Diagnosis of Pituitary Pars Intermedia Dysfunction: A Review of 1999 Versus 2008

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Since 1999, significant new information has been reported about pituitary pars intermedia dysfunction (PPID) in horses including its probable underlying cause, the role of the pars intermedia (PI) in the regulation of hypothalamic-pituitary-adrenal axis in health and disease, improved understanding of pathological changes in the PI associated with age and with disease, new diagnostic strategies, and improved understanding of the strengths and weaknesses of many older diagnostic tests. Whenever choosing one of the currently available diagnostic tests for PPID, it is important for the clinician to understand the strengths and weaknesses of each of the tests and tailor the selection of these tests to the clinical status of the patient at the time of testing with the understanding that it may be necessary to either repeat tests over time or utilize more than one test in a given patient. Authors’ address: Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211; e-mail: Messer@missouri.edu. © 2008 AAEP.

1. Introduction
Equine pituitary pars intermedia dysfunction (PPID; equine Cushing’s disease) is one of the most commonly diagnosed endocrine disorders in horses.1 The clinical signs of PPID are well recognized and have been described in detail elsewhere1–3; specific ante-mortem diagnosis of PPID through various endocrinologic testing methods can be difficult to interpret.6 The tests being used for diagnosis of PPID before 1999 and their relative merit have been reviewed.1–5 Since that time, considerable new evidence has emerged regarding the suspected underlying cause of PPID in horses,7 the role of the pars intermedia (PI) in regulation of hypothalamic-pituitary-adrenal axis in horses,6 and the pathological changes in the PI associated with age and with disease.8,9 This information has broadened the scope of testing alternatives available for equine practitioners, making the decision of which test to use more complex. Early diagnosis of PPID is important for long-term care and management of affected horses to minimize the complications that are often associated with the disease.1,2

2. Discussion
PPID occurs as a result of hyperplasia and hypertrophy of the melanotrope cell population in the PI of the pituitary gland secondary to loss of dopaminergic inhibition caused by neurodegeneration of the dopaminergic periventricular neurons.7 The resulting changes in the PI of affected horses have been variously referred to as pituitary hypertrophy, pituitary hyperplasia, or pituitary adenoma.1
In normal horses, melanotropes in the PI secrete relatively small quantities of the following protein hormones: α-melanocyte stimulating hormone (α-MSH), β-endorphin (β-END), adrenocorticotropin (ACTH), and corticotropin-like intermediate peptide (CLIP). These hormones are derived from a single large pre-pro-hormone called pro-opiomelanocortin (POMC) and are usually collectively referred to as POMC peptides. Secretion of the POMC peptides by melanotropes is normally under the tonic inhibitory influence of dopaminergic nerves originating in the periventricular nuclei (cell bodies) of the hypothalamus. Excessive secretion of POMC peptides and hyperplasia of melanotropes in the PI of PPID-affected horses results from the destruction of inhibitory dopaminergic nerves originating in the hypothalamus as a consequence of oxidative stress.

PPID is commonly suspected and diagnosed based on its characteristic physical appearance in older horses. Failure to shed out the hair coat in spring (hirsutism) is widely regarded as pathognomonic for PPID and may preclude the need for further diagnostic testing if present. The presence of chronic laminitis is also a strong indicator that PPID should be suspected in affected individuals. In one study, chronic laminitis was the most likely clinical expression of PPID, even in the absence of hirsutism.

The development of PPID commonly leads to the development of an abnormal body condition in older horses. Affected horses tend to appear thin overall because of loss of skeletal muscle mass, but they dichotomously acquire regional deposits of body fat in abnormal locations such as the crest of the neck, the supra-orbital fossae, and for males, the prepuce. In addition, PPID may also develop in overtly obese horses in which hirsutism is not present.

The clinical signs of PPID result from the effects of increased POMC-peptide secretion, physical enlargement of the PI, and stimulated secretion of cortisol by the adrenal glands (hypercortisolism). However, adrenocortical hyperplasia is identified in only ~20% of PPID-affected horses during postmortem examination. Although excess secretion of cortisol results from increased PI-derived ACTH, the steroidogenic properties of ACTH are significantly enhanced by other POMC peptides.

In 1999, the so-called “gold standard” test for PPID was the overnight dexamethasone suppression test (DST). Based on a study of 52 horses with clinical and pathologic evidence of PPID, all affected horses had plasma cortisol levels of >1 μg/dl 19 h after the administration of 40 μg/kg of dexamethasone, whereas all unaffected, normal, healthy horses had plasma cortisol levels of <1 μg/dl 19 h after the administration of 40 μg/kg of dexamethasone. Based on these criteria, the test was 100% sensitive and 100% specific in the population of horses included in the study. The test even identified clinically-normal-appearing horses as having PPID based on the presence of PI hyperplasia identified at necropsy.

Other tests in vogue at that time (1999) included measurement of basal plasma cortisol levels, basal plasma ACTH levels, basal plasma insulin levels, and plasma cortisol levels after administration of thyrotropin-releasing hormone (TRH). Preliminary studies were also conducted at that time on the use of a combined DST/TRH stimulation test. Several independent studies determined that measurement of basal plasma cortisol levels alone were of no value in diagnosing PPID. At the time, measurement of basal plasma ACTH levels in horses was not readily available in North America, but when performed, it was less sensitive and specific than the DST. Another study found that basal plasma insulin levels in horses with PPID were the same as those in young horses without PPID and that not all horses with high insulin levels had PPID. Although it was shown in one study that plasma cortisol levels increased in horses with PPID after administration of TRH, two other studies have subsequently concluded that it was not possible to distinguish normal horses from those with PPID after TRH administration. The first reporting of the measurement of plasma cortisol levels using a DST/TRH test indicated that it did distinguish horses suspected of having PPID from normal horses; however, this study did not confirm the presence of PI hyperplasia pathologically at necropsy.

A significant development in testing protocol that has been discovered since 1999 is that during the fall season (particularly September), there is an increase in both ACTH and plasma cortisol levels in normal horses. This apparent normal physiologic event adversely affects the results of diagnostic tests for PPID that use measurement of plasma ACTH and/or plasma cortisol such as the DST, basal plasma ACTH, TRH stimulation test, and DST/TRH test. For example, when horses are tested in September using a DST, the number of false-positive tests for PPID is greater than at other times of the year. Likewise, when measuring basal levels of ACTH in September, the number of false-positive tests for PPID is greater than other times of the year.

A few studies since 1999 have also shown lower sensitivity and specificity of the DST results than previously described. In one study, seven horses were all diagnosed with PPID at the beginning of the study based on the DST; in subsequent evaluations 30 and 60 days after the initial DST, they were found to have variable DST results. In that study, five of seven horses had normal DST results 60 days after all seven horses had showed abnormal DST results. This study was conducted before the time it was discovered that horses experienced a normal physiologic increase in hypothalamic-pituitary-adrenal axis activity in the fall of the year. Therefore, that finding could have influenced the results of the study. There was no mention
made as to the time of year that the initial DST was performed.

Two recent studies have also reported inconsistent results with the DST as originally described by Dybdal et al. The DST was also abnormal in 17 of 26 tests in these horses. In another study, the ACTH response to TRH administration was evaluated by itself and compared with DST results in 29 horses having variable involvement of the PI all the way from normal to abnormal. ACTH levels increased in all horses after TRH administration. However, the magnitude and duration of the increase was significantly greater in horses with PPID. The ACTH baseline concentration and response to TRH did not correlate with the DST results. Results of the DST were abnormal only in clinically abnormal horses or in horses that were clinically normal but afflicted with PI hyperplasia as determined by post-mortem examination histopathologic assessment. Results of the DST were considered normal in 17 of 26 tests in these horses.

The investigators in both studies made the assumption that using both the DST and TRH in the same horse within hours of one another would not affect the results. However, this is clearly different than the procedure used in the original highly specific and highly sensitive study. Further studies are warranted to verify that administration of dexamethasone and TRH in close proximity to one another does not affect the sensitivity and specificity of the DST results. There are also concerns associated with the use of dexamethasone, particularly in horses that may be afflicted with or at risk of developing laminitis; these risks are most likely quite small, and the studies using dexamethasone do not report that horses developed laminitis after the administration of dexamethasone.

A recent study looking at the response of pituitary melanotropes to TRH both in situ and in explants from the PI from normal horses and horses with PPID concluded that TRH administration is unlikely to be useful in the diagnosis of PPID. In that study, all horses with PPID had an increase in cortisol after TRH administration. However, unlike the first study reported by Beech et al., an increase in cortisol was also observed in normal horses. In fact, 10 of 16 normal horses had a cortisol increase of >30%, and of the 10, 7 had an increase of >50%, which would have meant that they would have been falsely diagnosed with PPID. In this same study, when a-MSH was used as a marker for PI response, it was shown that both healthy and PPID-affected horses had significant increases in a-MSH after administration of TRH and that both baseline and post-TRH a-MSH concentrations were significantly greater in PPID horses compared with normal horses. Unfortunately, routine testing for a-MSH in horses is not currently commercially available.

Measurement of basal (endogenous) levels of ACTH has become more widely available for clinical use since 1999 and is a convenient means of testing horses for PPID. One study, using a radioimmunoassay validated for use in horses, found that mean plasma ACTH levels for normal, healthy horses and ponies were significantly different and that mean levels for horses were significantly higher than for ponies. In addition, the mean plasma levels of ACTH for horses and ponies with PPID (based on clinical signs of hirsutism and one additional sign such as laminitis) were significantly higher than for control animals. Sensitivity of plasma ACTH levels as an indicator of PPID was 90.9% in horses and 81.8% in ponies. Specificity of the test for both groups was 100%. Another study, using a chemiluminescent ACTH assay validated for use in horses, reported that increased plasma ACTH levels (>35 pg/ml) were significantly associated with hirsutism and that the sensitivity and specificity for detecting hirsutism (clinical PPID) were 84% and 78%, respectively. This indicates that there were both false-positive and false-negative determinations made using this assay. Those investigators also evaluated the changes in plasma levels of ACTH and improvement in clinical signs in response to treatment with either pergolide or cyproheptadine. When horses were treated with either drug, there was a significant decrease in plasma ACTH levels from baseline to the first recheck, but no further changes occurred. There were also significant associations between a decrease in plasma ACTH levels and an improvement in hirsutism and laminitis.

Another promising diagnostic test for PPID in horses that is still under investigation is the domperidone-challenge test. Domperidone, an orally administered dopamine (D2) receptor antagonist, is commonly used for the management of endophyte-infected fescue agalactia in pregnant mares. In a preliminary study, the administration of domperidone did not affect the plasma ACTH concentration in normal horses. However, in PPID-affected horses, treatment with domperidone caused a significant elevation of the plasma ACTH concentration. This effect has been attributed to the fact that hypertrophied melanotropes in the PI of PPID-affected horses continue to be under some degree of dopaminergic inhibition; treatment with domperidone abolishes this residual inhibition, which leads to an elevation in the plasma ACTH concentration. Although these preliminary results regarding the domperidone-challenge test indicate that it is practical, safe, and highly accurate diagnostically, further work on a larger number of normal and PPID-affected horses must yet be undertaken to determine its true sensitivity and specificity. Similarly, it remains to be seen whether or not the test
might be affected by seasonal changes as described previously. The results from another study correlating pituitary histomorphometry to the results of the domperidone-challenge test in 33 horses donated for the purpose of humane euthanasia suggest that the domperidone-challenge test is promising for the diagnosis of PPID and will be particularly useful in detecting horses with mild PI lesions that have not developed classic clinical signs of PPID. This study also described the varying degrees and severity of pathologic involvement of the PI in horses with PPID and seems to confirm, to some extent, that PPID is a disease with a gradual and progressive onset that has subclinical stages that are difficult to definitively diagnose.

3. Summary

In summary, the discoveries made since 1999 about PPID in horses have been remarkable. These include its probable underlying cause, the regulation of hypothalamic-pituitary-adrenal function in both healthy horses and horses with PPID, and the varying degrees of PI pathological involvement. These discoveries have also fostered the development of new and promising diagnostic tests as well as resulted in reassessment of existing diagnostic tests. Although we have a much better understanding of the disease as a whole, this has not resulted in having a completely reliable antemortem diagnostic laboratory test for PPID. Whenever choosing one of the currently available diagnostic tests for PPID, it is important for the clinician to understand the strengths and weaknesses of each of the tests and tailor the selection of these tests to the clinical status of the patient at the time of testing. It may be necessary to either repeat tests over time or use more than one test in a given patient to achieve the best results.

References