

SOP No: AHP 91

SUBJECT: Induction of laminitis in horses using the euglycaemic hyperinsulinaemic clamp technique

REASON FOR USE: To enhance the understanding of the pathogenesis of laminitis in horses

POLICY: This procedure may only be performed by operators skilled in the technique. Horses must be monitored constantly throughout the experimental period. At the onset of any persistent clinical discomfort, horses must be euthanased immediately.

PRECAUTIONS: Rubber boots, overalls, gloved hands, face visor during operation of band saw

EQUIPMENT: Light harness with saddle bags to house data loggers
Data loggers
Thermistor probes and cables
Infusion pumps
Equine nappy for faeces and urine collection
Anaesthetic of choice

PROCEDURE:

1. Obtain healthy racehorses recently retired from racing and thus fit, in moderate-light body condition, from a reliable horse dealer.
2. The day before the experiment take lateral radiographs of both forelimb feet and only chose horses with normal anatomy and clinically normal hooves, free of the signs of laminitis. Videotape all horses while walking and trotting for evaluation of soundness and eliminate any unsound horse. Limit dietary intake in the 72 h preceding the experiment to hay and lucerne chaff.
3. Fit horses with an equine nappy to collect urine and faeces in separate compartments and to maintain stable hygiene.
4. Acclimatised horses for 24h in stables during which time they are clinically examined.

- 5. Confine horses to stocks in pairs (for companionship) for the duration of the 24 h experiment with free access to lucerne chaff, hay and fresh water.**
- 6. Prior to the experiment, clip the skin over both jugular veins of each horse. Surgically prepare the site and infuse with local anaesthetic (2% Lignocaine, Ilium, Troy). When desensitized incise the skin (2-3 mm incision) and insert a catheter (14G flexible, extended use intravenous catheters (Milacath, Mila International) into both veins and suture in place. The left catheter is used to administer the infusions while the right is capped (3-way stopcock, Mila International) 2 for blood sampling. Take blood (20 ml) from all horses to establish resting serum insulin and blood glucose concentrations, including three samples drawn 10 min apart for insulin sensitivity (SI) calculations in treatment horses. Also obtain 10ml blood samples for routine haematology and biochemistry. Assess free-catch urine samples for glycosuria by the glucose-oxidase method using a dipstick (Multistix-10SG, Bayer).**
- 7. Fit all horses with non-invasive, surface thermistor probe (Tinytag, Gemini) 9 placed on the dorsal midline of both forelimb hooves 25 mm below the hairline. Connect the probes to data-loggers that record the temperature of the hoof wall every minute throughout the experimental period.**
- 8. Infuse the horses in the treatment group with a combination of insulin and glucose via the prolonged-EHC clamp technique modified to last for up to 72 h (De Laat *et al.* 2010). A constant infusion of insulin is used to produce hyperinsulinaemia, while the rate of glucose infusion is adjusted throughout the experiment to maintain euglycaemia. The amount of glucose administered provides a proxy measure of the SI of muscle and adipose tissue. Deliver an initial bolus (45 mIU/kg) of insulin (Humulin-R, Eli Lilly) diluted in 50 ml of 0.9% saline to horses in the treatment group and administer over 60 sec. Immediately follow the bolus administration with an insulin infusion (6 mIU/kg/min) is commenced concurrently with an infusion of 50% glucose solution (Baxter) 5 (10 μ mol/kg/min). Blood samples for serum insulin**

concentration determination include three samples are obtained at 10 min intervals during the steady state period. The steady state period is defined as a 30 min period when euglycaemia (5 ± 1 mmol/L) had been achieved without the need to alter the glucose infusion rate, and occurs between 1.5 and 3.5 h after bolus administration in all treatment horses. Measure blood glucose using a pre-calibrated handheld glucometer (Accu-check, Roche). Take samples every 5 min during the first 3.5 h. Once a steady state is achieved, analyse the blood glucose every 30 min until euthanasia. The glucose infusion rate is adjusted to maintain euglycaemia. Draw samples for comparative blood glucose and serum insulin levels in all horses for the duration of the infusion period. Control horses receive an infusion of balanced, electrolyte solution (Hartmanns, Baxter) at a rate of 0.57 ml/kg/h for the same period of time as the treatment horse. Immediately place blood samples in plain plastic vacutainers (Vacuette, Greiner Bio-One) 7, and leave to clot at room temperature for 30 min and then centrifuge for 10 min at 3000 x g. Serum is pipetted into 1 ml aliquots and stored at -80°C until analysed. Measure serum insulin concentrations using a radio-immunoassay kit (Siemens, Coat-a-count) previously validated for use in horses. Where necessary, prepare sample dilutions using equine serum that contains undetectable levels of insulin, as this kit can suffer intolerance when assay buffer is used as the diluent. Perform assays in duplicate.

9. Development of clinical laminitis is the end point of the experiment so continue infusions until this point is reached in the treated horses. Monitor the horses continuously throughout the experimental period for changes in appetite, body temperature, heart and respiratory rates. Apart from the development of mild lameness (the end point of the experiment) insulin treated horses are NOT ill and remain clinically normal with good appetites throughout the experimental period.
10. Immediately at the onset of mild (Obel grade 2) lameness, take plain lateral radiographs of all forelimb hooves just before euthanasia. Standard measurements to assess changes in hoof and

distal phalangeal anatomy before and after treatment are performed on all radiographs.

- 11. Immediately after radiography anaesthetise the horses. Apply a rubber latex tourniquet to the fetlock of one limb and disarticulate the foot at the metacarpo-phalangeal joint and dissect to obtain blocks of lamellar tissue that are placed in liquid nitrogen for genomic and proteomic studies or 4% paraformaldehyde for 24 h prior for embedding in paraffin. Repeat this procedure for the other fore-foot and then euthanase the horse.**

RECOMMENDATIONS:

DATE ISSUED: 27/4/2011

REVISED:

REFERENCES

- 1. De Laat, M.A., McGowan, C.M., Sillence, M.N. and Pollitt, C.C. (2010) Equine laminitis: Induced by 48 h hyperinsulinaemia in Standardbred horses. *Equine vet. J.* 42, 129-135.**
- 2. Tinworth, K.D., Wynn, P.C., Harris, P.A., Sillence, M.N. and Noble, G.K. (2009) Optimising the Siemens Coat-A-Count Radioimmunoassay to measure insulin in equine plasma. *Proceedings of the Equine Science Society Congress, May 29 - June 1, 2009, Colorado, USA* In Press.**