

PITUITARY PARS INTERMEDIA DYSFUNCTION

(EQUINE CUSHING'S DISEASE)

Good evidence-based tests used in the diagnosis of pituitary *pars intermedia* dysfunction (PPID) comprise:

1. Plasma ACTH concentration
2. Overnight dexamethasone suppression test
3. Combined dexamethasone suppression - TRH stimulation test
4. TRH stimulation test

Further investigation into other pituitary peptides such as POMC, β endorphin and α MSH are ongoing and under development at the Liphook Equine Hospital Laboratory.

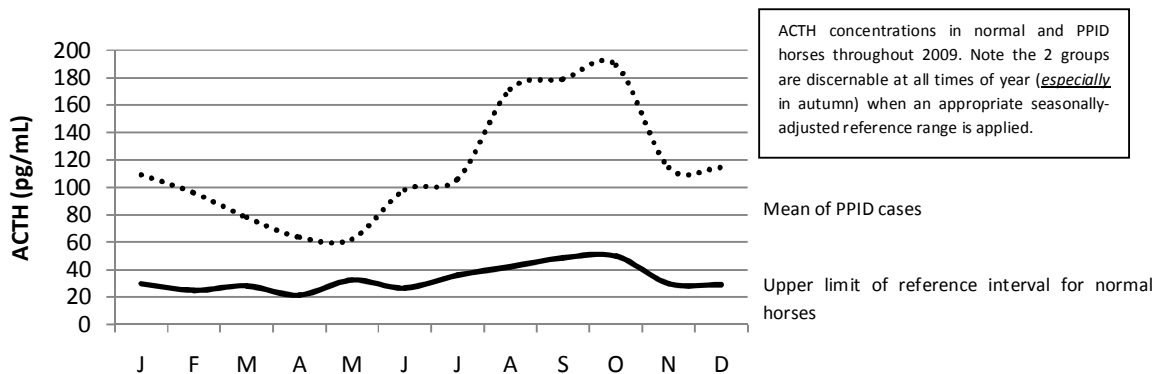
1. Plasma ACTH concentration

Technique

- a) Collect a single blood sample anytime **between 8.00 am and 5.30 pm**
- b) Collect sample in an **EDTA** (purple) tube (plastic or glass acceptable)
- c) The sample should be **chilled** within **3 hours** of collection.
- d) **Separate plasma** from the blood cells prior to posting either by **centrifugation or by gravity** (the timing of separation is unimportant as long as the sample is chilled within 3 hours of collection)
- e) **Chill** the sample *en route* to the laboratory using **specialized chiller packs** – supplied free on request (freezing is unnecessary although might help if the delivery is delayed)

Interpretation

Plasma ACTH is increased in the presence of pituitary hyperactivity and hypersecretion. This may be caused by PPID but is also known to occur in most normal horses and ponies in the autumn. Although this has been considered a reason to avoid testing for PPID in the autumn, this potential problem may be overcome by applying properly derived and calculated seasonally adjusted reference ranges (these will accompany all laboratory results from The Liphook Equine Hospital Laboratory). As can be seen from comparing the two lines on the following graph, it is apparent that testing in the autumn may actually allow the greatest differentiation between PPID cases from normal horses and there is no reason at all to avoid testing for PPID in the autumn. ACTH might also be affected by pain and stress (e.g. from laminitis) although this does not appear to have a large effect in most horses unless pain is marked.



2. Overnight dexamethasone suppression test (ODST)

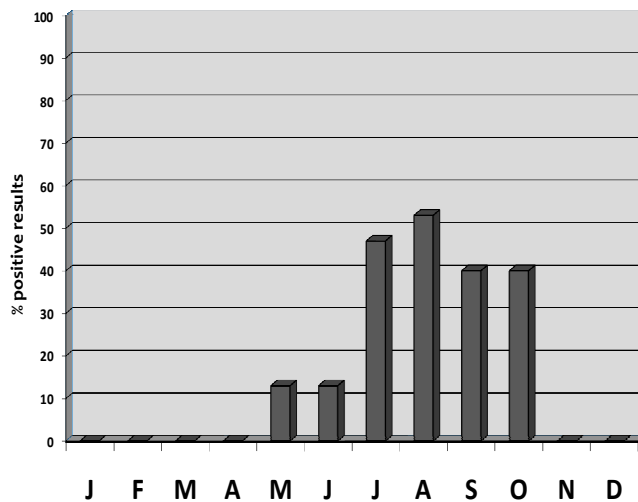
Technique

- a) Collect a plain serum sample for baseline cortisol at approximately 5.00 pm
- b) Following this, administer im or iv dose of 40µg/kg dexamethasone (10 mL of 2mg/mL solution per 500 kg)
- c) Collect a 2nd plain serum sample approximately nineteen hours later (11.00 am the following day) for analysis of the post-dexamethasone cortisol concentration (*the exact timing of this is not critical; 16 to 24 hours will probably suffice*).
- d) There are no special storage/postage requirements

Interpretation

A normal horse is expected to have almost complete suppression of endogenous cortisol in the 2nd sample and a cutoff of < 27nmol/L is generally applied. PPID is considered to be confirmed if the 19-hour sample contains a cortisol concentration greater than this.

As with plasma ACTH concentration, the ODST may also be liable to false positive results in the autumn. Unfortunately as the ODST is a qualitative test it is not currently possible to reliably interpret results of this test in the autumn. The exact period during which false positives may be common has not been determined in the UK although results from Michigan (below courtesy of Dr Hal Schott) suggest that July to October should be avoided.



Results of ODST repeated monthly in 18 normal horses at University of Michigan. Note an unacceptably high rate of false positives occur between July and October inclusive suggesting that this test should be avoided then (data courtesy of Dr Hal Schott)

3. TRH stimulation test

Although once advocated as a test wherein serum cortisol could be compared between samples taken before and 15 to 60 minutes following a TRH bolus, this test has more recently been shown to be unable to differentiate normal from PPID horses. However, the test appears still to be valid when comparing plasma ACTH (rather than serum cortisol) before and 30 minutes following a TRH bolus. As a single resting plasma ACTH concentration is well established as an excellent diagnostic test for PPID, the stimulation test is only recommended where borderline ACTH results are found from a single resting plasma sample. (N.B. collection and dispatch instructions should be followed as for ACTH described in previous section; this test is probably best avoided between July and October)

Technique

- a) Collect a baseline plasma sample for ACTH
- b) Inject 1mg TRH (5 x 200 µg vials "Protirelin") iv
- c) Collect a further plasma sample 30 mins later for assessment of post stimulation ACTH

Interpretation

PPID is indicated by either a baseline plasma ACTH value greater than the seasonally adjusted reference range (typically >30 pg/mL) and/or a post stimulation plasma ACTH value greater than 100 pg/mL.

4. The combined TRH stimulation and dexamethasone suppression test

Technique

- a) Collect a plain serum sample for basal cortisol (1) at 9.00 am
- b) Immediately following this, inject 40 µg/kg dexamethasone im/iv (10 mL of 2mg/mL solution per 500 kg)
- c) Collect a further plain serum sample for cortisol (2) at 12.00 noon
- d) Immediately following this, inject 1 mg TRH (5 x 200 µg vials "Protirelin") iv
- e) Collect a further plain serum sample for cortisol (3) 30 minutes after the TRH bolus (12.30 pm)
- f) Collect a further plain serum sample for cortisol (4) at 9.00 am the following day

Interpretation

PPID is confirmed if either or both of the following are found:

1. There is a >66% increase in serum cortisol between samples 2 and 3 (*large response to TRH stimulation*)
- AND/OR**
2. There is a cortisol concentration >27nmol/L in sample 4 (*not suppressed by dexamethasone*)

The combined dexamethasone suppression-TRH stimulation test interpretation

