Decoding the Equine Pituitary: Histological Insights into Clinical Expression of PPID

Isabelle Smith[1],Nicola Romano[1], Paul Le Tissier[1] & Ruth Morgan[1,2]

1. Institute for Neuroscience and Cardiovascular Research, The University of Edinburgh, Edinburgh, United Kingdom.

2. **Scotland's Rural College (SRUC)**, Edinburgh, United Kingdom

Introduction

- Pituitary pars intermedia dysfunction (PPID) is an endocrinopathology affecting >20% horses aged over 15 years old, characterised by hyperplastic or adenomatous pituitary intermediate lobe (IL) growth[1].
- PPID & IL expansion considered a primary hypothalamic disease, following neurodegeneration of dopaminergic innervation but little work views PPID as a pituitary-centric disease.
- PPID presents with varied clinical signs, with discordance between current ante-mortem diagnostics, clinical phenotypes and IL pathology grading[2].
- **We aim to determine how histological pituitary pathology is associated with diagnostic & clinical phenotypes.**

Methods

- 36 haematoxylin-eosin-stained equine pituitaries from (n= 21) healthy and PPID-confirmed (n= 15) horses were brightfield imaged.
- ImageJ & an AI trained algorithm were used to quantify vascularisation (fig 3a), pituitary size (fig 3b), nuclear phenotype proportions & average nuclear count per pituitary (fig 3c).

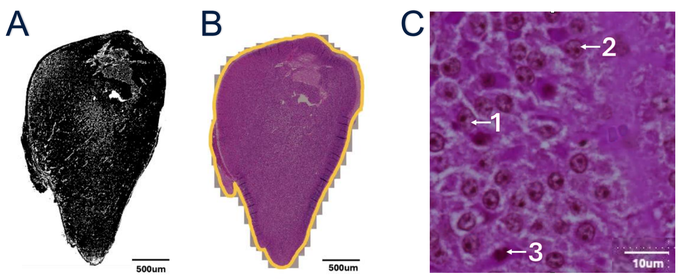

Fig 3-Representation of quantified histological features

- Principal component analysis (PCA) examined relationships between variables (fig 4).

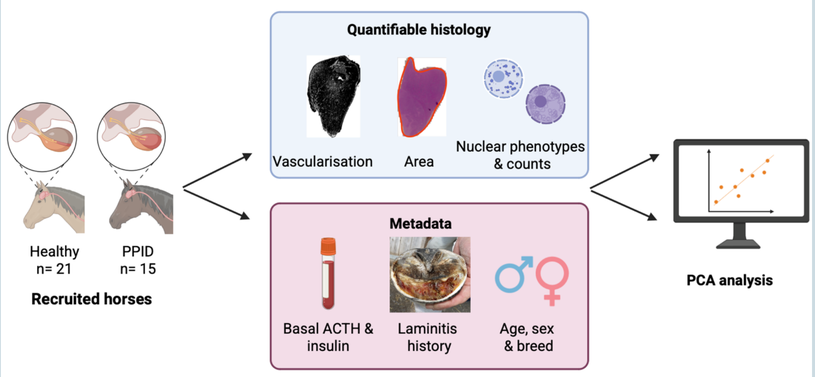

Fig 4 Variables entered for PCA analysis. .

Clinical characteristics

	Healthy (N= 20)	PPID (N= 16)
Age	14.9 ± 5.6	22.1 ± 3.4[a]
Sex	9 females	7 females
	11 castrated males	9 castrated males
Breed	7 TB/TBX	8 TB/TBX
	6 Pony	5 Pony
	4 Light breeds	2 Light Breeds
	2 Heavy breeds	1 Heavy breed
	1 Unknown	
Seasonality	3 spring	0 spring
	7 summer	6 summer
	6 autumn	9 autumn
	4 winter	1 winter
Body condition score, /5	2.0 ± 1.5	2.0 ± 2.0
Plasma ACTH (pg/mL)	27.25 ± 19.7	144 ± 208.7[a]
Plasma α-MSH (pmol/L)	25 ± 22.3	170.7 ± 87.2[b]
Serum insulin (IU/L)	2.0 ± 9.5	2.0 ± 11.2
Laminitis	4 diagnosed	4 diagnosed
	16 without	10 without
Pituitary grade, /5	2.0 ± 1.0	4.0 ± 2[a]

Table 1- Clinical characteristics of equine study group. Compared to healthy horses- A= P< 0.0001,B= P= 0.0009.

Horses with PPID…

1) Were **older** than healthy individuals but did not present different demographic populations.
2) Had significantly **higher plasma ACTH** and **α-MSH** concentrations.
3) Displayed **more advanced pituitary grading**- ie increased IL pathology.
4) Did not differ in body condition score, serum insulin or laminitis history.

Distinct Nuclear Landscapes in PPID

- Type 1 nuclei (large, pale, single nucleolus) showed no change between states (fig 5a).
- Type 2 nuclei (large, pale, double nucleoli) were linked to higher grades (fig 5b), whilst type 3 (condensed, single nuclei) appeared more in lower grades (fig 5c).
- No change in average nuclei number (fig 5d).

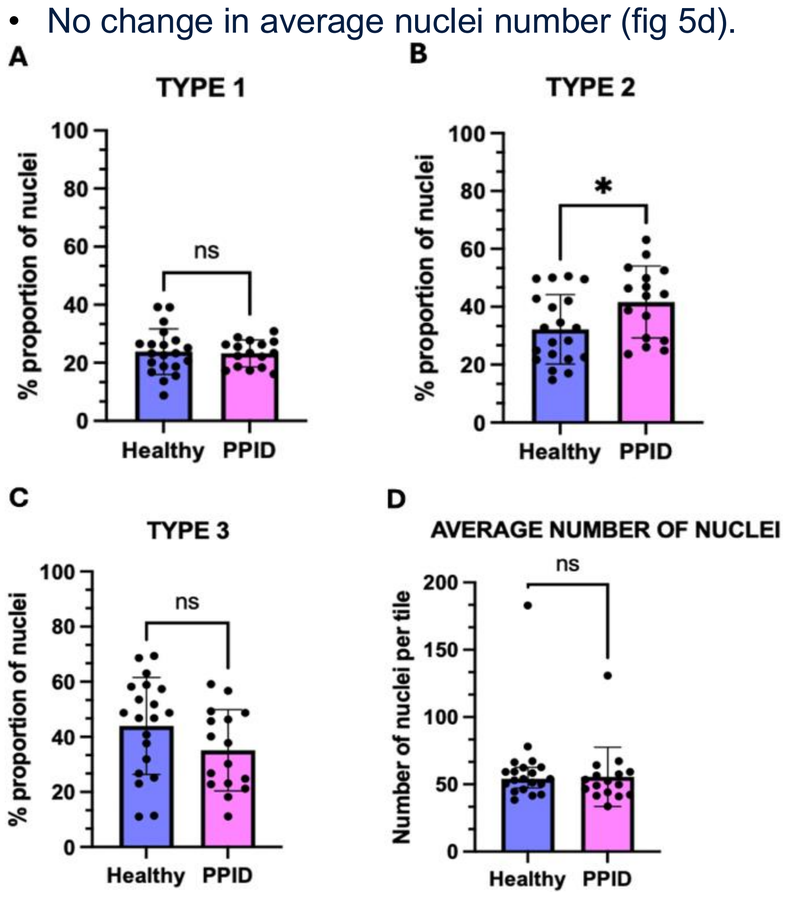

*Fig 5- Comparison of nuclear characteristics. *= p< 0.05. Data shown as mean ± SD or median ± IQR.*

Enlargement and Hypervascularity in PPID

- **Increasing pituitary size was highly associated with PPID** diagnosis & higher grades (healthy 8378um^2 ± 3437um^2 vs PPID 13117um^2 ± 6320um^2) .
- **Vascularisation strongly distinguished higher-grade** adenomatous pituitaries from healthy and hyperplastic tissue (healthy 4.0% ± 1.2% vs PPID 8.9% ± 2.7%) .

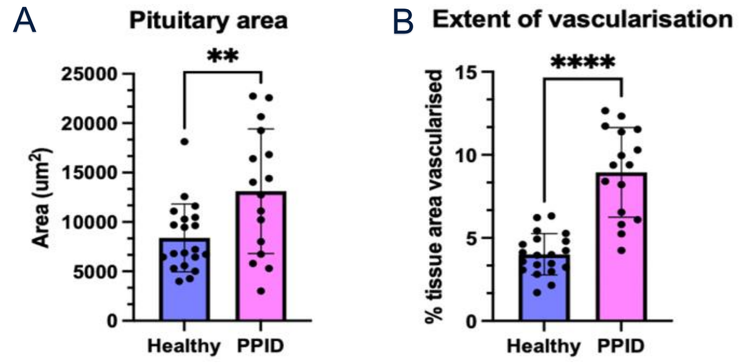

Fig 1- Comparisons of pituitary area and vascularisation. **= p< 0.01, ****= p < 0.0001. Data shown as mean ± SD.

- Both measurements strongly **coincide with increasing ACTH levels and age.**
- No link of breed, sex & laminitis history (associated with insulin status) with grade (fig 2).

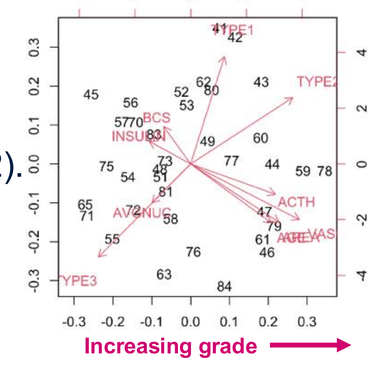

Fig 2- PCA biplot showing Influence of variables on spread of samples.

Discussion

- We demonstrate horses with **PPID and pathological pituitary IL growth have a histological, clinical and biochemical phenotype** distinct from hyperplastic change.
- Alterations in pituitary morphology offer new insight into possible mechanisms of pituitary adenoma pathogenesis.
- However, need to identify which processes cause these described nuclear phenotypes & drivers of vascularisation.
- More work is necessary to determine if this represents **a continuum of disease or if they are discrete pathologies** with independent drivers.

References

1. McGowan TW, Pinchbeck GP, McGowan CM. Prevalence, risk factors and clinical signs predictive for equine pituitary pars intermedia dysfunction in aged horses. Equine veterinary journal. 2013;45(1):74-9.
2. Miller MA, Pardo ID, Jackson LP, Moore GE, Sojka JE. Correlation of Pituitary Histomorphometry with Adrenocorticotrophic Hormone Response to Domperidone Administration in the Diagnosis of Equine Pituitary Pars Intermedia Dysfunction. Veterinary pathology. 2008;45(1):26-38.