic syndrome. In early cases of PPID, a sensitive and specific test is needed to make an accurate diagnosis. Testing is also indicated in PPID horses if pharmacologic treatment (e.g., pergolide) is going to be pursued. Test results are used to ensure that the administered dose of the drug is sufficient. Periodic rechecks are suggested every 6–12 mo, because the dose frequently needs to be increased over time to maintain hormonal concentrations in a normal range. Ideally, PPID diagnostic tests need to be suitable for field application.

3. Dexamethasone Suppression Test
The advantage of the dexamethasone suppression test (DST) is that it is the best validated antemortem diagnostic test. The disadvantages of DST are that it is not sensitive in early cases, it requires two farm visits, there is a perceived risk of laminitis, and there are seasonal effects on test results. For >20 yr, DST has been considered the gold standard antemortem diagnostic test for PPID. Early reports suggested that the test had a sensitivity and specificity of 100%; however, test validation was performed using horses pre-selected for having clinical signs consistent with very advanced stages.
of PPID. This likely skewed these results. More recent information has suggested that false-negative results may be common in early cases of the disease. In one study, only 56 of 95 (59%) horses with clinic signs consistent with PPID tested positive using DST. Presumably, the majority of the remaining 61% were false-negative test results, because clinical signs are highly predictive of the disease.

Other limitations of the DST include the need to make two farm visits, the concern for exacerbation of laminitis, and the influence of season on test results. Although induction of laminitis after administration of corticosteroids continues to be cited as a reason to avoid the DST, there is no evidence-based data to support this association. If it does occur, laminitis is a rare complication of the test. In a recent study, 150 DSTs were performed in horses, many of which had a history of laminitis, without report of adverse effects.

Season must be taken into account when interpreting DST results. False positives occur in ~40% of cases when the test is performed in the fall. Many practitioners now avoid testing in the fall, although a negative test performed at this time is very strong evidence that the animal does not have PPID. The exact extent of the seasonal period during which diagnostic tests are likely to yield false positives is still being investigated, but current data suggests that it is associated with shortening of day length and is evident by August.

The standard protocol for the overnight DST is as follows. A blood sample is drawn for serum cortisol measurement between 4:00 and 6:00 pm; 40 μg/kg dexamethasone is administered intramuscularly, and a second blood sample is collected the following morning, ~19–24 h after dexamethasone administration. When using the DST to assess response to therapy, it may be possible to forego the first sample and have the client administer the intramuscular dexamethasone injection. This limits the test to a single farm visit for the veterinarian and a single cortisol assay. This is not optimal, because the baseline cortisol will not be known; however, in many cases, the post-dexamethasone cortisol concentration is sufficient for test interpretation. This single-sample approach is not recommended when making an initial diagnosis, because interpretation of resting cortisol may be useful. For example, a very low cortisol concentration both before and after dexamethasone administration can indicate hypoactive adrenal-gland function. In this situation, an adrenocorticotropic hormone (ACTH) stimulation test would be indicated. This pattern of hormone response may be observed when the patient has received multiple doses of long-acting corticosteroids.

4. Endogenous Plasma ACTH Concentration

The advantages of measurement of the ACTH concentration is that it requires only a single sample and there is no risk to the horse. The disadvantages are that it is less sensitive than the DST, there are sample handling issues, and there are seasonal effects on test results.

The second most commonly used test for field diagnosis of PPID is the plasma ACTH concentration test. Limitations of this test include false-negative results in horses with early PPID. It is not clear how sensitive the measurement of plasma ACTH concentration is for diagnosing PPID. Several studies have been conducted to determine ability of plasma ACTH concentration to predict PPID; however, they have been limited by the lack of a suitable “gold standard” or a failure to account for the season when interpreting the test results. Combining the data from three studies in which horses were selected based on the presence of clinical signs and an abnormal DST, ACTH concentration results agreed with DST results in 72 of 95 (76%) horses. However, in one of these studies, 35 of 91 horses with clinical signs of PPID had a normal DST. Of those horses, 9 of 33 (27%) had an increased plasma ACTH concentration. This suggests that, on occasion, measurement of plasma ACTH may identify PPID cases missed by DST.

In humans and dogs, ACTH is highly unstable, and sample handling is a critical issue which seriously limits the usefulness of the test. In horses, ACTH is markedly more stable. Collection of samples in glass did not affect the concentration of the hormone as long as the plasma was separated within 8 h of collection. We found that samples collected in plastic EDTA-containing tubes could be stored upright at 4°C for up to 12 h before separating the plasma without a decrease in ACTH concentration. After plasma has been separated, it should be frozen until shipped to the laboratory. Samples can be shipped to the diagnostic laboratory on ice packs using overnight or next-day carriers, which avoids the expense of shipping on dry ice.

The season in which the testing is performed can have profound effects on the results obtained. In September, the false-positive rate in ponies and horses residing in Pennsylvania was >90%. Therefore, it is preferable to test a horse suspected of having PPID between late November and mid June. In contrast, if a negative result (an ACTH concentration within the normal reference range) is obtained when the testing was performed between August and October, it provides very strong evidence that the animal does not have PPID.

5. Thyrotropin-Releasing Hormone Stimulation Test

The advantages of the thyrotropin-releasing hormone (TRH) stimulation test are that it is a safe, dynamic test, it requires only one farm visit, and the serum cortisol is easily measured by most diagnostic laboratories. The disadvantages of this test are that it is not adequately validated, false positives are common, and it is not readily available.

The TRH stimulation test has been suggested as a safe alternative to the DST in those horses with a
history of laminitis. A 30% increase in serum cortisol 30 min after IV administration of 1 mg of TRH is considered diagnostic of PPID. This test has only been validated in a small number of horses. In one study, 6 of 11 (55%) healthy horses tested between November 14 and July 16 had a >30% increase in serum cortisol, falsely identifying them as having PPID. Evaluation of this test in a large number of horses may enable statistical adjustment of the cutoff values, which could result in greater accuracy. With the advent of veterinary compounding pharmacies, it is now easier to obtain TRH in practice.

6. Cortisol Circadian Rhythm

Determination of circadian cortisol rhythm has been advocated as a diagnostic test for PPID. It has been suggested that serum cortisol concentration in normal horses will be >30% greater in a sample collected in the morning (8 am) compared with one collected in the afternoon (4 pm). Examination of data in which serum cortisol concentration was measured every 4 h for a 24-h period in normal horses suggests that there is no difference in cortisol concentration between 8 am and 4 pm. To achieve a 30% difference, the second sample would need to be collected at 11 pm.

7. Fasting Serum-Insulin Concentration

Measurement of fasting insulin concentrations is not a selective test for identifying horses with PPID because several conditions can cause an increase in insulin, including equine metabolic syndrome. However, many horses (25–75%) with PPID will also have insulin resistance, and these horses are more likely to founder. Therefore, measurement of fasting insulin is recommended in horses suspected of PPID for its prognostic rather than diagnostic value. Although other provocative tests, such as the combined DST/TRH test or the domperidone challenge test, may perform moderately better than the tests discussed, their usefulness in the field is limited by the need for multiple farm visits, the numbers of samples needed, and/or the client’s cost.

References