Veterinary Review

CIRCADIAN CORTISOL RHYTHMICITY AND EQUINE CUSHING'S-LIKE DISEASE

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Equine Cushing's Syndrome was first described in the literature 40 years ago as caused by dysfunction of the pars intermedia (PI) of the pituitary gland. The initial lesion in the horse is not in the pituitary gland but likely nearby in the hypothalamus. By some as yet unknown mechanism, a loss of dopamine innervation in the PI results in a loss of negative control or feedback on the PI and ultimately hyperplasia and enlargement of the PI occurs. At the onset of this cascade of events resulting in Cushing's-like disease, there is an increase in the PI secretion of the proopiomelancortin(POMC) derived peptides.

This increase in POMC peptides decreases the sensitivity of the negative feedback effects of glucocorticoids on ACTH production. These peptide hormones also increase the effects of ACTH on adrenal steroidogenesis. The result is a decrease in circadian rhythmicity of cortisol secretion (below 30%?). There is a concomitant increase in the secretion of opiate-active Beta endorphin which may explain the lethargy seen in many horses thought to have Cushing's-like disease.

Moreover, it is well accepted that the most common clinical signs in horses with confirmed PI adenomas include: hirsuitism, weight loss, lethargy, laminitis, polyuria, and polydipsia. Other signs include decreased fertility, depressed immunity, hyperhidrosis, seizures, diabetes insipidus, anovulation, persistent endometritis, and pseudolactation. One must recognize that horses with

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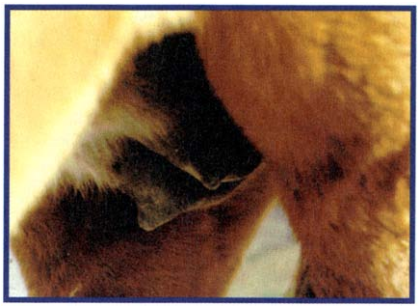

When barren mare presented with chronic pseudolactation, there is a high probability that Cushing's Disease is a cause of the subfertility.

Cushing's may exhibit only one or two of these clinical signs during the early onset of the disease.

Although clinical Equine Cushing's Disease has been recognized for almost half a century, our ability to diagnostically confirm that horses have PI dysfunction has not been very good with the exception of necropsy results. It has been suggested that PI dysfunction effects horses for many years before they become terminal and require euthanization. Cushing's Disease has been documented in horses as young as seven years of age but 85% of cases are reported to be greater than 15 years of age. These reults were based on histological findings at necropsy.

Han van der Kolk[1] included papers in an excellent review of equine PI dysfunction which demonstrated that clinical signs such as hirsuitism can occur ten years before other clinical signs such as polyuria-polydipisia and laminitis occur. Other papers in this review stated that the onset of clinical signs are generally insidious for 1.5 to four years before Cushing's Disease is suspected from the appearance of so-called classical signs. This is a key point in understanding the problems associated with diagnostic-hormonal testing to confirm Equine Cushing's Disease. The more we focus on the early hormonal events which precede the easily recognizable clinical signs, the more powerful tests we may have for early detection. For example, if the elevation in POMC peptides could accurately be measured routinely in a commercial laboratory setting, we would have a very powerful test to monitor onset of PI dysfunction. No such test is currently available.

The most commonly used hormonal diagnostic protocol utilizes the dexamethasone suppression test. The overnight dexamethasone suppression test is presently in vogue. To perform this test a blood sample for cortisol is collected prior to and 20 hours after 40 ug of dexamethasone/kg BW IM at 17000hrs. Two problems exist with this testing protocol. First it is insensitive and second it is rigid in terms of when it must be performed. In studies being conducted by our laboratory (BET Laboratories), Equine Cushing's Disease must be present for five or more years before this test is useful.

BET Laboratories has modified some of the previous protocols which have focused on circadian cortisol rhythmicity and the loss of this rhythm. We are basing this protocol on empirical observations from hormonal evaluations on approximately 1,000 horses suspected of having Cushing's Disease. The protocol in Figure 1 is used.

Two serum samples are taken eight to ten hours apart. The AM sample is taken first. No grain may be given within four hours prior to either sample since

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Figure **1.** Protocol for Cushing's Disease testing.

Time	Hormones Assayed		Cortisol Rhythm %
AM	TT4	Insulin	Cortisol
PM	TT4	Insulin	Cortisol

Cushing's Syndrome
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the concentrate will cause a transient rise in insulin in response to carbohydrate load. Hay or grass may be eaten as normal. We have arbitrarily used a cortisol rhythm of 30% or less as suggestive of PI dysfunction and thus the onset of Cushing's Disease. Keep in mind that this is only a benchmark, and there will be much better protocols for confirming the onset of PI dysfunction in the future. Stresses such as constant stall confinement will also cause a loss of circadian cortisol rhythm below 30%. A complete history should be taken and evaluated to identify any stress that may be present in the patient's environment.

Other researchers have reported the following:

1. Normal horses have a cortisol circadian rhythm of greater than 30% with levels often but not always higher in the morning.

2. Cortisol concentrations are not affected by: Breed, age, sex, or pregnancy.

3. Cortisol concentrations are elevated by: Exercise, fasting, hypoglycemia, disease, and surgery.

4. Cortisol binding globulin concentrations are very low in the horse. Clinically this means a very small increase in total plasma cortisol results in a large rise in bioactive free cortisol. This is opposite of the case with thyroid hormones where most of the hormone is bound to protein binding globulins.

Additional assays are performed on the AM and PM samples for total T4(TT4) and insulin. The literature suggests that TT4 is lower and insulin is higher than normal in horses with Cushing's Disease. Our laboratory has found 25% of horses have high insulin, above 80 micro-IU/ml, and 50% of horses have low TT4 , below 12.ng/ml, when their cortisol rhythm is below 30%. Empirically, we find a much better clinical response to drugs such as permax and cyproheptadine when thyroxine is used to elevate the TT4 levels to a normal range and when chromium or acupuncture is used to lower the insulin to a normal level.

BET Laboratories has performed this assay protocol on over 3,000 horses being evaluated for the presence of Cushing's Disease. We attempt to get good histories on each patient thus we have gotten some useful survey data. Some of our general observations are that circadian rhythmicity is less than 30% in approximately:

- 85% of horses with chronic laminitis.

• 90% of barren mares with pseudolactation present for more than six months.

• 70% of mares with chronic uterine mycotic infections.

• 60% of mares with chronic endometritis.

• 50% of stallions with significantly declining sperm numbers and declining fertility.

• 75% of mares which are anovulatory for six or more months.

• 60% of horses with chronic infections such as sole abscesses.

• 85% of fillies or mares which are stall confined and exhibiting aggressive behavior toward people and exhibiting a lack of interest in taking training. Sometimes exaggerated estrous behavior is present also. This is a stress manifestation and is not related to PI dysfunction. This abnormal behavior can be managed successfully with exogenous prednisone, usually 200 mg per day orally.

• 60% of weanlings and yearlings with slow growth rate, potbelly, poor hair coat, and poor muscle development where husbandry practices of deworming and vaccinations have been adequate.

• 60% of mares which have second or third trimester abortions in two or more consecutive years.

The two most well known drugs used to treat Cushing's Disease in the horse are cyproheptadine and permax (pergolide). There are some new human drugs, which are type dII dopamine receptor agonists, but their effects in the horse are unknown at present. The clinicians who have a great deal of experience in treating Equine Cushing's Disease generally feel that permax is more effective; however, no critical comparative trials have been conducted. The cost of permax is often too high to use in many cheaper horses, and cyproheptadine is affordable for most horses. We have focused a lot of research on using higher doses of cyproheptadine. When a dose of 0.25mg/kg was used, the effective rate was reported to be only about 35%. We have found a response rate approximately double of that rate when 0.50 to 1.0 mg/kg is used once per day. We advise repeating the AM/PM testing protocol every three weeks to monitor the efficacy of the dose of permax or cyproheptadine being given and adjusting the dosage according to how close the circadian cortisol rhythmicity is rising toward 30%. Observations in the field suggest clinical improvement closely parallels the rise in cortisol rhythm. Some degree of synergy appears to exist by combining

cyproheptadine and permax in treatment protocols. Per 400 kg BW, 0.5mg permax and 200 mg cyproheptadine has worked better than higher doses of either compound given alone. There is great room for advances in treatment of PI dysfunction in the horse. Another disadvantage seen with permax is that over a period of years, the horse seems to become refractory to this drug. It could also be argued that the disease is becoming more advanced as well. For this reason, we suggest the dosage of permax be kept as low as possible. More effective and cheaper drugs perhaps in sustained release vehicles such as SABER are needed.

Another interesting area in which our basic understanding of endocrinology must assist in clinical decisions is in management of the chronically barren broodmare with Cushing's. Empirically we have made observations in over 50 such mares which have carried pregnancies successfully to term. One important thing we have learned is that since pergolide and other dopamine agonists elevate dopamine and thus have a negative effect on prolactin production, agalactia can be a problem in these mares. We now suggest dopamine-active drugs such as permax and cyproheptadine be withdrawn three weeks before the date of parturition and that domperidone is used if udder development is inadequate. The protocol for Cushing's screen is repeated about five days after birth and the mare is again placed on medication for Cushing's Disease if indicated. Most horses must be treated for the remainder of their lives; however, some eight to 13- year-old horses may remain normal after therapy with cyproheptadine and/or permax is terminated even though they were showing clinical evidence of Cushing's Disease and tested positive in the AM/PM protocol. It appears these animals may somehow reset their PI function and remain normal for at least several years.

It should be emphasized that much remains to be learned about the diagnosis and treatment of PI dysfunction in the horse and the associated symptoms of Cushing's Disease.

Two excellent reviews are to be found in:

1. van der Kolk, H. Diseases of the pituitary gland, including hyperadrenocorticism. In: Watson TD(ed). Metabolic and endocrine problems of the horse. W B Saunders. 1998; 41-59.

2. Dybdal, N. Pituitary pars intermedia dysfunction (equine cushing's –like disease). In: Robinson, NE(ed). Current therapy in equine medicine. W B Saunders. 1997; 499-503.