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Pituitary *pars intermedia* dysfunction (Cushing's syndrome) associated with pituitary adenoma in a mare

Disfunção da pars intermedia *da pituitária (síndrome de Cushing) associada a adenoma de hipófise em égua*

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ABSTRACT: Pituitary *pars intermedia* dysfunction (PPID), also known as Cushing's syndrome (CS) or hyperadrenocorticism, is a condition characterized by loss of dopaminergic inhibition of the pituitary *pars intermedia*. A Crioulo mare was presented with progressive weight loss, incoordination when walking, polydipsia, and a coat with a hirsute appearance. Loss of tail tone and muscle mass in the lumbar and gluteal regions, as well as long hair with a hirsute, opaque, and brittle appearance, were observed on inspection. In addition, the mare had small abscesses in the thoracolumbar region, and there were no other abnormalities on physical examination. The low-dose dexamethasone suppression test (DST) was performed to confirm the suspicion of CS, demonstrating a level of 4.6 µg/dL higher than baseline after 19h, confirming the diagnosis of PPID. The treatment consisted of cyproheptadine, and the animal showed improvement, being discharged from the hospital after 10 days. Continuation of the treatment was recommended on the farm. According to the veterinarian on the farm, the animal had been showing improvement, but it developed respiratory complications 10 days after discharge and died three days later. The main findings of the necropsy included severe diffuse granulomatous pneumonia, in addition to pituitary *pars intermedia* adenoma, which confirmed the clinical suspicion of PPID.

KEYWORDS: pituitary adenoma; corticosteroid therapy; endocrinopathy; equine; hyperadrenocorticism.

RESUMO: A disfunção da *pars intermedia* da pituitária (PPID), também conhecida por síndrome de Cushing (CS) ou hiperadrenocorticismo é uma afecção caracterizada pela perda de inibição dopaminérgica da *pars intermedia* da hipófise. Foi atendida uma égua da raça crioula, que foi relatado emagrecimento progressivo, incoordenação ao caminhar, polidipsia e pelagem com aparência hirsuta. Na inspeção foi visualizado perda do tônus da cauda e de massa muscular na região lombar e glútea, pelos longos de aparência hirsuta, opaca e quebradiça. Além disso, apresentava pequenos abscessos na região tóraco-lombar, sendo que não apresentava outras alterações no exame físico. A fim de confirmar a suspeita de SC foi realizado o teste de supressão com dexametasona (DST) em dose baixa, o qual demonstrou nível de de 4,6 µg/dL maior que o basal, após 19h, confirmando o diagnóstico de PPID. O tratamento utilizado foi com ciproeptadina, o animal demonstrou melhora, por isso após 10 dias a paciente recebeu alta hospitalar, recomendando a continuação do tratamento na propriedade. Segundo o médico veterinário da propriedade o animal vinha apresentando melhora, porém 10 dias após a alta ela apresentou complicações respiratórias e após três dias morreu. Os principais achados da necropsia incluíam pneumonia granulomatosa difusa acentuada, além de adenoma de *pars intermedia* da hipófise, o que confirmou a suspeita clínica de PPID.

PALAVRAS-CHAVE: adenoma hipofisário; corticoterapia; endocrinopatia; equino; hiperadrenocorticismo.

INTRODUCTION

Endocrinopathies in equines are rarely reported but have become increasingly relevant, as equine medicine has also focused on older animals (Ireland *et al.*, 2011; Welsh *et al.*, 2016). Dysfunction of the pituitary *pars intermedia*, also known as Cushing's syndrome or hyperadrenocorticism, is one of the endocrine disorders that affect this species (Millington *et al.*, 1988; Orth *et al.*, 1982).

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PPID in equines results from the loss of dopaminergic inhibition of the pituitary *pars intermedia*, possibly due to oxidative stress and degeneration of dopaminergic neurons in the hypothalamus. As a result, there is a decrease in dopamine production and, consequently, an increase in the synthesis of proopiomelanocortin (POMC) and plasma concentrations of adrenocorticotropic hormone (ACTH) (Mcgowan; Pinchbeck; Mcgowan, 2013; Millington *et al.*, 1988; Orth *et al.*, 1982).

Currently, some tests are available to diagnose pituitary *pars intermedia* dysfunction, such as the low-dose dexamethasone suppression test, plasma adrenocorticotropin (ACTH) concentration, thyrotropin-releasing hormone (TRH) stimulation test, adrenocorticotropin stimulation test, and domperidone-response test (Mcfarlane, 2011; Hart *et al.*, 2021; Kirkwood; Hughes; Stewart, 2022a; Stewart *et al.*, 2023).

In addition to nursing care, the management of this condition requires the use of some medications, such as pergolide mesylate or cyproheptadine (Mcfarlane, 2019; Hart *et al.*, 2021).

Given the relevance of describing clinical cases to aid in future diagnoses, the present study aimed to report a case of PPID in a Crioulo mare, characterizing the observed clinical manifestations and laboratory and pathological findings.

CASO REPORT

A 10-year-old, 400 kg Crioulo mare used in sports activities presented a history of progressive weight loss (body condition score of 4) with pronounced muscle mass loss in the lumbar and gluteal regions for 15 days, as well as incoordination when walking, polydipsia, and loss of tail tone. The coat had a hirsute, opaque, brittle appearance with long hair, especially in the jugular groove region, along with alopecia in the perianal region. There were also small abscesses in the thoracolumbar region (Figure 1). No changes were observed on physical examination. Cauda equina syndrome was suspected, due to decreased tail tone, or an endocrine disorder, such as diabetes mellitus or pituitary *pars intermedia* dysfunction.

Source: Author's collection.

Figure 1. Body score of 4 (A and B), opaque, brittle, and long hair (C), muscle mass loss in the lumbar and croup regions (D), and abscess in the thoracolumbar region (E).

Laboratory tests (Table 1) performed on the first day of hospitalization revealed leukopenia, lymphopenia, hyperproteinemia, hyperglobulinemia, hyperglycemia, hypercholesterolemia, and increased GGT and ALP enzymes. Urinalysis revealed glycosuria (1573 mg/dL), the presence of leukocytes (++), red blood cells (++), and bacteria (+++). The other parameters were within the physiological range. Urinalysis was repeated after 24 hours. The findings were similar, with glycosuria (1559 mg/dL), slight presence of bacteria (+), slight presence of leukocytes (++), and red blood cells (++). The presence of amorphous material (+++) and clusters of transitional epithelial cells in small quantities were also observed.

Treatment consisted of antibiotic therapy with enrofloxacin (4 mg/kg), VO, BID, for eight days, combined with benzathine penicillin (30,000 IU/kg), every 48 hours, IM, for 6 days.

A case of PPID was suspected, considering the clinical signs and laboratory test findings. The low-dose dexamethasone (40 mg/kg) suppression test was performed, as described by Burns *et al.* (2017). The samples were sent for evaluation at an external laboratory and the results were received after 48 hours. The obtained curve showed an increase in plasma cortisol levels even after the application of dexamethasone, confirming the clinical suspicion of PPID (Figure 2).

New laboratory tests were performed while the results of the low-dose dexamethasone suppression test were awaited. The hematologic evaluation revealed normocytic normochromic anemia, thrombocytosis, hyperproteinemia, hyperglobulinemia, hyperglycemia, hypercholesterolemia, and increased AST and GGT enzymes (Table 1).

The imaging tests consisted of radiographic evaluation of the hoof in both thoracic limbs using lateral-medial and dorsopalmar projections, in which no changes suggestive of laminitis were observed. An ultrasound evaluation of the thorax was performed, in which no images suggestive of pleuritis and/or pleuropneumonia were found. An abdominal ultrasound was also performed, evaluating especially the kidneys for changes in the cortical-medullary relationship, in addition to observing the adrenal glands, given a possible increase in volume in these organs, which was not visualized. During this period, spontaneous drainage of the abscess located in the lumbar region and a new abscess in the region of the coronary band of the hoof also occurred. Both were cleaned and hydrogen peroxide and 0.1% iodine solution were applied for three days. The use of 0.1% iodine solution was continued until the site healed. After cleaning, antibiotic and healing ointment was applied to the abscess in the lumbar region, and the abscess located on the coronary band of the hoof was bandaged. A new radiographic evaluation was performed to verify the extent of the latter, which showed no damage to adjacent structures.

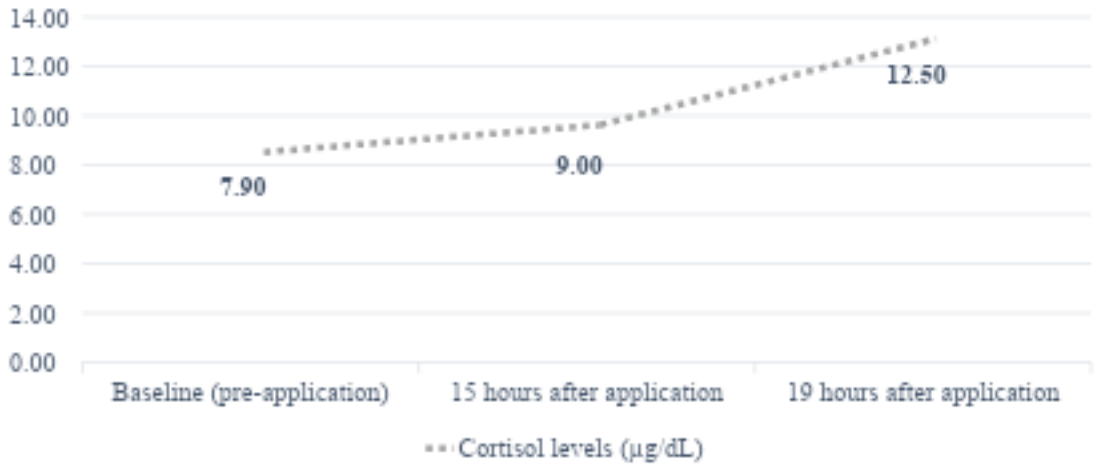
The diagnosis of PPID was confirmed after the results of the low-dose dexamethasone suppression test.

Table 1. Presentation of hematological and biochemical analysis parameters.

Examination	1st examination	2nd examination	Reference values
Hematological			
Erythrocytes	6.8	4.8	5.9–9.4
Hematocrit	38.0	28.0	30–47
Hemoglobin	13.8	9.8	10.7–16.7
MCV	56	59	40–61
MCHC	36	35	32–39
Metarubricytes	0	0	
Anisocytosis	Negative	Negative	Mild
Polychromasia	Negative	Negative	Mild
Howell-Jolly	Negative	Negative	Mild
Platelets	330,000	394,000	90,000–210,000
Leukocytes	4,200	5,500	5,000–11,000
Basophils	0	0	0–300
Eosinophils	0	0	100–800
Segmented neutrophils	3,822	4,950	2,200–6,100
Band neutrophils	0	0	0–200
Lymphocytes	210	550	1,500–6,500
Monocytes	168	0	0–600
Unclassified	0	0	0
Total proteins	10.4	9.8	6.8–8.4
Fibrinogen	0.4	0.2	0.1–0.5
P/F	26	49	>15
Biochemical			
Urea	30	40	21.6–51
Creatinine	1.02	0.98	0.96–1.3
Total protein	7.82	7.98	6.8–8.4
Albumin	3.23	3.04	2.6–3.8
Globulin	4.6	4.34	2.5–4.1
A/G	0.7	0.70	
AST	350	1600	120–480
GGT	568	492	10–62
ALP	669.7	-	170–530
Cholesterol	269	274.5	70–178
Glucose	362	290	59–95
Observation	Mildly lipemic serum		

Previously, clinical and blood evaluation helped to elucidate the clinical conditions of the animal, that received care directed at the consequences of the condition, such as treatment of secondary infections.

The treatment for PPID was cyproheptadine (0.25 mg/kg), VO, SID. The drug was used for four days at the veterinary hospital, which was not enough time to detect changes in the clinical condition. The animal was discharged after a 10-day hospitalization period, and continuation of the treatment was recommended on the farm. Contact was made with the veterinarian responsible for the farm, who reported that the patient had been showing clinical improvement, evidenced by the complete healing of the abscesses and improvement in its external appearance. However, treatment with cyproheptadine had been interrupted due to lack of medication, 5 days after returning to the farm. The patient began to present respiratory complications on the 10th day after discharge, such as increased respiratory rate and dyspnea, as well as elevated temperature.



Source: Author's collection.

Figure 2. Cortisol curve obtained after performing the low-dose dexamethasone suppression test in the equine patient with suspected PPID.

Antibiotic therapy based on ceftiofur (2.2 mg/kg), IM, SID, was started at that time. Despite this, the patient died 3 days after presenting these clinical changes.

Changes in different organs were observed at necropsy, characterized by clots with focally extensive areas of hemorrhage in the parenchyma of the left adrenal gland, which was also enlarged, similar to infarctions (Figure 3A), as well as moderate enlargement of the cortical region in both adrenal glands. The lungs presented multiple yellowish nodules that, upon cut, were friable and contained whitish necrotic material inside, similar to fungal lesions. The pituitary gland was diffusely and firmer, approximately 2 cm in diameter, and protruded into the cranial cavity, with slight compression of the brainstem, in the hypothalamic region (Figure 3B). Mild splenomegaly was also observed. Fragments of the organs were collected and sent for histopathological examination.

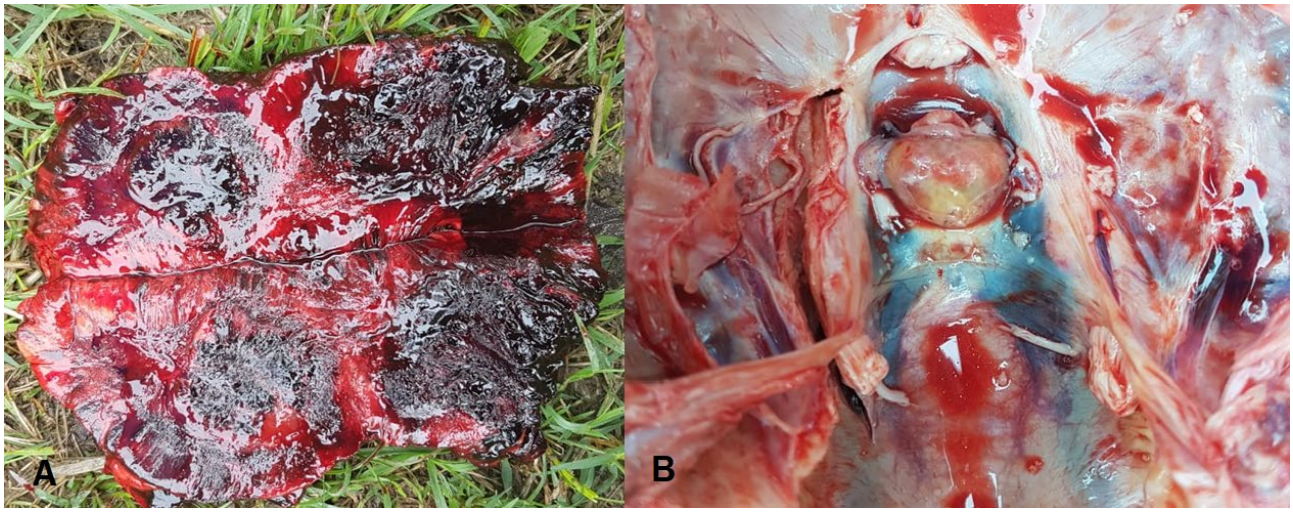
Histopathological examination of the left adrenal gland revealed marked multifocal coagulation necrosis, dense thrombus formation, and a focally extensive hemorrhagic area, in addition to hyperplasia of cells in the cortical layer. A marked multifocal inflammatory infiltrate was observed in the lungs, composed mainly of macrophages, Langerhans-type giant cells, and intact and degenerated neutrophils associated with bacteria and fungal hyphae. Type 2 pneumocytes and marked diffuse edema were also observed. The pituitary gland showed proliferation of well-differentiated neoplastic cells originating from the *pars intermedia*, arranged in small nests and bundles supported by a thin and well-defined fibrovascular stroma. The cells presented round or oval nuclei with eosinophilic cytoplasm and mild pleomorphism, with the formation of pseudorosettes, allowing the establishment of the diagnosis of pituitary adenoma.

RESULTS AND DISCUSSIONS

Hyperadrenocorticism in equines is most commonly reported in older animals, aged 15 years or older, with a history of hirsutism being the most reported by owners (Mcgowan; Pinchbeck; Mcgowan, 2013; Kirkwood; Hughes; Stewart, 2022b). In the clinical history, owners of horses may report poor performance, apathy, and lethargy (Frank, 2018). Several clinical signs can be observed in PPID, the most common of which include hypertrichosis, laminitis, polyuria, polydipsia, loss of muscle, redistribution of body fat, and pendulous abdomen (pot-bellied appearance) (Mcfarlane, 2011; Franco *et al.,* 2021). The animal in this report was 10 years old, which differs from the literature, but presented most of the most observed clinical signs, except for laminitis, redistribution of body fat, and pendulous abdomen.

This condition may present neutrophilia, lymphopenia, and hyperglycemia, in addition to an increase in the alkaline phosphatase enzyme in laboratory tests and glycosuria in the urine test (Mccue, 2002). A study with animals showing clinical signs of Cushing's syndrome, animals without clinical signs but with Cushing's syndrome, and control animals without Cushing's syndrome showed an increase in insulin and glucose in animals with clinical signs (Keen *et al.*, 2004). In this report, the animal presented lymphopenia, glycosuria, and hyperglycemia. However, insulin was not evaluated.

There are some tests for diagnosing pituitary *pars intermedia* dysfunction, the most recognized being the low-dose dexamethasone suppression test, plasma adrenocorticotropin (ACTH) concentration, thyrotropin-releasing hormone (TRH) stimulation test, adrenocorticotropin stimulation test, and domperidone-response test (Schott, 2002; Mcfarlane, 2011; Tatum; Mcgowan; Ireland, 2021; De Souza; De Zoppa, 2022; Kirkwood; Hughes; Stewart, 2022a; Bamford *et al.*, 2023). According to Horn *et al.* (2021), the ACTH concentration



Source: Author's collection.

Figure 3. Enlarged left adrenal gland with a focally extensive area of hemorrhage and thickening of the cortical region (A). Diffusely enlarged pituitary gland with yellowish nodule with protrusion of the parenchyma into the cranial cavity (B).

test is reliable but recommended to use associated with the TRH test to confirm the diagnosis of Cushing's syndrome.

The low-dose dexamethasone suppression test is an appropriate diagnostic method although it is not widely used and has lower sensitivity than other tests (Mcfarlane, 2019). In this study, the low-dose dexamethasone suppression test was used because it was an available and easily accessible test. Animals with PPID require therapeutic management, especially geriatric animals, establishing care with body score, dental health, parasite control, and hoof care (Mcfarlane, 2019). Pergolide mesylate and cyproheptadine are some of the drugs used to treat PPID. Pergolide is a dopamine D2 receptor agonist, while cyproheptadine is a serotonin antagonist, antihistamine, and antimuscarinic (Mcfarlane, 2019). Studies with pergolide have shown that it is effective in improving clinical signs, but the improvement in plasma ACTH concentrations has been variable (Tatum; Mcgowan; Ireland, 2020).

Perkins *et al.* (2002) observed no association between changes in ACTH and improvement in clinical signs of horses with PPID treated with cyproheptadine. In another study, the owners reported an improvement in the clinical signs of animals treated with pergolide compared to owners whose animals were treated with cyproheptadine (Donaldson *et al.*, 2002). Cyproheptadine was the choice for PPID treatment in this case report, which demonstrated an improvement in the animal's clinical condition. Discontinuation of treatment may have aggravated the condition and predisposed the animal to worsening of the infectious condition and consequent death.

A retrospective study of pathological findings in horses with PPID showed that 66% of the cases presented adenoma and the other 34% had adenomatous hyperplasia, and diffuse adrenocortical hyperplasia was observed in 44% of the horses (Glover *et al.*, 2009). Miller *et al.* (2008) observed that horses with and without clinical signs of PPID presented normal findings or pituitary adenomas, hypertrophy or focal hyperplasia, diffuse adenomatous hyperplasia or microadenomas. In this report, the histopathological examination using microscopy showed well-differentiated neoplastic cells in the pituitary *pars intermedia*, which is characteristic of adenoma, and an area of marked diffuse coagulation necrosis, dense thrombus formation, and a focally extensive hemorrhagic area in the left adrenal gland.

CONCLUSIONS

PPID is an important endocrine disorder that affects the equines. However, it is still rarely reported due to the lack of diagnosis or lack of understanding of clinical signs. Understanding clinical signs and laboratory findings is essential to reach a definitive diagnosis and appropriate treatment. Cyproheptadine used as a treatment in this case initially obtained a positive response regarding the improvement of the clinical signs presented by the animal.

REFERENCES

BAMFORD, N. J. *et al.* Investigation of breed differences in plasma adrenocorticotropic hormone concentrations among healthy horses and ponies. **The Veterinary Journal**, v. 296, p. 105995, 2023.

DE SOUZA, A. F.; DE ZOPPA, A. L. V. Basal plasma adrenocorticotrophic hormone concentration for diagnosing pituitary *pars intermedia* dysfunction in horses: Comparative analysis of two systematic reviews. **Applied Veterinary Research**, v. 1, n. 2, p. 2022008-2022008, 2022.

DONALDSON, M. T. *et al.* Treatment with pergolide or cyproheptadine of pituitary *pars intermedia* dysfunction (equine Cushing's disease). **Journal of veterinary internal medicine**, v. 16, n. 6, p. 742-746, 2002.

FRANCO, F. A. *et al.* Diagnostic Process in a Crioulo Horse with Cushing's Syndrome. **Acta Scientiae Veterinariae**, v. 49, 2021.

FRANK, N. Endocrine disorders of the equine athlete. **Veterinary Clinics**: Equine Practice, v. 34, n. 2, p. 299-312, 2018.

GLOVER, C. M. *et al.* Extrapituitary and pituitary pathological findings in horses with pituitary *pars intermedia* dysfunction: a retrospective study. **Journal of Equine Veterinary Science**, v. 29, n. 3, p. 146-153, 2009.

HART, K. *et al.* Recommendations for the diagnosis and treatment of pituitary *pars intermedia* dysfunction (PPID). **Equine Endocrinology Group**, 2021.

HORN, R. *et al.* 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**, v. 35, n. 1, p. 560-570, 2021.

IRELAND, J. L. *et al.* A crosssectional study of geriatric horses in the United Kingdom Part 1: Demographics and management practices. **Equine Veterinary Journal**, v. 43, p. 30-36, 2011.

KEEN, J. A. *et al.* Biochemical indices of vascular function, glucose metabolism and oxidative stress in horses with equine Cushing's disease. **Equine Veterinary Journal**, v. 36, n. 3, p. 226-229, 2004.

KIRKWOOD, N. C.; HUGHES, K. J.; STEWART, A. J. Prospective case series of clinical signs and adrenocorticotrophin (ACTH) concentrations in seven horses transitioning to pituitary *pars intermedia* dysfunction (PPID). **Veterinary Sciences**, v. 9, n. 10, p. 572, 2022a.

KIRKWOOD, N. C.; HUGHES, K. J.; STEWART, A. J. Pituitary *pars intermedia* dysfunction (PPID) in horses. **Veterinary Sciences**, v. 9, n. 10, p. 556, 2022b.

MCCUE, P. M. Equine Cushing's disease. **Veterinary Clinics**: Equine Practice, v. 18, n. 3, p. 533-543, 2002.

MCFARLANE, D. Equine pituitary *pars intermedia* dysfunction. **Veterinary Clinics**: Equine Practice, v. 27, n. 1, p. 93-113, 2011.

MCFARLANE, D. Diagnostic testing for equine endocrine diseases: confirmation versus confusion. **Veterinary Clinics**: Equine Practice, v. 35, n. 2, p. 327-338, 2019.

MCGOWAN, T. W.; PINCHBECK, G. P.; MCGOWAN, C. M. Prevalence, risk factors and clinical signs predictive for equine pituitary *pars intermedia* dysfunction in aged horses. **Equine Veterinary Journal**, Filadélfia, v. 45, n. 1, p. 74-79, 2013.

MILLER, M. A. *et al.* Correlation of pituitary histomorphometry with adrenocorticotrophic hormone response to domperidone administration in the diagnosis of equine pituitary *pars intermedia* dysfunction. **Veterinary Pathology**, v. 45, n. 1, p. 26-38, 2008.

MILLINGTON, W. R. *et al.* Equine Cushing's disease: Differential regulation of beta-endorphin processing in tumors of the intermediate pituitary. **Endocrinology**, v. 123, p. 1598– 1604, 1988.

ORTH, D. N. *et al.* Equine Cushing's disease: Plasma immunoreactive proopiomelanocortin peptide and cortisol levels basally and in response to diagnostic tests. **Endocrinology**, v. 110, n. 4, p. 1430–1441, 1982.

PERKINS, G. A. *et al.* Plasma adrenocorticotropin (ACTH) concentrations and clinical response in horses treated for equine Cushing's disease with cyproheptadine or pergolide. **Equine Veterinary Journal**, v. 34, n. 7, p. 679-685, 2002.

SCHOTT, H. C. Pituitary *pars intermedia* dysfunction: equine Cushing's disease. **Veterinary Clinics**: Equine Practice, v. 18, n. 2, p. 237-270, 2002.

STEWART, A. J. *et al.* Diagnosis of equine pituitary *pars intermedia* dysfunction. **The Veterinary Journal**, v. 300, p. 106036, 2023.

TATUM, R. C.; MCGOWAN, C. M.; IRELAND, J. L. Efficacy of pergolide for the management of equine pituitary *pars intermedia* dysfunction: A systematic review. **The Veterinary Journal**, v. 266, p. 105562, 2020.

TATUM, R. C.; MCGOWAN, C. M.; IRELAND, J. L. 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**, v. 275, p. 105695, 2021.

WELSH, C. E. *et al.* Prevalence, survival analysis and multimorbidity of chronic diseases in the general veterinarian-attended horse population of the UK. **Preventive Veterinary Medicine**, v. 131, p. 137-145, 2016.