Equine Endocrine Testing: Webinar Questions & Answers
Wednesday | April 27, 2016 | 7:00 p.m. EDT

Presenter: Lisa Tadros, DVM, PhD, DACVIM
Endocrinologist, Diagnostic Center for Population and Animal Health
Michigan State University College of Veterinary Medicine

Abbreviations:
ACTH: Adrenocorticotropic hormone
EMS: Equine Metabolic Syndrome
PPID: Pituitary pars intermedia dysfunction
TRH: Thyrotropin-releasing hormone
TSH: Thyroid-stimulating hormone

Questions from Chat and Q&A, Not Answered Live:

1) For which tests do you advise fasting?

The benefit of testing for insulin dysregulation in the fasted versus fed state is currently under investigation as we work to optimize protocols and maximize test sensitivity for detecting metabolic derangements. It appears that fasting may affect the results of some insulin dysregulation tests, but not others. For now, we recommend the following:

Fasting is only necessary prior to the oral sugar (Karo® syrup) test and other dynamic oral glucose challenge tests. This entails leaving 1 flake of hay in the stall or dirt pen after 10:00 p.m. the night prior to testing. Fasting is not necessary prior to measurement of resting insulin and glucose concentrations. If measured in the fed state, a resting insulin concentration > 360 pmol/L (50 μU/mL) supports insulin dysregulation and an insulin concentration between 145 – 360 pmol/L (20 – 50 μU/L) suggests possible insulin dysregulation. If fasted, a resting insulin concentration > 145 pmol/L (20 μU/mL) supports the presence of insulin dysregulation. The TRH stimulation test may be performed either fasted or after feeding hay (but not grain). Fasting is not necessary prior to measuring endogenous ACTH concentration or performing the overnight dexamethasone suppression test.

2) Back to equine TSH, is there interest by any researchers to develop an equine TSH assay? It seems like there is a market for it. But too small of a market?

Currently, the market to offer a commercial equine TSH assay is not likely large enough to support the cost of assay development and ongoing reagent production. Although equine TSH assays have been developed in the research setting, I am not aware of any of these being offered commercially at this time.

3) Any comments on leptin testing for insulin resistance (dysregulation)?

A number of studies have demonstrated that leptin concentration is associated with fat mass and insulin dysregulation in horses. Hyperleptinemia is a component of EMS. However, it is not yet known whether including leptin as part of equine endocrine diagnostic test panels improves the sensitivity for detecting insulin dysregulation and EMS over testing glucose and insulin dynamics alone.
4) **Standard practice in my practice is to test for ACTH, cortisol, insulin, and leptin. I've always felt as though resting insulin and cortisol were relatively clinically irrelevant. I felt as though you validated my concerns regarding cortisol. Curious as to your thoughts regarding resting (non-fasting) insulin.**

Although dynamic tests such as the oral sugar (Karo® syrup) test may be more sensitive for detecting insulin dysregulation, measuring resting (fasted or non-fasted) serum insulin and glucose concentration is also acceptable, provided that the limitations of the latter test are recognized. Logistically, screening large numbers of horses using resting insulin and glucose measurement may be easier than screening with dynamic tests. Horses with more severe insulin dysregulation are often identified in this way, but it must be recognized that animals with subtle insulin dysregulation can be missed because hyperinsulinemia (with or without an excessive glucose response) may only occur following an oral or intravenous glucose challenge. Therefore, abnormal resting insulin and glucose concentrations can confirm the presence of insulin dysregulation, but normal concentrations cannot rule it out.

Measuring resting cortisol concentration is not a valid diagnostic test for PPID in horses for a number of reasons. The excess ACTH released by the abnormal pars intermedia is less bioactive with regard to stimulating cortisol secretion than normal pars distalis-derived ACTH. Commercially available assays detect both normal ACTH and biologically inert ACTH-like peptides; as a result, endogenous blood ACTH and cortisol concentrations are dissociated, rendering cortisol measurement invalid for assessing pituitary dysfunction. While abnormal adrenal function likely plays a role in the pathophysiology of PPID, overt adrenal hyperplasia only occurs in 20% of PPID-affected horses; hypercortisolemia is therefore not a feature of PPID in all cases.

5) **How long does the TRH last after purchasing and opening the multi-dose vial? There seems to be a question of stability.**

There is currently one published study examining the stability of the compounded TRH product (protirelin solution) available from Wedgewood Pharmacy. In that study, 1-mL vials of the TRH product (1 mg/mL) were stored at room temperature for a maximum of 18 days and the product retained its efficacy during that time. Long-term stability beyond 18 days, as well as the stability of other compounded TRH products, requires further investigation.

6) **In what percentage of PPID horses do ACTH levels lower with pergolide therapy?**

Studies indicate that endogenous ACTH concentration decreases in the majority of PPID-affected horses receiving pergolide therapy, and that endocrine tests eventually improve (typically defined as reduction of endogenous ACTH concentration by ≥ 50%) or normalize (return of ACTH concentration to within the seasonal reference range and/or normalization of the overnight dexamethasone suppression test) in approximately 60-80% of these animals. Normalization of endocrine tests usually occurs within the first 1-3 months of treatment, but cases where tests normalize after several years of pergolide therapy without a dose increase occur on occasion. An important point to note is that some evidence, as well as clinical impressions, suggests that the rate of clinical improvement may actually be higher than that for normalization of endocrine test results, at least in certain treated populations.

Current recommendations state that endogenous ACTH concentration (or TRH stimulation, if used for initial diagnosis of PPID) should be measured 1-2 months after starting pergolide therapy. If the dose is adequate, endogenous ACTH is expected to be within or near the normal range for the season and clinical improvement should be seen. If laboratory tests are abnormal and clinical signs persist, the pergolide dose should be increased and the horse reassessed monthly until an appropriate pergolide dose is determined or the maximum dose has been reached. The same approach should be taken in situations where laboratory test results are normal, yet the clinical response remains poor (this includes the presence of ongoing insulin dysregulation). Dilemmas arise when laboratory tests are abnormal, yet the patient is responding well clinically to pergolide therapy. In such cases, the author generally advocates remaining on the current pergolide dose and reassessing the horse in 3-6 months. However, abnormal laboratory values imply ongoing pituitary dysfunction. We do not yet know which pathophysiologic abnormality confers the greatest risk of
developing laminitis, and it is conceivable that the laminitis risk does not decrease if pituitary function remains abnormal, even when other signs such as hypertrichosis improve. For this reason, some experts recommend increasing the pergolide dose until laboratory tests normalize. Once the proper pergolide dose has been established and the clinical signs are stable, laboratory tests should be repeated every 6 months to monitor therapy, with one test performed during fall season (mid-July to mid-November). It should also be noted that, in some horses that initially fail to respond to pergolide therapy, prolonged administration over several months to years may eventually result in normalization of laboratory tests and clinical improvement.

7) How long after an active case of laminitis gets controlled is it okay to test for PPID?

Interpreting PPID test results during an active laminitis episode involves a degree of subjectivity. While pain, stress, and systemic illness are known to increase endogenous ACTH concentration, heighten hypothalamic-pituitary-adrenal axis responses, and cause false-positive PPID test results, the degree of overlap between PPID-affected horses and those with other disorders is not clearly defined. It is likely that pain and systemic illness must be severe before rendering PPID test results uninterpretable. Although mildly elevated endogenous ACTH concentrations or exaggerated ACTH responses to TRH stimulation in the face of active laminitis could be a result of pain, values falling far outside the reference ranges are more likely to be caused by PPID. Therefore, the decision to test for PPID during a laminitic episode versus waiting until the episode resolves depends on the animal’s degree of pain.

8) What do you recommend for duration of a pergolide therapeutic trial?

A minimum of 2-3 months is required before conclusions can be drawn about a given pergolide dose’s efficacy in improving the horse’s clinical signs. In horses with hypertrichosis, haircoat changes may not be observed until the next seasonal shedding cycle.

9) A patient with an ACTH of 546 pmol/L (ref interval 2-10) is this unusual in your experience?

Based on historical data from a large number of sample submissions to the MSU Diagnostic Center, the majority of PPID-affected horses have endogenous ACTH concentrations below 50 pmol/L. Concentrations between 50-100 pmol/L are uncommon and values above 100 pmol/L are only occasionally encountered. An endogenous ACTH concentration of 546 pmol/L is most likely an erroneous result, therefore I would recommend that the test be performed again on a separate plasma sample from that animal to ensure that the finding is repeatable.

10) Why can’t the oral glucose challenge and the TRH stimulation test be done on same day?

It is currently recommended to perform these tests on different days. Although the mechanism is not fully understood, TRH stimulation testing should not be performed immediately after the oral sugar (Karo® syrup) test, as the latter has been shown to blunt pituitary ACTH responses to exogenous TRH administration. Conversely, the TRH stimulation test cannot be performed immediately prior to the oral sugar test, as activation of the hypothalamic-pituitary-adrenal axis alters glucose and insulin dynamics.

Questions Answered Live:

11) Does treatment of PPID increase immune response?

Immunosuppression is a feature of PPID, making affected animals susceptible to infections and endoparasitism. Horses with PPID also respond differently to vaccination than normal aged horses, although potential effects of PPID on vaccine efficacy and duration of immunity are not yet known. The immunological dysfunction in PPID is complex, involving over-secretion of several immunosuppressive hormones and altered inflammatory responses. Various abnormalities have been identified in neutrophil and peripheral blood mononuclear cell responses in PPID-affected horses. Clinical impressions are that management of PPID with pergolide therapy improves affected horses’ overall health and reduces susceptibility to infections. However, there is currently no experimental data examining immunological responses to therapy in PPID-affected...
animals. Whether normalization of the hormone profile as a result of therapeutic intervention also normalizes immune function is yet to be determined.

12) Recommended source of TRH & cost?

Two TRH products are currently available for TRH stimulation testing in horses. One is a compounded synthetic TRH product called “protirelin solution,” which is produced by Wedgewood Pharmacy and is stable at room temperature; the current cost is $66 for a 5-dose vial. The other is reagent-grade lyophilized TRH powder; although cheaper, this product must be reconstituted and handled properly and stored correctly by the customer before used for testing.

13) Reference endocrine ranges for donkeys?

Hormone concentrations frequently differ between normal horses and donkeys; therefore reference ranges developed for horses cannot always be extrapolated to other equids. Differences have been identified in thyroid function, glucose and insulin dynamics, and energy homeostasis involving hormones such as leptin and adiponectin. Development of donkey-specific endocrine reference ranges is an area of current research, but there is still much work to be done to fully characterize the unique aspects of endocrinology in donkeys.

14) Anorexia related to pergolide?

The most commonly reported adverse effect of pergolide therapy is transient anorexia during the initial few weeks of treatment. If this occurs, pergolide administration should be discontinued for a few days until the appetite returns. After that, the medication can be reintroduced gradually by starting with a partial dose and slowly increasing to the desired dose over several days. Alternatively, some horses with anorexia may initially tolerate the drug better if the dose is split, with half administered in the morning and half in the evening, until the appetite returns to normal.

15) How consistently is hypercalcemia seen with neoplasia?

Humoral hypercalcemia of malignancy has been documented in association with several types of cancer in the horse, including lymphoma, squamous cell carcinoma, adrenocortical carcinoma, multiple myeloma, and ameloblastoma. Malignancy should always be on the differential diagnosis list when hypercalcemia is identified. However, clinical impressions suggest that hypercalcemia is not a consistent or particularly common finding in horses with cancer; the absence of hypercalcemia cannot be used to eliminate a diagnosis of malignancy.

16) Is there any evidence that pergolide prevents laminitis in PPID horses?

The most likely mechanism by which pergolide therapy would prevent laminitis in horses with PPID is by normalizing the metabolic state, particularly insulin and glucose homeostasis. It is currently believed that PPID-affected horses with concurrent insulin dysregulation and hyperinsulinemia are at the highest risk of developing laminitis. Consequently, controlling PPID with pergolide may reduce the risk of laminitis if insulin dysregulation improves. Although clinical impressions support this theory, there is currently no direct experimental evidence conclusively demonstrating that pergolide therapy either prevents the development of laminitis in PPID-affected horses or reduces the frequency and/or severity of future laminitis episodes. This question, along with the efficacy of pergolide to normalize glucose and insulin metabolism, is the focus of current research.

When considering this question, it must also be remembered that pergolide is not a direct medical treatment for laminitis. The efficacy of pergolide in preventing or improving laminitis will also depend on the severity of laminar damage already present at the time of PPID diagnosis. Horses with severe laminar damage will likely continue to experience chronic pain from abnormal hoof growth and recurrent abscesses, as the normal tissue architecture cannot be restored. Lifelong therapeutic farrier care will remain essential to successfully managing these cases. Judicious intermittent use of anti-inflammatory drugs may also be necessary during painful episodes.
17) I have a hard time when many horses backing off feed even after being on pergolide for many months; eating fine then stops eating. Is it building up in the system?

This brings up some interesting points. Clinically, the most common time to encounter anorexia is during the first few weeks of pergolide therapy, although that is not to say that it could not develop later in some animals. The pharmacokinetics and pharmacodynamics of pergolide in horses are still being studied and are not well understood for all dosing protocols, routes of administration, and types of patients. Preliminary investigation of long-term (6 months) pergolide administration in horses with PPID showed that the drug can remain detectable in the serum for up to 2 weeks after discontinuing the medication in some animals. The endogenous ACTH concentration may also remain suppressed for up to 6 days after pergolide discontinuation. Additionally, endocrine test results can sometimes normalize after years of pergolide therapy, even if there is no increase in drug dosage. Taken together, these observations suggest some degree of cumulative drug effect; however, this likely represents a physiological response to long-term drug exposure rather than accumulation of the drug over time and increasing concentrations within the body.

18) For those case horses you discussed where there was doubt with the resting ACTH would you do the TRH response to help make that decision?

Both endogenous ACTH concentration and the TRH stimulation test are appropriate first-line diagnostics when there is clinical suspicion of PPID. If endogenous ACTH is measured and the concentration is normal, it is recommended to follow up with TRH stimulation testing. Until seasonal reference ranges are established, the TRH stimulation test should only be performed from mid-November to mid-July. Alternatively, a therapeutic trial of pergolide may be considered.

19) What about Urine Cortisol/Creatinine Ratio as a screening?

The urine cortisol/creatinine ratio is not a useful screening test for PPID. One reason is its lack of specificity, as upregulation of the hypothalamic-pituitary-adrenal axis occurs in response to the metabolic stress of many systemic illnesses; this often elevates the urine cortisol/creatinine ratio. Additionally, endogenous blood ACTH and cortisol concentrations are dissociated in PPID-affected horses because of differences in the bioactivity of normal pars distalis-derived ACTH and abnormal ACTH produced by the diseased pars intermedia. This renders cortisol measurement in both blood and urine invalid for assessing pituitary dysfunction.