

# ENDOCRINOLOGY

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The following information is a guide for veterinary surgeons, giving specific details about our Laboratory endocrinology testing.

## PREGNANCY TESTS

Serum gonadotrophins (eCG) may be detected in mares where functional endometrial cups are present. For accurate results, serum samples should be collected between 45 and 95 days since the last date of mating. False negatives are unusual inside this period, but can occur in rare cases where eCG levels are below test 'threshold'. False positives are more common and may occur where early foetal death has occurred after endometrial cup formation, leaving residual functional cup tissue which remains for the normal lifespan of the cups (up to 100 days).

Oestrone sulphate may be detected in the serum/plasma of mares over 120 days pregnant. At that time, levels of >100 ng/ml are usually found (0-20 ng/ml in non-pregnant mares). Most of the oestrone sulphate peak originates from the foetal gonads so this may be a useful test of foetal viability as well as a pregnancy test. Levels fall during the last few weeks of pregnancy.

Urinary oestrogens, of placental origin, may be detected in mares after 150 days of gestation. Urine samples are required.

## PROGESTAGENS

The analysis of peripheral progestagen levels is a useful guide to diagnosis and treatment in the acyclic or irregularly cyclic mare. In the non-pregnant mare, levels >2 ng/l (6.3 nmol/l) indicate functional luteal tissue and suggest that prostaglandin treatment should induce luteolysis, providing that the corpus luteum is not refractory (less than 4 days old).

In the pregnant mare, there is no proven relationship between progestagen levels and the integrity of pregnancy. Mares with levels <2 ng/ml (6.3 nmol/l) are unlikely to be pregnant. Most normally pregnant mares have levels >4 ng/ml but there is considerable daily and individual variation. The progesterone assay is in no way an accurate pregnancy test but may be helpful or reassuring to owners and managers in monitoring individual mares with histories of repeated pregnancy failure.

Assays are run Monday to Friday throughout the year.

## 'RIG' TESTS

Testosterone stimulation tests are used to detect cryptorchidism in horses and donkeys of all ages.

Testosterone levels should be measured in serum (clotted) or heparinised plasma samples taken before and 30-120 minutes after the intravenous injection of 6000 iu HCG.

In horses (not donkeys) over 3 years old, the diagnosis of cryptorchidism may be made by measuring oestrone sulphate on a single serum sample.

## THYROID FUNCTION

T4 and T3 assays are measured on the Immulite analyser. As diurnal rhythms are involved, two samples should be collected ideally early in the morning and early evening. Low levels may indicate hypothyroidism, which is rare, but is sometimes seen in overweight, lethargic horses, and ponies that may be prone to laminitis.

## ADRENOCORTICAL FUNCTION

The most common indications to assess pituitary gland function are equine Cushings syndrome and equine Metabolic Syndrome. Plasma ACTH, Cortisol levels, DXM suppression tests (2 x cortisol), TRH stimulation tests (3 x cortisol), Combined DXM suppression/TRH stimulation tests (4 x cortisol) are run. Cortisol: Creatinine ratio can be run on urine samples.

## PLASMA ACTH

Resting plasma ACTH has become the test of choice for diagnosis of PPID, and for monitoring response to treatment. ACTH shows seasonal variation with higher values being obtained in late summer and autumn. This does not pose a problem for interpretation provided that normal ranges appropriate to the season are used. Pain / stress may affect the ACTH level, but this rise is not usually a significant factor unless the level of pain is severe.

### Sampling procedure:

ACTH is labile and careful sample collection is required:

- 1) Collect a blood sample into an EDTA tube (glass or plastic), mix well by inversion and keep the sample cool while transporting the sample back to your practice. The sample must be chilled within 3 hours of collection.
- 2) Separate the plasma by centrifugation into a plain plastic tube. If no centrifuge is available, place the sample upright in a fridge for at least 1 hour, and then carefully remove the plasma.
- 3) The plasma must be kept cool. It can be stored frozen and, although not necessary, freezing will help to keep the sample cool for longer while in transit.
- 4) Send the sample to Beaufort Cottage Laboratories using ice packs and a Next Day Delivery Service. Beaufort Cottage Laboratories can supply ice packs and pre-paid Next Day Delivery labels on request (01638 663017 or [laboratory@rossdales.com](mailto:laboratory@rossdales.com)).
- 5) It is advisable not to send samples over the weekend or Bank Holidays in case they are delayed in the post.

## OVERNIGHT DEXAMETHASONE SUPPRESSION TEST

This test is based upon the fact that dexamethasone administration suppresses plasma cortisol in normal horses because of feedback on ACTH release. A resting serum sample is collected at 5 pm and 40 µg/kg DXM (Azium, Gist-Brocades, 20 mg/500 kg horse) is injected im. A follow-up serum sample is collected at 12 noon the following day. Normal horses show a suppression of serum cortisol levels to <10% of baseline levels (to <27 nmol/l) whereas cushingoid horses show no significant suppression. There is significant seasonal variation using this test and false positive results can be obtained during late summer and autumn (July to October).



## INSULIN

Serum Insulin is sometimes a useful screening test for Equine Cushing's Syndrome and Metabolic Syndrome. Results  $<50\mu\text{IU/ml}$  are considered normal in grazing or fasting animals. Samples should not be collected from stabled horses within two hours following a feed. Raised insulin levels are seen in horses with peripheral insulin resistance which is a feature of Equine Cushing's Syndrome and Metabolic Syndrome. Many classical cases have marked elevations in serum insulin levels, often  $>1000\mu\text{IU/ml}$ .

Troponin assays are also run on our Immulite Chemiluminescent Auto analyser.

Samples submitted for testing on the Immulite Analyser (progesterone, testosterone, cortisol, T4, T3, Insulin and Troponin), should preferably be plain serum or heparinised samples. Gel tubes are not recommended as prolonged contact with the gel may depress values obtained. The presence of EDTA may interfere with the assay reactions therefore sample tubes containing this should not be used. Haemolysis may adversely affect assay results. Serial samples should be taken into the same type of tube.

## OTHER TESTS

Also performed in this department are ELISA assays for the detection of Clostridial toxins from faeces samples (*C.difficile* and *C.perfringens*) and an ELISA screening test for Equine Infectious Anaemia