

Exploring the Dynamics of Physiological and Environmental Factors for Horse Welfare

최 연 주

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(Equi-path Healing & Leadership)

Curriculum Vitae

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마연구회 심포지엄

Exploring the Dynamics of Physiological and Environmental Factors for Horse Welfare

2025/06/26 | EXCO 그랜드볼룸

Yeonju Choi

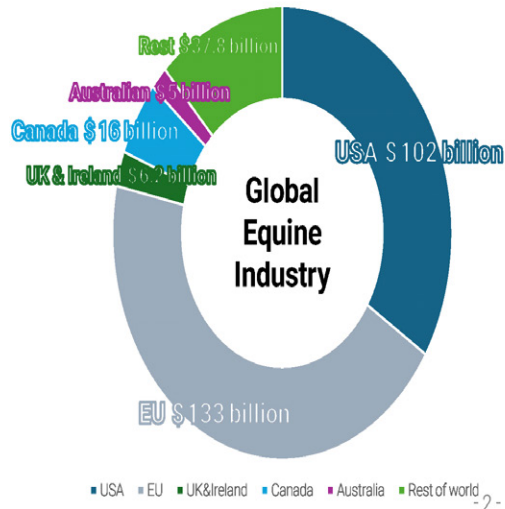
Equi-path Healing & Leadership



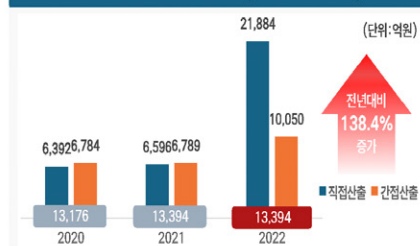
Background

The global equine industry is valued at approximately

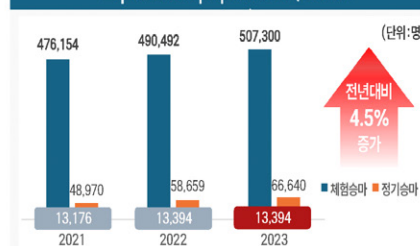
\$300 billion^{1,2}



Economical value (South Korea)



Equestrian population (South Korea)

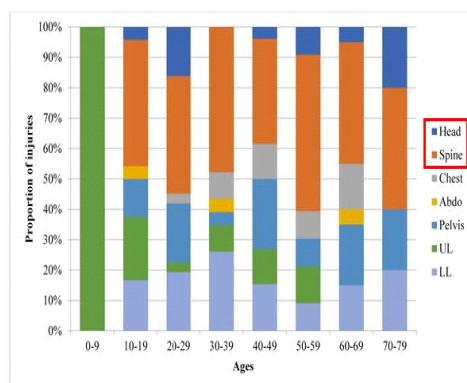


※2023 말산업실태조사 보고서

Background

81% of equestrians get injured at some point in their riding career and 21% are injured seriously⁴.

Horse trauma incurs significant costs at several levels.



⁵Proportion of injuries by age group and body zones.

Economical impacts of horse-related accidents

Country	Annual costs	
Sweden	€3.2 million	Meredith et al., 2014
USA	\$6.5 million	Adler et al., 2013
New Zealand	\$2.6 million	Jones et al., 2016



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Background

Unpredictability of a horse is highly associated with horse-related injuries⁶.



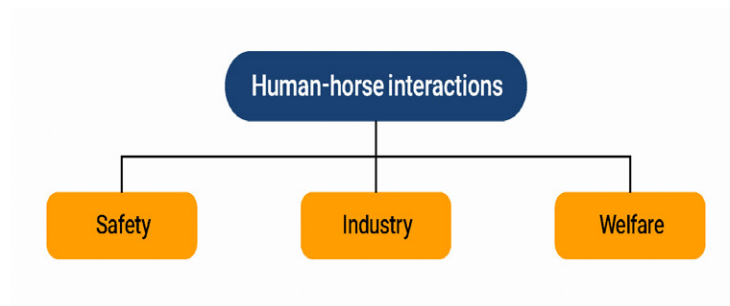
- Accidents are more rely on the **frequency and amounts** of interactions with horses than the level of competency^{7,8}.
- Fear reactions in horses are often unpredictable and are recognized as a major cause of accidents⁹.
- The unpredictability is affected by choice of **horse-human combination** and choice of **training methods**¹⁰.



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Background

A deeper understanding of human-horse interactions and enhancing positive relationships are important for both human-horse safety, welfare, and industrial development.



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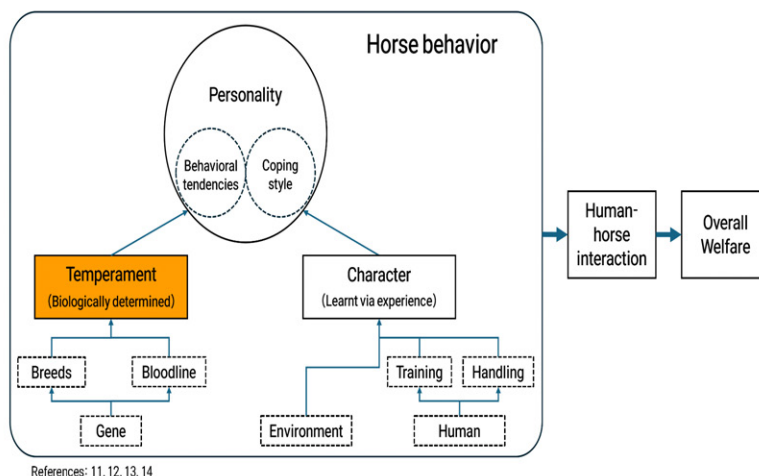
경북대학교(Kyungpook National University)

Study 1

Development of the method for evaluating equine temperament

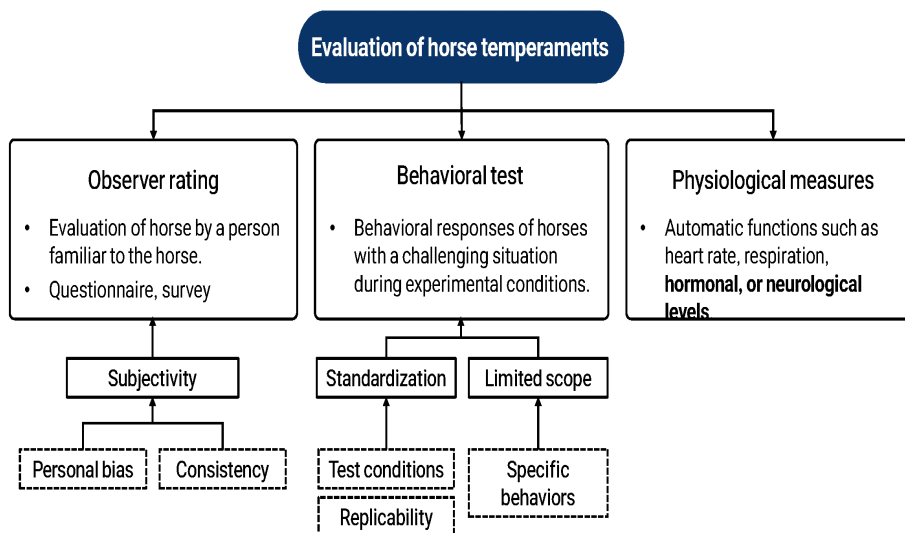
Introduction

Evaluating horse temperament is important for effective training, management, and safety during the interactions between horses and their handlers¹¹.



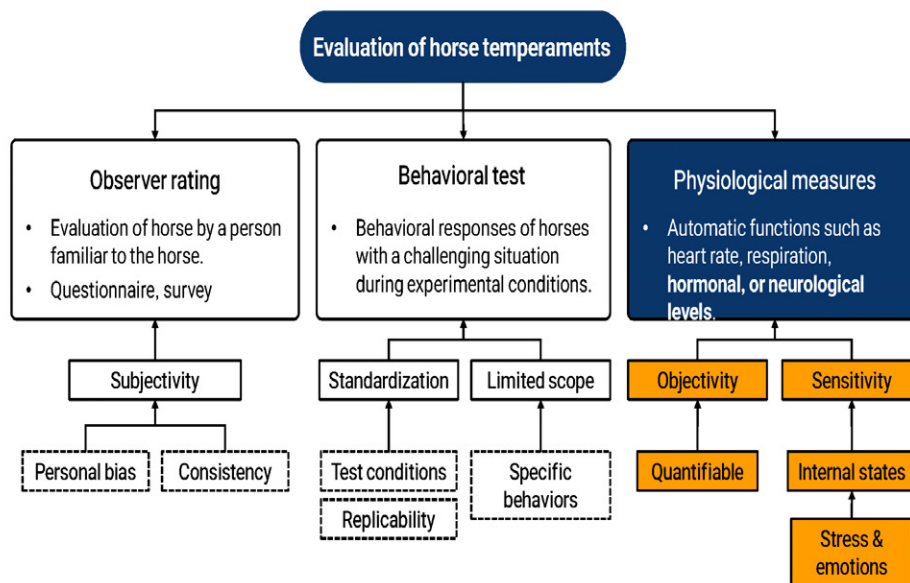
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Introduction



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Introduction

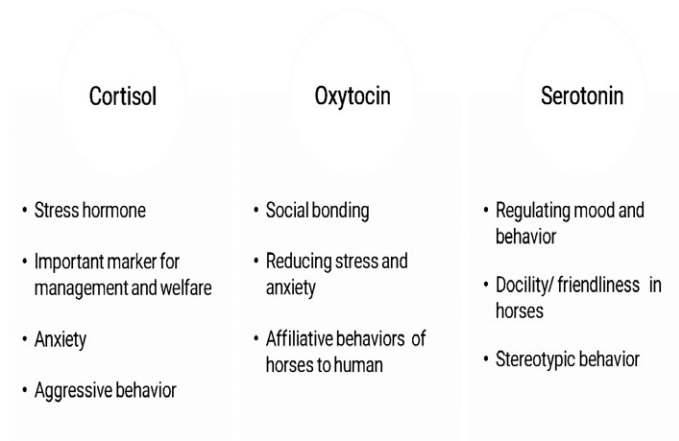


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Introduction

Hormones and neurotransmitters can be a reliable marker for assessing animal welfare and behavior¹².

- **Cortisol, oxytocin, and serotonin** are significant in assessing animal welfare and behavior, serving as biomarkers^{13, 14, 15}.



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Introduction

Hormones and neurotransmitters can be a reliable marker for assessing animal welfare and behavior¹².

- Cortisol, oxytocin, and serotonin are significant in assessing animal welfare and behavior, serving as biomarkers^{13, 14, 15}.

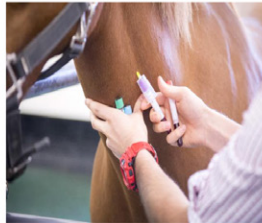
Cortisol

- Recent research has explored using saliva as a non-invasive method to measure hormone and neurotransmitter levels.

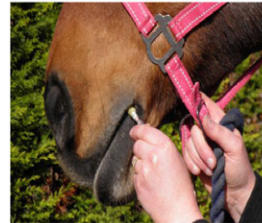
- The reliability of salivary hormone levels have not fully established.

Oxytocin

Serotonin



VS



- Reduced stress
- Safety and convenience

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Objectives

To evaluate the relationship between blood and salivary cortisol, oxytocin, and serotonin as indicators of equine temperament.

✓ Hypothesis

- 1 There is a significant correlation between the concentrations of cortisol, serotonin, and oxytocin in saliva and plasma.
- 2 The levels of these biomarkers are reliable indicators of equine temperament, and they can provide objective, quantifiable data that correlates with observed behavioral traits.

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Materials and Methods



Animals

- 40 horses (10 Warmbloods, 21 Thoroughbreds, 5 Haflingers, and 4 Halla ponies)
- Average age of 12.3 ± 4.2 years
- Approved by the Animal Experimentation Ethics Committee (2023-0191-2)

Trained, available horses

20

- ✓ Horse Industry Complex Center, Jeonju Kijeon College, Jeonju
- ✓ Utilized in horse riding sessions for college students
- ✓ Casually handled and exercised

Untrained, unutilized horses

20

- ✓ Man-geum Farm, Anseong
- ✓ Prince Riding Center, Andong
- ✓ Considered as **untrained and dangerous**
- ✓ **Rarely handled, attack or run away from people**

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Materials and Methods



Temperament assessment survey

- Temperament assessment was performed by a group of college students and trainers (n=10, 4).
- 11 temperament traits of each horse was assessed.

Table 1. Questionnaire items on horse temperament

Temperament traits	Description	Scale (Not at all ↔ Very much so)
Calm	Remains composed in new or startling situations	1 ↔ 5
Concentrate	Maintains concentration without being distracted by the environment	1 ↔ 5
Trainable	Learns and adapts to training easily and quickly	1 ↔ 5
Cooperative	Willingness to work with and follow instructions from handlers	1 ↔ 5
Gentle	Overall disposition of being gentle and easily handled	1 ↔ 5
Patient	Ability to tolerate various stimuli without distress	1 ↔ 5
Friendly	Friendly and approachable and interacts with others in a friendly manner	1 ↔ 5
Excitable	Tendency to become easily excited	1 ↔ 5
Stubborn	Shows resistance or reluctance to follow commands	1 ↔ 5
Competitive	Displays competitive behavior with other horses	1 ↔ 5
Fearful	Tendency to get scared easily	1 ↔ 5

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Materials and Methods



Blood and saliva sampling

- Samples were collected between 9:00 and 11:00 AM.
- Blood samples were collected from jugular vein
- Saliva was collected using synthetic swabs, which was affixed to a bit and inserted into the mouth for 1-2 min until fully saturated.



Enzyme-linked immunosorbent assay

- Cortisol ELISA kits (ADI-900-071, Enzo Life Sciences).
- Oxytocin ELISA kits (MBS2700454, Mybiosource).
- Serotonin ELISA kits (ADI-900-175, Enzo Life Sciences).



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Materials and Methods



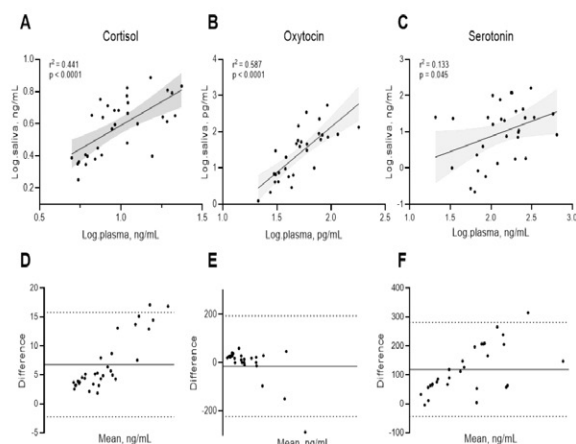
Statistical analysis

- Prism 9 (GraphPad Software)
- Shapiro-Wilk test for the normality test
- Spearman's correlation coefficient and linear regression analysis
- Interclass correlation coefficient analysis
- A p-value less than 0.05 was considered as a statistical significance.

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Results

Relationship between plasma and saliva samples



Cortisol

- $r^2 = 0.441$
- $p < 0.0001$
- Discrepancy: 6.75 ng/mL

Oxytocin

- $r^2 = 0.587$
- $p < 0.0001$
- Discrepancy: 14.48 pg/mL

Serotonin

- $r^2 = 0.133$
- $p = 0.045$
- Discrepancy: 118.7 ng/mL

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Results

Reliability of temperament assessment survey

Table 3. Reliability of scores in assessing horse temperament

	95%		95%	F test with true value 0				P-Value
	ICC	CI	CI	Value	df1	df2		
	(1,1)	Lower Bound	Upper bound					
Calm	.893	.160	.541	9.367	13	266	<0.001	
Concentrate	.917	.839	.969	12.101	13	266	<0.001	
Trainable	.922	.848	.970	12.832	13	266	<0.001	
Cooperative	.952	.907	.982	20.936	13	266	<0.001	
Gentle	.921	.845	.970	12.620	13	266	<0.001	
Patient	.880	.766	.954	8.353	13	266	<0.001	
Friendly	.943	.890	.978	17.670	13	266	<0.001	
Competitive	.861	.729	.947	7.199	13	266	<0.001	
Stubborn	.891	.787	.958	9.164	13	266	<0.001	
Excitable	.898	.606	.923	4.958	13	266	<0.001	
Fearful	.833	.674	.936	5.985	13	266	<0.001	

Average measures, one-way random effects model, absolute agreement definition.

df, degrees of freedom

Consistency of response among different evaluators

- The strength of agreement of each temperament trait all exceeded 0.8.
- This indicates the concordance in the assessments across surveys.

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Results

Relationship of Cortisol, oxytocin, and serotonin levels and temperament traits

Table 3. Correlation coefficient matrix of neurotransmitters and horse temperaments

Variables	PCor	SCor	POxt	SOxt	PSer	SSer
Calm	-0.345	-0.306	-0.161	-0.003	0.260	0.028
Concentrate	-0.038	0.213	0.284	0.350	-0.160	-0.404
Trainable	-0.421	-0.259	0.044	0.336	-0.334	0.069
Cooperative	-0.371	-0.310	-0.268	-0.110	0.383	0.126
Gentle	-0.716**	-0.554*	-0.251	0.110	0.535*	0.497*
Patient	0.442	0.395	0.298	0.218	-0.450	-0.461*
Friendly	0.017	0.015	0.576**	0.729**	-0.268	-0.173
Competitive	0.528*	0.361	0.263	0.175	-0.404	-0.372
Stubborn	0.603*	0.420	0.306	0.093	-0.388	-0.420
Excitable	0.659**	0.451*	0.366	0.063	-0.363	-0.536*
Fearful	0.796***	0.524*	0.370	0.105	-0.363	-0.743**

***p < 0.0001 **p < 0.001, *p < 0.05. PCor, plasma cortisol; SCor, saliva cortisol; POxt, plasma oxytocin; SOxt, saliva oxytocin; PSer, plasma serotonin; SSer, saliva serotonin

Cortisol

- **Plasma:** gentleness, Competitiveness, Stubbornness, Excitability, Fearfulness
- **Saliva:** gentleness, excitability, fearfulness

Oxytocin

- **Plasma:** friendliness
- **Saliva:** friendliness

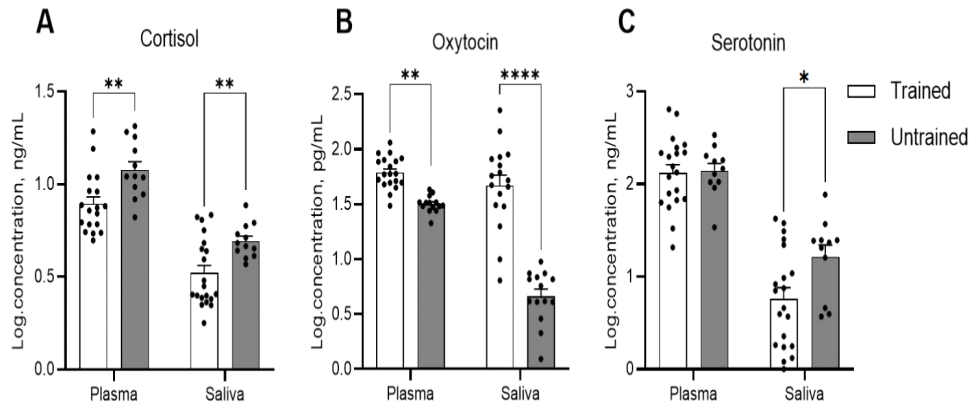
Serotonin

- **Plasma:** gentleness
- **Saliva:** gentleness, patience, excitability, fearfulness

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Results

Hormone levels in trained and untrained horse groups



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Discussion

Saliva can be used as a non-invasive method for measuring **cortisol and oxytocin** levels in horses.

Hormonal markers can serve as reliable indicators of specific temperament traits in horses



Challenges and future directions

- The weak correlation observed between salivary and plasma serotonin levels suggests limitations in using saliva to assess central serotonin levels.
- Further research involving larger sample size and standardized methods is needed to clarify the roles of the biomarkers in assessing horse temperament and behavior.

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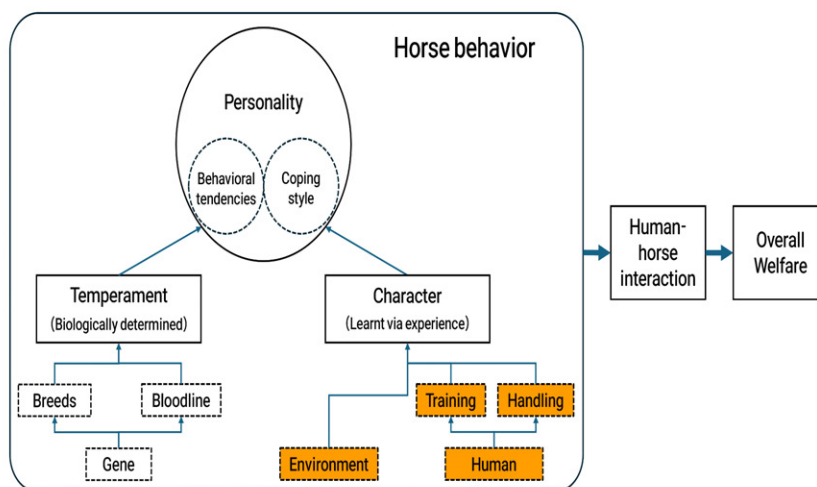
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Study 2

Evaluation of the effect of environmental factors for development of equine behavior and temperament.

Introduction

The way humans treat horses can influence the horse's behavior and personality^{16, 17}.



References: 11, 12, 13, 14

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Introduction



- Food rewards can encourage horses to spend more time near trainers or to approach trainers more quickly¹⁸.
- Natural horsemanship can reduce stress in young horses¹⁹.

Early life experiences are important for the psychological and behavioral development of young horses²⁰.

Social experiences are key in developing social skills, establishing social hierarchies, and forming emotional states in young horses^{21, 22}.



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Objectives

To explore how positive interactions and positive foundational training influence the hormonal levels and overall horse behavior and temperament after weaning.

✓ Hypothesis

- 1 Levels of specific hormones, such as cortisol and oxytocin, are associated with the behavioral and temperament traits of young horses, reflecting their stress responses and social interactions.
- 2 Positive foundational training can influence the development of temperament in young horses.

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Materials and Methods



Animals

- 17 Quarter horse foals (10 fillies and 7 colts), with an average age of 6 months.
- Foals were born between January and April 2022 at the Horse Research Center, University of Florida.
- After weaning, foals were relocated to the Horse Teaching Unit of the University of Florida.
- Approved by the University of Florida IACUC committee (IACUC2022000000363).

Group 1



Group 2

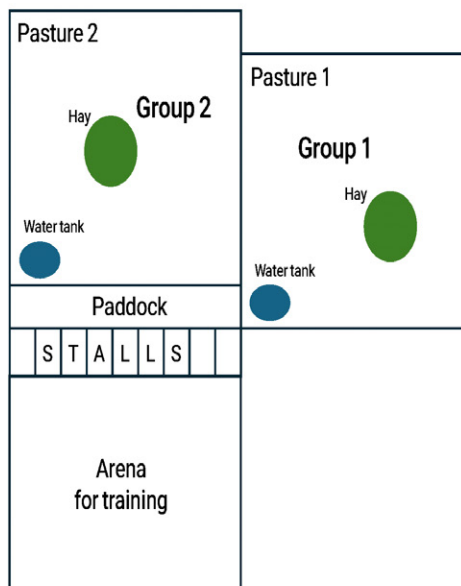


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Materials and Methods



Animals



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Materials and Methods

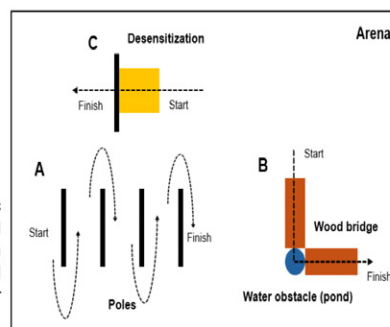


Training

- The study was conducted from September to December 2022.
- Foals participated in a training program that included **fundamental exercises**, such as **haltering, leading, and desensitizing** in an outdoor arena.
- Training sessions lasted 2 hours per day, twice a week.
- Each foal was paired with a specific handler (students).



Diagram for horse training arena. Three types of obstacles were used: poles arranged in a line (A), a combination of wood bridges and water pond (B), and a yellow tarp for desensitization (C). Foals first trotted a serpentine path around the poles led by handlers, then walked across the wood bridges and water pond, and finally were introduced to the yellow tarp to observe their responses.



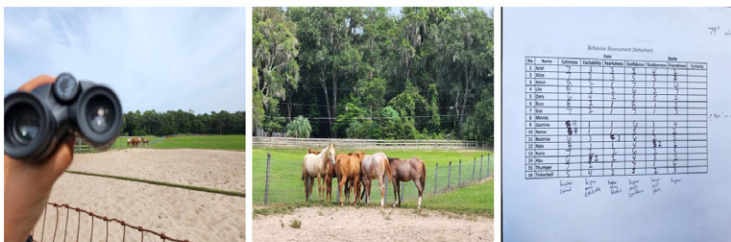
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Materials and Methods



Behavior and temperament assessment

- Behavior observation and temperament assessments were conducted at three points: **the beginning, middle, and the end** of the training program.
- Observations were made twice daily, from 9:00 to 11:00 and from 13:00 to 15:00.
- Temperament assessments were conducted **2 times** at each observation point while handlers were working with foals.
- The adeptness of handlers was also evaluated at the end of the training program.



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Materials and Methods



Behavior and temperament assessment

Affiliative behaviors

Behavior	Description
Mutual groom	Members of a herd positioned adjacent to one another, typically aligning head-to-shoulder or head-to-tail, engage in reciprocal grooming through gentle nipping, nuzzling, or rubbing actions
Huddle	Two or more companions within a herd positioned closely together, often adopting a similar stance or posture without displaying any aggressive behaviors
Follow	To move along the path of another horse, typically matching its gait without attempting to direct the movement, challenge, or overtake the leading horse
Approach	A horse approaching another horse, halting within reaching distance, and standing still for at least 5 seconds without displaying any aggressive behavior
Olfactory investigation	Exploratory smelling, involving sniffing different areas of another's head and/or body, often beginning with mutual sniffing while facing each other
Snapping	Chewing with the lower jaw moving up and down, usually with an open mouth and exposed incisors. Typically, the head and neck are extended, and the ears are relaxed and positioned back or to the side
Head on neck, back, or rump	Resting the chin or entire head on the upper surface of the neck, body, or rump of another horse

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Materials and Methods



Behavior and temperament assessment

Agonistic behaviors

Behavior	Description
Strike	Swiftly extending one or both front limbs, aiming towards another horse with the intention of making contact
Strike threat	Quick extension of one or both front limbs towards another horse, stopping just short of making contact
Bite	Stretching the neck towards another horse, with jaws opening and closing rapidly as if attempting to grasp the flesh
Bite threat	Mimicking a bite motion with an open mouth and exposed teeth, and extending the neck towards another horse, but without actually making contact
Kick	Lifting one or both hind legs off the ground and thrusting them backward towards another horse, while the forelegs support the body's weight
Kick threat	Similar to a kick, but with hind legs lifted slightly off the ground and tensed, ready to strike, without actually making contact
Nip	Resembling a bite, but with the mouth less widely opened and teeth closing on only a small area of flesh
Rear	Elevating the forequarters high into the air while the hind legs remained grounded, creating a nearly vertical stance
Chase	One horse chasing another, often at a gallop, displaying aggressive behavior such as pinning the ears, baring teeth, and attempting to bite the pursued horse's rump and tail
Displace	Horse advancing toward another and occupying the exact spot the other horse vacated
Push	Applying pressure with the head, neck, shoulder, chest, body, or rump to displace or pin the target horse against an object
Ear pinned back	Flattening or positioning the ears behind vertical or flat against the head
Avoidance	Engaging in movements to maintain or increase distance from an approaching horse

Materials and Methods



Behavior and temperament assessment

Table 2. The parameters used for evaluation of the temperament of the horse and related score

Temperaments	Description	Scale (Not at all ↔ Very much so)
Calmness	Remains composed in new or startling situations	1 ↔ 10
Excitability	Maintains concentration without being distracted by the environment	1 ↔ 10
Fearfulness	Tendency to get scared easily	1 ↔ 10
Confidence	Manifests as calm assurance in handling new or challenging situations	1 ↔ 10
Stubbornness	Shows resistance or reluctance to follow commands	1 ↔ 10
Friendliness	Friendly and approachable and interacts with others in a friendly manner	1 ↔ 10

Materials and Methods



Saliva collection

- Saliva collection was performed at the **beginning, middle, and the end** (9:00 to 11:00 AM).
- Before saliva collection, all foals were acclimated with the process.
- The synthetic swabs (Salivette, Sarstedt) was used.
- The swabs were attached to a soft rope and gently introduced into the mouths for a duration of 1-2 minutes.



Enzyme-linked immunosorbent assay

- Cortisol ELISA kits (ADI-900-071, Enzo Life Sciences).
- Oxytocin ELISA kits (MBS2700454, Mybiosource).



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Materials and Methods



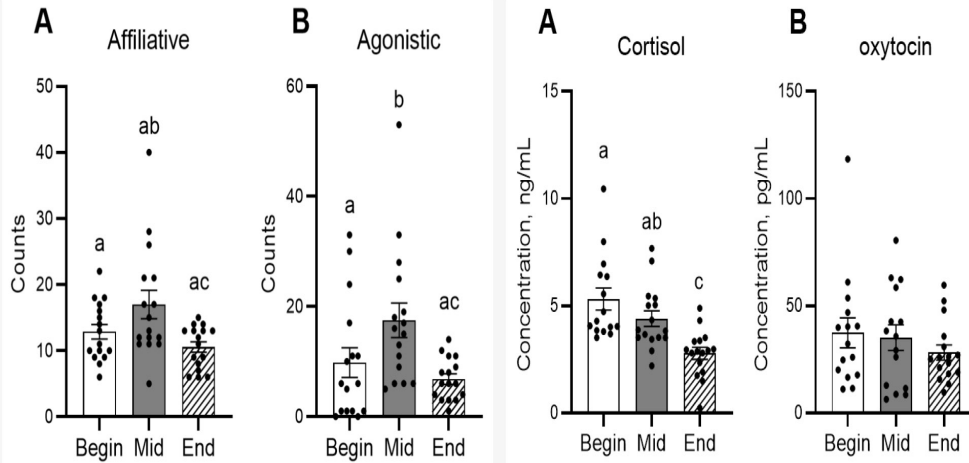
Statistical analysis

- R Studio (version 4.3.3)
- Shapiro-Wilk test for the normality test
- Mixed model for repeated measurements
- Tukey tests for post-hoc analysis
- Linear regression
- A p-value less than 0.05 was considered as a statistical significance.

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Results

Behavior and hormone level changes of foals



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Results

The relationships of hormones, behaviors, and temperament traits

Table 3. Linear regression results between hormone levels and interactive behavior

DV	IV	Coefficients	Standard error	t-value	p-value	VIF
Cortisol	(Intercept)	0.045	0.234	0.193	0.847	
	Affiliative	0.517	0.203	2.549	0.014	1.012
	Agonistic	0.002	0.026	0.089	0.930	1.012
R ² = 0.140, adjusted R ² = 0.097						
F = 3.280 P = 0.048, Durbin-Watson = 1.895						
Oxytocin	(Intercept)	1.953	0.252	7.734	<0.0001	
	Affiliative	0.501	0.231	2.168	0.035	1.044
	Agonistic	0.039	0.088	0.442	0.660	1.044
R ² = 0.039, adjusted R ² = -0.018						
F = 0.678, P = 0.514 Durbin-Watson = 2.494						

DV = dependent variable; IV = independent variable; VIF = variance inflation factor

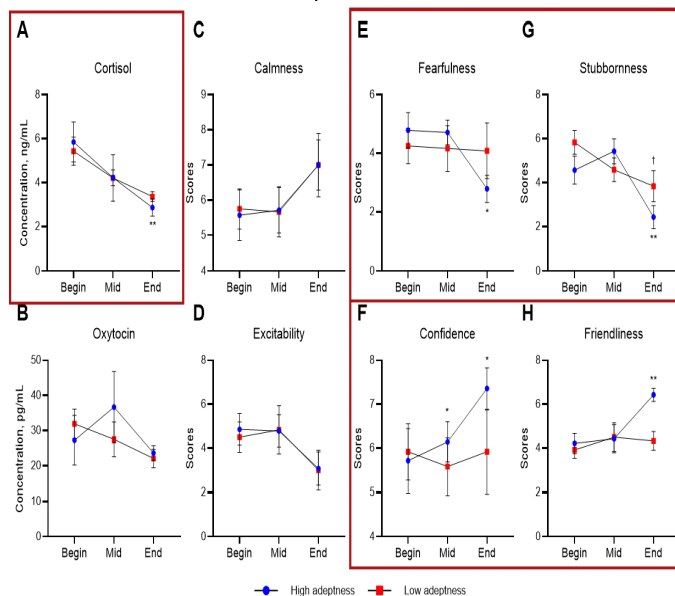
Table 4. Linear regression results of hormone levels and temperament traits

DV	IV	Coefficients	Standard error	t-value	p-value	VIF
Cortisol	(Intercept)	-4.567	3.793	-1.204	0.237	
	Calmness	-0.129	0.226	-0.571	0.571	2.986
	Fearfulness	0.812	0.320	2.561	0.015	6.260
	Confidence	0.570	0.314	1.811	0.079	5.832
	Stubbornness	0.391	0.151	2.582	0.014	1.160
	Friendliness	0.236	0.288	0.819	0.418	2.325
R ² = 0.357, adjusted R ² = 0.257						
F = 3.562, P = 0.011, Durbin-Watson = 1.453						
Oxytocin	(Intercept)	-28.333	26.58	-1.066	0.295	
	Calmness	1.0545	1.620	0.651	0.520	3.056
	Fearfulness	3.708	2.216	1.673	0.104	5.962
	Confidence	-0.399	2.209	-0.181	0.857	5.667
	Stubbornness	2.419	1.053	2.297	0.128	1.156
	Friendliness	4.930	2.053	2.401	0.022	2.336
R ² = 0.449, adjusted R ² = 0.357						
F = 4.896, P = 0.002, Durbin-Watson = 1.379						

- 36 - DV = dependent variable; IV = independent variable; VIF = variance inflation factor

Results

Adeptness of handlers, hormone levels, and temperament traits

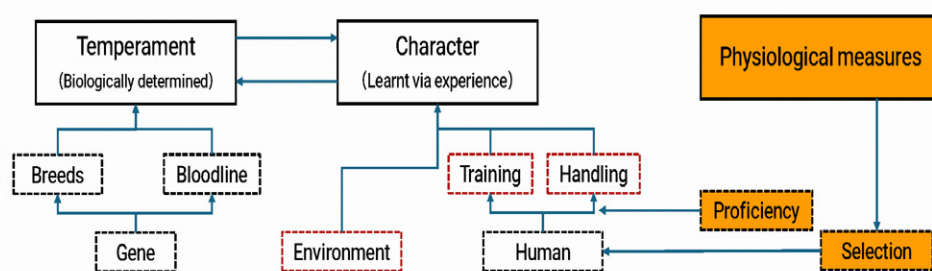


Discussion

The study investigate the effects of socialization and training on the behavior, temperament, and hormonal responses of weaned Quarter Horse foals.

The study highlights the importance of social interactions within the herd and the skill of handlers in shaping the foals' development.

Effective management practices can enhance the welfare and growth of young horses by considering these factors.



Study 3

Assessing the influence of stress level of human on horse behaviors

Introduction

Horses are known for their sensitivity to human cues and emotions

Human-horse interactions can be influenced by the emotional and physiological states of human



Functionally relevant responses to human facial expressions of emotions in the domestic horse²¹

Objectives

To assess the influence of human stress levels on horse behaviors by evaluating physiological and behavioral responses in both humans and horses



Hypothesis

- 1 Human stress leads to observable changes in horse behavior, reflecting the sensitivity of horses to their handlers' emotional states
- 2 Human stress influences the quality of human-horse interaction

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Materials and Methods



Animals

- 7 horses (5 Thoroughbreds, 1 pony, and 1 Warmblood) of Gyeongbuk Natural Science High School, Sangju.
- All horses were managed at the Gyeongbuk Natural Science High School, Sangju.



Human subjects

- 7 human participants (4 females and 3 males), with an average age of 27.8 years.
- All participants were healthy adults with no reported mental illness.



Ethical consideration

- Approved by the Experimentation Ethics Committee of Kyungpook National University (2024-0024, 2024-0043).

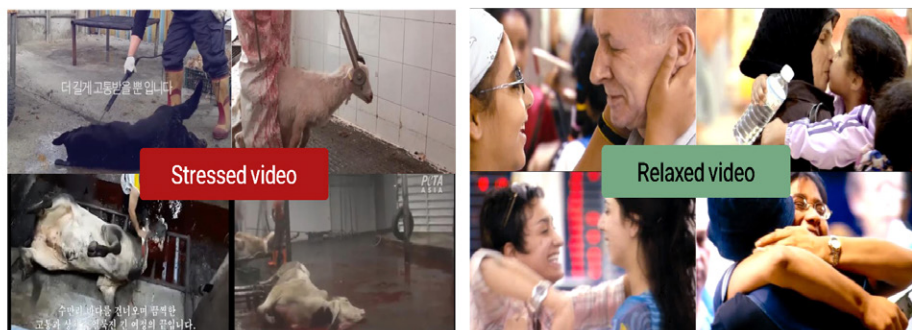
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Materials and Methods



Experimental design

- The experiment was conducted using a **crossover design**.
- Each participant was exposed to two different conditions: a **stressful video** and a **relaxed video**.



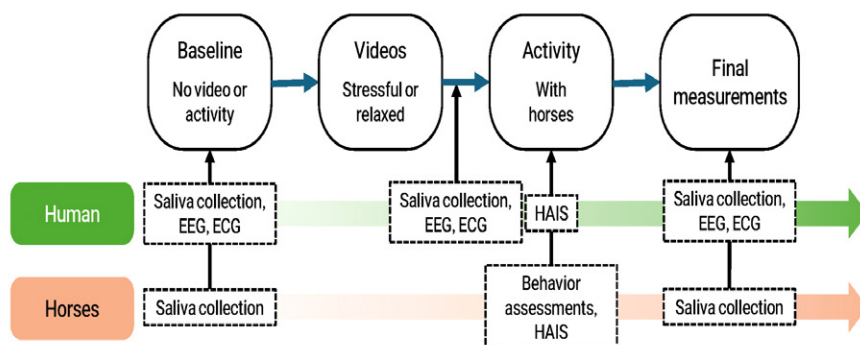
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Materials and Methods



Experimental design

- The experiment was conducted using a **crossover design**.
- Each participant was exposed to two different conditions: a **stressful video** and a **relaxed video**.



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Materials and Methods



Activities

- The activities with horses were conducted in 40 m X 60 m indoor arena.
- Activities included leading, backing up, walking side-to-side, crossing over tarps, and touching gym ball (Lundberg et al., 2020)



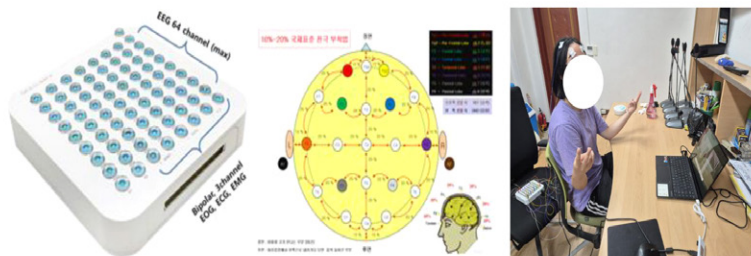
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Materials and Methods



Electroencephalography (EEG) and Heart Rate Variability (HRV)

- EEG data was collected using QEEG-8FX (Laxtha) and processed using Telescan software (CD-TS-2.2)
- Data preprocessing: band-pass filtering (0.5-50 Hz)
- Absolute power was calculated: alpha (8-12 Hz), beta (13-30 Hz), high-beta (23-40 Hz), theta (4-7.5 Hz)^{22, 23}
- ECG data was analyzed using Kubios HRV software (Kubios Oy) including HR, LF, HF, and LF/HF ratio



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Materials and Methods



Behavior observations and assessments

- Horse behaviors and the time taken to cross over obstacle course was measured during activities.
- The scores of interactions between handlers and horses were measured via Human-Animal Interaction Scale (HAIS).

Table 1. Ethnogram of horse behaviors during interactions with handler.

Behaviors	Description
Ear pinned back	Flattening or positioning the ears behind vertical or flat against the head
Tail swishing	Lateral, dorsoventral or circular motion of the tail
Push	Applying pressure with the neck, shoulder, chest, body to displace the handler
Bucking	Sudden arching of the back and leaping, and kicking with hind legs
Resisting	Ignoring or resisting the cues provided by the rider
Body shaking	Shaking its entire body, starting from the head and moving down to the tail
Lip-licking	Moving its tongue to lick the upper or lower lips
Blowing	Short non-vocal sound given during exhalation

Human-Animal Interaction Scale (HAIS) - Observer Form

Observer: _____ Date: _____

Subject: _____

Rating Key: _____

Observation: After observing an episode or series of human-animal interactions, rate each behavior according to the % of the episode the behavior was present.

Behavior	Operational Definition	Yes	No	Never	Def
1. Did the animal...?	Did the animal...?	0	1	2	3
2. Did the animal...?	Did the animal...?	0	1	2	3
3. Did the animal...?	Did the animal...?	0	1	2	3
4. Did the animal...?	Did the animal...?	0	1	2	3
5. Did the animal...?	Did the animal...?	0	1	2	3
6. Did the animal...?	Did the animal...?	0	1	2	3
7. Did the animal...?	Did the animal...?	0	1	2	3
8. Did the animal...?	Did the animal...?	0	1	2	3
9. Did the animal...?	Did the animal...?	0	1	2	3
10. Did the animal...?	Did the animal...?	0	1	2	3
11. Did the animal...?	Did the animal...?	0	1	2	3
12. Did the animal...?	Did the animal...?	0	1	2	3
13. Did the animal...?	Did the animal...?	0	1	2	3
14. Did the animal...?	Did the animal...?	0	1	2	3
15. Did the animal...?	Did the animal...?	0	1	2	3
16. Did the animal...?	Did the animal...?	0	1	2	3
17. Did the animal...?	Did the animal...?	0	1	2	3
18. Did the animal...?	Did the animal...?	0	1	2	3
19. Did the animal...?	Did the animal...?	0	1	2	3
20. Did the animal...?	Did the animal...?	0	1	2	3
21. Did the animal...?	Did the animal...?	0	1	2	3
22. Did the animal...?	Did the animal...?	0	1	2	3
23. Did the animal...?	Did the animal...?	0	1	2	3
24. Did the animal...?	Did the animal...?	0	1	2	3
25. Did the animal...?	Did the animal...?	0	1	2	3
26. Did the animal...?	Did the animal...?	0	1	2	3
27. Did the animal...?	Did the animal...?	0	1	2	3
28. Did the animal...?	Did the animal...?	0	1	2	3
29. Did the animal...?	Did the animal...?	0	1	2	3
30. Did the animal...?	Did the animal...?	0	1	2	3
31. Did the animal...?	Did the animal...?	0	1	2	3
32. Did the animal...?	Did the animal...?	0	1	2	3
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36. Did the animal...?	Did the animal...?	0	1	2	3
37. Did the animal...?	Did the animal...?	0	1	2	3
38. Did the animal...?	Did the animal...?	0	1	2	3
39. Did the animal...?	Did the animal...?	0	1	2	3
40. Did the animal...?	Did the animal...?	0	1	2	3
41. Did the animal...?	Did the animal...?	0	1	2	3
42. Did the animal...?	Did the animal...?	0	1	2	3
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48. Did the animal...?	Did the animal...?	0	1	2	3
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60. Did the animal...?	Did the animal...?	0	1	2	3
61. Did the animal...?	Did the animal...?	0	1	2	3
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64. Did the animal...?	Did the animal...?	0	1	2	3
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66. Did the animal...?	Did the animal...?	0	1	2	3
67. Did the animal...?	Did the animal...?	0	1	2	3
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71. Did the animal...?	Did the animal...?	0	1	2	3
72. Did the animal...?	Did the animal...?	0	1	2	3
73. Did the animal...?	Did the animal...?	0	1	2	3
74. Did the animal...?	Did the animal...?	0	1	2	3
75. Did the animal...?	Did the animal...?	0	1	2	3
76. Did the animal...?	Did the animal...?	0	1	2	3
77. Did the animal...?	Did the animal...?	0	1	2	3
78. Did the animal...?	Did the animal...?	0	1	2	3
79. Did the animal...?	Did the animal...?	0	1	2	3
80. Did the animal...?	Did the animal...?	0	1	2	3
81. Did the animal...?	Did the animal...?	0	1	2	3
82. Did the animal...?	Did the animal...?	0	1	2	3
83. Did the animal...?	Did the animal...?	0	1	2	3
84. Did the animal...?	Did the animal...?	0	1	2	3
85. Did the animal...?	Did the animal...?	0	1	2	3
86. Did the animal...?	Did the animal...?	0	1	2	3
87. Did the animal...?	Did the animal...?	0	1	2	3
88. Did the animal...?	Did the animal...?	0	1	2	3
89. Did the animal...?	Did the animal...?	0	1	2	3
90. Did the animal...?	Did the animal...?	0	1	2	3
91. Did the animal...?	Did the animal...?	0	1	2	3
92. Did the animal...?	Did the animal...?	0	1	2	3
93. Did the animal...?	Did the animal...?	0	1	2	3
94. Did the animal...?	Did the animal...?	0	1	2	3
95. Did the animal...?	Did the animal...?	0	1	2	3
96. Did the animal...?	Did the animal...?	0	1	2	3
97. Did the animal...?	Did the animal...?	0	1	2	3
98. Did the animal...?	Did the animal...?	0	1	2	3
99. Did the animal...?	Did the animal...?	0	1	2	3
100. Did the animal...?	Did the animal...?	0	1	2	3

HAIS: The Human-Animal Interaction Scale & Manual²⁴

Materials and Methods

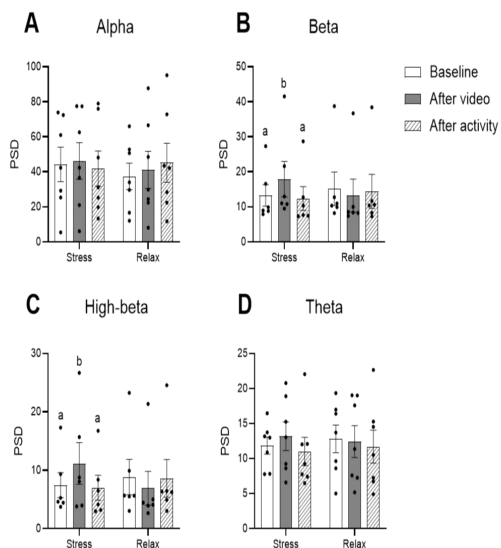


Statistical analysis

- Prism 10 (Graphpad Software, CA, USA)
- Mixed model for repeated measures
- Tukey's multiple comparison test for post-hoc analysis
- Paired t-test
- A p-value less than 0.05 was considered as a statistical significance.

Results

Brainwaves changes of handlers



Alpha

- Alpha wave activity did not show significant changes across the different conditions

Beta

- Beta wave activity increased after watching stressful videos

High-beta

- High-beta wave activity increased after watching stressful videos

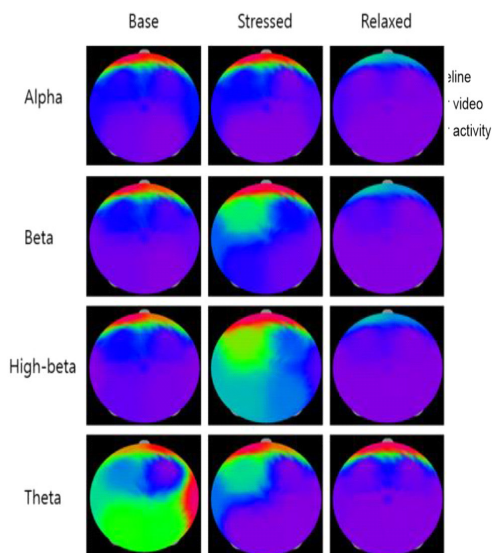
Theta

- Theta wave activity remained stable

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Results

Brainwaves changes of handlers



Alpha

- Alpha wave activity did not show significant changes across the different conditions

Beta

- Beta wave activity increased after watching stressful videos

High-beta

- High-beta wave activity increased after watching stressful videos

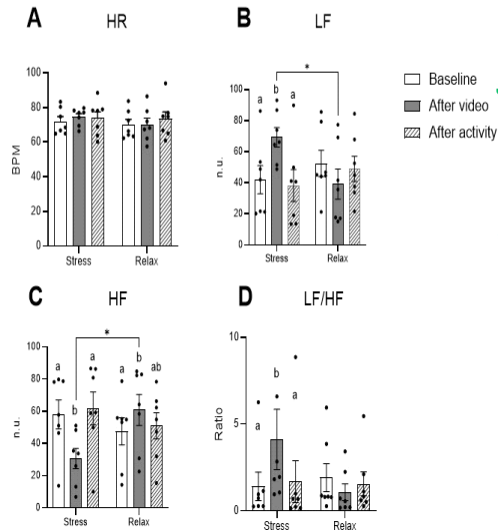
Theta

- Theta wave activity remained stable

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Results

Heart rate variability changes of handlers



HR

- HR did not show significant changes

LF

- LF power increased after watching stressful videos

HF

- HF power decreased after watching stressful videos

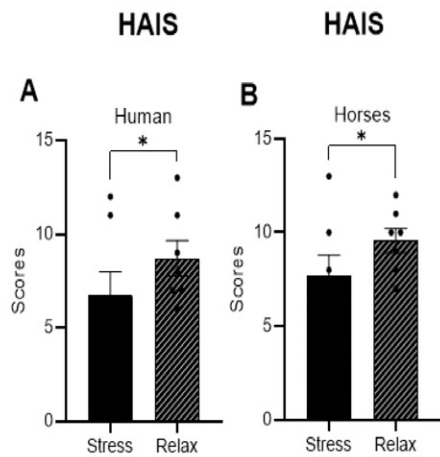
LF/HF

- LF/HF ratio increased after watching stressful videos

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Results

Changes in human-horse interaction scales of handlers and horses



Human-Animal Interaction Scale

- HAIS for both handlers and horses significantly lower when handlers watched stressful videos



Human & horses (relaxed videos)

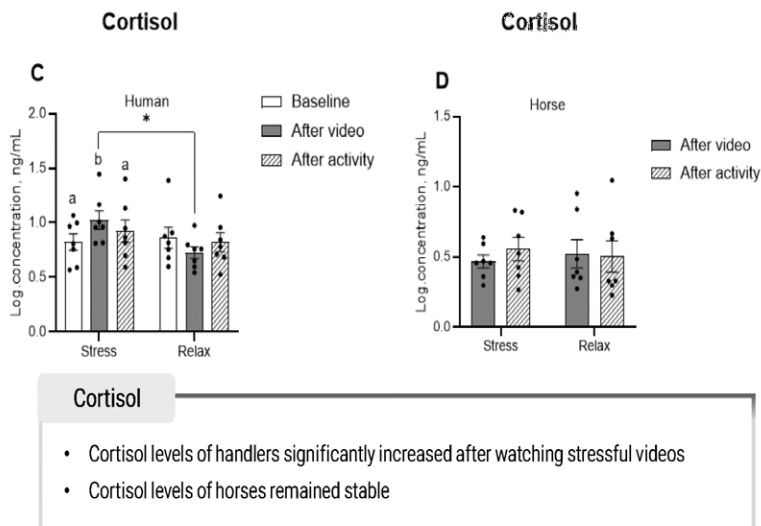


Human & horses (stressed videos)

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Results

Changes in cortisol levels of handlers and horses



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Results

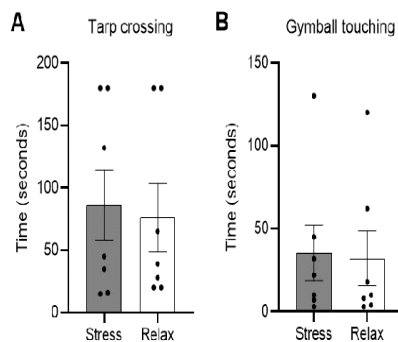
Changes in horse behaviors

- Horses tended to push the handler more and showed less lip-licking behaviors when the handler had watched the stressful video.
- The time taken for horses to complete the tarp obstacle and to touch the gym ball did not show differences.

Table 2. The frequencies of behaviors that horses showed during interaction with handlers

Behaviors	Stress	Relax	<i>p</i>
Ear pinned back	9.71±2.81	8.00±2.30	0.053
Tail swishing	16.86±7.34	12.14±4.57	0.160
Push	4.00±0.87	3.00±0.62	0.017
Bucking	0.86±0.59	0.14±0.14	0.253
Resisting	4.00±0.65	3.00±0.53	0.134
Head shaking	0.29±0.18	0.71±0.18	0.078
Lip-licking	0.29±0.18	1.29±0.18	0.003
Blowing	0.43±0.20	1.29±0.29	0.078

The results are expressed as the mean ± standard error of mean.

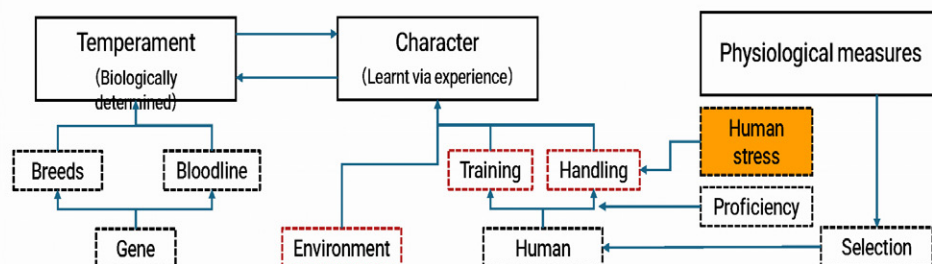


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Discussion

The study demonstrates the negative impact of human stress on the quality of human-horse interactions and horse behavior.

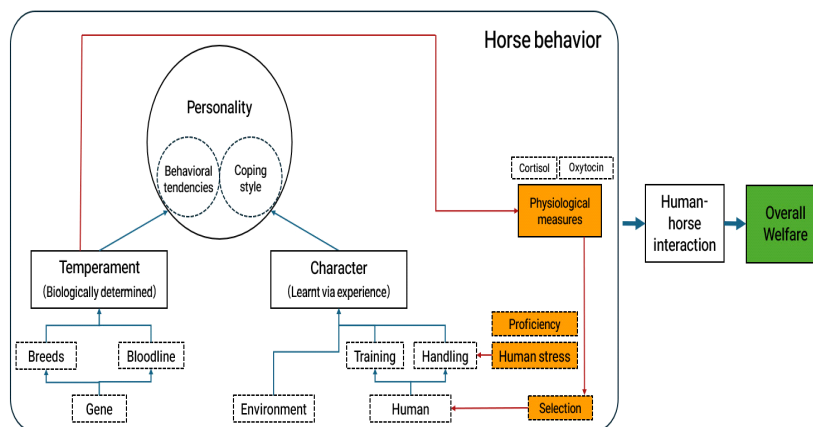
The study emphasizes the importance of managing handler stress to foster positive interactions, particularly in therapeutic settings and equine training.



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Conclusion

The study concludes that effective socialization and training significantly enhance the behavioral and hormonal development of horses, underscoring the importance of human-horse interactions for improving equine welfare.



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Thank you for listening

