Investigating the relationship of cortical frequencies and blood biomarkers using surface EEG for lameness detection in adult horses

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USDA NIFA TARLETON STATE UNIVERSITY Member of The Texas A&M University System

About me:

Jesús A. Cortés Mercado

Home Town:

Canóvanas, Puerto Rico

TSU Role:

MS. Animal Sciences

BSc. Biology
2019-2024

Vet Admissions Boot Camp "Champs" Alumni
April 2023

USDA REEU Equine Research Summer Program
June 2024

Graduate School:
MSc. Animal Sciences
Fall 2024

Vet School:
Equine Specialist

Internship Setting

Dr. Jessica Leatherwood

Associate Professor

Equine Nutritionist and Physiologist

Outstanding Young Professional Award from the Equine Science Society

Personal Development

- Adapting to a new environment
- Increased Self-Confidence
- Meeting Like-Minded People
- Exploring New Places

Professional Development

- Improve Laboratory Techniques
- Horse Handling and Management
- Problem-Solving in Research
- Data Interpretation and Analysis
- Desire to pursue a Graduate Career

Equine Welfare

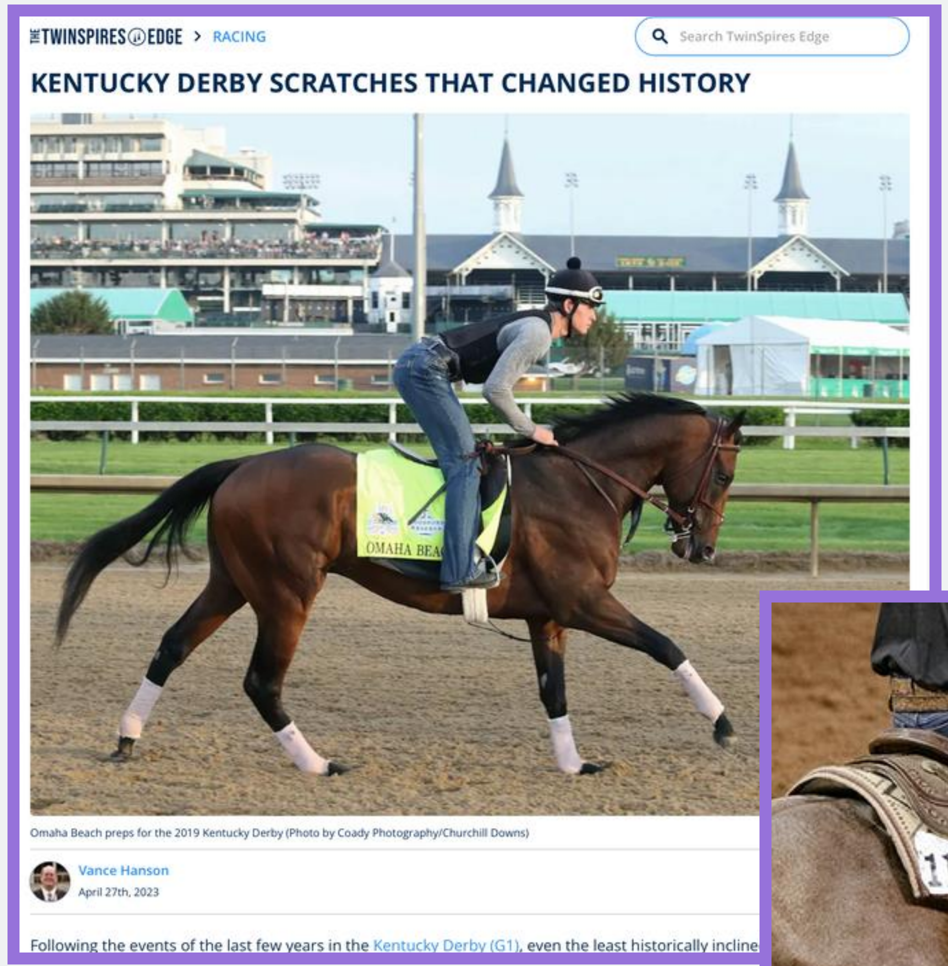
- Major cause for enforcement due to musculoskeletal injuries (MSI) and lameness
  - MSI accounts for over 70% of equine fatalities (Crawford, 2021)

Lameness

- Alterations in gait pattern
  - Linked to abnormalities within the whole musculoskeletal system is used

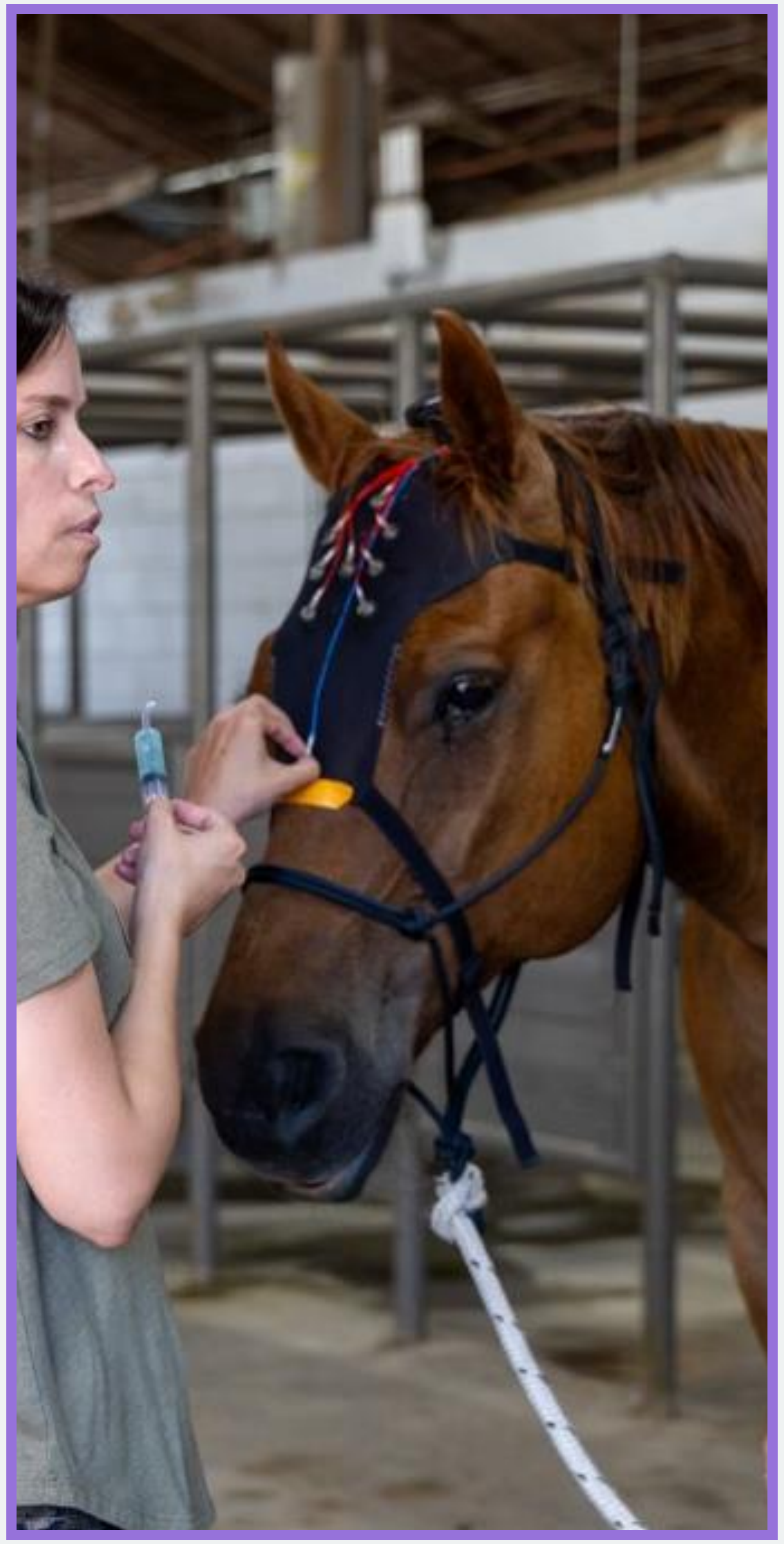
- Caused by pain

- Compensatory alterations overload and damage muscles, joints, and ligaments (Hafsa Zaneb, 2009)



Electroencephalography (EEG) in Horses

- Measures electrical activity in the brain
- Used to distinguish behavioral states (Jensen, 2013)
- Novel potential tool to assess cognition and welfare
- Pain activates CNS and PNS neuronal cells that can be detected (Aijia Liu et al., 2021)
- Used in other species for pain detection, but their evaluation in horses remains limited
    - Castration procedures in calves (Coetzee, 2013) and acute pain in sheeps (Ong, 1997)



Low Frequency Brain Waves

Delta (0-3 Hz)

- Increase of comfort and reduction of pain (Mir, 2021)
- Used to ease headaches and muscle contractions (Mir, 2021)

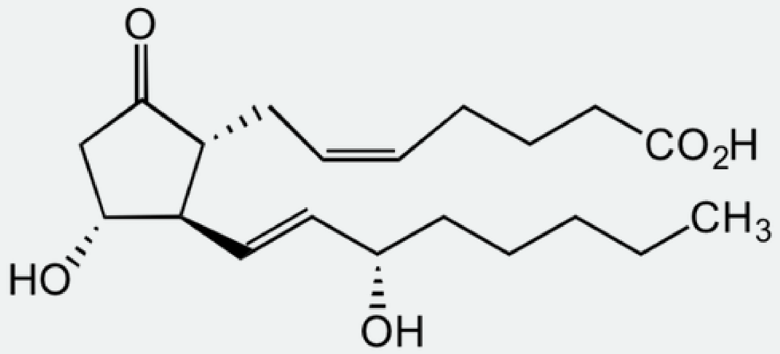
Theta (4-7 Hz)

- Related to pain in different chronic pain conditions (Simis, 2022)
- EEG activity was characterized by more theta waves activity in horses with chronic pain along spine (Stomp et al., 2020)

Biomarkers

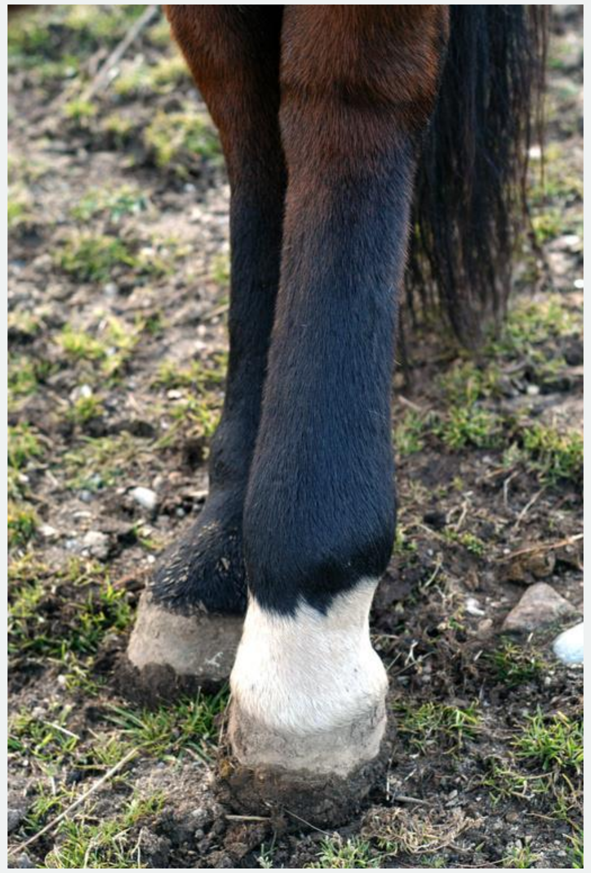
Prostaglandin E2 (PGE2)

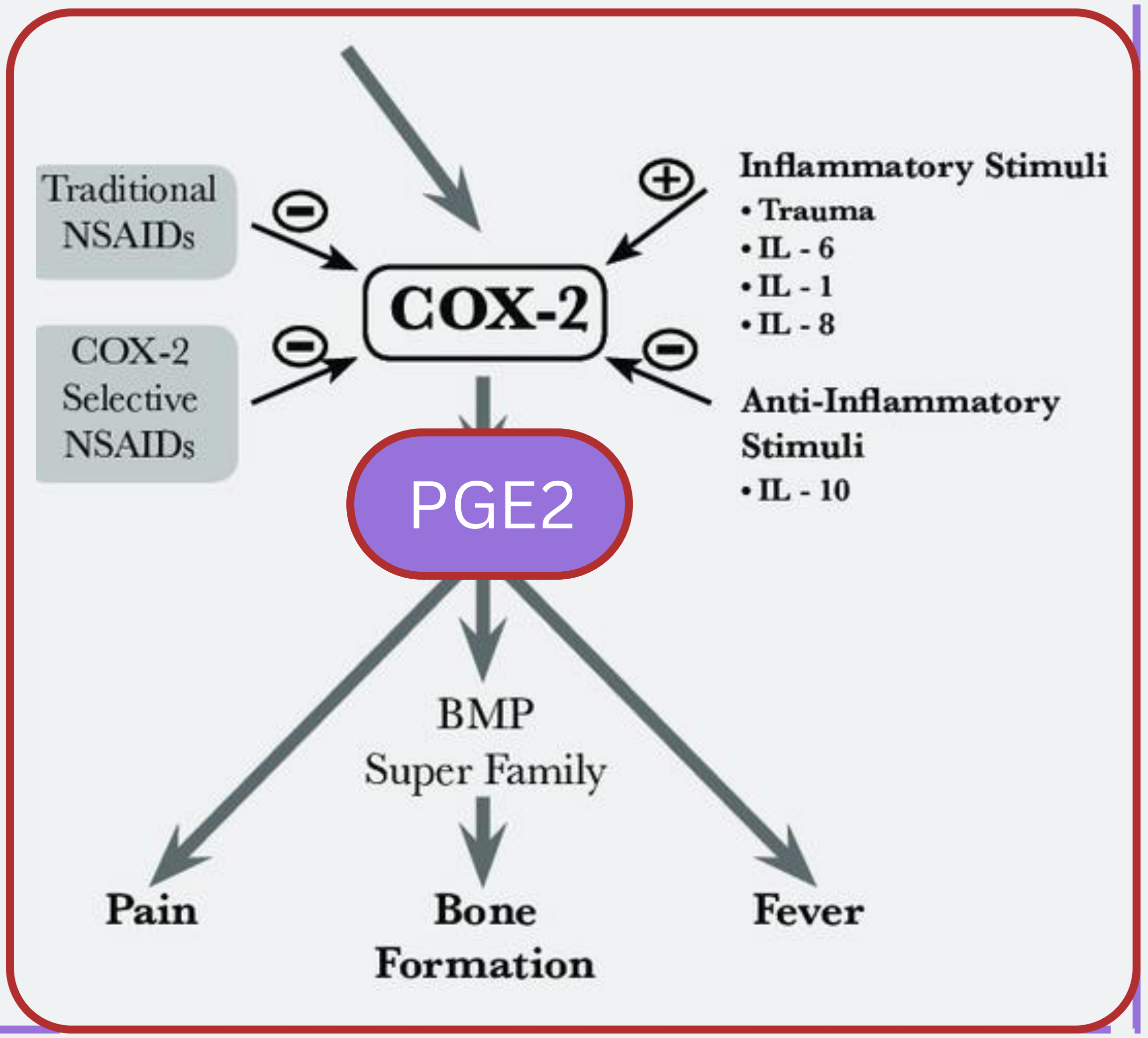
- Important eicosanoid in joint diseases (Niemela, 2018)
- Inflammatory mediators implicated in joint disorders (Wang, 2015)
- Cartilage matrix components and degradation fragments can trigger synovitis and release inflammatory mediators (Glant and Olah 1980; Kato et al., 2004)



Prostaglandin E2 Molecule

Arachidonic Acid





Objective

Investigate the relationship of sEEG and blood biomarkers related to lameness in mature horses.

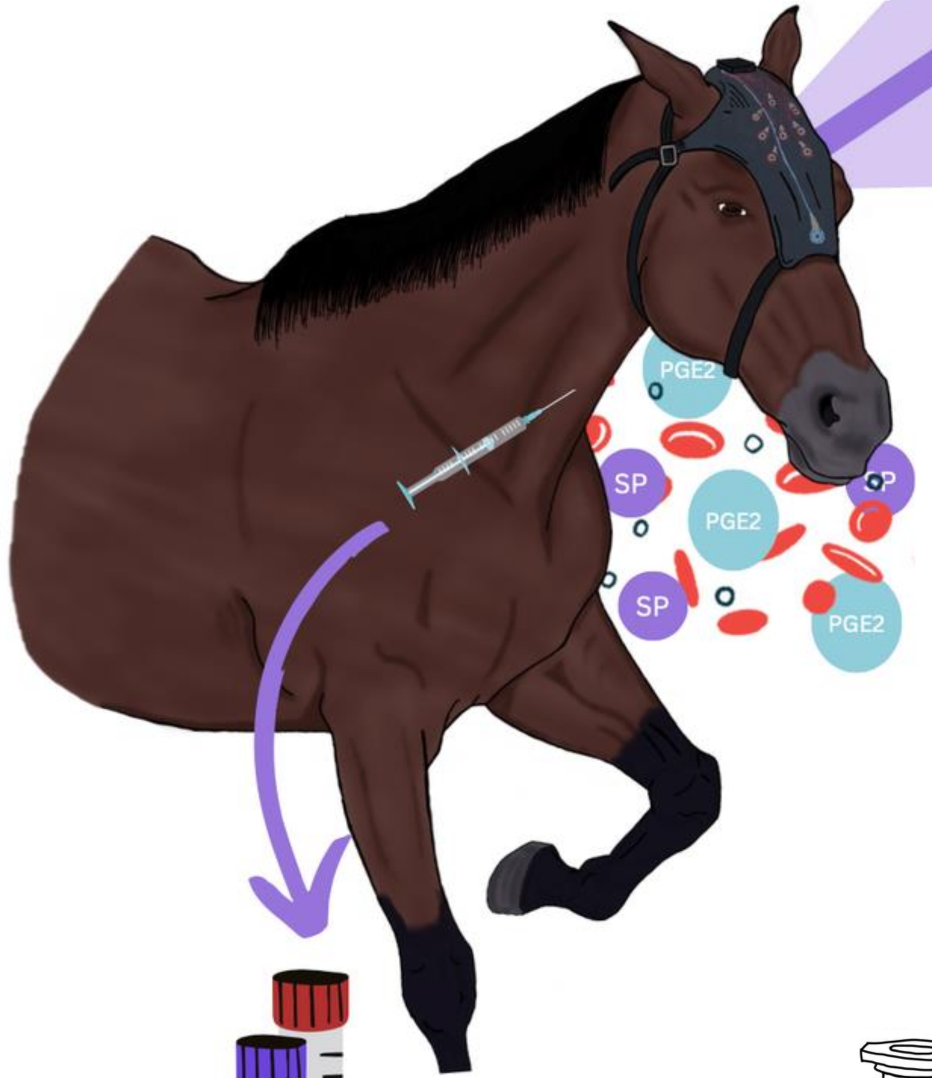
Hypothesis

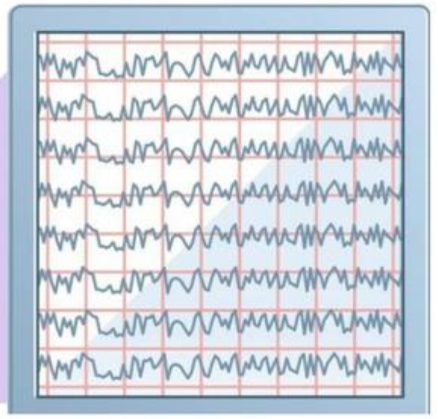
Low sEEG brainwaves will correlate with an established biomarker of inflammation (PGE2) contingent on lameness scores.

Materials and Methods

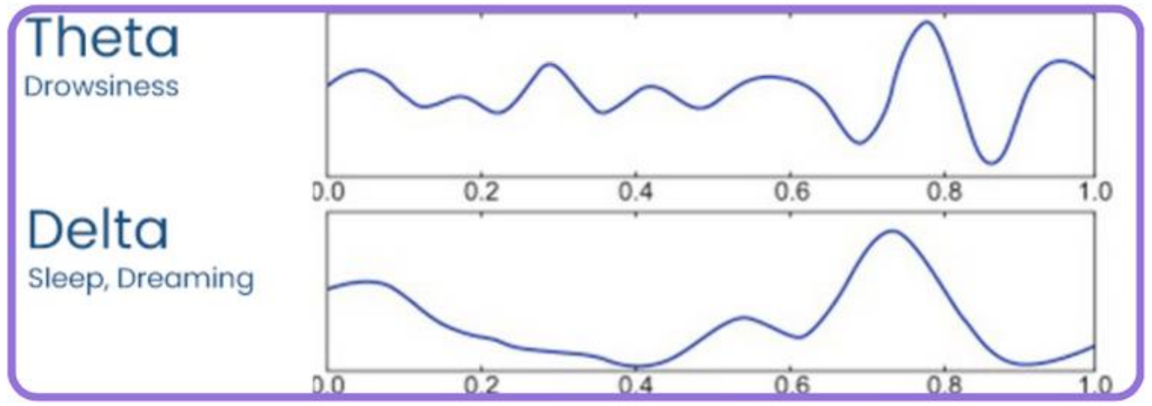
- **Quarter Horses (n = 8)**
  - 3 mares and 5 geldings
  - 16.5 ± 3.6 years
  - 573 ± 54 kg BW
- **Tarleton State University**
  - Equine Center Teaching Herd
  - Texan Therapeutic Riding
- **Groups assigned by:**
  - Lameness categories (LCat)
    - Non-Lame: NL (0-1)
    - Lame: L (2-3)

Methodology

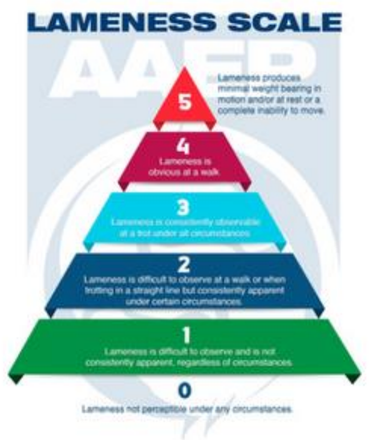




EEG Reading for 5 min Pre & Post LE



Resting state sEEG (Mentalab) data were recorded at a minimum 250 Hz sampling



Lameness Evaluation by D.V.M

Plasma & Serum Tubes prior and post LE

Serum and Plasma harvested & aliquoted for storage at -80 °C

PGE2 high sensitivity ELISA kit for Plasma & Serum matrices

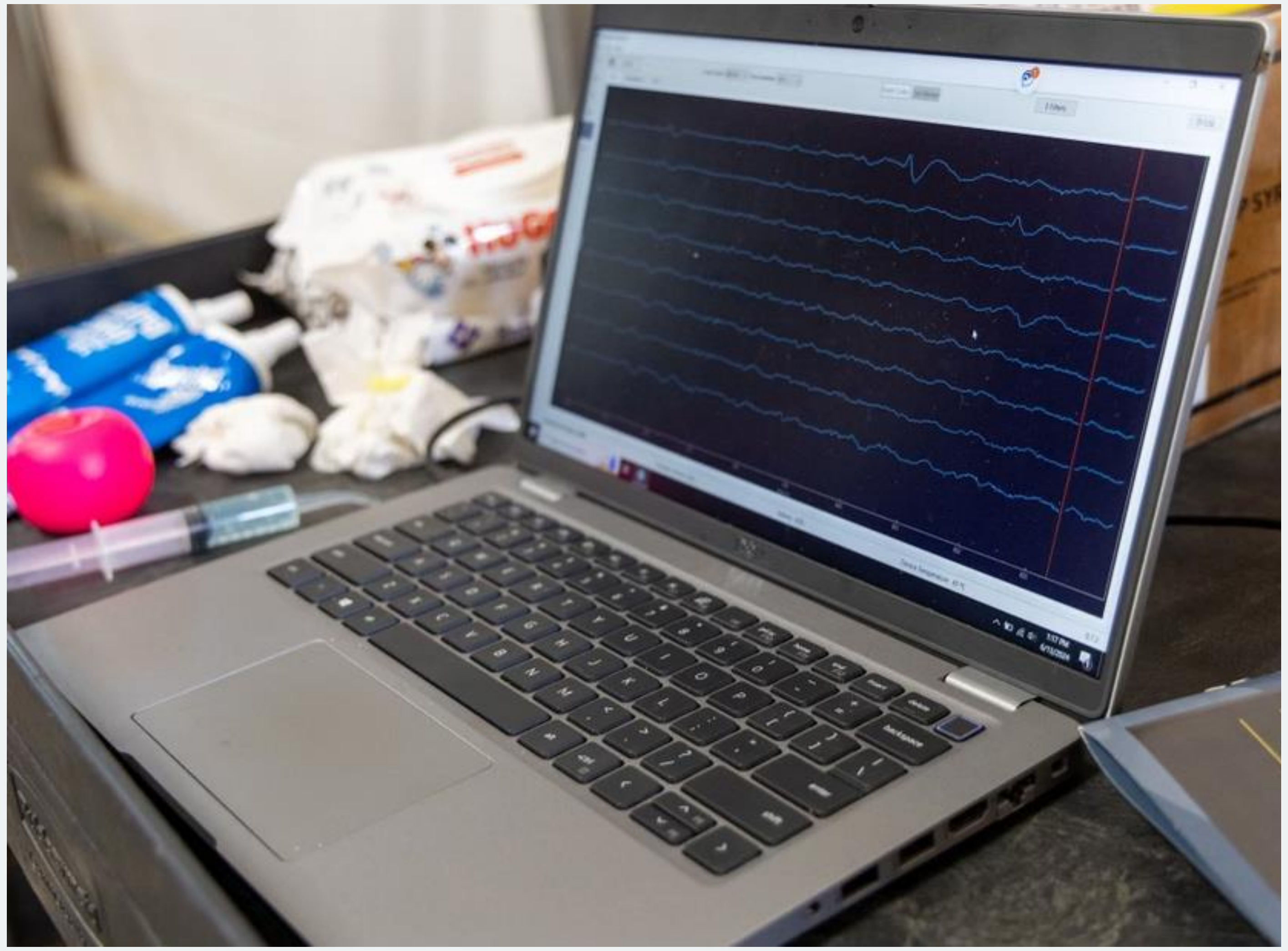
Concentrations analyzed with BioTek CYTATION|5 imaging reader

Surface EEG Cap Placement

1

2

3



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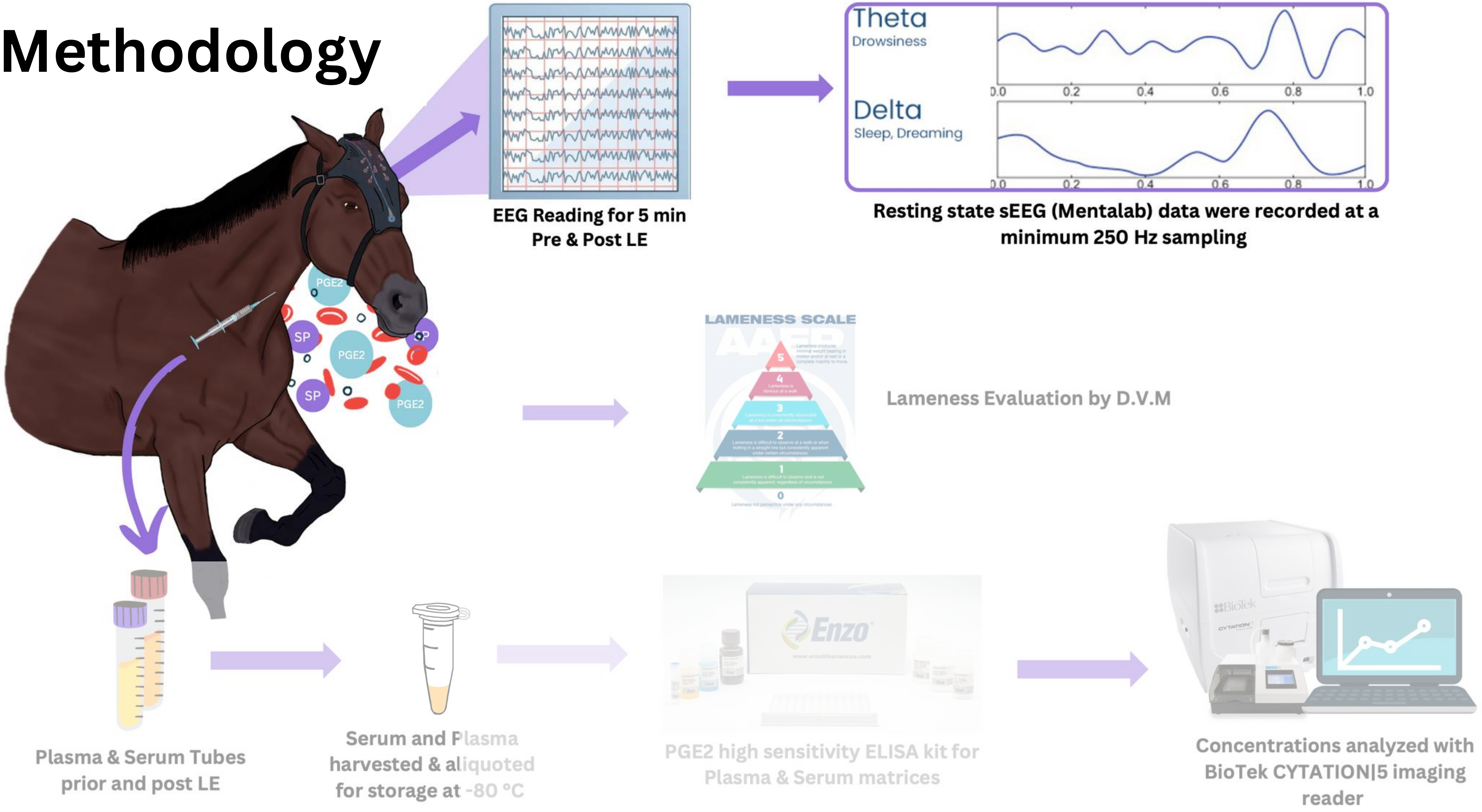
Methodology



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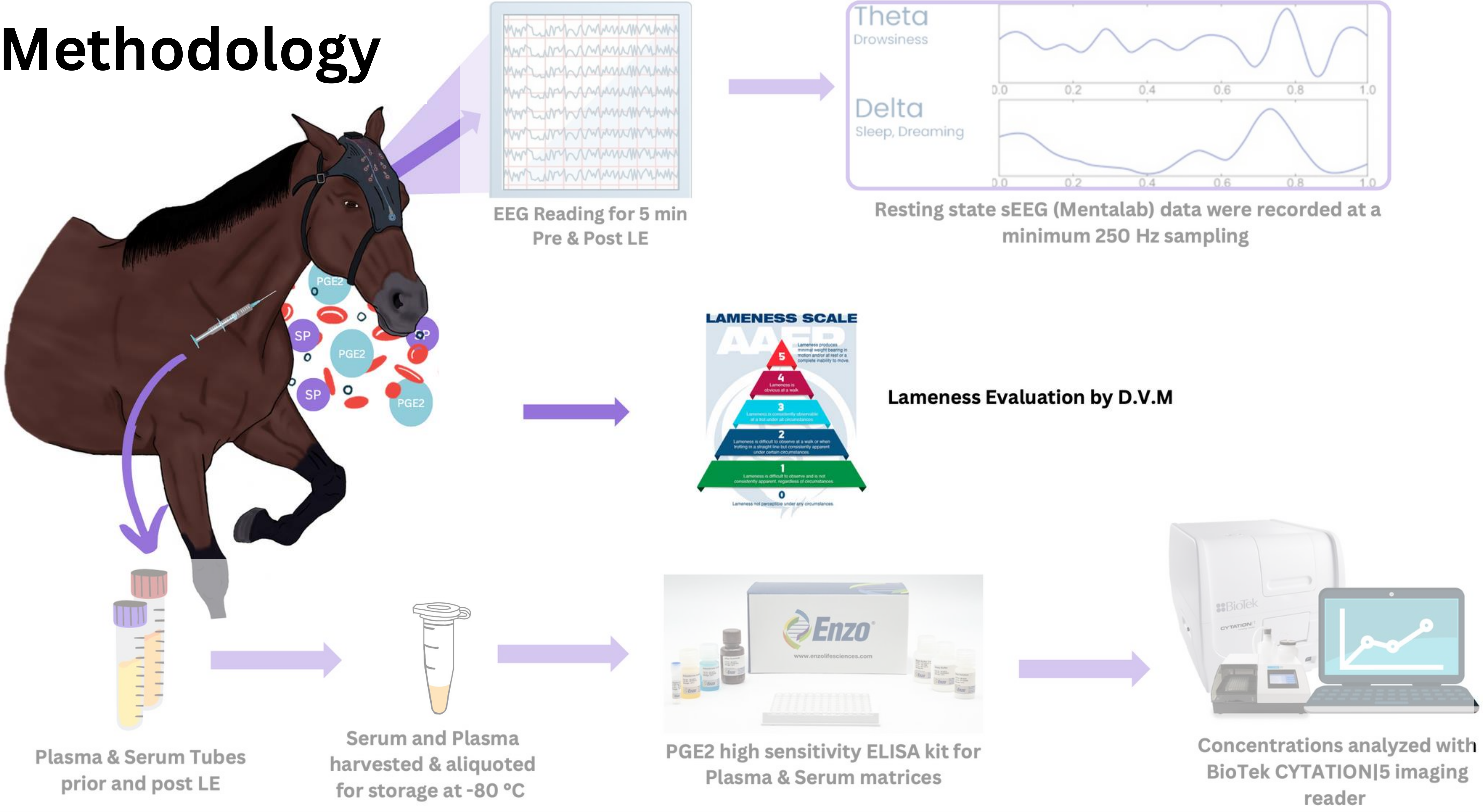
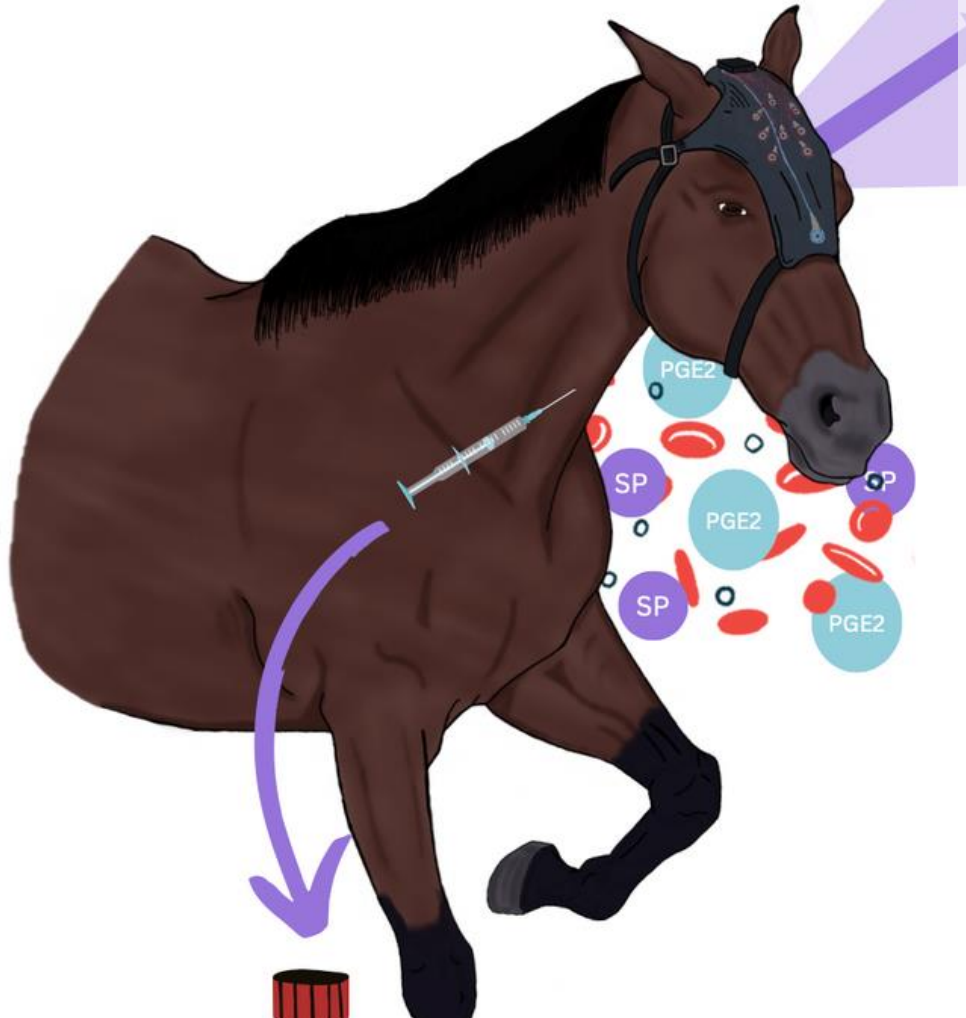
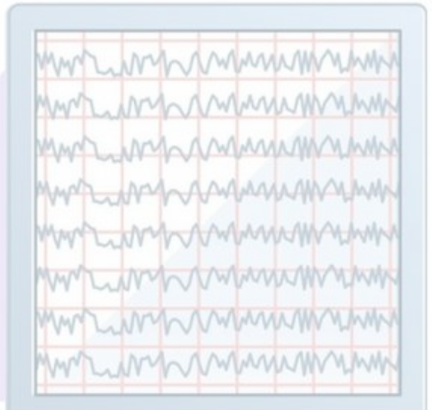


Lameness Evaluation

- Walked in a straight line, figure eight and trotting
    - Circles (left and right)
- Backed up and moving forward with head held high
- Palpated within the back, loin, croup and limbs, flexed the limbs and joints
- Lameness scores were assigned

Methodology





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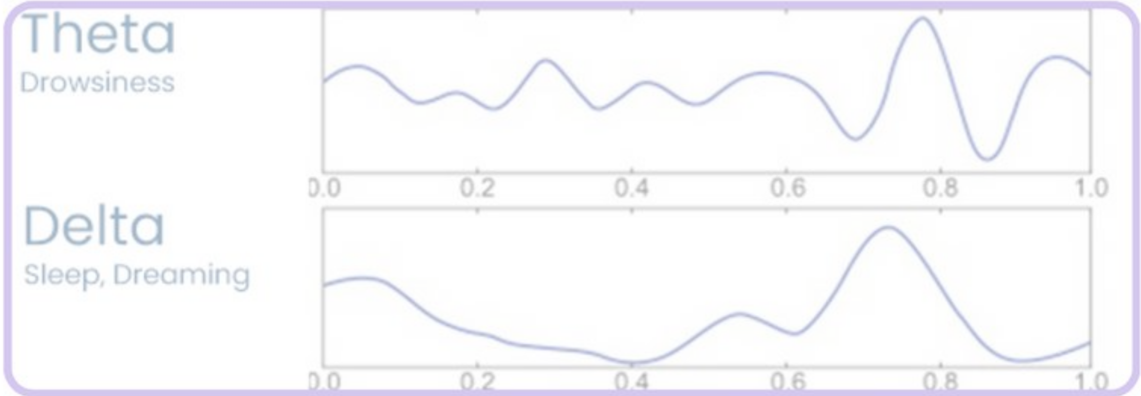
Pre & Post LE



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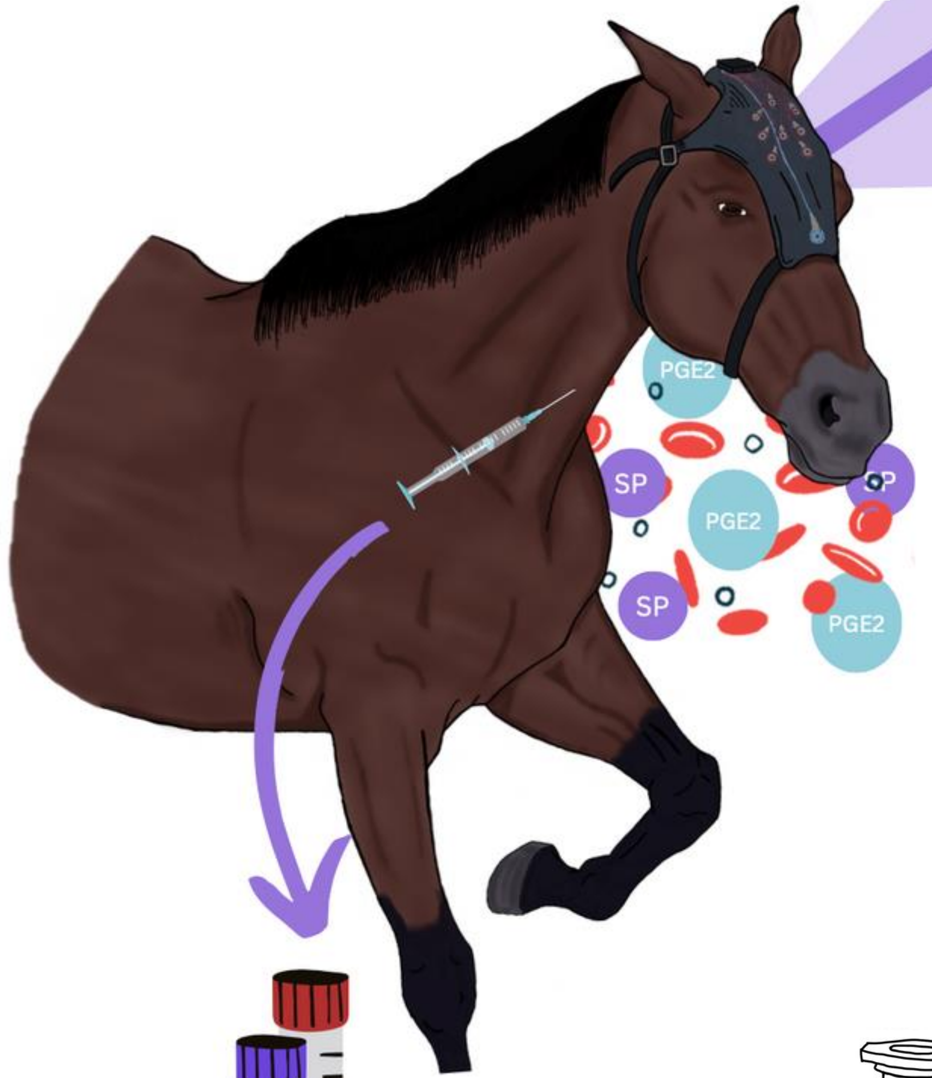

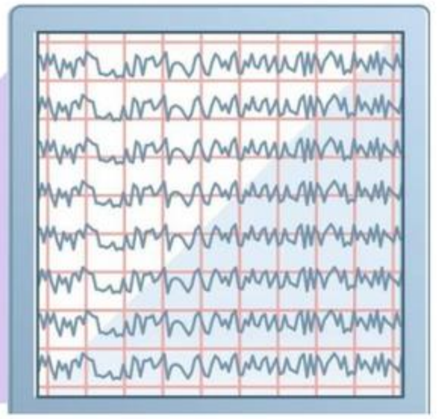

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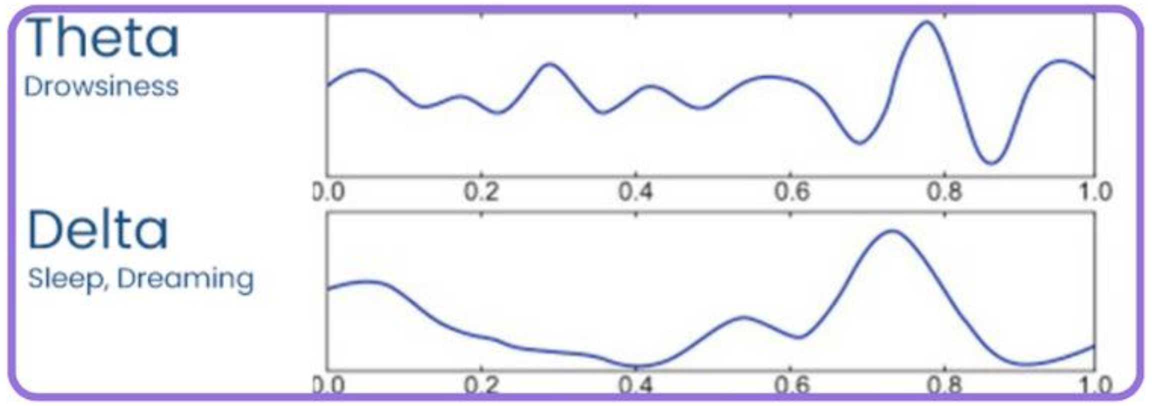

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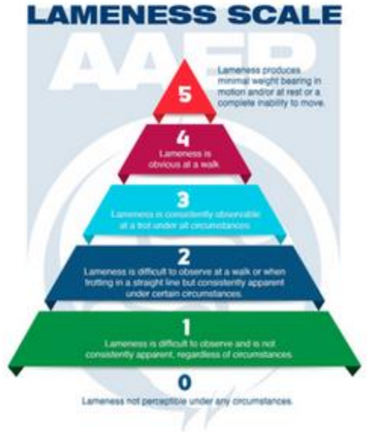

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Results for EEG

Table 1. Delta frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre		$P \geq 0.115$		Post			$P \geq 0.160$
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.05006	0.14286	0.16667	0.40476	-0.025	0.2381	0.54762	0.42857
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Table 5. Gamma frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre		$P \geq 0.0856$		Post			$P \geq 0.160$
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.55069	0.35714	0.64286	-0.0238	-0.025	-0.2381	0.54762	0.42857
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Table 4. Beta frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre		$P \geq 0.115$		Post			$P \geq 0.160$
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.35044	0.45238	0.30952	0.04762	-0.025	-0.2381	0.54762	0.42857
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Results for EEG

Table 2. Theta frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre			$P \geq 0.115$	Post			$P \geq 0.139$
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.17522	0.2381	0.33333	0.28571	-0.1001	-0.1905	0.57413	0.54762
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Table 3. Alpha frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre			$P \geq 0.115$	Post			$P \geq 0.160$
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	-0.1001	0.40476	-0.0714	0	-0.1001	-0.1905	0.57143	0.54762
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

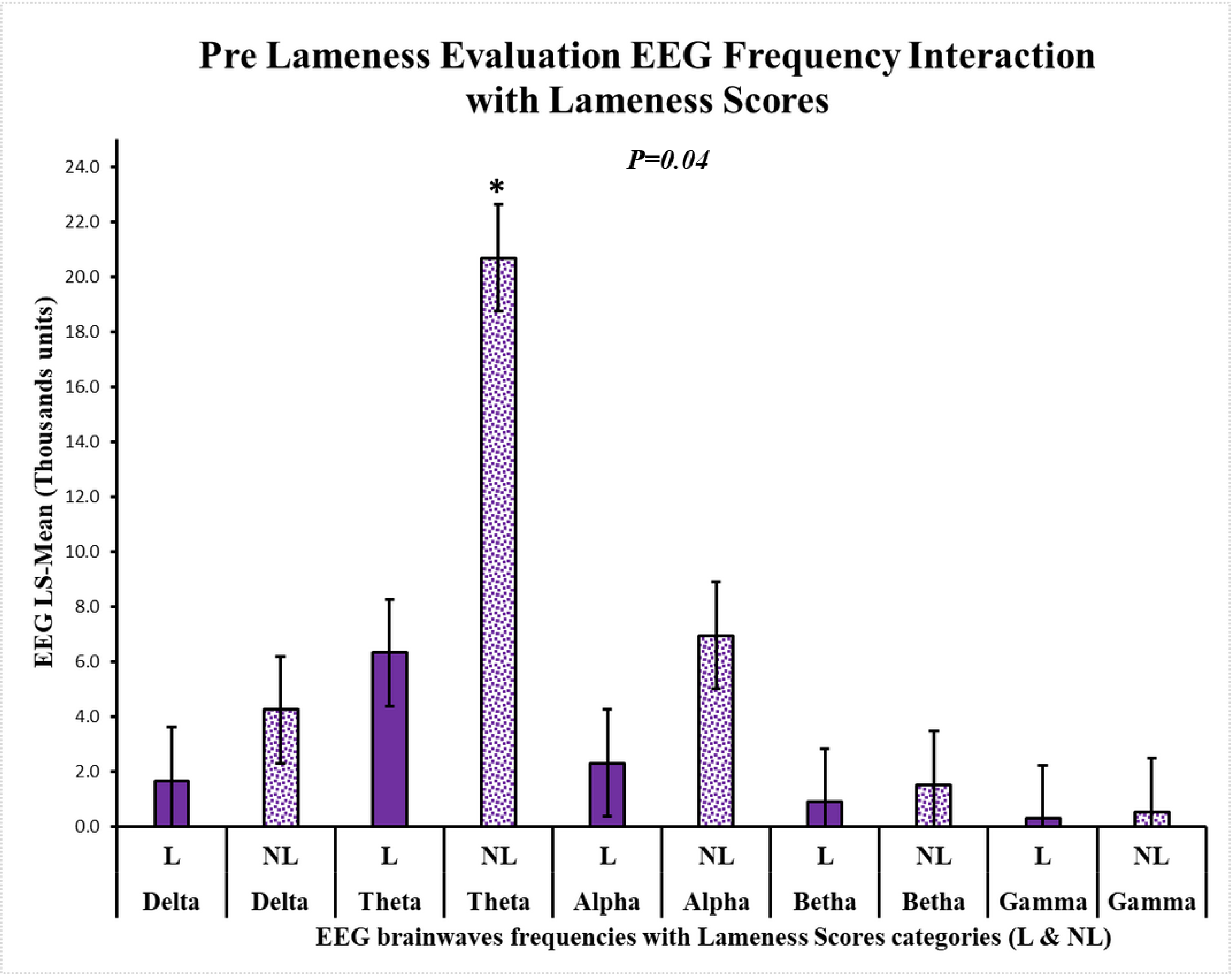
Results for EEG

Table 6. Main effect interactions of Electroencephalography (EEG) frequencies and Lameness Score (LS) at Pre-Lameness Evaluation.

Effect	NumDF	DenDF	Fvalue	Pr>F
EEG	4	22	579.31	<0.0001*
LS	1	6	0.34	0.58
EEG*LS	4	22	3.06	0.04*

Electroencephalography activity measurements were taken before lameness evaluation (Pre) for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). EEG frequencies effect contain the five brainwaves' measurements (delta, theta, alpha, beta and gamma). Asterisks (*) represent significant differences ($P \leq 0.05$) within main effect or interaction.

Results for EEG



Results for Biomarkers

Figure 2

Concentration differences of Prostaglandin E2 matrices by time

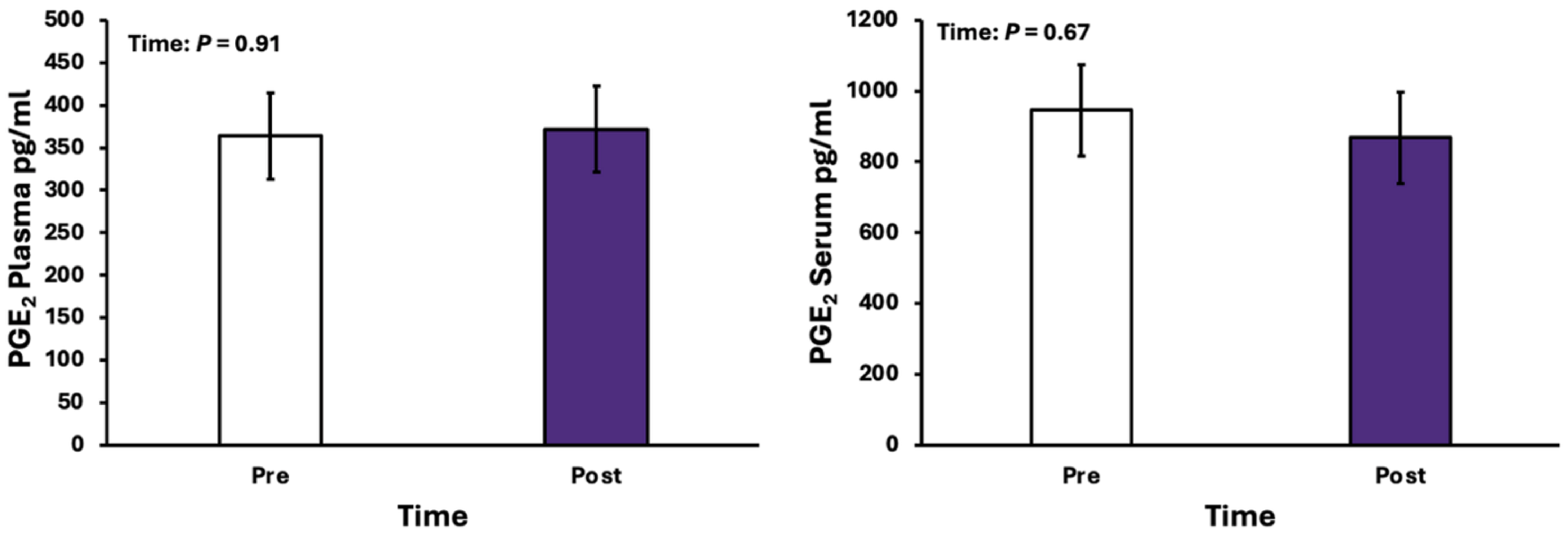


Figure 2. Concentration differences of Prostaglandin E_2 matrices by time. Blood samples were draw before lameness evaluation (Pre) and after (Post). This samples were aliquoted as plasma and serum. Means with different letter subscripts represent significant differences in PGE2 matrices by time ($P \leq 0.05$).

Results for Biomarkers

Figure 3

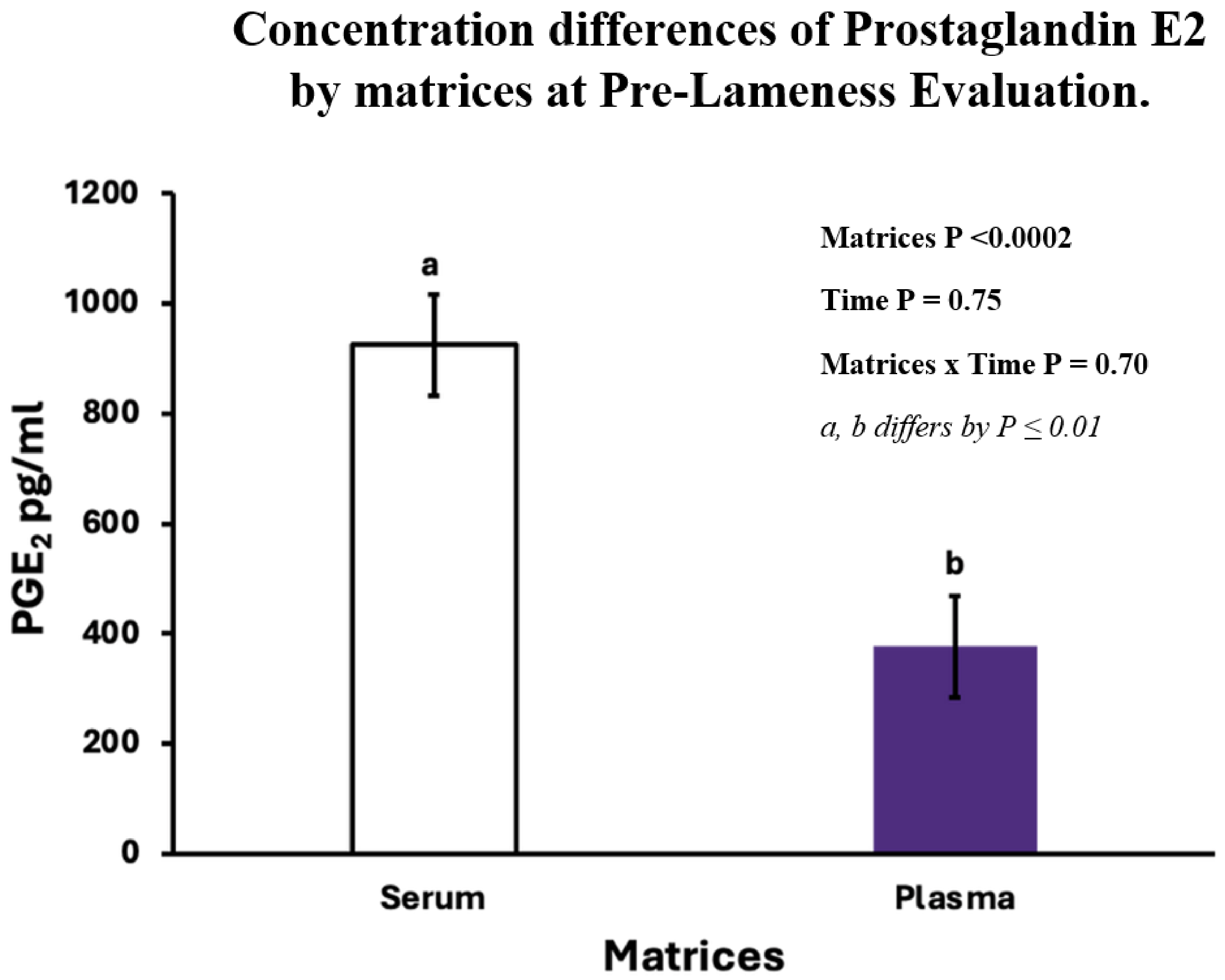


Figure 3. Concentration differences of Prostaglandin E_2 by matrices at Pre-Lameness Evaluation. Blood samples were draw before lameness evaluation (Pre) and after (Post). This samples were aliquoted as plasma and serum. Means with different letter subscripts represent significant differences in PGE2 concentration by matrices ($P \leq 0.05$).

Conclusion

In conclusion, this study highlights the potential relationship between sEEG wave frequency and pain presence in horses, particularly in the delta and theta bands, as they varied between lame and non-lame categories. The investigation into blood biomarkers, such as PGE2, showed moderate, though non-significant, correlations with sEEG data, suggesting a possible avenue for early lameness detection.




Recommendations

- To confirm the utility of sEEG, a larger sample size of horses is needed, with an even distribution across the lameness scale to cover a wider range of categories.

- Additionally, modifying the existing sEEG cap prototype to better fit the horse’s head shape and more effectively detect frontal cortex brain activity could improve the accuracy of the research findings.

Acknowledgments

- Thanks to Dr. Leatherwood’s graduate and undergraduate students
- Tarleton State University Animal Science Department and Faculty
- Dr. Lauren Hanna for helping with the Statistics
- United States Department of Agriculture (USDA)
- National Institute of Food and Agriculture (NIFA)
- Agriculture and Food Research Initiative (AFRI)
- Research and Extension Experience for Undergraduates (REEU)
- USDA-NIFA-AFRI-009041; Award Number 2023-68018-40320
- The Equine Lameness Center - Dr. Jessica Hamilton D.V.M

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Questions ?