Equine Cushing's Disease or Equine Pituitary Pars Intermedia Dysfunction (PPID)
Keeping up with Evolution

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In 1932, Pallaske reported a condition in geriatric horses that resulted in a long, curly hair coat and an enlarged pituitary gland [1]. This disease, known as Equine Cushing's Disease, or re-named as **equine pituitary pars intermedia dysfunction (PPID)** is now commonly recognized in equine practice, partly because of the increasing geriatric equine population. PPID is a primary problem of the **pituitary gland**, located beneath the brain. This gland is like a dispatch center, where hormones and other chemical mediators called proopiomelanocortin peptides (POMC) are produced and then released into the body to control body functions. PPID is a naturally occurring, progressive condition characterized by hypertrophy and hyperplasia of the pars intermedia of the pituitary gland resulting in an increased expression of POMC peptides [2]. Horses with PPID have a breakdown of the control of the pituitary gland – it literally doesn't shut down and continues to produce POMC. Affected horses have an increased circulating concentration of POMC-derived peptides, ultimately leading to the development of clinical signs through a sequence of events that was poorly understood until recently.

The name **Cushing's Disease** is derived from human literature for a disease syndrome described by Dr. Harvey Cushing in 1932. In people, Cushing's Disease affects mostly females ages 20 to 50 years old, with typical clinical signs including excessive weight (especially in the upper torso and face), elevated body temperature, depressed immune function with potential for increased incidence of infections, thin and visible damaged skin/bruising, decreased bone density leading to fractures, anxiety, irritability, depression, polyuria (increased urination), polydipsea (increased thirst) and hyperglycemia. Women show excessive hair growth on the face, neck, chest, abdomen and thighs. These symptoms in people are due to exposure to elevated circulating blood cortisol (hypercortisolism).

In humans (and dogs also), Cushing's Disease is most commonly attributed to a corticotrophin adenoma in the pars distalis (anterior lobe; people do not have a pars intermedia) of the pituitary gland with primary or secondary involvement of the adrenal glands. In contrast, this disease in horses is almost exclusively attributed to hyperplasia or adenoma formation in the pars intermedia (intermediate lobe); consequently, **pituitary pars intermedia dysfunction (PPID)** is the preferred term for the disorder in the horse. Similarly, while Cushing's Disease in people and dogs is really attributable directly to excess cortisol, the condition in horses is distinct in that excess cortisol is not always significant, but the production and release of excessive quantities of pars intermedia-derived POMC peptides is inescapably significant. This represents an equine-specific difference.

The classic sign of PPID in horses is **hirsutism**, a long and curly hair coat that fails to shed. The presence of late-onset hirsutism in an aged horse or pony is essentially diagnostic for PPID. In some affected horses, coat-color changes can also occur. The pathogenesis of these coat changes, characterized by arrest of hair follicle in telogen, is poorly understood but theoretically is caused by increased pituitary-derived POMC. This peptide in normal horses stimulates increased coat growth prior to winter. Hyperhidrosis (excessive sweating) is also observed in 60% of horse with PPID, most commonly over the neck and shoulders, and has been attributed to a thermoregulatory response to the long-hair coat. Altered energy metabolism (protein catabolism with the increase in hormone activity) may result in muscle wasting, weight loss or abnormal fat distribution. Lethargy and decreased performance has also been reported in horses with PPID. Loss of muscling across the spine and rump can be noticed in more advanced cases. Often preceding the loss of muscle mass can be deposition of fat along the crest of the neck, over the tail head, and in the sheath of male horses.

Other signs include elevated body temperature, depressed immune system (increased incidence of infections – respiratory, sinus, periostitis, bronchopneumonia, skin infections, foot abscesses, buccal ulcers, gingivitis, periodontal disease, delayed wound healing), hampered protein and fat metabolism, dental abnormalities, polyuria, polydipsea, and hyperglycemia. Another clinical sign reported in horses with PPID is persistent lactation and infertility, which are probably a consequence of altered release of prolactin and gonadotrophin hormones. A sometimes disastrous musculoskeletal complication that may develop in the occasional horse with PPID is suspensory ligament desmitis and subsequent ligament breakdown. Horses with PPID have been described as overly docile and more tolerant of pain than normal horses. They often are found to have increased concentrations of plasma and spinal fluid beta-endorphin (60-100xs) compared to normal horses. Signs of central nervous system dysfunction, including ataxia, blindness, and seizure-like activity, are also occasionally observed in horses with PPID, but the cause of these neurological deficits is poorly understood.

Chronic, insidious-onset laminitis is perhaps the major clinical complication of PPID with >50% of horses affected in most reports [3]. Most PPID horses, but not all, are insulin resistant. It is speculated that the excess cortisol and high circulating blood glucose increases the risk of laminitis through several mechanisms: reducing blood supply to the lamellar tissue, weakening hoof lamellar attachments, ongoing hoof lamellar restriction, and reducing glucose delivery to the hoof cells. Chronic or recurrent pain with exacerbation of laminitis and associated foot abscesses is often the reason euthanasia is pursued.

In the case of horses, the over-active pituitary gland of the PPID horse can grow in size. Pituitary glands of PPID-affected horses may enlarge up to 5x their normal size. It was once thought that benign tumors in the intermediate lobe of the horse's pituitary gland resulted in an inappropriate secretion of hormones. This tumor-enlargement compresses the adjacent pituitary tissues and

hypothalamus of the brain, resulting in further functional loss in these tissues. However, the enlarged pars intermedia remained active in horses with PPID, secreting relatively large quantities of POMC-derived peptides into the peripheral circulation. Looking at the **pars intermedia** itself, it is comprised of a single cell type, the melanotrope, which is under inhibitory control by dopamine. **Dopamine** is released by local nerve terminals in the hypothalamus of the brain located near the pituitary gland [4]. Based on studies in rats (where the nerve terminals in the brain were surgically disrupted), it was deduced that equine PPID results from a loss of inhibition of the pars intermedia because of degeneration of the periventricular hypophyseal dopaminergic neurons. In other words, the enlargement and hyperplasia in the pars intermedia was secondary and not primary, and the enlargement was caused by degeneration in the hypothalamus of the brain, and not the other way around. This discovery redefined the syndrome and the research focus shifted to looking at the brain, instead of the pituitary gland itself.

If PPID is a dopaminergic neurodegenerative disease, that raised some questions. What caused the neurons in the brain to degenerate? And why are only some horses affected? Based on the literature, it was speculated by researchers that PPID may have similar pathologic mechanisms as other dopaminergic neurodegenerative diseases, specifically **Parkinson's disease** in people. Although the cause of Parkinson's disease is not well understood, accumulation of oxidative stress damage has been shown to be a cause. Oxidative stresses are caused by excessive exposure of cells to exogenous or endogenous sources of oxidants. Cellular damage to the hypothalamus ultimately leads to cell death, or in the case of these dopaminergic neurons, neurodegeneration. Chronic exposure to oxidants in excess of an animal's anti-oxidant capacity results in accumulation of functionally impaired cellular components. Investigations into the potential role of oxidative stress and protein mis-folding as a cause of PPID in the horse then began. Now, it has been determined that degeneration of the hypothalamic dopaminergic neuron is the primary cause for PPID. This theory fits with what is known; both in how horses with PPID respond to treatment with **pergolide**, a drug that also replaces dopamine, and also from the older work from the 1980's showing decreased pituitary dopamine concentrations.

The role of oxidative stress in the pathophysiology of PPID is a new finding. Oxidative stress may result from excessive exposure to environmental oxidants, increased metabolism, inflammation or deficiency in anti-oxidant capacity. The sporadic nature of PPID makes exposure to environmental toxicants a less likely cause; however, it may be a contributing factor in development of disease in individuals with poor anti-oxidant capacity. Chronic inflammation is also an unlikely cause of PPID, because there is no histological evidence of this in any studies performed. Therefore researchers deduced that horses with PPID may be predisposed to oxidative stress because of either a deficiency in anti-oxidant capacity or altered glucose metabolism. Chronic hyperglycemia and type II diabetes are both associated with oxidative stress in other species [5]. It's now been shown that horses chronically affected by obesity and insulin resistance are predisposed to PPID [6]. Therefore, **prevention is possible and treatment during the early stages of the disease is recommended**, before the neurons are irreparably damaged.

How is PPID diagnosed? The best indication of PPID is the presence of hirsutism in an aged horse. The sensitivity of this unusual clinical sign in predicting whether a horse has a dysfunctional pituitary is better than that of the available diagnostic tests [7]. The primary reasons to test a hirsute, aged horse for PPID are to facilitate the dosing of therapeutics and to confirm the diagnosis for the owner. But testing for PPID has been its own form of controversy. A drawback for many of the currently described tests for PPID is that they do not test the pars intermedia cells directly.

On routine laboratory tests, abnormalities in horses with PPID may include mild anemia, an absolute or relative neutrophilia (increased white cells) and an absolute or relative lymphopenia (decreased lymphocyte count) [8]. The most common abnormality detected on serum biochemical evaluation is mild to moderate hyperglycemia. Additional abnormal biochemical findings may include elevations in hepatic enzyme activities, and higher than normal cholesterol and triglyceride concentrations. Urine specific gravity may be elevated, but glucosuria is generally not detected unless hyperglycemia is also present [9]. Silent urinary tract infection can be found in the occasional horse.

In 1994, Dybdal [2] published a study that evaluated tests of the pituitary –adrenal axis for their specificity and sensitivity in diagnosing PPID. In the study, the overnight **Dexamethasone Suppression Test (DST)** showed 100% sensitivity and specificity and was subsequently adopted as the gold standard. More recently, however, the ability of the overnight DST to differentiate normal aged horses from horses with PPID has been challenged. In 2003 Miesner [10] reported on the serial use of the overnight DST in seven clinically affected horses. When DST was repeated three times at 30-day intervals in the same seven horses with clinical signs of PPID, only one of the seven horses tested positive for disease on all three days. This suggested that the test was inaccurate in early cases of PPID. The high sensitivity and specificity that was originally reported reflected a case-selection bias toward horses with advanced disease, as the horses with PPID in Dybdal study were selected based on the presence of overt clinical signs, including hirsutism. This was consistent with the common finding that horses with clinical signs consistent with early disease (such as slow or incomplete shedding and severe epaxial muscle atrophy) would have postmortem evidence of PPID despite a normal response to the DST.

A second common test for PPID is measurement of the endogenous plasma **adrenocorticotropic hormone (ACTH)** concentration. This test is relatively simple to conduct and avoids administration of dexamethasone to animals at risk for laminitis. Studies [11] have suggested that the endogenous ACTH test has a sensitivity of 80-90% and a specificity of 100% when the DST is used as the gold standard. Based on data, however, it is unlikely that the ACTH test is better than the overnight DST for early recognition of PPID. In addition, significant variations in the plasma ACTH concentration may be observed in normal animals, even

when serial samples are collected less than 30 minutes apart. Season also affects ACTH concentration. In 2004, it was first reported that the equine pars intermedia is seasonally regulated with greater hormone production occurring in the fall [12]. This seasonal change is presumably part of a physiologic process that prepares horses for winter conditions, including harsh weather and sparse food. Ponies have a more profound increase than horses in pituitary hormone production during the fall, possibly resulting from the genetic thriftiness of ponies [12]. Endogenous hormones are greater in the fall than in other seasons. Donaldson [13] explored the impact of seasonal hormonal regulation on diagnostic testing and determined that false-positive overnight DST results were common when horses or ponies were tested in the fall. Therefore if horses are tested for PPID in the fall, a positive test result must be interpreted very cautiously.

Another assay that has been suggested for diagnosing PPID is measurement of diurnal cortisol rhythm. This test is based on the observation that horses with PPID have a loss in diurnal cortisol rhythm [2]. The current recommendation is to measure the serum cortisol concentration at 8 am and 4 pm [14]. A difference of less than 30% between the two values is considered suggestive of PPID. This test, however, has not been appropriately validated. In normal horses there is no significant difference in serum cortisol concentration in samples collected at 8 am and 4 pm [2]. For this testing method to be effective a later sample (8-12 pm) would need to be collected and the assay appropriately validated with additional research.

A thyroid releasing hormone (TRH) test was adapted from human literature and experimented with. The advantage of the TRH stimulation test over the DST was that it was considered safer to perform in laminitic horses, avoiding dexamethasone administration. Unfortunately, because the test measured a percentage increase in cortisol concentration, interpretation of the results were complicated by variations in baseline cortisol concentration. Further, McFarlane [15] recently found increases in cortisol concentration in normal horses, which indicates that the TRH stimulation test was not an appropriate diagnostic test for PPID.

Experiments were conducted combining the TRH and DST tests to diagnose PPID. In a recent report in which the combined DST/TRH stimulation test results were compared with histological findings in the pituitary glands of 42 horses, the combined test was more accurate than either the DST or TRH component of the test alone [7]. Unfortunately, all endocrinologic test results were less accurate than hirsutism alone. As a consequence, use of this combined test has not gained wide acceptance.

It has long been recognized that PPID horses and ponies are less able to utilize orally or intravenously administered glucose because of insulin sensitivity [3]. Hyperinsulinemia is a common finding, along with hyperglycemia [3]. Because cortisol and insulin have antagonistic metabolic effects, hyperinsulinemia has been attributed to excess circulating cortisol in PPID-affected animals. As a consequence, measurement of serum insulin concentration has also been investigated as a potential single-sample endocrine test for a diagnosis of PPID. It is very accurate; however, hyperinsulinemia can accompany other disorders such as the recently described equine metabolic syndrome [16] and insulin resistance. McGowan [17] recently reported that the long term survival for horses with PPID was poorer when concurrent hyperinsulinemia was detected before treatment, so it seem insulin levels would be an important measurement to have.

Extrapolating from human disease, radiology, commuted tomography (CT) and magnetic resonance imaging (MRI) was experimented with to see if pituitary enlargement and/or changes in the hypothalamus could be detected. There were no consistent findings to help with early detection of disease.

It was discovered from postmortem examinations that changes in pars intermedia can be present for years before clinical signs of PPID become apparent. Thus, in actuality, histopathologic evidence of disease may not be the most appropriate gold standard either. To evaluate this supposition, McFarlane [18] recently evaluated the degree of agreement between seven veterinary pathologists asked to examine histological sections of pars intemedia tissue collected from 10 horses with mild signs of PPID. They found that post-mortem assessment was in agreement with ante-mortem endocrinologic test results 79% of the time, but they also reported that for 5 of 10 tissues samples examined histologically [18]. Another recent study documented that the size and histological anatomy of the pituitary gland varies with age and gestation status in mares and also found histological lesions in the pars intermedia of nearly 50% of horses without clinical signs of PPID [19]. These data further call into question the validity of histopathologic evidence of pars intermedia disease as a gold standard, at least until uniform diagnostic criteria are established by veterinary pathologists.

So practically, the diagnosis of PPID is most commonly made by observation of hirsutism and other supportive clinical signs in older horses and ponies [8]. In fact, in a recent study that compared the sensitivity, specificity and positive and negative predictive values of hirsutism with results of a combined dexamethasone suppression/thyrotropin-releasing hormone stimulation test, presence of hirsutism had greater diagnostic accuracy than endocrinologic test results [7]. Because hirsutism seems to be a pathognomonic clinical feature of PPID, an 'over the fence' diagnosis from this finding alone is often made. However, to provide the best service to the horse and its owner, a complete physical exam, including good oral exam and determination of body condition score and weight, should be performed when patients are initially evaluated for suspected PPID. In addition, collection of blood samples for a complete blood count, serum chemistry profile and baseline endocrine tests are also recommended if the clients desire to provide the highest level of care for their animal.

The most challenging question regarding PPID is the issue of mild/early disease. It is relatively easy to diagnose advanced PPID because horses with hirsutism are easily recognized. **Can PPID be diagnosed before it is clinically obvious?** Early diagnosis can facilitate early intervention and avoidance of complications such as laminitis and infections. However, early clinical signs of PPID can be subtle and can often overlap with the phenotype of the aged horse. One of the first indicators that a horse is developing PPID is the loss of epaxial (topline) muscle mass. A second early change is slow or incomplete shedding of the winter coat. Unfortunately and obviously, the available tests for PPID fall short in making an early diagnosis. The desire to accurately identify

geriatric horses with early PPID has led to a search for new diagnostic strategies. One new approach is based on the concept that secretion of ACTH after administration of domperidone should be greater in horses with PPID than in aged horses without the disease [20]. Because domperidone is a dopamine antagonist, horses with PPID are predicted to have a greater sensitivity to the drug and a more marked increase in their plasma ACTH concentration. Early results in these studies have been promising [21].

Detection of early disease still leaves equine practitioners trying to decide whether to be aggressive and try to prevent the disease or wait until it gets severe enough to diagnose. Because hirsutism can be effectively managed for years by body clipping alone, should all horses with hirsutism receive drug treatment? The answer to this question is dependent on both the owner's concerns and finances. A clear argument can be made in support of medical treatment when clinical signs are initially recognized, because this can minimize progression of PPID and prolong the life of the older horse. It is still unclear whether drug treatment needs to be continuous or if it can be intermittent; only long-term longitudinal studies comparing various treatment regimens would answer this question and such studies are unlikely to be performed. Some researchers advocate the early use of medication (pergolide) in horses that are suspected to develop the condition at a younger age (chronically obese, insulin-resistant) and the provision of adequate antioxidants in the diet primarily in the form of vitamin E. This approach shifts the focus to slowing the development of disease or even prevention, much like in human Parkinson's disease.

Individual management becomes essential. This should include quality nutritional support and aggressive deworming protocols (routine fecal testing should be done to ensure that there has been an adequate response). Providing frequent, high-quality hoof and dental care is critical, and affected horses should have their hair coat clipped according to the weather.

Pharmacologic treatment with pergolide may improve the horse's clinical signs and lower the risk for complications such as laminitis and secondary infections. **Pergolide**, used to treat Parkinson's disease in humans, stimulates dopamine release, which in turn tells the pituitary gland to shut down. There are good anecdotal results and very limited side effects. Because loss of hypothalamic dopaminergic innervations seems to be a critical pathophysiologic mechanism for PPID, treatment with dopaminergic agonists represents a logical approach to therapy. The most common adverse effect of pergolide, recognized in 5-10% of horses, is a mild decrease in appetite during the first few days after treatment has been initiated [22]. When this problem develops, treatment is stopped for a couple of days and reinstituted at one-half the previous dose; most horses seem to tolerate this approach.

Cyproheptidine, a drug with anti-serotonin actions, was one of the initial drugs used for treatment of PPID, because serotonin has been shown to be a secretagogue of ACTH in isolated rat pars intermedia tissue. Furthermore, the drug was effectively used to treat human patients with Cushing's disease [23]. This drug is also beneficial in horses. Adverse effects of cyproheptidine seem to be minimal; because the drug also has anti-histamine actions, mild sedation may be noticed when higher dosages are used. Although some reserved the use of cyproheptidine for horses that fail to respond to the maximal dose of pergolide, cyproheptidine used with pergolide can be very effective.

A human drug called **trilostane** offers promise for treating PPID. This drug works at the level of the adrenal gland to slow down cortisol production. Current research has shown that this drug has reversed some of the symptoms of PPID. Trilostane is available in the United Kingdom and also to veterinarians that have it compounded (expensive).

As with many chronic diseases in the horse, specific nutrient supplementation and complementary or alternative therapies have been recommended and used in horses with PPID. Both magnesium and chromium supplementation have been advocated for supportive treatment of this condition. **Magnesium** supplementation (to achieve a dietary calcium to magnesium ratio of 2:1) has been recommended, because magnesium deficiency seems to be a risk factor for insulin insensitivity and type-2 diabetes in humans. Additionally, anecdotal reports suggest that supplementation may help horses with obesity-associated laminitis. Similarly, **chromium** supplementation is recommended to improve carbohydrate metabolism (specifically glucose uptake) and improve insulin sensitivity in type-2 diabetes in people, and supplementation with chromium tripicolinate has been shown to increase glucose uptake during a glucose tolerance test in normal yearlings [24]. Over the past few years, an herbal product made from Chaste Berry has also been advocated for treatment of PPID. *Vitex agnus castus* (**Chaste Berry**) extracts have been used medicinally for centuries for various human female cycle disorders, and lay articles claim its therapeutic efficacy in horses with pituitary hyperplasia and other hormonal dysfunction [25]. Ethanol extracts of the seeds are an organic source of dopamine stimulation and have been demonstrated to bind to the D_2 receptor of rat pituitary cells and inhibit prolactin secretion [26]. While it hasn't completely stood the rigors of scientific testing, many researchers are still looking into it as a source of treatment for PPID. **Vitamin E** is often supplemented as an anti-oxidant, and **vitamin C** given for immune support.

Horses with PPID do well on the same low-sugar, low-starch diet fed to horses with insulin resistance. It is generally agreed to avoid alfalfa and grain, leaving grass hay, grass hay pellets and rice bran as the primary feed sources. PPID can be difficult to keep weight on, because they are generally geriatric, needing extra proteins that are difficult to safely deliver. It takes dedication to balance diet with exercise.

Once present, PPID is a lifelong condition, and the prognosis for correction of the disorder is poor. However, PPID can be effectively treated with a combination of management factors and medications. When PPID is caught early, treatment is very successful in reducing clinical signs and allowing affected horses to live almost normal lives. For those horses in advanced stages of the disease, treatment still offers improved quality of life and longevity. In one report, survival time from initial diagnosis to development of complications necessitating euthanasia ranged from 120-368 days in 4 untreated horses [9]. However, there are

numerous anecdotal reports of horses being maintained for several years as long as response to medical treatment was favorable and close patient monitoring and follow-up was performed. Because a recent case series found that concurrent presence of hyperinsulinemia with PPID was a negative prognostic factor [17], measurement of fasting insulin concentration in the initial evaluation and ongoing management of horses with PPID is also recommended.

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