# Equine Pituitary Pars Intermedia Dysfunction

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**KEYWORDS**

• Cushing's • Horse • Pituitary • Laminitis
• Hypothalamus • Adrenal

Equine pituitary pars intermedia dysfunction (PPID), also known as equine Cushing's syndrome, is a widely recognized disease of aged horses. Over the past two decades the aged horse population has expanded significantly and in addition, client awareness of PPID has increased. As a result, there has been an increase in both diagnostic testing and treatment of the disease. This review focuses on the pathophysiology and clinical syndrome, as well as advances in diagnostic testing and treatment of PPID, with an emphasis on those findings that are new since the excellent comprehensive review by Schott in 2002.[1]

## ANATOMY AND PHYSIOLOGY OF THE EQUINE PITUITARY PARS INTERMEDIA

### *Anatomy*

The equine pituitary gland lies within the sella turcica, separated from the brain by a fold of dura mater known as the diaphragma sellae, suspended ventral to the hypothalamus by the infundibular stalk. The pituitary gland can be divided into 4 lobes: pars distalis, pars intermedia, pars tuberalis (collectively known as the adenohypophysis), and pars nervosa (neurohypophysis). The pars distalis is a collection of endocrine cells that synthesize, store, and release hormones in response to hypothalamic releasing and inhibiting factors. These factors reach the pars distalis by way of the hypophyseal portal system, which connects the capillaries of the median eminence to the capillaries of the pars distalis. The pars tuberalis is a thin band of endocrine cells enveloping the infundibular stalk. It is dense in the melatonin receptors through which it reads and decodes daily melatonin concentrations to coordinate the output of reproductive hormones with season.[2] The pars nervosa is a collection of axons and nerve terminals that originate in the paraventricular and superoptic nuclei of the hypothalamus. The pars nervosa stores and releases oxytocin and arginine vasopressin. The pars intermedia of the horse consists of a single endocrine type cell, the melanotrope, that

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produces pro-opiomelanocortin (POMC) derived peptides. The pars intermedia is directly innervated by the dopaminergic neurons of the periventricular nucleus of the hypothalamus. It is unknown if neurons other than dopaminergic neurons directly innervate equine melanotropes.

### *Physiology*

Melanotropes of the pars intermedia and corticotropes of the pars distalis both produce a hormone precursor protein, POMC. POMC undergoes extensive tissue-specific posttranslational processing to yield adrenocorticotropin (ACTH), melanocyte-stimulating hormones (MSHs), β-endorphin, corticotropin-like intermediate lobe peptide (CLIP), lipotropins, and several other small peptides. Prohormone convertases 1 and 2 (PC1 and PC2, respectively) are serine proteases that cleave the larger POMC into smaller peptides (**Fig. 1**). PC1 is expressed in both corticotropes and melanotropes, whereas PC2, which cleaves ACTH into α-MSH and CLIP, is only expressed in melanotropes. As a result, nearly all plasma ACTH in the healthy horse is produced in the pars distalis.[3] Prohormone convertase activity is inhibited by dopamine. In mice lacking the dopamine receptor, PC1 activity increases 4- to 5-fold and PC2 activity increases 2- to 3-fold.[4] This relative difference in the magnitude of increase in expression that the 2 enzymes display when dopamine is absent may explain why horses with PPID produce pars intermedia–derived ACTH; PC2 cannot keep pace with the relatively more abundant PC1.

Following cleavage by the prohormone convertases, POMC peptides are further processed by *N*-acetylation and carboxy terminal proteolysis yielding a population of peptides with altered bioactivity.[5–7] For example, initial cleavage of β-lipotropin by PC2 yields β-endorphin,[1–31] which is a highly potent opioid agonist. In the presence of dopamine, β-endorphin may be further modified to β-endorphin (1-27), acetylated

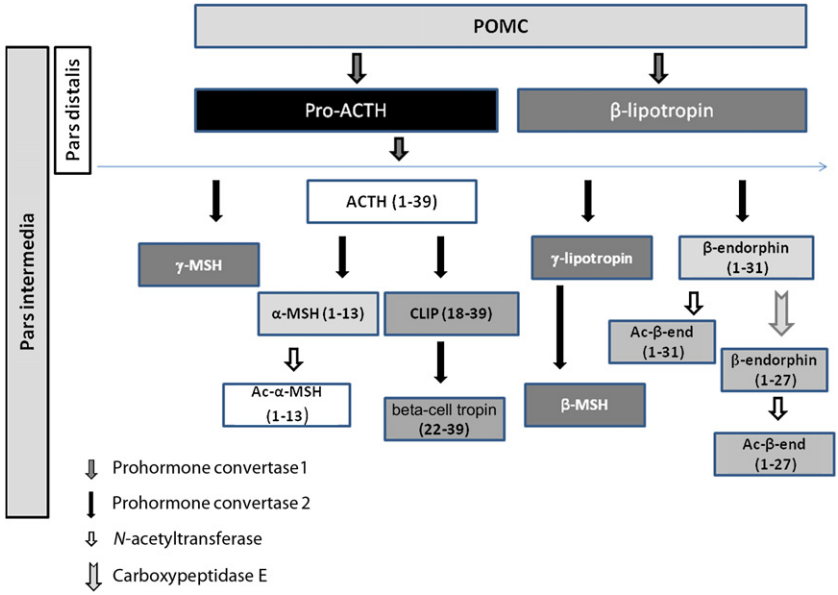


**Fig. 1.** POMC processing pathway. POMC is processed differently in the corticotropes of the pars distalis than in the melanotropes of the pars intermedia because of the differential expression of the enzymes involved in the posttranslational processing steps. Ac-α-MSH, acetyl-α-MSH; Ac-β-end, acetyl-β-end.

β-endorphin (1-27), and acetylated β-endorphin (1-31), all of which have minimal opioid agonist activity (see **Fig. 1**).[5–7]

The products of POMC are diverse and highly pleiotropic in function. Melanocortins exert a biologic effect through their interaction with a family of 5 G-protein coupled melanocortin receptors,[8,9] each with its own anatomic location and biologic activity. α-MSH is a primary product of POMC cleavage in the pars intermedia. It has a role in metabolism and obesity and is a potent antiinflammatory hormone. It is an antipyretic that is 25,000 times more potent than acetaminophen in reducing fever.[10,11] It has broad antiinflammatory effects that include decreasing the production of cytokines, costimulatory molecules, and other factors contributing to inflammation.[12] α-MSH also reduces neutrophilic oxidative burst, chemotaxis, and adhesion.[13,14] Little is known about the function of CLIP, the cleavage product generated from the C-terminal portion of ACTH. However, both CLIP and its cleavage product, beta-cell tropin, stimulate the release of insulin from rodent pancreatic beta cells.[15,16] β-Endorphin is a potent endogenous opioid agonist that functions in analgesia and in reduction of pain-associated inflammation.

Activity of the equine pars intermedia has been shown to be inhibited by dopamine and stimulated by thyrotropin-releasing hormone (TRH).[17,18] Both the dopamine $D_2$ receptor and the TRH receptor are expressed in the equine pars intermedia. Dopamine is released at the pars intermedia from the nerve terminals of the hypothalamic periventricular neurons that synapse directly to the melanotropes.[19] In the presence of dopamine, there is a decrease in POMC transcription and translation and secretion of POMC-derived peptide hormones. It is unknown whether TRH neurons directly innervate equine melanotropes as in amphibians[20,21] or TRH reaches the equine pars intermedia via the circulation. It is likely that in addition to dopamine and TRH, there are unidentified regulatory factors that modify equine pars intermedia function.

Similar to other species such as hamsters and sheep, activity of the pars intermedia in horses has a robust seasonal rhythm, with increased output as day length shortens (**Fig. 2**).[22–26] As a result, the plasma concentrations of α-MSH and ACTH are greatest in the autumn (August–October).[22–29] This adaptation helps animals to prepare for the metabolic and nutritional pressures of winter.[23–25] Because of the increase in pars intermedia activity, false-positive diagnostic test results are common when testing is performed in autumn and if reference ranges are not adjusted for season.[22,27] In addition, clinical signs of PPID may follow a seasonal pattern. Laminitis occurs most frequently in autumn.[30] Because pasture composition also changes significantly with season, studies are needed to determine the role of hormone level increase in seasonal development of laminitis.[31]

## EPIDEMIOLOGY

PPID is a common endocrinopathy of aged horses and ponies. Recent epidemiologic investigations have suggested a disease prevalence of 15% to 30% in aged equids. According to data from owner surveys, hair coat abnormalities were present in 14% to 30% of aged horses.[32,33] Using the determination of plasma ACTH and α-MSH concentrations as a diagnostic test, 20% of aged horses showed positive test result for PPID by one or the other test, with 80% of the horses with positive test result having historical or concurrent clinical signs of disease.[33]

Similar to other neurodegenerative diseases, the most important risk factor for the development of PPID is age. Typically, recognition of clinical signs occurs in animals aged 18 to 20 years, with only rare reports in horses younger than 10 years.[1,17] Several breeds have been considered to be at greater risk for the development of PPID based

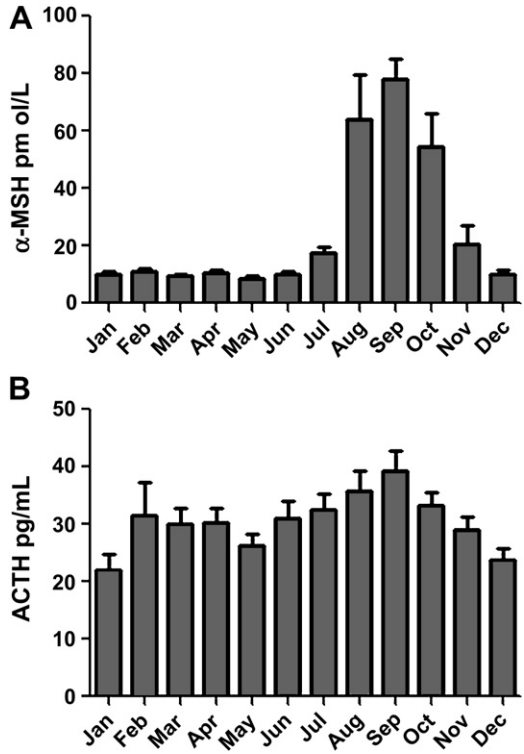


**Fig. 2.** Seasonal variability of POMC-derived hormones in horses. Mean (±SEM) monthly concentrations of plasma α-MSH (*A*) or ACTH (*B*) in 22 normal horses.

on clinical case reports, including ponies and Morgan horses. In the literature, 100 of 242 (42%) equids diagnosed with PPID were ponies.[34–44] However, in a recent study of 340 aged equids, neither breed nor height was associated with an increased risk for PPID, despite the ponies being significantly older than the horses.[33] Morgan horses were not part of the study population. One explanation for the conflicting results is that all pony breeds may not have a similar risk of PPID. Additional epidemiologic studies are needed to clarify the role of breed in the risk of PPID. Although early reports suggested that mares are at greater risk of developing PPID,[45] the current literature suggests that the distribution of PPID does not differ between males (n = 161) and females (n = 153).[34–44,46,47]

Geographic distribution of PPID has not been studied. Parkinson disease, a dopaminergic neurodegenerative disease of aged people, has been shown to have a regional distribution in the United States, with fewer cases in the south and more cases in agricultural areas.[48–50] It would be interesting to assess geographic data for PPID in the horse population. A shared pattern would suggest that similar environmental exposures may predispose to dopaminergic neurodegeneration in both diseases.

## PATHOPHYSIOLOGY

Horses with PPID have hyperplasia of the pars intermedia with a single large adenoma or multiple small adenomas. PPID was previously characterized as a benign neoplasia

of the equine pituitary gland. However, clinical, pharmacologic, biochemical, and histologic data indicate that PPID is a neurodegenerative disease with loss of dopaminergic inhibitory input to the melanotropes of the pars intermedia.[51] The clinical course of PPID is typical of a neurodegenerative disease; it is a slowly progressive disease that affects primarily aged animals. In response to administration of a dopamine agonist, plasma concentrations of POMC-derived peptides decrease in horses with PPID,[17] and treatment of horses with PPID with the dopamine agonist pergolide results in improvement of both the clinical signs and biochemical abnormalities associated with disease.[37–39] Furthermore, pars intermedia tissue from PPID horses was shown to have 8-fold less dopamine concentration than the tissue from age-matched controls,[52] and hypothalamic and pituitary tissues from horses with PPID were found to have a 6-fold reduction in levels of dopaminergic nerve terminals in the pars intermedia and a 50% reduction in levels of dopaminergic cell bodies in the periventricular nucleus.[53] An absence of dopamine is known to cause proliferation of melanotropes with increased production of POMC and POMC-derived peptides in culture,[54] dopamine receptor–deficient mice,[4] and surgically hypothalamic pituitary gland–disconnected animal models.[55,56] Considered collectively, these data strongly support the hypothesis that PPID is a dopaminergic neurodegenerative disease.

Although the precise cause of PPID is unknown, evidence suggests that oxidative stress may contribute to neuronal damage and cell death. Histologic examination of the pituitary gland of horses with PPID revealed a 16-fold increase in the levels of oxidative stress marker 3-nitrotyrosine in the nerve terminals of the periventricular dopaminergic neurons compared with healthy adult horses.[53] Lipofuscin pigment is also abundant in the pituitary neurons of horses with PPID.[57] Lipofuscin is an accumulation of oxidized cellular debris. Systemic oxidative stress or antioxidant failure does not seem to contribute to the development of PPID. The only indicator of systemic oxidative stress that has been shown in horses with PPID is a mild decrease in plasma thiol levels.[58] Peripheral total glutathione, malondialdehyde, glutathione peroxidase, and superoxide dismutase activities are unchanged in horses with PPID.[58,59] Although pituitary antioxidant capacity has not been shown to be impaired in horses with PPID, pituitary manganese superoxide dismutase activity has been found to decrease with age in horses.[59] Impairment of the activity of this mitochondrial antioxidant could contribute to the increased risk of PPID that occurs with age.[59] Further evaluation of the role of mitochondrial dysfunction and mitochondrial reactive oxygen species production in the pituitary gland of horses with PPID is warranted.

Neuronal accumulation and aggregation of misfolded proteins is a mechanism that contributes to the pathogenesis of most neurodegenerative diseases including Parkinson disease. In Parkinson disease, the protein that accumulates in the dopaminergic neurons is α-synuclein, a natively unfolded soluble monomeric protein that is expressed in nerve terminals and leukocytes. Under certain cellular conditions, α-synuclein can misfold and aggregate disrupting cellular function and triggering cell death.[60] Conditions that promote accumulation of α-synuclein include excessive concentration because of increased production or decreased clearance; posttranslational modifications, such as oxidation or nitration; and primary gene mutations.[61,62] Similar to what is observed in the brain of patients with Parkinson disease, α-synuclein was found to be more abundant in the pars intermedia of horses with PPID.[53] Increased gene expression of α-synuclein suggests that enhanced production has a role in α-synuclein accumulation in the horse with PPID (Dianne McFarlane, unpublished data, 2010). In addition to being more abundant, pars intermedia α-synuclein seems to be excessively nitrated, a modification known to promote α-synuclein aggregation.[53,63] It is unknown if failure of protein clearance also contributes to

α-synuclein accumulation in horses with PPID. Misfolded proteins are removed primarily through autophagy, the process by which damaged proteins or organelles are recycled by the lysosome. Impaired autophagy has been suggested to play a critical role in the pathogenesis of protein-misfolding diseases, including Parkinson disease.[64] Assessment of autophagy in the periventricular neurons of horses with PPID is needed.

## CLINICAL SIGNS OF PPID

The clinical signs of PPID have been discussed in detail in previous reviews.[1] However, the mechanistic cause of these signs remains largely unknown. In addition, the interrelationship of the clinical signs present in individual animals is not well described. For example, it is not clear in the literature if the horses with PPID with abnormal fat deposits also have insulin resistance, hyperglycemia, polyuria/polydipsia (PU/PD), and laminitis. It is conceivable that PPID is a collection of syndromes each with a unique set of clinical signs and hormone profiles. Knowing how the clinical signs are related to each other and to an array of hormones would improve the understanding of the pathologic mechanisms of PPID.

### *Hirsutism*

The most unique and specific clinical sign associated with PPID is the development of an abnormal hair coat, including hirsutism, delayed shedding, incomplete shedding, and lightening of coat color in aged horses.[46] Aged horses with hirsutism or with a history of hirsutism and those that fail to shed completely have been shown to be 5 times more likely to have a positive PPID test result than aged horses with normal coats,[33] and hirsutism was found to have a positive predictive value of 90% for PPID using postmortem examination as the gold standard.[46] The pathologic mechanisms responsible for hair coat abnormalities in PPID have not been studied.

### *Muscle Atrophy*

A common sign of PPID is muscle wasting or sarcopenia, affecting most prominently the epaxial and gluteal musculature. Sarcopenia is also a characteristic of aging in horses and people in the absence of disease.[33,65,66] Characterization of muscle changes in horses with PPID revealed atrophy of type 2 fibers, sarcoplasmic lipid accumulation, increased myofiber size variation, and subsarcolemmal accumulation of swollen mitochondria.[67] These findings are consistent with those of glucocorticoid excess in other species.[68,69] However, other hormone derangements, including insulin resistance, and chronic inflammation can also cause sarcopenia.[65,70] Further studies are needed to clarify the mechanism underlying muscle wasting in PPID.

### *Laminitis*

Endocrinopathy is the most common cause of laminitis in the horse. Both equine metabolic syndrome and PPID are associated with an increased risk of laminitis.[30,71,72] Although the cause of laminitis remains elusive, recent work has suggested that high serum insulin concentration both predicts and provokes laminitis. Studies have documented that horses and ponies with high fasting insulin concentrations are more likely to founder.[40,72,73] Recently, induction of clinical, radiographic, and histologic signs of laminitis in normal horses and ponies was achieved using a model that creates hyperinsulinemia while maintaining normal insulin sensitivity and blood glucose concentration.[74,75] High nonphysiologic doses of insulin were used in the model. Alterations in cortisol metabolism may also have a role in the

development of laminitis. Preliminary data suggest that tissue-specific variation in 11β-hydroxysteroid dehydrogenase activity occurs in the neck adipose tissue of horses with acute laminitis and equine metabolic syndrome.[76,77] Tissue activity of 11β-hydroxysteroid dehydrogenase has not been evaluated in horses with PPID.

### *PU/PD*

PU/PD occurs in approximately 30% (80 of 260) of horses with PPID.[30,34,36–42,45,47,72] Proposed mechanisms for the development of PU/PD include loss of antidiuretic hormone because of compression of the pars nervosa, increased thirst because of central actions of hypercortisolemia, and osmotic diuresis because of hyperglycemia and glucosuria. The observation that horses can have marked hyperglycemia without an increase in voluntary water intake suggests that a mechanism other than osmotic diuresis is responsible in at least some cases of PPID.[35]

### *Hyperhidrosis*

Excessive sweating has been reported to occur more frequently in horses with PPID.[1] Using a quantitative intradermal terbutaline sweat test,[78] 4 of 8 horses with PPID were observed to sweat excessively (Dianne McFarlane, unpublished data, 2010). Although some horses with PPID may sweat only because of a long hair coat, other horses with PPID-associated hyperhidrosis continue to sweat excessively even in a cool environment or when body clipped.

### *Abnormal Fat Distribution and Insulin Resistance*

Abnormal fat distribution is present in 15% to 30% of horses with PPID,[34–38,40,47] and insulin resistance, defined by increased fasting insulin level, is present in 60% (61 of 103) of horses with PPID.[1,33,58,79] Fat pads are typically located above the eyes in the supraorbital fossa, along the crest of the neck, over the tail head, and in the sheath or mammary region.[80,81] It is unclear whether fat deposition occurs as a result of PPID or abnormal fat accumulation is a predisposing condition for the development of PPID. Adiposity and insulin resistance cause chronic inflammation and mitochondrial impairment resulting in oxidative stress, which may have a role in the development of PPID. Longitudinal population studies are needed to determine how frequently horses with obesity and its related conditions progress to develop PPID.

### *Opportunistic Infections and Immunosuppression*

Opportunistic or secondary infections occur in approximately 35% (63 of 180) of horses with PPID compared with 11% (4 of 33) of healthy aged horses (Dianne McFarlane, unpublished data, 2010).[34,36,38,40–42,45,47,57] Common infections include dermatophilosis, sinus infection, pneumonia, and abscesses. Horses with PPID are also likely to have occult infections presumably because of the absence of a significant inflammatory response to pathogens. Horses with PPID often have a pathologic evidence of chronic pneumonia at necropsy without a history of clinical disease.[57] Horses with PPID also have been shown to have higher fecal strongyle egg counts, suggesting that they are more susceptible to endoparasitism.[82]

Previous literature has suggested that high serum cortisol concentration is responsible for immunosuppression in PPID; however, this view is likely oversimplified. As discussed earlier, blood concentrations of several immunosuppressive hormones are increased in horses with PPID, including α-MSH, β-endorphin, and ACTH. These hormones may function in concert to alter the immune response and create a pathogen permissive environment. Aging, in the absence of disease, is associated with changes in immune function characterized by a loss in the ability to respond appropriately to

challenges and an increased baseline inflammatory state. Horses with PPID have a leukocyte proinflammatory cytokine profile typical of adult rather than aged horses.[44] In contrast, cytokine response to endotoxin stimulation is greater in peripheral blood mononuclear cells from horses with PPID than from adult horses. Neutrophil function also seems to be impaired with PPID; chemotaxis and oxidative burst are decreased compared with age-matched controls.[83] Equine neutrophilic oxidative burst activity was found to be strongly correlated to α-MSH/insulin ratio but not correlated to serum cortisol concentration from the same sample.[83]

### *Behavioral Abnormalities*

Horses are often described as becoming more lethargic or docile with the development of PPID. Lethargy may result due to metabolic abnormalities, such as insulin resistance; concurrent disease; or high plasma β-endorphin concentrations. Occasionally, a horse is perceived as lethargic because of reluctance to move secondary to laminitis. Clinical signs of laminitis secondary to PPID can be subtle, possibly because of high pain tolerance secondary to increased β-endorphin concentration.

### *Reproductive Infertility*

PPID should be considered in the differential diagnosis of aged mares that fail to conceive or have abnormal estrous cycles. Decreased dopaminergic regulation of reproductive hormonal output and chronic uterine infections may contribute to infertility in mares with PPID. Treatment of infertile mares with PPID with pergolide may restore reproductive function and normal cycling.[38,42] Administration of pergolide to pregnant mares does not seem to be associated with adverse effects. Discontinuing pergolide administration a month before foaling to avoid periparturient complications such as agalactia is recommended.

### *Neurologic Disease*

Neurologic impairment, including ataxia, blindness, seizures, and narcolepsy, has been suggested to occur in 6% to 50% of PPID cases. In a herd of 37 aged horses, neurologic impairment was observed more commonly in horses with PPID (27%) than in aged horses without PPID (5%) (Dianne McFarlane, unpublished data, 2010); however, larger studies are needed.

### *Clinical Pathology*

Routine hematologic and serum biochemical analyses should be part of a complete health examination of an aged horse with or without PPID. In most horses with PPID, routine blood analysis is nondiagnostic but it may provide information regarding general health and PPID-associated secondary diseases. The most common abnormality identified by serum biochemical evaluation in the laboratory in a horse with PPID is hyperglycemia. Although nonspecific, when hyperglycemia is present in routine blood analysis of an aged horse, PPID should be considered. Other abnormalities in horses with PPID may include increased liver enzyme activities, which may be an indication of steroid-induced hepatopathy. Histologic findings consistent with steroid-induced hepatopathy, specifically swollen vacuolated hepatocytes, were reported in 73% of horses with PPID, and 71% of the horses with hepatocellular swelling also had adrenocortical hyperplasia.[57]

## DIAGNOSTIC TESTS FOR PPID

With the past decade has come the realization that diagnosis of PPID is not straightforward. Rather, it is complicated by the slow progressive nature of PPID, seasonal variation in hormone output, and overlapping endocrine response to various diseases and pathologic events. In addition, the lack of a true gold standard has impeded the ability to adequately validate traditional or novel diagnostic tests. Hence, there is confusion regarding the best way to diagnose PPID, and many equine clinicians have been frustrated trying to arrive at an accurate diagnosis in patients. Testing for PPID in the autumn is associated with false-positive test results regardless of the diagnostic method used. Studies that were conducted before 2004 validating diagnostic tests must be interpreted cautiously because the investigators would have not accounted for the potential influence of season in either the study design or the data interpretation.[31,37] In all methods of testing, false-negative test results are common early in disease. As with other neurodegenerative diseases, the slow progressive nature of PPID makes it likely that significant pathologic effects have already occurred before diagnostic testing can identify the animal as having PPID. Repeated testing of horses with negative test results but with clinical signs compatible with PPID is recommended.

### *Dexamethasone Suppression Test*

Dexamethasone suppression test has long been considered the gold standard antemortem test, although its superiority over other diagnostic methods remains unproven.[84] In the normal horse, inhibition of ACTH release from the pars distalis by dexamethasone results in suppression of cortisol release from the adrenal gland (**Table 1**). Horses with PPID cannot suppress cortisol release because of ACTH secretion from the pars intermedia, which is not subject to glucocorticoid feedback. The dexamethasone suppression test was originally reported to have 100% sensitivity and specificity using postmortem examination as the gold standard.[84] However, horses in this study were selected based on the presence or absence of overt signs of PPID, which would have favorably biased test performance. There are at present no unbiased studies that compare the overnight dexamethasone suppression test directly with other diagnostic tests using clinical signs and postmortem examination as the gold standard. In the author's experience, some patients with confirmed PPID have an abnormal plasma ACTH concentration before an abnormal dexamethasone suppression test result, whereas in others the converse is observed. At present, data are insufficient to say which diagnostic method is the best at different stages of disease. It is the author's opinion that PPID is a clinical syndrome of different causes, all culminating in dysfunction of the pars intermedia. Therefore, it is unlikely that 1 testing strategy will be optimal in all cases.

### *Endogenous Plasma ACTH or $\alpha$-MSH Concentration*

Measurement of plasma concentrations of ACTH and $\alpha$-MSH has also been shown to be useful in the diagnosis of PPID.[22,36] In the healthy horse, $\alpha$-MSH is primarily a product of the pars intermedia, whereas ACTH is produced by the corticotropes of the pars distalis. In PPID, ACTH is also released from the pars intermedia. Measurement of plasma ACTH concentration for diagnosis of the disease may be confounded by many factors. ACTH levels have been shown to increase in response to stress, competition, and exercise.[85,86] The effect of disease, debilitation, inflammation, or trauma on ACTH concentration in horses has not been extensively investigated. It is

**Table 1**
**Diagnostic testing methods for PPID**

| Diagnostic Test | Procedure | Sample | Interpretation | Comments (Also See Text) |
|---|---|---|---|---|
| Overnight DEX Suppression | Collect serum between 4–6 PM. Administer DEX at 40 μg/kg BW IM. Collect serum 19–20 h later | 2 serum samples, 1 mL each: 1 preDEX administration and 1 post-DEX administration | Serum cortisol of >1 μg/dL at 19 h post-DEX administration suggests PPID | A mildly decreased resting cortisol (pre-DEX administration) is typical of a PPID-affected horse. A resting cortisol of <1.8 μg/dL is suggestive of iatrogenic adrenal insufficiency |
| Endogenous Plasma ACTH Concentration | Collect EDTA plasma, preferably in plastic blood collection tube. Separate plasma by centrifugation, and freeze for submission to laboratory. Avoid hemolysis and heat. Process sample within 8 h of collection | EDTA plasma sample, 1 mL | Normal reference range depends on methodology and laboratory. Typically an ACTH concentration <35 pg/mL (chemiluminescent immunoassay) or <45–50 pg/mL (radioimmunoassay) is considered normal | ACTH is likely affected by many biologic events, all of which are not well documented at present. Seasons can have a profound effect, with higher concentrations seen in autumn |
| Endogenous Plasma α-MSH Concentration | Collect EDTA plasma, preferably in plastic blood collection tube. Separate plasma by centrifugation, and freeze for submission to laboratory. Avoid hemolysis and heat. Process sample within 8 h of collection | 1 EDTA plasma sample, 1 mL | Nonautumn reference range: >35 pmol/L suggests PPID | Plasma α-MSH concentration is extremely seasonal. High concentrations are observed in autumn |

| | | | | |
|---|---|---|---|---|
| TRH Stimulation Assay | Collect serum. Administer TRH, 1 mg IV. Collect serum 30–60 min after TRH | 2 serum samples, 1 mL each: pre-TRH administration and 30–60 min post-TRH administration | 30%–50% increase in serum cortisol 30 min after TRH administration suggests PPID | Pharmaceutical TRH is expensive, TRH compounded for this use may be difficult to obtain. False-positive results may be common |
| Combined DEX Suppression/ TRH Stimulation Test | Collect plasma between 8 and 10 AM Administer DEX at 40 μg/kg BW IM. Administer TRH, 1 mg IV, 3 h after DEX administration. Collect serum 30 min after TRH and 24 h after DEX administration | 3 plasma samples, 1 mL each: pre-DEX administration, 30 min post-TRH administration, and 24 h post-DEX administration | Plasma cortisol >1 μg/dL at 24 h post-DEX administration or ≥66% increase in cortisol levels 3 h after TRH administration suggests PPID | Some diagnostic laboratories prefer to use serum for measurement of cortisol levels. The effect of season on the combined test has not been assessed but would likely result in false-positive results as each of the component tests do |
| Domperidone Response Test | Collect EDTA plasma at 8 AM. Administer domperidone at 3.3 mg/kg BW po. Collect EDTA plasma at 2 and 4 h after domperidone administration | 3 EDTA plasma samples, 1 mL each | A 2-fold increase in plasma ACTH concentration suggests PPID | Higher doses (5 mg/kg po) may improve response. The 2-h sample is more diagnostic in the summer and autumn, and the 4-h sample is best in the winter and spring |

*Abbreviations:* BW, body weight; DEX, dexamethasone; IM, intramuscularly; IV, intravenously.

likely other diseases or events may confound interpretation of plasma ACTH concentration for the diagnosis of PPID.

Plasma α-MSH concentration is a direct product of the pars intermedia, and an increased plasma α-MSH concentration is highly suggestive of PPID. α-MSH has been shown to be strongly influenced by season, and seasonal reference ranges are needed to maximize the discriminatory ability of this test. Measuring α-MSH concentration has been shown to have improved diagnostic accuracy than measuring ACTH concentration.[28] Although not offered as a commercial test at present, measurement of plasma α-MSH concentration may provide a slight improvement as a single sample test for PPID compared with tests available at present.

#### *TRH Stimulation Test*

The TRH stimulation test is based on the observation that horses with PPID have a 30% to 50% increase in serum cortisol concentration following administration of TRH, whereas normal horses do not respond.[87] TRH directly stimulates equine melanotropes; plasma α-MSH concentration increased more than 400% in healthy horses following TRH administration.[20] The TRH stimulation test has the advantage of being both a safe and expedient (30 minutes) test. However, false-positive test results are common, with 1 of 3 healthy horses being falsely identified as having PPID in one study.[20] Adrenal gland release of cortisol may be modulated by several poorly defined physiologic and pathologic events. In addition, the disparity between plasma ACTH and cortisol concentrations in horses with PPID suggests that the excessive ACTH produced in horses with PPID may be immunologically active but biologically inert.[88] To circumvent this problem, Beech and colleagues[89] investigated the measurement of ACTH following TRH administration and suggested that this measurement may be a more discriminating test for PPID than the measurement of cortisol release.

#### *Combined Dexamethasone Suppression/TRH Stimulation Test*

The combination of the 2 provocative tests described earlier improves the sensitivity and specificity compared with either test considered alone.[46] The disadvantage of this approach is the need for multiple samples within 24 hours.

#### *Domperidone Response Test*

The domperidone response test measures ACTH release in response to administration of domperidone, a dopamine receptor antagonist. Because healthy horses are thought to have minimal ACTH production from the pars intermedia and because their corticotropes are not regulated by dopamine, only horses with PPID should produce ACTH when relieved of dopaminergic inhibition.[90,91]

#### *Serum Insulin Concentration*

Fasting serum insulin concentration is increased in approximately 60% of horses with PPID.[1,33,58,79] Conditions other than PPID may also increase fasting insulin levels, most notably, equine metabolic syndrome. A high percentage of false-positive and false-negative test results provides limited value to this test for the diagnosis or screening of PPID. However, monitoring fasting insulin concentration is recommended in all horses suspected of having PPID because it has been shown to be predictive for the development of laminitis.[72,73]

#### *Cortisol Circadian Rhythm Loss*

Loss of cortisol circadian rhythm occurs in horses with PPID.[84] It has been suggested that monitoring the circadian rhythm of cortisol may be useful for the diagnosis of

PPID.[92] However, loss of circadian rhythm is a common manifestation of a generalized disease and it also occurs as part of normal aging. In a study of 50 healthy horses, 64% had a difference of less than 30% in morning and evening serum cortisol levels, which is the suggested cutoff for the diagnosis of PPID (Dianne McFarlane, unpublished data, 2010).[92] This very low specificity makes this test unsuitable as a diagnostic test.

### *Urinary Cortisol/Creatinine Ratio*

The utility of the measurement of cortisol/creatinine concentration in a single urine sample to diagnose PPID has been evaluated[43] in healthy horses, horses with PPID, and horses with non-PPID illness (grass sickness). Although urinary cortisol/creatinine ratio was higher in horses with PPID than in healthy controls, there was not a significant difference in the ratio among the 3 groups, and the diagnostic sensitivity (85%) and specificity (55%) for PPID was poor. This poor performance was likely the result of the nonspecific cortisol response that occurs as part of a general sickness syndrome.

### *ACTH Stimulation Test*

Administration of ACTH results in release of cortisol from the adrenal gland. The magnitude of the cortisol response is correlated to the adrenal gland size. Therefore, an equal dose of ACTH elicits a greater cortisol response in animals with hyperadrenocorticism than in normal animals. The performance of this test for diagnosing PPID has been assessed in 3 small studies that used postmortem examination as the gold standard. Although the test had 75% sensitivity for the diagnosis of PPID in 2 of the studies,[34,79] the third study found no difference in ACTH-stimulated cortisol response between PPID-affected and healthy aged horses.[84] Adrenal gland hyperplasia is observed in only 20% to 30% of horses with PPID; therefore, an exaggerated response to ACTH in horses with PPID is unlikely to be a consistent finding.[45,47,57]

### *Advanced Imaging Modalities*

The use of advanced diagnostic imaging may not ever prove practical for the diagnosis of PPID because of the expense and the need for general anesthesia. However, recent studies have demonstrated that contrast-enhanced magnetic resonance imaging has the capability to image the equine pituitary gland in enough detail such that the pars intermedia can be differentiated from the adjacent lobes.[93] This advancement will enable the monitoring of dynamic changes of the pituitary with season, with disease, and in response to treatment and therefore has a strong potential to enhance the understanding of the development and progression of PPID.

### *Necropsy*

Postmortem examination of the horse with PPID reveals a grossly enlarged pituitary, often 2 to 5 times the normal size. This enlargement is caused by hypertrophy and hyperplasia of the pars intermedia with microadenomas (<1 cm) or macroadenomas (>1 cm). Large adenomas may contain areas of hemorrhage and necrosis. Affected melanotropes are pleomorphic (polyhedral or spindle shaped) with eosinophilic granular cytoplasm. Cells are organized into nodules or follicular structures separated by fine septal tissue.[35,47,57,90,94] Lipofuscin deposition is common and often severe in the region of the pars nervosa adjacent to the pars intermedia. Other lesions include compression of the adjacent structures, including the pars distalis, pars nervosa, pars tuberalis, or in rare cases, the optic chiasm or hypothalamus. Other, nonpituitary lesions related to disease complications such as laminitis or pneumonia are commonly present.[35,47,57] Evidence of inflammation and oxidative damage may be observed in multiple organs, including the heart, liver, kidney, and lungs.[57]

## TREATMENT

Horses with PPID benefit from the provision of an optimized geriatric health management program and the pharmaceutical treatment of the disease. Similar to other aged horses, PPID-affected animals require aggressive preventative health care. The importance of excellent dental care, hoof care, nutrition, and parasite control cannot be overemphasized. PPID-affected horses typically benefit from a processed senior-type concentrate that is easy to masticate and digest. In theory, feeds high in antioxidants could slow the neurodegenerative process associated with PPID, although evidence for this action is lacking. The specific amount and type of feed needs to be individualized to the horse's weight and hormone profile. In horses with insulin resistance, a highly soluble carbohydrate feed should be avoided. However, unlike the horses with equine metabolic syndrome, the PPID-affected insulin-resistant horses often also have concurrent muscle wasting, complicating their nutritional requirements. Fortunately, many commercial feeds are now available designed specifically for the aged or endocrine-impaired horse. Fecal egg counts should be routinely performed in horses with PPID because of their predisposition to strongyle infections.[82] Horses with PPID may have difficulty with thermoregulation, so ample fresh water, shelter, and shade should be provided, with body clipping and blanketing as needed. Medical conditions are more frequent in aged horses, especially in those with PPID; therefore, careful and frequent observation for the evidence of secondary illness is important. When well cared for, horses with PPID can live into their 30s and even 40s.

The drug of choice in the treatment of PPID is pergolide mesylate. Pergolide is an ergot-derived dopamine $D_2$ receptor agonist, which downregulates POMC peptide production. The efficacy of pergolide at improving clinical signs of disease and diagnostic test response has been documented in several studies.[37–39,41,42] Pergolide, which was used to treat Parkinson disease, was voluntarily withdrawn from the market in 2007 for use in humans because of the reports of cardiac regurgitation and vegetative valvular lesions.[95,96] Heart lesions have not been reported in horses receiving pergolide. After its removal from market, the Food and Drug Administration's Center for Veterinary Medicine issued a limited exemption from the Animal Medicinal Drug Use Clarification Act regulations, allowing pergolide to be compounded for veterinary use from bulk sources until a new animal drug application for the product is approved. Care must be taken, however, when using compounded pergolide because it is unstable in aqueous vehicles and needs to be stored refrigerated in the dark.[97] Aqueous drug form should not be used more than 30 days after formulation.[97]

Most clinicians start with a dose of 1 mg per horse then titrate to effect in increments of 0.5 to 1.0 mg units. Clinical and diagnostic test improvement is typically not apparent for several months, so recheck examinations with recommendations for dose adjustments every 6 to 12 months are appropriate. Adverse effects are uncommon at this dose; however, anorexia can occur. Anorexia usually can be resolved by abruptly decreasing the dose, then slowly increasing the dose over time until the desired dose is achieved. Some horses improve if the total dose is split and administered twice daily. Lifelong treatment with pergolide is recommended. There are reports of horses that have been maintained on pergolide treatment for more than 10 years (Schott, personal communication, 2010). Anecdotal reports suggest that dose requirements increase slowly over time in treated animals, likely because of a continued progression of disease or the development of drug tolerance. Because of the seasonal fluctuation in hormone production by the pars intermedia, it may be possible to strategically treat mild cases of PPID for only 6 months of the year (eg, June–December); however, this therapeutic

approach has not yet been critically assessed. In vitro studies have suggested that pergolide may also have antioxidant activity and may provide neuroprotective benefits.[98,99] These properties could be beneficial in slowing the progression of PPID. Further work is needed to determine the benefit of early therapeutic intervention in the time course and ultimate outcome of the disease.

Recent improvements in the methodology to measure serum pergolide levels have allowed pharmacokinetic studies to be performed in humans and horses.[100,101] Preliminary work has shown that pergolide is rapidly absorbed following oral administration in horses. Exact bioavailability was not determined.[101] As expected, the pharmacodynamic effects of pergolide (change in hormone concentration) were not predicted by the pharmacokinetic parameters of the drug.[101]

Cyproheptadine has been suggested as a second-line drug used in combination with pergolide when maximal doses of pergolide alone are insufficient to achieve resolution of clinical signs. As a monotherapeutic agent, cyproheptadine has limited efficacy.[38,39] Cyproheptadine is a mixed-action drug, with serotonin antagonist, antihistamine, and antimuscarinic effects. Cyproheptadine lowers seizure threshold in mice, so it should be used cautiously in horses with a history of seizures or central neurologic disease.[102]

Trilostane is a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, the enzyme responsible for production of cortisol from cholesterol. Trilostane was reported to improve clinical signs of PPID but not the dexamethasone suppression test results.[40] Trilostane may be beneficial to those horses with PPID with adrenal gland hyperplasia and hypercortisolemia; however, it would have no effect on the excessive production of pituitary-derived hormones.

Many nutraceuticals, botanicals, and natural remedies are available for the treatment of PPID. To date, only 1 such product has been tested in horses with PPID. In this study, a commercial *Vitex agnus castus* (chasteberry, Vitex) extract failed to resolve clinical signs or improve diagnostic test results in 14 horses.[103] In fact, several animals' condition worsened, and use of the extract was discontinued early. In contrast, 8 of 9 horses subsequently treated with pergolide improved. The lack of efficacy and safety evidence makes these remedies contraindicated in horses with PPID.

## SUMMARY

Much has been learned about equine PPID over the past decade; however, far more remains to be accomplished. There is a critical need for accurate and early testing methods with season-specific reference ranges. Disease risk factors need to be clarified and preventative strategies developed. Understanding the complexity of the hormonal derangements in PPID and using this knowledge to formulate more individualized and targeted therapy plans may help avoid the life-threatening complications that occur with PPID.

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