PITUITARY PARS INTERMEDIA DYSFUNCTION - DIAGNOSIS

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Although the frequency of diagnosis and treatment of pituitary pars intermedia dysfunction (PPID) in horses has clearly increased over the past decade, there is no evidence that the prevalence of PPID is actually increasing. Increased recognition of the disease is likely a consequence of clients maintaining their horses to more advanced ages as well as improved health care (e.g., diet and dentistry) being provided to older horses. Recent surveys of horse owners in Queensland, Australia and the United Kingdom revealed prevalences of 15-20% of PPID in horses and ponies 15 years of age and older, increasing to nearly 30% in horses over 30 years of age. There is no gender predilection and average age of affected horses is around 20 years. All breeds and types of equids can be affected with PPID but ponies appear to be at greater risk.

Pathophysiology: In humans and dogs, Cushing’s disease is most commonly attributed to a corticotroph adenoma in the pars distalis of the pituitary gland. These adenomas are thought to arise spontaneously. In contrast, Cushing’s disease in horses is almost exclusively attributed to melanotrope hyperplasia or adenoma formation in the pars intermedia (PI) that appears to be due to loss of hypothalamic innervation. Abnormal PI tissue in horses contains markedly reduced amounts of dopamine, about 10% that of normal pars intermedia tissue, consistent with a specific loss of hypothalamic dopaminergic innervation. Further, tyrosine hydroxylase activity, the rate limiting enzyme for production of dopamine, is also markedly reduced in equids with PPID. Recent evidence suggests that this loss of dopaminergic innervation is due to oxidant-induced injury to hypothalamic tissue. Thus, a risk factor for affected horses may be reduced anti-oxidant defense mechanisms in neural tissue. Further, insoluble aggregates of the neural protein α-synuclein have been found in dopaminergic nerve terminals of PPID-affected horses. These protein aggregates are also found in humans with Parkinson’s disease suggesting that the two neurodegenerative disorders may share a similar pathogenesis. However, the population of neurons affected in horses, as compared to humans, appears to be somewhat different leading to the difference in clinical signs observed in each species.

In normal equids, hypothalamic dopaminergic neurons exert a tonic inhibitory action on PI melanotropes. Progressive loss of dopaminergic innervation over many years leads to unregulated enlargement of the PI in equids with PPID, initially by melanotrope hyperplasia and progressing to micro- and macroadenoma formation within the PI. A consequence of loss of PI dopaminergic innervation is excess production of the prohormone pro-opiomelanocortin (POMC, 241 amino acids). Processing of POMC by prohormone convertase 1 and prohormone convertase 2 leads to increased amounts of many POMC-derived peptides, including ACTH1-39 (POMC138-176) and α-MSH (ACTH1-13) in jugular venous plasma. Excess amounts of POMC peptides are
thought to be responsible for development of the variable clinical signs of PPID, although actual pathophysiology remains poorly understood.

Also unlike Cushing’s disease in humans and dogs, adrenocortical hyperplasia accompanying equine Cushing’s disease is relatively uncommon, occurring in ~20% of affected horses. These differences in location and pathophysiology between human, canine, and equine pituitary adenomas have lead several authors to suggest that the disease in horses should not be called equine Cushing’s disease; rather, pituitary pars intermedia dysfunction (PPID) has been advanced as a more appropriate descriptor.

**Clinical signs:** The classic clinical sign of PPID in horses is hypertrichosis, a long and curly hair coat that fails to shed. In some affected horses, coat color changes have also been observed. The pathogenesis of hypertrichosis, characterized by arrest of hair follicles in anagen, remains unclear. Abnormal sweating, both hyperhidrosis and anhidrosis, is also observed in up to two-thirds of horses with PPID. Weight loss and lethargy, or poor performance, can also be observed in horses with PPID. In addition to true weight loss, protein catabolism due to increased cortisol activity leads to loss of muscle mass. This is most notable in advanced cases as a loss of epaxial and rump musculature. Despite weight loss, appetite in affected horses is normal or even increased (polyphagia). However, dental abnormalities, leading to painful mastication and quidding, may compromise feed intake and contribute to weight loss in some horses. Combined with, or often preceding, loss of muscle mass in some horses is insulin resistance and deposition of fat along the crest of the neck, over the tail head, and in the sheath of male horses. Another area where abnormal fat deposition may occur is above and behind the eyes (supraorbital area). Horses with PPID have also been described as overly docile and more tolerant of pain than normal horses. The latter signs have been attributed to increased plasma and cerebrospinal fluid concentrations of β-endorphin that are 60- and more than 100-fold greater, respectively, in horses with PPID than in normal horses.

Chronic, insidious-onset laminitis is perhaps the major clinical complication of PPID with more than 50% of horses affected in most reports. Although the condition is more amenable to management in ponies due to their lower body weight, chronic or recurrent pain with exacerbation of laminitis or associated foot abscesses is often the reason for euthanasia. Polydipsia and polyuria (PU/PD) develops in about one-third of horses with PPID. Equids with PPID tend to have delayed wound healing and are frequently affected with secondary infections. Commonly recognized infections include skin infections (e.g., refractory “scratches” and fistulous tracts), recurrent subsolar abscesses, conjunctivitis, sinusitis, gingivitis, alveolar periostitis, and bronchopneumonia.

Other signs that have been reported in horses with PPID include persistent mammary secretions and infertility. Central nervous system (CNS) dysfunction, including ataxia, blindness, and seizure-like activity, are occasionally observed in equids with PPID. A major complication of hypercortisolism in affected human patients is osteoporosis. Although occurrence of this complication has not been investigated in horses, it is interesting to note that euthanasia of horses with PPID has been reported due to development of pelvic, pedal bone, mandibular, and multiple rib fractures.
Clinicopathologic findings: Abnormal laboratory data in horses with PPID may include mild anemia, an absolute or relative neutrophilia, and an absolute or relative lymphopenia. As well as being increased in number, neutrophils in affected animals may appear hypersegmented. This finding reflects maturity of neutrophils and can be attributed to a longer half-life of circulating neutrophils because cortisol excess limits diapedesis from the vasculature. The most common abnormality detected on serum biochemical evaluation is mild to moderate hyperglycemia, reported in 25-75% of cases, depending on the upper end of the reference range used. Additional abnormal biochemical findings may include elevations in liver enzyme activities, hypercholesterolemia, and hypertriglyceridemia.

Diagnosis: Practically, the diagnosis of PPID is most commonly made by observation of hypertrichosis and other clinical signs in older equids. However, establishing a diagnosis of PPID in less severely affected animals can be challenging. As a result, a number of endocrinologic tests have been used to evaluate horses with suspected PPID. Recently, the PPID Diagnosis Working Group of the Equine Endocrine Society developed a consensus statement (http://sites.tufts.edu/equineendogroup/files/2013/11/EEG-recommendations-downloadable-final.pdf) for the approach to diagnosis of PPID. Testing involves Tier 1 tests (screening tests) and Tier 2 tests for further evaluation of horses with inconclusive Tier 1 test results. Tier 1 tests include measurement of plasma ACTH concentration and the overnight dexamethasone suppression test and the currently recommended Tier 2 test is assessment of the ACTH response to thyrotropin releasing hormone (TRH). It warrants emphasis that the vast majority of experimental work evaluating diagnostic tests for PPID has been performed on horses; thus, extrapolation of findings to ponies and other equids has not been well validated.

Plasma ACTH concentration (Tier 1). Equids with PPID have excessive amounts of ACTH and ACTH-like peptides in abnormal PI tissue and increased amounts are released into plasma. Thus, measurement of plasma ACTH concentration is a logical choice for a single sample screening test for initial evaluation of equids suspected to have PPID. Earlier reports indicated that appropriate sample handling was critical for accurate determination of ACTH concentration; however, it is now recognized that the peptide is more stable. Collection of a blood sample into a plastic EDTA tube and placing it in a cooler or refrigerator with centrifugation and separation of plasma with 6-8 hours appears adequate. The plasma should then be frozen and sent on ice packs to the testing laboratory. Of interest, it has been shown that the “ACTH” measured in plasma from PPID-affected horses is less bioactive than ACTH in normal horses. This finding suggests that the assays used by testing laboratories may be measuring both endogenous ACTH and ACTH-like peptides that can also bind to the antibodies used in the assays. Thus, it should not be surprising that different ACTH assays (used in different labs) can yield different results. Which assay may be most “accurate” remains to be determined but a “take-home message” is that you should be consistent with the laboratory you use and interpret results using that labs reference intervals. A limitation of using plasma ACTH concentration is seasonal variation in test results. In normal ponies and horses without signs of PPID, plasma ACTH concentrations measured in the fall are often above the threshold for diagnosis of PPID. This “seasonal problem” initially led to a recommendation that testing in the late summer and fall months (late July through November in the northern hemisphere) should be avoided due to the for potential false-positive test results. Recently, however, it has been shown that PPID-affected horses have a more dramatic seasonal increase in ACTH in the fall (Figure 1) and that testing at this time of year,
interpreting results with seasonally adjusted reference ranges, may actually be a more sensitive test to detect PPID in the earlier stages of the disease, when plasma ACTH concentration may remain normal in non-fall months.

**Figure 1.** Plasma ACTH concentrations measured monthly in groups of normal horses (filled squares) and PPID-affected equids (open circles) demonstrating greater seasonal increases in ACTH in fall months in PPID-affected horses; the hatched line is the seasonally-adjusted upper limit of the reference interval (from Copas VEN, Durham AE. *Equine Vet J* 2012;44:440).

**Dexamethasone suppression test (Tier 1):** The overnight dexamethasone suppression test (ODST) is still considered by some equine clinicians to be the “gold standard” endocrinologic test to support a diagnosis of PPID. However, this statement is not without controversy and there is concern, although poorly documented, that administration of dexamethasone may induce or exacerbate laminitis in PPID-affected equids. In its most simple form, the ODST consists of measuring cortisol in the late afternoon (typically 5 pm) followed by administration of dexamethasone (40 µg/kg, IM = 20 mg to a 500 kg horse) and subsequently measuring plasma cortisol concentration between 17 and 19 h hours later (between 10 am and noon the following day) (Figure 2). The major limitation of the ODST for ambulatory practitioners is that it requires two visits to the horse. However, considering the fact that the most important value is the cortisol concentration following dexamethasone administration, the ODST can be simplified by dispensing dexamethasone to the client for administration and limiting the test to one visit the following morning. When using this test, it is probably wise to consider dexamethasone as a “sledgehammer” in terms of feedback to the hypothalamic-pituitary axis. In other words, failure of dexamethasone to induce suppression of circulating endogenous cortisol concentration is strongly supportive of PPID. Unfortunately, as with plasma ACTH concentration, the ODST may be less effective in diagnosis of PPID in the earlier stages of the disease when test results remain normal.

**Figure 2.** Overnight dexamethasone suppression test (ODST results in 43 horses with pituitary pars intermedia dysfunction (PPID) confirmed at necropsy and 18 non-PPID horses. Endogenous cortisol was measured prior to dexamethasone administration (40 µg/kg, IM) and again 15 and 19 hours later. Only 2 of 43 PPID-affected horses had an endogenous cortisol concentration <1.0 µg/dL (≈30 pmol/L, dashed line) at 15 hours and all 43 horses had an endogenous cortisol concentration >1.0 µg/dL at 19 hours. In contrast, all 18 non-PPID horses had suppression of endogenous cortisol concentration to <1.0 µg/dL at both 15 and 19 hours. (adapted from Dybdal NO, Hargreaves KM, Madigan JE, et al. *J Am Vet Med Assoc* 1994;204:627).
Seasonal variation can also affect ODST results but to a lesser extent than ACTH concentration. To examine the effect of season on ODST results, the author performed the test monthly for a year in a group of 18 aged horses (>19 years) without clinical signs of PPID. Seven of 18 horses had normal overnight DST results throughout the year while 11 horses had overnight DST results supportive of PPID from 1 to 9 months of the year. Test results from late July through late October were most commonly affected by seasonal variation. Thus, results of tests performed from July through November, if abnormal, should be interpreted with caution. However, it warrants emphasis that normal ODST results during late summer to fall are valid and can be useful in case assessment. A further observation in the author’s study that warrants mention is that no signs of laminitis were induced in this group of older horses during performance of 216 ODSTs.

Response of ACTH to TRH (Tier 2). TRH is a releasing hormone for several pituitary hormones. Nearly 30 years ago, administration of TRH was shown to increase plasma cortisol concentration when administered to horses and ponies with PPID. More recently, administration of TRH has also been demonstrated to result in greater increases in plasma ACTH concentration in PPID-affected equids than in normal aged equids (Figure 3). Because melanotropes in the PI have TRH receptors while corticotrophs in the pars distalis do not, the increase in ACTH following TRH administration can be attributed solely to release of ACTH and ACTH-like peptides from the pars intermedia. This difference has led to renewed interest in using the TRH stimulation test to support a diagnosis of PPID, especially when basal plasma ACTH concentration or results of an ODST are equivocal. The test is performed by measuring plasma ACTH concentration before and 10 minutes after administration of 1 mg of TRH IV and a positive result is an increase in ACTH above 110 pg/mL. Although the TRH stimulation test is currently being advocated as a “more sensitive” test for detection of PPID in the earlier stages of the disease, the true value of this test remains uncertain and needs to be assessed in a larger group of equids. A further reason that this test may be pursued, rather than an ODST, would be to alleviate owner concerns about possible exacerbation of laminitis following dexamethasone administration. As with the Tier 1 tests, the increase in plasma ACTH concentration after TRH administration is also greater in fall months and the ACTH threshold value, above which supports a diagnosis of PPID, has yet to be established.

Figure 3. Median plasma ACTH responses to 1 mg TRH IV in 44 horses with PI hyperplasia (PH+, open circles and solid line) and 22 horses with normal pituitary glands (PH-, + and dashed line) (from Durham AE et al. *Equine Vet Educ* 2014;26:216).
Serum insulin concentration. Measurement of basal insulin concentration may be of benefit in initial evaluation of equids with suspected PPID, not because insulin concentration is either sensitive or specific for diagnosis of PPID, but because it may offer prognostic information. Specifically, one case series found poorer long-term survival in PPID-affected equids with hyperinsulinemia as compared to PPID equids with a normal insulin concentration. This makes sense because insulin dysregulation has been associated with laminitis and it is also logical that prognosis may be poorer with multiple endocrine abnormalities than with dysfunction of the hypothalamic-pituitary-adrenal axis alone.

References

General reviews

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