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## HORSE SPECIES II

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**1206 (W134) Trotting stride variables of the North American Akhal-Teke Horse.** M. C. Nicodemus\*<sup>1</sup> and J. Beranger<sup>2</sup>, <sup>1</sup>Mississippi State University, Starkville, <sup>2</sup>American Livestock Breeds Conservancy, Pittsboro, NC.

Although the population is less than 350 horses placing it on the threatened breed list of the American Livestock Breed Conservancy (ALBC), the Akhal-Teke Horse of North America is as a source and a reservoir for genetic diversity for the ancient breed as it includes bloodlines that are unique. Preservation of the breed and its distinctive characteristics such as its smooth and elastic gaits is of top priority to the ALBC and the Akhal-Teke Association of America, and to accomplish this, a better understanding of the breed is needed. Therefore, the objectives of this study were to measure and describe the stride timing found in the trot of the Akhal-Teke Horse of North America. Subjects ( $n = 6$ ) were selected through the direction of the ALBC and the Akhal-Teke Association of America based on bloodlines and performance history. Each horse was filmed at 60 Hz being ridden under saddle by a rider familiar with the horse at a trot with hoof contact and lift-off documented using frame-by-frame analysis. Means (SD) were determined for 10 strides for each horse with variables given as % of stride and variability of measurements indicated using the coefficient of variation (CV) expressed as a % of mean. The trot was performed in a diagonal footfall pattern that alternated between periods of unipedal (Hind:  $10 \pm 1\%$ , CV 6%; Fore:  $10 \pm 2\%$ , CV 19%) and bipedal (Diagonal:  $80 \pm 2\%$ , CV 3%) supports. The trotting velocity ( $4.34 \pm 0.15$  m/s, CV 3%) was achieved using a stride length of  $2.96 \pm 0.25$  m (CV 8%), rate of  $1.47 \pm 0.11$  strides/s (CV 7%), and duration of  $683 \pm 49$  ms (CV 7%) with the limbs spending the majority of the stride in the swing phase (Fore:  $55 \pm 2\%$ , CV 6%; Hind:  $54 \pm 2\%$ , CV 4%). The diagonal limbs moved as couplets, both at hoof contact (Advanced Placement:  $5 \pm 1\%$ , CV 15%) and lift-off (Advanced Lift-Off:  $5 \pm 1\%$ , CV 19%), creating a 4-beat rhythm. Coupling, rather than pairing of limbs, was similar to the stride timing reported in previous research for Dutch Warmblood and Standardbred horses, but the unpairing of diagonal limbs occurred at dissimilar velocities. In addition, the absence of suspension that has been reported for other trotting breeds such as the Dutch Warmblood and Morgan horse was also produced at a velocity distinctive from the other breeds suggesting velocity may account for the uniqueness of the trot of the Akhal-Teke Horse of North America.

**Key Words:** Akhal-Teke

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**1207 (W135) Development of an objective on-farm equine temperament scoring system.** J. N. Foley\*<sup>1</sup>, J. L. Lucia<sup>2</sup>, and K. W. Walter<sup>1</sup>, <sup>1</sup>Truman State University, Kirksville, MO, <sup>2</sup>Sam Houston State University, Huntsville, TX.

To evaluate interest in development of an objective equine temperament scoring system (TSS), an online survey was distributed via hyperlink to faculty at three public universities and their equine interest groups and social media pages. Of 123 respondents, 71.4% were familiar with existing TSS, 81.1% of those familiar with existing TSS felt they did not accurately represent the horse's temperament, and 70.5% were interested in an improved TSS to replace subjective scales currently used on equine websites (equinenow.com and horsetopia.com most frequently cited). Existing TSS rank the horse from 1 to 10 (1 = calm, 10 = spirited), however no further clarification is provided. To assess equine temperament more objectively, 27 horses were utilized in a two phase test. Phase 1 incorporated 10 min of isolation while tied. Frequency of vocalization, defecation, urination, lateral movements, forelimb pawing, and blatant pulling on lead were recorded and used to develop a 0 to 6 scoring system (score assigned based on frequency of aforementioned behaviors). Phase 2 tested willingness to cross an unfamiliar obstacle. Horses were allowed 1 min to cross the obstacle. If the obstacle was refused, horses were trotted in a 5 m circle for 1 min. This procedure was repeated up to 5 times, and horses were assigned a score from 0 to 6 based on time required to cross with little resistance or fear. For both phases, vital signs of heart rate (HR), respiration rate (RR), and rectal temperature (RT) were evaluated immediately before and after each test. Blood samples were taken via jugular venipuncture and later analyzed for cortisol concentration utilizing a commercially available ELISA kit. Pre-test values of HR, RR, RT and cortisol concentrations were subtracted from post-test values to calculate the difference. Pearson's correlations were analyzed for all variables within phase, and Spearman's rank test performed to compare phases. Strong positive correlation existed between difference in cortisol and assigned score in both phase 1 and 2 ( $r \geq 0.61$ ;  $P < 0.001$ ). A strong positive correlation between assigned score in both phases and difference in vital signs (HR, RR, RT;  $r \geq 0.4387$ ,  $P \leq 0.02$ ) was noted. However, there was no correlation between phase 1 and phase 2 scores ( $r = 0.25$ ,  $P = 0.20$ ). This suggests each phase successfully evaluated different aspects of equine behavior using clearly defined 0 to 6 point scales, and both phases could be used together to evaluate equine temperament.

**Key Words:** behavior evaluation, equine, temperament scoring

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**1208 (W136) Cooling of equine semen at 16°C for 36 h with the addition of cysteine in different concentrations.** R. A. De Oliveira<sup>1</sup>, L. S. Murata<sup>1</sup>, M. A. D. O. Viu<sup>2</sup>, and M. L. Gambarini<sup>2</sup>, <sup>1</sup>University of Brasilia, Brazil, <sup>2</sup>Federal University of Goiás, Goiânia, Brazil.

Equine semen manipulation during the cooling process reduces sperm viability and fertility in consequence to, among others, membrane lipid peroxidation, because of the high content of polyunsaturated fatty acids, which makes cells highly susceptible to free radicals and reactive oxygen species. The objective of the present study was to evaluate the effect of in vitro addition of cysteine in four concentrations (0, 1, 1.5, and 2.5 mM) for cooling spermatozoa of 12 stallions at 16°C for 36 h. Evaluated variables were motility, vigor, viability and plasmatic and acrosomal membrane integrity in four different moments (0, 12, 24, and 36 h). With the exception of acrosomal integrity, it was verified a reduction in motility, vigor and plasmatic membrane integrity in all samples, during cooling. In the evaluations at 36 h of cold storage, motility (mot) and viability (viab) were greater in groups treated with 1 mM (mot:46,5 ± 6,1/viab:76,5 ± 6,9) and 1.5 mM (mot:46,0 ± 4,6/viab:76,9 ± 3,7) cysteine, respectively, compared to control (mot:35,5 ± 18,4/viab:68,1 ± 13,4) and 2.5 mM (mot:39,7 ± 12,4/viab:66,0 ± 17,2) ( $P < 0.05$ ). As for vigor (vig) and plasmatic membrane integrity (plasm), 1 mM cysteine (vig:3.6 ± 0.5/plasm: 57.2 ± 9.5) showed greater results compared to control (vig:3.2 ± 1.1/plasm:54.1 ± 11.8), 1.5 mM (vig:3.5 ± 0.6/plasm:52.2 ± 13.3) and 2.5 mM (vig:3.2 ± 1.1/plasm:55.8 ± 12.5) ( $P < 0.05$ ). Regarding acrosomal membrane integrity, in general, there was no loss of integrity (70.5 ± 10.4; 69.4 ± 4.4; 68.0 ± 7.2 and 70.3 ± 0.5), control, 1 mM, 1.5 mM and 2.5 mM, respectively. The concentration of 1 mM cysteine was more effective for the protection of sperm cells in the commercial system of passive cooling at 16°C for 36 h, with greater values for motility, vigor, viability and plasmatic membrane integrity.

**Key Words:** antioxidant, cooled semen, stallion

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**1209 (W137) Administration of bioactive proteins to mature horses improves gait kinematics.**

J. Coverdale\*, and J. M. Campbell<sup>2</sup>, <sup>1</sup>Texas A&M University, College Station, <sup>2</sup>APC, Inc., Ankeny, IA.

Thirty mature quarter horses (439 to 684 kg and 5 to 22 yr) were utilized in a randomized complete block design to evaluate the effect of a supplement consisting of a proprietary blend of bioactive proteins on gait kinematics. These bioactive proteins are isolated from plasma. Horses were blocked by age and BW and randomly assigned to treatment within block for the 28-d trial. Treatments consisted of a commercial pelleted concentrate administered with no supplement (CON), 240 g/d of a pelleted supplement containing 66 g of bioactive proteins (Low; Lifeline, APC, Inc.), or 240 g of a pelleted supplement

containing 132 g of bioactive proteins (High; Lifeline, APC, Inc.). Concentrate was fed at 0.5% BW (as-fed) daily in addition to ad libitum coastal bermudagrass hay. Each horse was exercised by a single assigned student 5 d/wk focusing on horsemanship skills at the walk, trot, and canter approximately 60 min/d. Gait kinematic analysis was performed on d 0, 14, and 28 with video footage collected and analyzed using gait analysis software (EquineTec). Horses were trotted in hand for three passes over a 10 m flight path while wearing reflective markers at each joint of the right limbs. Stride length was measured as distance the right foreleg traveled during the swing phase. Additionally, range of motion (ROM) of the knee was determined using the difference between the maximum and minimum angles observed during each frame of the swing phase. All data were analyzed using PROC GLM of SAS. Mean stride length of the front limb tended to increase linearly at d 14 as increasing levels of bioactive proteins were added to the diet ( $P = 0.07$ ). Similarly, at d 28 stride length of the front limb increased linearly with increasing inclusions of bioactive proteins ( $P = 0.05$ ). Stride length at the trot of the hind limb tended to increase at d 14 ( $P = 0.10$ ) and increased linearly at d 28 ( $P = 0.02$ ) with increasing levels of bioactive proteins. When evaluated at the trot, knee range of motion increased linearly at d 14 and d 28 with increasing levels of bioactive proteins in the diet ( $P < 0.01$ ). In conclusion, supplementation of bioactive proteins in mature, exercising horses resulted in improved gait kinematics.

**Key Words:** bioactive proteins, gait, horse, stride, supplement

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**1210 (W138) The effect of skim milk as an equine semen extender.** M. L. Freitas, C. S. Bouéres,

F. J. G. De Oliveira, L. S. Murata\*, and R. A. De Oliveira, University of Brasilia, Brazil.

Cryopreservation constitutes the best method to conserve the genetic material of great zotechnic value stallions. However, the expenses of this procedure are high, becoming an obstacle to the spread of this biotechnology. One possible way to reduce spending on the process of cryopreservation is to search for alternative and less onerous extender media. The current study aimed at comparing the commercial semen extender (control group) to UHT skim milk (treatment group) used during centrifuging for subsequent cryopreservation of equine semen. After thawing of semen, parameters such as computerized spermatic kinetics and acrosome and plasmatic membrane integrity using fluorescent dyes were assessed. No differences ( $P > 0.05$ ) were observed in what concerns to total sperm motility (42.71 × 38.29%); progressive sperm motility (12.29 × 7.86%); plasmatic membrane integrity (53.43 × 60.14%) and acrosomal membrane integrity (93.29 × 93.71%) between the control and the treatment groups. Considering that UHT skim milk has a much lower cost than the commercial semen extender, this may henceforth skim milk is an

option for extending equine semen, which decreases the expenses of the equine semen cryopreservation process.

**Key Words:** equine, sperm, UHT skim milk

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**1211 (W139) Reproductive activity in quarter horse mares with artificial light.** J. A. Ramírez-Godínez\*, J. Delgado-Laphond, A. Flores-Mariñelarena, and E. Santellano-Estrada, *Universidad Autónoma de Chihuahua, México.*

The objective was to determine the effect of artificial light during the winter on the reproductive activity in quarter horse (QH) mares in México. Sixty seven QH mares, 5 to 12 yr old with an average weight of  $480 \pm 20$  kg, and body condition between 5 and 7 were randomly divided into a control group (G0,  $n = 15$ ), exposed to natural light and an artificial light group 1 (G1,  $n = 52$ ), exposed to artificial light to complete 16 h light from November 15 to February 15. Both groups were alternately monitored (every other day) with a teaser stallion to assess the presence of estrus, and the reproductive tract ultrasounded to monitor follicular growth and ovulation. Means for days to estrus, ovulation, pregnancy, services per conception and size of the ovulatory follicle were compared between treatments using the GLM procedure of SAS. The effect of treatments on ovulation rate per month was analyzed by Chi Square ( $\chi^2$  test) using the FREQ procedure of SAS. The use of artificial light accelerated ( $P < 0.001$ ) the onset of estrus, ovulation and gestation considerably. The average time from exposure of artificial light to onset of estrus was  $47 \pm 3.21$  d ( $P < 0.001$ ) in G1 and  $105 \pm 6.13$  d in G0, and to ovulation  $81.6 \pm 3.7$  d with artificial light (G1) and  $134.5 \pm 7.0$  d control group (G0). The highest rate of ovulations ( $P < 0.1$ ) occurred in February (0.46) in G1 and in April (0.66) in G0, respectively. Diameter of the ovulatory follicle was similar ( $P > 0.05$ ) in mares in the control group ( $40.7 \pm 1.11$  mm) than under artificial photoperiod ( $41.7 \pm 0.58$  mm). The interval from the onset of artificial light to pregnancy was  $96.54 \pm 4.46$  d (G1) and  $141.28 \pm 8.53$  d with natural light (G0;  $P < 0.001$ ). The services per conception (SPC) were similar ( $P < 0.05$ ) between treatments ( $1.6 \pm 0.123$  SPC for artificial photoperiod and  $1.7 \pm 0.236$  SPC for controls, respectively). The use of artificial light from November to February in QH mares accelerated the presence of estrus and the ovarian activity (ovulation) which resulted in a higher proportion of earlier gestations in the year.

**Key Words:** estrus, ovulation, mares

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**1212 (W140) Composition of follicular fluid and serum, ovarian dynamics, and IGF-1 concentrations following n-3 fatty acid supplementation in mares.**

S. E. Buist<sup>1</sup>, M. J. Schmidt<sup>1</sup>, D. M. Grieger<sup>1</sup>, C. A. Blevins<sup>1</sup>, S. K. Weibel<sup>2</sup>, T. L. Douthit<sup>1</sup>, L. Murray<sup>1</sup>, and J. M. Kouba<sup>1</sup>, <sup>1</sup>*Kansas State University, Manhattan*, <sup>2</sup>*JBS United, Baylis, IL.*

The effects of a marine-derived n-3 fatty acid supplement on ovarian dynamics and follicular fluid and serum composition were evaluated during the estrous cycle in mares. Fifteen mares were assigned to a control diet (CONT,  $n = 7$ ) or a fish oil-enriched diet (FO,  $n = 8$ ) providing 18.48 g eicosapentaenoic acid (EPA) and 10.08 g docosahexaenoic acid (DHA) daily. Follicular activity was determined via transrectal ultrasonography at the initiation of the treatment diet. Estrous cycles were synchronized in all mares concurrently with initiation of treatment diets using a progesterone and estradiol protocol. Following ovulation post-synchronization, mare monitoring continued through the second estrous cycle. Ovarian activity, timing of ovulation, and presence of a corpus luteum were recorded. Mares were scanned during the third estrous cycle until a 35-mm follicle was detected, at which time hCG was administered. A transvaginal ultrasound-guided follicular aspiration (TUGA) was performed on the largest preovulatory follicle 14 to 16 h post-hCG. Follicular fluid was analyzed for fatty acids, estradiol 17- $\beta$ , LH, progesterone, PGF<sub>2 $\alpha$</sub> , PGE<sub>2</sub>, and IGF-1 concentrations. Serum samples were collected at the onset of treatment diet and every 2 wk until termination of the study to determine fatty acid concentrations. Additional serum samples were obtained before hCG administration and before TUGA procedure for measurement of IGF-1. Arachidonic acid (ARA), EPA, docosapentaenoic acid (DPA), and DHA in mare serum and EPA, DPA, and DHA in follicular fluid were increased ( $P < 0.01$ ) in the FO group. Serum IGF-1 was decreased ( $P < 0.05$ ) in the FO group immediately before aspiration. Concentrations of IGF-1 were decreased ( $P < 0.05$ ) in follicular fluid in the FO group compared with controls. No other differences in follicular fluid hormone concentrations were detected. Follicular growth rate, ovulation interval, and timing of ovulation were similar between groups. These data indicate that in addition to incorporation into serum, dietary n-3 fatty acids can also be incorporated into follicular fluid, and may have an inhibitory effect on serum and follicular fluid IGF-1 concentrations in the cycling mare.

**Key Words:** mare, n-3, follicular fluid