The Veterinary Journal 300-302 (2023) 106036

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Diagnosis of equine pituitary pars intermedia dysfunction

Allison J. Stewart [a], Joanne L. Ireland [b], Andy E. Durham [c], Catherine M. McGowan [b,*]

[a] The University of Queensland, Gatton, QLD 4343, Australia
[b] School of Veterinary Science, Faculty of Health and Life Sciences, The University of Liverpool, Neston CH64 7TE, UK
[c] Liphook Equine Hospital, Liphook, Hampshire GU30 7JG, UK

ARTICLE INFO

Keywords:
ACTH
Laboratory test
PPID
Sensitivity
TRH

ABSTRACT

Equine pituitary pars intermedia dysfunction (PPID) is common in aged horses. The majority of horses respond well to treatment, but treatment is lifelong, meaning accurate diagnosis of PPID is important. Similar to any condition, there is no perfect laboratory test to diagnose PPID and accuracy is affected by the characteristics of the population in which the test is being evaluated. This review details the importance of consideration of clinical factors and diagnostic test accuracy. Basal adrenocorticotrophic hormone (ACTH) concentration is used most frequently in practice and has very good diagnostic accuracy when used in combination with clinical judgement and the correct application of diagnostic thresholds. The thyrotropin-releasing hormone stimulation test can be used in horses with equivocal test results following basal ACTH testing, or to evaluate subtle cases due to its improved accuracy.

Introduction

The first stage in the diagnostic reasoning process is consideration of signalment, history and clinical signs (Croskerry, 2009). Diagnostic hypotheses should be generated based on these factors, as these have a large impact on the accuracy of subsequent testing. The interpretation of diagnostic tests is influenced by the pre-test probability (the probability of the suspected disease in an individual given their clinical signs). Positive test results are likely to be inaccurate in animals with a low pre-test probability of pituitary pars intermedia dysfunction (PPID), whereas test accuracy is far higher when applied to those considered likely to have PPID. Therefore, considering the initial likelihood of PPID prior to diagnostic testing is a vital initial step in diagnosis.

Factors associated with pre-test probability of PPID

Age

Knowledge of disease prevalence in the population can be used to give an initial estimate of pre-test probability. In a large study of horses of all ages, PPID prevalence was 2.9% (Welsh et al., 2016). PPID is rare in younger equids, and only 3% of cases in a multi-centre retrospective cohort study were aged < 10 years (Rohrbach et al., 2012). Median age at PPID diagnosis was reported as 21 years (interquartile range 15–26 years; Welsh et al., 2016), and an 18% increase in odds of PPID was reported in horses/ponies for every year > 15 years of age (McGowan et al., 2013). In horses/ponies aged ≥ 15 years, two field-based studies conducted in Denmark and Australia reported an identical prevalence of 21.2%, despite utilising different sample selection and diagnostic testing strategies (Christiansen, 2009; McGowan et al., 2013). Pars intermedia (PI) adenomatous hyperplasia or adenoma was identified at post-mortem examination in 27.0% of equids aged > 15 years (Miller et al., 2016). Taken together, these data support that PPID is more prevalent in older individuals. However, when PPID testing is based on age alone, pre-test probability is moderate (i.e. approximately 21–27% based on the above studies) and further pre-selection is important.

Sex and breed

There are conflicting findings regarding sex predisposition (Rohrbach et al., 2012; McGowan et al., 2013; Ireland and McGowan, 2018), therefore sex is unlikely to be helpful for estimating pre-test probability. While results of a systematic review demonstrated pony breeds were overrepresented in several studies (Ireland and McGowan, 2018), there was no difference in odds of PPID between horses and ponies in the only study to date that has evaluated pony phenotype as a risk factor (McGowan et al., 2013). As there is no evidence supporting a breed predisposition to PPID, breed or type is of limited use for estimating

* Corresponding author.
E-mail address: cmcgowan@liverpool.ac.uk (C.M. McGowan).

https://doi.org/10.1016/j.tvjl.2023.106036
Received 23 December 2022; Received in revised form 25 September 2023; Accepted 4 October 2023
Available online 6 October 2023

pre-test probability. However, breed differences in basal adrenocorticotrophic hormone (ACTH) concentrations have been reported and should be considered in interpretation of laboratory test results for PPID diagnosis (Durham et al., 2022; Bamford et al., 2023).

Clinical signs and co-morbidities

As found in a systematic review, data pertaining to clinical signs have come mainly from small case series, often including many advanced PPID cases (Ireland and McGowan, 2018), whereas early cases may present with few or subtle signs. Hair coat abnormalities are frequently reported, ranging from abnormal shedding patterns to overt regional or generalised hypertrichosis (Ireland and McGowan, 2018); however, comparison of research studies is challenging due to lack of consistency and definition of the coat changes. Hypertrichosis, defined simply as excessive hair growth (Saleh et al., 2022), is a subjective clinical sign, which is more frequently observed in ponies than horses (McGowan et al., 2010; Ireland et al., 2012). Observation of delayed shedding is influenced by season, and a clinical examination at a single time point is unlikely to detect all coat abnormalities exhibited over time. Therefore, owner-reported changes are important to provide a complete assessment (McGowan et al., 2013). Generalised hypertrichosis is considered to indicate advanced disease (Hart et al., 2021), and while few studies specifically separate hypertrichosis and delayed shedding, it is logical that delayed shedding may be more frequently observed in earlier cases as was recently supported by a case series (Kirkwood et al., 2022). In a small population of 36 horses, where PPID prevalence was 39%, hypertrichosis (defined as a long curly coat, delayed winter coat shedding relative to other horses on the same premises, or retained hair on physical examination) was reported to be highly specific for PPID (95%), with a positive predictive value of 91% (Frank et al., 2006). Using similar criteria (owner-reported long hair coat and/or delayed shedding), hypertrichosis was associated with increased odds of PPID (odds ratio 7.8; McGowan et al., 2013). Therefore, in equids with characteristic hair coat changes, the pre-test probability of PPID can be considered to be high.

Laminitis is a prevalent co-morbidity in PPID cases (Ireland and McGowan, 2018; Horn et al., 2019; Tadros et al., 2019) and PPID has been diagnosed in 20–34% of equids with laminitis (Karikoski et al., 2011; Welsh et al., 2016). Other common signs in PPID-affected equids include epaxial muscle atrophy, lethargy/docility, weight loss, recurrent/opportunistic infections, hyperhidrosis, polyuria and polydipsia, and abnormal fat distribution/regional adiposity (Rohrbach et al., 2012; Ireland and McGowan, 2018; Horn et al., 2019). Although these signs are frequently reported, there is limited evidence directly associating them with PPID. While greater proportions of PPID cases had muscle atrophy, lethargy, polyuria and polydipsia compared to unaffected aged horses/ponies, only owner-reported coat abnormalities were associated with PPID on multivariable analysis (McGowan et al., 2013). In a study including 2989 horses with at least one 'typical' sign, odds of PPID were not increased with recurrent/opportunistic infections, polydipsia or abnormal fat distribution, but delayed shedding and hyperhidrosis were positively associated with PPID (Grubbs et al., 2020). Less frequently reported signs include poor wound healing, pot-bellied appearance, bulging supraorbital fat, inappropriate lactation, infertility, lordosis, corneal ulceration, anhidrosis/heat stress, polyphagia, ataxia and seizures (McGowan and Neiger, 2003; Rohrbach et al., 2012; Spelta and Axon, 2012; Ireland and McGowan, 2018; Horn et al., 2019). Unfortunately, there is a paucity of evidence regarding the association between these signs and PPID and many may simply be related to age. The combination of age and clinical signs, most notably hair coat abnormalities, provides the most effective means by which to estimate pre-test probability in an individual, which is vital both for selecting appropriate cases for diagnostic testing and for interpretation of test results.

Pathophysiology of PPID in relation to diagnosis of PPID

PPID is an age-related neurodegenerative disorder that ultimately results in a loss of dopaminergic inhibition of the pituitary PI, increasing melanotrope activity and plasma concentrations of the peptide derivatives from the PI (McFarlane, 2011). The increased PI activity also results in changes to the melanotrope cells histologically, with hyperplastic, micro adenomatous and adenomatous change (Miller et al., 2008).

PPI endocrine products

The endocrine products in PPID remain poorly understood in terms of secretory patterns, molecular identities and functional characteristics. Melanotropes of the PI are known to secrete peptides cleaved from the 241-amino acid peptide pro-opiolipomelanocortin (POMC; McFarlane, 2011). Work in other species indicates processing of POMC in the PI results from the action of the endopeptidases prohormone convertase 1 (PC1, also known as PC3 and PC1/3), PC2 and carboxypeptidase A (CPE-A), with further modifications including amidation and acetylation of some products (Lindberg and Fricker, 2021).

The initial peptide products resulting from PC1 cleavage of POMC comprise pro-gamma-melanocyte stimulating hormone (pro-gamma-MSH), ACTH and beta-lipotropin (beta-LPH; Fig. 1). Subsequent action by PC2 and CPE-A cleaves pro-gamma-MSH into gamma-MSH, ACTH into alpha-MSH ($ACTH_{1–17}$) and corticotrophin-like intermediate lobe peptide (CLIP, $ACTH_{18–39}$), beta-LPH into beta-MSH (beta-$LPH_{41–58}$) and beta-endorphin (beta-$LPH_{61–91}$; Harno et al., 2018). CLIP may be further cleaved to beta-cell tropin ($ACTH_{22–39}$) in some species (Beloff-Chain et al., 1983; Fig. 1). Conclusions from equine studies are largely in accordance with this peptide cascade describing upregulation of POMC, PC1 and PC2 activities in PPID cases (Carmalt et al., 2018). Additionally, plasma and PI tissue from PPID cases have been shown to contain greater concentrations of POMC end products (alpha, beta, gamma-MSH, beta-END and CLIP) than intermediate products (ACTH, beta-LPH) although unidentified peptides also exist (Wilson et al., 1982; Orth et al., 1982; Heinrichs et al., 1990; Carmalt et al., 2018).

Laboratory tests for PPID

Many laboratory tests have been used to support a diagnosis of PPID including basal concentrations of PI peptides and several dynamic tests, with measurement of basal plasma ACTH continuing to be the most popular diagnostic biomarker (Durham et al., 2014). It is generally accepted that the diagnostic usefulness of plasma ACTH concentration is further enhanced by measurement following thyrotropin-releasing hormone (TRH) administration although problems exist with autumnal interpretations (Beech et al., 2007; Beech et al., 2011a, 2011b; Funk et al., 2011). Given the relative abundance of POMC peptides in

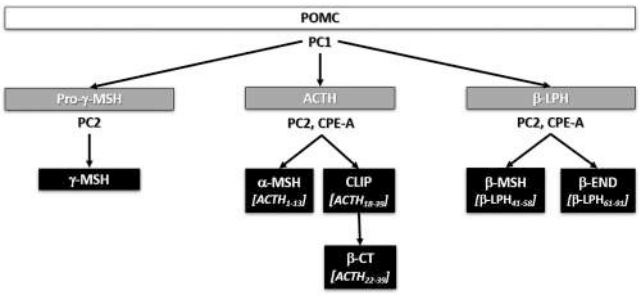

Fig. 1. Illustration of proteolytic cleavage of proopiolipomelanocortin into intermediate peptides following the action of prohormone convertase 1, and end-product peptides following the action of prohormone convertase 2 and carboxypeptidase A.

plasma described above (Orth et al., 1982; Wilson et al., 1982; Heinrichs et al., 1990; Carmalt et al., 2018), the choice of ACTH as the preferred biomarker for PPID does not appear entirely logical but likely came about due to ACTH assays being readily available commercially and, also possibly an assumption of analogy between PPID in equids and pituitary-dependent hyperadrenocorticism in other species where ACTH was already an established biomarker (Bennaim et al., 2019). With hindsight, neither of these reasons is robust, but nevertheless, basal plasma ACTH is a test of good sensitivity and high specificity (Tatum et al., 2021). Furthermore, currently no further simple biomarkers have been found to outperform basal plasma ACTH. Plasma ACTH and alpha-MSH concentrations are highly correlated suggesting that the latter peptide is also worthy of consideration as a diagnostic marker for PPID (McGowan et al., 2013). However, although alpha-MSH had greater specificity than ACTH through most of the year, sensitivity was lower; and the two peptides had similar diagnostic value in the autumn (McGowan et al., 2013). Additionally, there is currently no validated assay for equine alpha-MSH (or any PI peptide other than ACTH) suitable for rapid testing of large numbers of clinical samples.

Assays for ACTH concentration

Measurement of equine plasma ACTH concentration has been validated using radioimmunoassays (Van Der Kolk et al., 1995; Couetil et al., 1996), a radioimmunometric assay (Lee et al., 2010), chemiluminescent assays (CLAs; Perkins et al., 2002; Carmalt et al., 2018; Humphreys et al., 2022) and an immunofluorescent assay (IFA; Irvine et al., 2016). The CLAs and IFA offer significant advantages in being automated and avoiding radiation safety issues. It is clear that the various immunoassays generate discordant ACTH values which cannot be directly compared, reflecting important technical differences including antibody types, target sequences and binding affinity. The majority of publications and diagnostic laboratories reporting equine plasma ACTH concentration have used a CLA method of which there are three generations known as Immulite 1000, 2000 and 2000XPi (Siemens Healthcare Limited, Camberley, UK). Although results on these three platforms are proportionate to one another, they do differ (higher concentrations measured with Immulite 1000 compared with the newer generations, AED unpublished data) and therefore this should also be taken into account when results are considered.

It is also important to recognise that although laboratories report measured values as plasma ACTH concentration, all immunoassays quantify 'immunoreactive ACTH' and not necessarily true 'bioactive ACTH'. That is, any peptides that bind to the antibodies in the particular immunoassay used will contribute to the measured ACTH value whether or not these peptides exactly correspond to the 39 amino-acid ACTH peptide. This was illustrated by one study which compared plasma ACTH concentrations measured in ponies using CLA and IFA methods (Knowles et al., 2018). The CLA method consistently measured higher values than the IFA, with the difference in measured ACTH concentrations being small in the spring but much larger in the autumn. This was considered to be a consequence of the CLA detecting a proportion (approximately 13–17%) of CLIP ($ACTH_{18–39}$) which also partially bound the antibody in the ACTH CLA and was presumably secreted in greater abundance in the autumn. This might be viewed as a disadvantage of the CLA as the measured ACTH values are falsely increased in the autumn (CLA generates false positive results for plasma ACTH), or possibly an advantage in that the CLA detected further diagnostically useful PI peptides which the IFA did not (IFA generates false negative results for PPID). This question remains unanswered with some studies suggesting diagnostic accuracy of the CLA assay is greatest in the autumn (Copas and Durham, 2012; McGowan et al., 2013; Durham et al., 2021), presumably as a consequence of this cross-reactivity, whereas others have found CLA to be less accurate in autumn (Horn et al., 2021). A further consequence of assay specificity is that the IFA, being a more ACTH-specific assay, was less able to detect plasma ACTH which had begun to degrade post-sampling, suggesting that the CLA has an advantage in this respect in clinical samples shipped with some delay to a testing laboratory (Knowles et al., 2018). Nevertheless, as ACTH is relatively unstable, blood samples should be kept chilled, centrifuged and ideally analysed within 24 h of collection. Freezing and thawing of plasma significantly decreases measured ACTH values (Hu et al., 2020).

Basal ACTH concentration measurement in the diagnosis of PPID

Diagnostic test accuracy

Basal ACTH has a consistently good test accuracy, with a tendency for a better test specificity than sensitivity. It is the most frequently used test in practice for both diagnosis and monitoring of PPID (Ireland and McGowan, 2021; Rumfola et al., 2022). Based on systematic review, summary estimates for sensitivity were 75.5% or 72% and 95.2% or 88% for specificity (Tatum et al., 2021 and Meyer et al., 2022 respectively). The sensitivity of a test is a measure of the ability to correctly identify horses with PPID (true positives; Shreffler and Huecker, 2022). Conversely, specificity is the ability of the test to correctly identify horses without PPID (true negatives; Shreffler and Huecker, 2022). While the reported sensitivity of basal ACTH is good, it could return false negative results in horses with PPID, thus missing the diagnosis. The high specificity of basal ACTH means false positives are unlikely.

It is important to note that these sensitivity and specificity values were derived from different studies with varying reference standards or criteria for diagnosis or exclusion of PPID using different cut-off values for a positive and negative diagnosis, relevant to the analytical technique and, in some studies, season (Tatum et al., 2021; Meyer et al., 2022). Despite the differences, and the difficulty in defining a reference standard for PPID, both reviews yielded similar estimates for sensitivity and specificity, supporting that these numbers are likely accurate, yet subject to bias.

The cut-off value and sensitivity and specificity are linked, where a higher cut-off value for basal ACTH concentration produces a higher specificity and lower sensitivity. Until recently, most laboratories have used cut-offs based on the upper limit of laboratory reference intervals (Tatum et al., 2021), which by definition, err towards higher specificity (Ireland and McGowan, 2023).

Interpreting basal ACTH using disease prevalence

Sensitivity and specificity are not the only indicators of test accuracy, and test accuracy can be improved by incorporating disease prevalence (Barrett and Fardy, 2021). Including estimation of disease prevalence allows calculation of positive and negative predictive values. A high positive predictive value (PPV) represents confidence that a positive test is truly positive and a high negative predictive value (NPV) confidence that a negative test is truly negative (Barrett and Fardy, 2021).

As prevalence increase PPV increases and NPV decreases (Table 1). At a prevalence of 21% in horses 15 years and older (McGowan et al., 2013), using median sensitivity and specificity (Tatum et al., 2021), the PPV would be 80.7% and NPV 93.6%. At a prevalence of 64%, reported

Table 1
Relationship between prevalence of pituitary pars intermedia dysfunction (PPID) and positive (PPV) and negative (NPV) predictive values. A high PPV represents confidence that a positive test is truly positive and a high NPV confidence that a negative test is truly negative.

	Prevalence of PPID 3%	Prevalence of PPID 21%	Prevalence of PPID 64%
PPV	32.7%	80.7%	96.5%
NPV	99.2%	93.6%	68.6%

PPV and NPV calculated using sensitivity of 75.5% and specificity of 95.2% (Tatum et al., 2021)

in equids with hair coat abnormalities (hypertrichosis and/or delayed shedding recorded in laboratory submissions; Durham et al., 2021), the PPV increases to 96.5% and NPV decreases to 68.6%. At a prevalence of 3%, representing the likelihood of PPID in the general veterinarian-attended horse population (Welsh et al., 2016), a high NPV is anticipated as a negative test result is expected in the vast majority of horses, while the PPV is approximately 33% indicting low certainty that a positive result is truly positive.

Interpreting basal ACTH using clinical presentation

The evidence for test accuracy can be used with clinicians' ability to estimate disease prevalence based on age and clinical signs of disease to maximise diagnostic ability in horses with suspected PPID. Defined as pre-test probability, it represents the probability of the suspected disease in an individual given their clinical presentation (Fanshawe et al., 2018). Other factors such as month and breed (Durham et al., 2022), handling and testing conditions will also need to be factored in (Durham et al., 2014).

Basal ACTH concentration in aged horses showing specific clinical signs of PPID will have a high PPV (Table 1) based on a high pre-test probability (due to high disease prevalence), meaning positive results are highly likely to be true positives and the clinician can be confident of the result. Similarly, in horses showing clinical signs of PPID the NPV is lower and a negative result provides less assurance that the horse does not have the disease and repeat testing or use of a lower cut-off value (use of the lower threshold; see below and Table 2) in horses where the clinician is still suspicious of disease may be appropriate (Durham et al., 2021).

Basal ACTH concentrations in young horses showing few or non-specific signs of PPID will have a lower PPV (Table 1) based on a low pre-test probability (due to low disease prevalence). This means positive test results may be false positives and clinicians cannot be confident in the test result. The lack of confidence in basal ACTH in younger horses not showing signs of PPID has led experts to recommend not testing horses without clinical signs of disease (Hart et al., 2021).

Diagnostic thresholds and equivocal zones for basal ACTH concentration results

Diagnostic thresholds are a form of clinical decision limits defined as thresholds above or below which a specific medical decision is recommended (Ozarda et al., 2018). Research involving large datasets to determine diagnostic thresholds throughout the year have been derived from a clinical laboratory population (Durham et al., 2021). The derived thresholds were based on 95% sensitivity (low threshold) or 95% specificity (high threshold) and varied throughout the year to incorporate circannual changes. These thresholds can be applied to different clinical case presentations to aid interpretation of test results (Durham et al., 2021; Table 2). This research has also been used to define an equivocal zone (where results fall in between 95% sensitivity and 95% specificity thresholds) in expert recommendations (Hart et al., 2021). Where basal ACTH results fall in the equivocal zone, the TRH stimulation can be used for further evaluation.

Use of the thyrotropin stimulation test in the diagnosis of PPID

The TRH stimulation test is recommended as a dynamic test to further evaluate horses with suggestive clinical signs and normal or equivocal basal ACTH concentrations or in cases with subtle signs (Hart et al., 2021). It has increased accuracy in the diagnosis of PPID (Horn et al., 2021; Adams et al., 2023). There is also evidence that the TRH stimulation test can detect subclinical disease (Beech et al., 2007; Horn et al., 2021; Kirkwood et al., 2022). However, in practice, testing in the absence of clinical signs is not recommended.

TRH receptors are expressed in the PI, with TRH administration resulting in POMC-derived peptide secretion from melanotropes of normal horses, and an exaggerated response from the hyperplastic PI of PPID horses (McFarlane et al., 2006). The TRH stimulation test involves administration of 1 mg of synthetic TRH (0.5 mg for ponies) intravenously, with blood collected before and after either 10 or 30 min (Beech et al., 2007). The 10 min post-TRH assessment of plasma ACTH is more convenient for veterinary practitioners (Hart et al., 2021). However, it is essential that samples are collected precisely at 10 min as a deviation of only 1 min, with sampling at 9 or 11 min can change the median ACTH concentration by 10% (range 0–92%). In one report, sampling 1 min early or late resulted in deviation of the ACTH concentration by $\geq$ 10% in 75% (n = 18/24) of horses and led to an erroneous diagnosis of PPID status in 21% (n = 5/24) horses (Thane et al., 2022). For this reason, other groups have preferred to use a 30-min post TRH administration time point, especially when sampling multiple horses as the gradient of the ACTH response curve to TRH administration is flatter at this time

Table 2
Use of measures of diagnostic test accuracy in combination with clinical judgement to maximise confidence in clinical diagnosis of pituitary pars intermedia dysfunction using basal adrenocorticotrophic hormone concentrations.

Clinical presentation	Signs of PPID	Test considerations	Confidence of positive result being truly positive (PPV;Table 1)	Confidence of negative result being truly negative (NPV;Table 1)	What to do if equivocal or non-supportive result
Confirmation of disease or 'rule in'	Advancing age and at least one clinical sign e.g. hypertrichosis, delayed shedding, muscle wastage, sweating, polydipsia	Use most sensitive threshold (lower value 'cut-off')	High	Moderate – does not match clinical presentation and NPV can be < 70%	Retest at a later date, or retest in the autumn, or use the TRH stimulation test
Confirmation of absence of disease or 'rule out'	Non-specific sign such as laminitis or weight loss in a younger animal	Testing may be contraindicated. Use the most specific threshold (higher value 'cut-off')	Low; PPV can be low (<33%) in absence of clinical signs	High, particularly during autumn months (using the appropriate seasonal threshold)	Use the TRH stimulation test
Unsure of confirmation of presence or absence of disease	Advancing age and a non-specific sign such as laminitis or weight loss; At least one clinical sign e.g. hypertrichosis, delayed shedding, muscle wastage, sweating, polydipsia	Requires clinical judgement as well as diagnostic test result; With increasing clinical suspicion of disease, use more sensitive threshold (lower value 'cut-off'); With decreasing clinical suspicion use the most specific threshold (higher value 'cut-off')	High (using the appropriate threshold)	High (using the appropriate threshold)	Retest at a later date, or retest in the autumn, or use the TRH stimulation test

PPID, pituitary pars intermedia dysfunction; PPV, positive predictive value; NPV, negative predictive value; TRH, thyrotropin-releasing hormone

resulting in less minute-to-minute variation than at a 10 min time point (Beech et al., 2007; Funk et al., 2011; Horn et al., 2021; Kirkwood et al., 2022).

Peptides measured as basal immunoreactive ACTH appear distinct from the ACTH fragments measured after TRH stimulation, with differences identified when samples were concurrently measured on CLA versus IFA assays (McGilvray et al., 2020). Season also affects basal and post-TRH ACTH concentrations in horses with and without PPID, and seasonally specific thresholds for diagnosis of PPID specific for each type of analyser should always be utilized (Funk et al., 2011; Horn et al., 2021). The storage of samples for later measurement of ACTH concentration does not appear to be affected by TRH stimulation (Hinrichsen et al., 2022; Hu et al., 2020).

There is considerable individual variability in the magnitude of increase in ACTH concentration post TRH stimulation, with variability between animals influenced by season especially around the end of summer and beginning of autumn (Kam et al., 2021; Kirkwood et al., 2022). Similar to basal ACTH, seasonal changes should be considered, either utilising circannual diagnostic thresholds or avoiding TRH stimulation testing in late summer and autumn (Horn et al., 2021; Durham et al., 2022).

All equids suspected of having PPID should also be assessed for insulin dysregulation, ideally using dynamic testing. Simultaneous identification of PPID and insulin resistance can be performed by combining a TRH stimulation test and a 2-step insulin sensitivity test by simultaneous injection of TRH and insulin in the same syringe (Horn and Bertin, 2019). The TRH stimulation test can also be performed immediately before an oral sugar test to identify hyperinsulinemia (Hodge et al., 2019). This allows assessment of PPID status and insulin dysregulation on a single day.

Basal and TRH stimulated ACTH concentrations at the 10 min time point decrease after treatment with pergolide and the TRH stimulation test can be used to monitor response to therapy, however basal and TRH stimulated ACTH concentrations may remain positive or in the equivocal zone (Durham, 2022). Further, high inter- and intra-animal variability has led to the recommendation for caution in interpretation of the TRH stimulation test if used for monitoring PPID (Bennet and McGowan 2021).

Although a compounded TRH solution is available in the USA that can be stored at room temperature (Goodale et al., 2013), most investigators have utilised a chemical grade powder product (Sigma-Aldrich Co) that is made into a solution and stored in aliquots at − 20 °C for later use (Beech et al., 2011). In the UK and most of Europe, use of unlicensed chemicals (extemporaneous preparations) is restricted (GOV.UK, 2021), which limits use of the TRH stimulation test in these geographic regions. The TRH stimulation test has been associated with transient side effects such as yawning, muscle fasciculation, transient coughing, lip-smacking and the flehmen response.

Conclusions

Diagnosis of PPID should always include consideration of clinical presentation that affects the pre-test probability of PPID. Basal ACTH concentration is used most frequently in practice and has very good diagnostic test accuracy when used in combination with clinical judgement and the correct application of diagnostic thresholds. The TRH stimulation test can be used in horses with equivocal test results following basal ACTH testing, and also to evaluate subtle cases due to its improved accuracy.

Conflict of interest statement

AED is employed by the Liphook Equine Hospital which offers a commercial clinical laboratory service including endocrine testing. CM, JI and AS have represented their Universities, as key opinion leaders and researchers for which the respective institution has received support from Boehringer Ingelheim Animal Health. None of the authors of this paper has any other financial or personal relationships with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Adams, A.A., Siard-Altman, M.H., Reedy, S.E., Barker, D., Elzinga, S., Sanz, M.G., Urschel, K., Ireland, J.L., 2023. Evaluation of seasonal influences on adrenocorticotropic hormone response to the thyrotropin-releasing hormone stimulation test and its accuracy for diagnosis of pituitary pars intermedia dysfunction. The Veterinary Journal.

Bamford, N.J., Stewart, A.J., El-Hage, C.M., Bertin, F.R., Bailey, S.R., 2023. Investigation of breed differences in plasma adrenocorticotropic hormone concentrations among horses and ponies. The Veterinary Journal. https://doi.org/10.1016/j.tvjl.2023.105995.

Barrett, B.J., Fardy, J.M., 2021. Evaluation of Diagnostic Tests. Clinical Epidemiology. Humana, New York, NY, pp. 319–333.

Beech, J., Boston, R., Lindborg, S., 2011. Comparison of cortisol and ACTH responses after administration of thyrotropin releasing hormone in normal horses and those with pituitary pars intermedia dysfunction. Journal of Veterinary Internal Medicine 25, 1431–1438.

Beech, J., Boston, R., Lindborg, S., Russell, G.E., 2007. Adrenocorticotropin concentration following administration of thyrotropin-releasing hormone in healthy horses and those with pituitary pars intermedia dysfunction and pituitary gland hyperplasia. Journal of the American Veterinary Medical Association 231, 417–426.

Beech, J., McFarlane, D., Lindborg, S., Sojka, J.E., Boston, R.C., 2011. alpha-Melanocyte–stimulating hormone and adrenocorticotropin concentrations in response to thyrotropin-releasing hormone and comparison with adrenocorticotropin concentration after domperidone administration in healthy horses and horses with pituitary pars intermedia dysfunction. Journal of the American Veterinary Medical Association 238, 1305–1315.

Beloff-Chain, A., Morton, J., Dunmore, S., Taylor, G.W., Morris, H.R., 1983. Evidence that the insulin secretagogue, β-cell-tropin, is ACTH22–39. Nature 301, 255–258.

Bennaim, M., Shiel, R.E., Mooney, C.T., 2019. Diagnosis of spontaneous hyperadrenocorticism in dogs. Part 2: Adrenal function testing and differentiating tests. Veterinary Journal 252, 105343.

Carmalt, J.L., Mortazavi, S., Mconie, R.C., Allen, A.L., Unnippan, S., 2018. Profiles of pro-opiomelanocortin and encoded peptides, and their processing enzymes in equine pituitary pars intermedia dysfunction. PLoS One 13, e0190796.

Christiansen, A., 2009. Prevalence of equine Cushing's syndrome among older horses in Southern Vestsjælland. Denmark Dansk Veterinærtidsskrift 92, 24–29.

Copas, V.E., Durham, A.E., 2012. Circannual variation in plasma adrenocorticotropic hormone concentrations in the UK in normal horses and ponies, and those with pituitary pars intermedia dysfunction. Equine Veterinary Journal 44, 440–443.

Couetil, L., Paradis, M.R., Knoll, J., 1996. Plasma adrenocorticotropin concentration in healthy horses and in horses with clinical signs of hyperadrenocorticism. Journal of Veterinary Internal Medicine 10, 1–6.

Croskerry, P., 2009. A universal model of diagnostic reasoning. Academic Medicine 84, 1022–1028.

Durham, A.E., 2022. The effect of pergolide mesylate on adrenocorticotrophic hormone responses to exogenous thyrotropin releasing hormone in horses. The Veterinary Journal 285, 105831.

Durham, A.E., Potier, J.F.N., Huber, L., 2022. The effect of month and breed on plasma ACTH concentrations in equids. The Veterinary Journal 286, 105857.

Durham, A.E., McGowan, C.M., Fey, K., Tamzali, Y., Van Der Kolk, J.H., 2014. Pituitary pars intermedia dysfunction: Diagnosis and treatment. Equine Veterinary Education 26, 216–223.

Durham, A.E., Clarke, B.R., Potier, J.F., Hammarstrand, R., Malone, G.L., 2021. Clinically and temporally specific diagnostic thresholds for plasma ACTH in the horse. Equine Veterinary Journal 53, 250–260.

Fanshawe, T.R., Power, M., Graziadio, S., Ordóñez-Mena, J.M., Simpson, J., Allen, J., 2018. Interactive visualisation for interpreting diagnostic test accuracy study results. BMJ Evidence-Based Medicine 23, 13–16.

Frank, N., Andrews, F.M., Sommardahl, C.S., Eiler, H., Rohrbach, B.W., Donnell, R.L., 2006. Evaluation of the combined dexamethasone suppression/thyrotropin-releasing hormone stimulation test for detection of pars intermedia pituitary adenomas in horses. Journal of Veterinary Internal Medicine 20, 987–993.

Funk, R.A., Stewart, A.J., Wooldridge, A.A., Kwessi, E., Kemppainen, R.J., Behrend, E.N., Zhong, Q., Johnson, A.K., 2011. Seasonal changes in plasma adrenocorticotropic hormone and alpha-melanocyte-stimulating hormone in response to thyrotropin-releasing hormone in normal, aged horses. Journal of Veterinary Internal Medicine 25, 579–585.

Goodale, L., Hermida, P., Oench, S.D., Frank, N., 2013. Assessment of compounded thyrotropin releasing hormone for the diagnosis of pituitary pars intermedia dysfunction in horses. Journal of Veterinary Internal Medicine 27, 656-656.

GOV.UK, 2021. The cascade: Prescribing unauthorised medicines available from: ⟨https://www.gov.uk/guidance/the-cascade-prescribing-unauthorised-medicines#about-the-cascade⟩ (Accessed 3 October 2023).

Grubbs, S.T., Neal, D.L., Keefe, T.J., 2020. Development and Evaluation Of A Clinical Sign Scoring System For Pituitary Pars Intermedia Dysfunction In Horses. In: Proceedings of the 4th Global Equine Endocrine Symposium, 2020. Gut Ising, Bavaria, p. 20.

Harno, E., Gali Ramamoorthy, T., Coll, A.P., White, A., 2018. POMC: the physiological power of hormone processing. Physiology Review 98, 2381–2430.
Hart, K.A., Durham, A.E., Frank, N., McGowan, C.M., Schott, H.C., Stewart, A.J., 2021. Equine Endocrinology Group (EEG) Recommendations for the diagnosis and treatment of pituitary pars intermedia dysfunction (PPID). Available at: ⟨https://sites.tufts.edu/equineendogroup/files/2021/12/2021-PPID-Recommendations-V11-wo-insert.pdf⟩ (Accessed 3 October 2023).
Heinrichs, M., Baugartner, W., Capen, C.C., 1990. Immunocytochemical demonstration of proopiomelanocortin-derived peptides in pituitary adenomas of the pars intermedia in horses. Veterinary Pathology 27, 419–425.
Hinrichsen, S.L., Yuen, K.Y., Dryburgh, E.L., Bertin, F.R., Stewart, A.J., 2022. Short-term effects of temperature and thyrotropin-releasing hormone stimulation on adrenocorticotropin stability in horses. Animals 12 (324), 2030324.
Hodge, E., Kowalski, A., Torcivia, C., Lindborg, S., Stefanovski, D., Hart, K., Frank, N., van Eps, A., 2019. Effect of thyrotropin-releasing hormone stimulation testing on the oral sugar test in horses when performed as a combined protocol. Journal of Veterinary Internal Medicine 33, 2272–2279.
Horn, R., Bertin, F.R., 2019. Evaluation of combined testing to simultaneously diagnose pituitary pars intermedia dysfunction and insulin dysregulation in horses. Journal of Veterinary Internal Medicine 33, 2249–2256. https://doi.org/10.1111/jvim.15617.
Horn, R., Stewart, A.J., Jackson, K.V., Dryburgh, E.L., Medina-Torres, C.E., Bertin, F.R., 2021. Clinical implications of using adrenocorticotropic hormone diagnostic cutoffs or reference intervals to diagnose pituitary pars intermedia dysfunction in mature horses. Journal of Veterinary Internal Medicine 35, 560–570.
Horn, R., Bamford, N.J., Afonso, T., Sutherland, M., Buckerfield, J., Tan, R.H.H., Secombe, C.J., Stewart, A.J., Bertin, F.R., 2019. Factors associated with survival, laminitis and insulin dysregulation in horses diagnosed with equine pituitary pars intermedia dysfunction. Equine Veterinary Journal 51, 440–445.
Hu, K., Stewart, A.J., Yuen, K.Y., Hinrichsen, S., Dryburgh, E.L., Bertin, F.R., 2020. The effect of freeze-thaw cycles on determination of immunoreactive plasma adrenocorticotrophic hormone concentrations in horses. Journal of Veterinary Internal Medicine 34, 1350–1356.
Humphreys, S., Kass, P.H., Magdesian, K.G., Goodrich, E., Berryhill, E., 2022. Seasonal variation of endogenous adrenocorticotropic hormone concentrations in healthy non-geriatric donkeys in Northern California. Frontiers in Veterianry Sciences 9, 981920.
Ireland, J.L., McGowan, C.M., 2018. Epidemiology of pituitary pars intermedia dysfunction: a systematic literature review of clinical presentation, disease prevalence and risk factors. The Veterinary Journal 235, 22–33.
Ireland, J.L., McGowan, C.M., 2021. Translating research into practice: adoption of endocrine diagnostic testing in cases of equine laminitis. The Veterinary Journal 272, 105656.
Ireland, J.L., McGowan, C.M., 2023. Deciphering reference intervals and clinical decision limits in equine endocrine diagnostic testing. The Veterinary Journal. https://doi.org/10.1016/j.tvjl.2023.106037.
Ireland, J.L., Clegg, P.D., McGowan, C.M., McKane, S.A., Chandler, K.J., Pinchbeck, G.L., 2012. Disease prevalence in geriatric horses in the United Kingdom: veterinary clinical assessment of 200 cases. Equine Veterinary Journal 44, 101–106.
Irvine, K.L., Burt, K., Hill, A.J., Shaw, S., Papasouliotis, K., 2016. Initial analytic quality assessment and method comparison of an immunoassay for adrenocorticotropic hormone measurement in equine samples. Veterinary Clinical Pathology 45, 154–163.
Kam, Y.N., McKenzie, K., Coyle, M., Bertin, F.R., 2021. Repeatability of a thyrotropin-releasing hormone stimulation test for diagnosis of pituitary pars intermedia dysfunction in mature horses. Journal of Veterinary Internal Medicine 35, 2885–2890.
Karikoski, N.P., Horn, I., McGowan, T.W., McGowan, C.M., 2011. The prevalence of endocrinopathic laminitis among horses presented for laminitis at a first-opinion/referral equine hospital. Domestic Animal Endocrinology 41, 111–117.
Kirkwood, N.C., Hughes, K.J., Stewart, A.J., 2022. Prospective case series of clinical signs and adrenocorticotrophin (ACTH) concentrations in seven horses transitioning to pituitary pars intermedia dysfunction (PPID). Veterinary Sciences 9 (572), 9100572.
Knowles, E.J., Moreton-Clark, M.C., Shaw, S., Harris, P.A., Elliott, J., Menzies-Gow, N.J., 2018. Plasma adrenocorticotropic hormone (ACTH) concentrations in ponies measured by two different assays suggests seasonal cross-reactivity or interference. Equine Veterinary Journal 50, 672–677.
Lee, Z.Y., Zylstra, R., Haritou, S.J., 2010. The use of adrenocorticotrophic hormone as a potential biomarker of pituitary pars intermedia dysfunction in horses. The Veterinary Journal 185, 58–61.
Lindberg, I., Fricker, L.D., 2021. Obesity, POMC, and POMC-processing enzymes: surprising results from animal models. Endocrinology 162, Bqab155.
McFarlane, D., 2011. Equine pituitary pars intermedia dysfunction. Veterinary Clinics of North America: Equine Practice 27, 93–113.
McFarlane, D., Beech, J., Cribb, A., 2006. Alpha-melanocyte stimulating hormone release in response to thyrotropin releasing hormone in healthy horses, horses with pituitary pars intermedia dysfunction and equine pars intermedia explants. Domestic Animal Endocrinology 30, 276–288.
McGilvray, T.A., Knowles, E.J., Harris, P.A., Menzies-Gow, N.J., 2020. Comparison of immunofluorescence and chemiluminescence assays for measuring ACTH in equine plasma. Equine Veterinary Journal 52, 709–714.
McGowan, C.M., Neiger, R., 2003. Efficacy of trilostane for the treatment of equine Cushing's syndrome. Equine Veterinary Journal 35, 414–418.
McGowan, T.W., Pinchbeck, G.P., McGowan, C.M., 2013. Prevalence, risk factors and clinical signs predictive for equine pituitary pars intermedia dysfunction in aged horses. Equine Veterinary Journal 45, 74–79.
McGowan, T.W., Pinchbeck, G., Phillips, C.J.C., Perkins, N., Hodgson, D.R., McGowan, C.M., 2010. A survey of aged horses in Queensland, Australia. Part 2: clinical signs and owners' perceptions of health and welfare. Australian Veterinary Journal 88, 465–471.
Meyer, J.C., Hunyadi, L.M., Ordóñez-Mena, J.M., 2022. The accuracy of ACTH as a biomarker for pituitary pars intermedia dysfunction in horses: A systematic review and meta-analysis. Equine Veterinary Journal 54, 457–466.
Miller, M.A., Moore, G.E., Bertin, F.R., Kritchevsky, J.E., 2016. What's new in old horses? Postmortem diagnoses in mature and aged equids. Veterinary Pathology 53, 390–398.
Miller, M.A., Pardo, I.D., Jackson, L.P., Moore, G.E., Sojka, J.E., 2008. Correlation of pituitary histomorphometry with adrenocorticotrophic hormone response to domperidone administration in the diagnosis of equine pituitary pars intermedia dysfunction. Veterinary Pathology 45, 26–38.
Orth, D.N., Holscher, M.A., Wilson, M.G., Nicholson, W.E., Plue, R.E., Mount, C.D., 1982. Equine Cushing's disease: plasma immunoreactive proopiolipomelanocortin peptide and cortisol levels basally and in response to diagnostic tests. Endocrinology 110, 1430–1441.
Ozarda, Y., Sikaris, K., Streichert, T., Macri, J., IFCC Committee on Reference intervals and Decision Limits (C-RIDL), 2018. Distinguishing reference intervals and clinical decision limits – A review by the IFCC Committee on Reference Intervals and Decision Limits. Critical Reviews in Clinical Laboratory Sciences 55, 420–431.
Perkins, G.A., Lamb, S., Erb, H.N., Schanbacher, B., Nydam, D.V., Divers, T.J., 2002. Plasma adrenocorticotropin (ACTH) concentrations and clinical response in horses treated for equine Cushing's disease with cyproheptadine or pergolide. Equine Veterinary Journal 34, 679–685.
Rohrbach, B.W., Stafford, J.R., Clermont, R.S.W., Reed, S.M., Schott, H.C., Andrews, F.M., 2012. Diagnostic frequency, response to therapy, and long-term prognosis among horses and ponies with pituitary par intermedia dysfunction, 1993–2004. Journal of Veterinary Internal Medicine 26, 1027–1034.
Rumfola, E., Banse, H.E., Atkins, M., McGowan, C.M., Ireland, J.L., 2022. Approaches to endocrinopathic laminitis in the field: Results of a survey of veterinary practitioners in North America. Journal of Equine Veterinary Science 110, 103856.
Saleh D., Yarrarapu S.N.S., Cook C., 2022. Hypertrichosis. In: StatPearls, StatPearls Publishing. ⟨https://www.ncbi.nlm.nih.gov/books/NBK534854/⟩ (Accessed 27 September 2023).
Shreffler J., Huecker M.R., 2022. Diagnostic testing accuracy: Sensitivity, specificity, predictive values and likelihood ratios. In: StatPearls, StatPearls Publishing ⟨https://www.ncbi.nlm.nih.gov/books/NBK557491/⟩ (Accessed 27 September, 2023).
Spelta, C.W., Axon, J.E., 2012. Case series of equine pituitary pars intermedia dysfunction in a tropical climate. Australian Veterinary Journal 90, 451–456.
Tadros, E.M., Fowlie, J.G., Refsal, K.R., Marteniuk, J., Schott, H.C., 2019. Association between hyperinsulinaemia and laminitis severity at the time of pituitary pars intermedia dysfunction diagnosis. Equine Veterinary Journal 51, 52–56.
Tatum, R.C., McGowan, C.M., Ireland, J.L., 2021. Evaluation of the sensitivity and specificity of basal plasma adrenocorticotrophic hormone concentration for diagnosing pituitary pars intermedia dysfunction in horses: a systematic review. The Veterinary Journal 275, 105695.
Thane, K., Uricchio, C., Frank, N., 2022. Effect of early or late blood sampling on thyrotropin releasing hormone stimulation test results in horses. Journal of Veterinary Internal Medicine 36, 770–777.
Van Der Kolk, J.H., Wensing, T., Kalsbeek, H.C., Breukink, H.J., 1995. Laboratory diagnosis of equine pituitary pars intermedia adenoma. Domestic Animal Endocrinology 12, 35–39.
Welsh, C.E., Duz, M., Parkin, T.D., Marshall, J.F., 2016. Prevalence, survival analysis and multimorbidity of chronic diseases in the general veterinarian-attended horse population of the UK. Preventive Veterinary Medicine 131, 137–145.
Wilson, M.G., Nicholson, W.E., Holscher, M.A., Sherrell, B.J., Mount, C.D., Orth, D.N., 1982. Proopiolipomelanocortin peptides in normal pituitary, pituitary tumor, and plasma of normal and Cushing's horses. Endocrinology 110, 941–954.