282 ASAS Centennial Presentation: Historical review and future outlook of equine reproductive technology. D. Sharp*, *University of Florida*, *Gainesville*.

The twentieth century was a rich and fascinating time for those with an interest in equine reproductive biology. The birth of equine reproductive research might properly be marked as the first Symposium on Equine Reproduction held at Kingâ€™s College, Cambridge, 1973. Seen as a summation of equine reproductive research up to that point, the symposium also provided moral support for those daring to venture into the sometimes unpopular field. In the more-than a quarter of a century elapsed since then, equine reproductive research has benefited and grown as a result of technological advances that touched all areas of biological research. Key to the growth of that research was the introduction and utilization of but five major technological advances; radioimmunoassay, novel surgical procedures, ultrasound, molecular biological approaches, and acquisition of the equine genome. The latter has yet to prove its worth, but likely will pass the test. The allotted space cannot serve to point out the many milestones and conceptual leaps accomplished in our understanding of equine reproduction. Nor can this short abstract list the players who contributed. The rate of knowledge gain has roughly paralleled that of other domestic species; sometimes lagging, sometimes ahead, but definitely advancing. Suffice it to say here, that horses deserve a place in the texts and in the universities of those who would understand the underlying mechanisms of reproductive biology. The many departures by horses from central scientific dogma provide perspective on the problems of reproduction, and often prove the rule by their exception. Often, the unique anatomical and physiological features of horses, mares especially, lend themselves to development of new approaches which return the investment manifold in knowledge. Certainly one cannot long work with these animals without a sense of awe at their invention. New technologies alone cannot guarantee progress. Those who employed new technologies in the study of equine reproduction, and those yet to do so have a responsibility to ask intelligent, pertinent, questions of the fundamental principles, and direct new technologies appropriately.

Key Words: Equine, Reproduction, Technology

283 Pituitary responsiveness to continuously-administered native GnRH at the winter solstice in anovulatory mares and mares with residual ovarian activity. I. C Velez*[1,2], M Amstalden[1,2], J. D Pack[1,2], and G. L Williams[1,2], [1]*Texas AgriLife Research*, *Beeville, TX*, [2]*Texas A&M University*, *College Station*.

Mares exhibit profound reproductive seasonality in North America, and varying strategies have been used to regulate this phenomenon in order to promote earlier foaling during the calendar year. Previous work in our laboratory has shown that low doses of continuously-administered, native GnRH (2.5-5 μg/h s.c.) are unable to prevent the development of or to reduce the length of winter anovulation. However, similar doses in April successfully induce follicular development and ovulation in approximately 80 % of persistently anovulatory mares, suggesting that the anterior pituitary may be less responsive before or near the winter solstice than after. Moreover, results of other studies in our laboratory have questioned whether GnRH secretion is limiting during the anovulatory season. Because of these limited and controversial findings, we examined dose-related effects of GnRH on anterior responsiveness just before the winter solstice (December 6 through 20). Fifteen mares (5/grp) were used, with 12 of 15 confirmed anovulatory and assigned randomly to: 1) Control, saline; 2) GnRH-20, 20 μg/h GnRH; 3) GnRH-100, 100 μg/h GnRH. Three mares with residual ovarian activity were distributed equally across groups. Treatments were administered s.c. for 14 d using Alzet osmotic minipumps (model 2ML2; 5 μL/h). Repeated measures ANOVA was used to analyze data. Mean concentrations of serum LH (0.3 ± 0.08 ng/mL) did not differ among groups on d 0, but increased ($P < 0.001$) in a dose-dependent manner within 24 h. On d 14, least squares mean concentrations of LH averaged 0.1, 0.8, and 1.8 ng/mL (SEM = 0.14) for control, GnRH-20 and GnRH-100 groups, respectively ($P < 0.001$). Maximum follicle sizes did not exceed 25 mm in controls and ranged from 21 to 38 mm in GnRH groups. By d 14, 0/5 control, 1/ 5 GnRH-20 and 4/5 GnRH-100 had ovulated. Anterior pituitary responsiveness to GnRH at the winter solstice appeared adequate to support follicle development and ovulation.

Key Words: Equine, LH, Seasonality

284 Patterns of pituitary venous LH release in the luteal and follicular phase mare: Effects of continuous treatment with native GnRH. I. C. Velez*[1,2], M. Amstalden[1,2], J. D. Pack[1,2], and G. L. Williams[1,2], [1]*Texas AgriLife Research*, *Beeville, TX*, [2]*Texas A&M University*, *College Station*.

Continuous administration of high-dose GnRH to most mammals results in GnRH receptor down-regulation and reduced secretion of LH. However, in the horse, continuous GnRH exposure remains stimulatory. Little is known about the hypothalamic-pituitary interrelationships that subserve this unique phenomenon. Herein, we tested the hypothesis that endogenous circulating patterns of LH, as measured in pituitary venous effluent of the intercavernous sinus (ICS), retain their pulsatile character in the face of continuous GnRH exposure. Twelve cyclic mares were assigned randomly to one of two groups (n = 6/group): 1) Control, saline, and 2) GnRH, 100 μg/h. Between 3 and 6 d after ovulation (d 0 of expt.), Alzet osmotic minipumps (Model 2ML1) containing saline or GnRH were placed s.c. and connected to a jugular infusion catheter. On d 3, the ICS of 10 of 12 mares (5/group) were catheterized for 5-min sampling during 8 h on d 4, followed immediately by treatment with prostaglandin F2-alpha to cause luteal regression. A similar intensive sampling period (6 h) was performed 36 h later (d 6). A pulse detection algorithm was used to identify pulses. Data were analyzed by one-way and repeated measures ANOVA. Mean jugular concentrations of LH on d 0 (2.2 ± 0.7 ng/mL; n=12) did not differ between groups; however, within 24 h, a 2.8-fold increase in mean LH was observed in GnRH-treated mares ($P < 0.001$). Values in treated mares remained greater ($P <0.001$) than controls in both daily peripheral (4.1 vs 1.6 ng/mL; SEM = 0.3) and ICS plasma of luteal (6.9 vs 1.3 ± 0.1 ng/mL) and follicular phases (5.5 vs 1.2 ± 0.1 ng/mL). The luteal phase pattern of pituitary venous LH in 4/5 controls was characterized by infrequent (0.175 ± 0.07/h), secretory episodes of long-duration, punctuated by small secondary pulses. Continuous GnRH exposure eliminated this pattern, producing a chronically-elevated baseline of high-frequency, low-amplitude pulses. Moreover, follicular phase pulse frequency (1.2 ± 0.3 episodes/h) was accelerated (1.6 ± 0.3 episodes/h; $P < 0.05$) further by GnRH treatment. Results provide additional insight into unique mechanistic features of the equine hypothalamic-hypophyseal axis.

Key Words: Equine, LH, GnRH

285 Effect of centrifugation technique on post storage characteristics of stallion spermatozoa. M. M. Dean and G. W. Webb*, *Missouri State University*, *Springfield*.

Three ejaculates were collected from each of 4 stallions and used to compare the effects of 3 centrifugation methods on motility, percent harvest and intact acrosomes following both storage at 5°C for 48 h and freezing in liquid nitrogen. Following collection 2 aliquots per treatment were diluted with skim milk-glucose (SKMG) extender to a final concentration of 50×10^6, placed in 50 ml conical bottom tubes, and centrifuged at either 700 × g for 15 minutes or 600 × g for 12 minutes. A third aliquot was diluted 1:1 with SKMG, placed in 15 ml conical tubes and centrifuged at 400 × g for 7 minutes. Following centrifugation aliquots where again split with subsamples from each stored at 5°C in Equine Express® shippers (Exodus Breeders Supply, York, PA) for evaluation after 24 and 48 h. Another aliquot of each centrifugation treatment was diluted with INRA 96® (IMV International) supplemented with 4% egg yolk and 4% glycerol, loaded into 0.5 ml straws and frozen. Following storage, aliquots were evaluated for motility using CASA (CEROS®; Hamilton Research Inc, Beverly, MA). Data were analyzed using a GLM procedure with a one-way ANOVA and Tukey′s used to test differences between centrifuge treatments. Post storage percentages of intact acrosomes and detached heads were evaluated after staining with Spermac Stain™ (FertiPro, Beernem, Belgium). Percentage of spermatozoa harvested was determined by hematocytometer and was higher ($P \leq 0.05$) for aliquots centrifuged in 15 ml tubes at 400 × g for 7 min vs. 600 × g for 12 min in 50 ml tubes. Spermatozoal damage, measured by percentage of detached heads or intact acrosomes, was not different between treatments. Following cold storage total motility was higher ($P \leq 0.05$) for aliquots which were centrifuged in 15 ml tubes compared to those centrifuged in 50 ml tubes at higher speeds and longer amounts of time. Post thaw motility of frozen spermatozoa was not different between treatments. Use of 15 ml vs. 50 ml centrifugation tubes allows centrifugation at slower speeds and shorter times without affecting the % harvest of available spermatozoa. This may result in less damage to the spermatozoa due to centrifugation.

Key Words: Stallion, Semen, Centrifugation

286 Effect of selenium supplementation and dietary energy manipulation on mares and their foals: Selenium concentrations and glutathione peroxidase activity. B. J. Karren*[1], J. F. Thorson[2], C. A. Cavinder[1], C. J. Hammer[2], and J. A. Coverdale[1], [1]*Texas A&M University*, *College Station*, [2]*North Dakota State University*, *Fargo*.

To investigate maternal plane of nutrition and role of Se yeast on muscle Se concentration, plasma glutathione peroxidase (Gsh-Px) activity, and colostrum Se concentration in mares and their foals, 28 Quarter Horse mares were utilized in a randomized complete block design. Mares were blocked by expected foaling date and assigned randomly within block to dietary treatments. Dietary treatments were arranged as a 2x2 factorial with two levels of nutrition, pasture or pasture plus grain (fed at 0.75% BW) and two levels of Se supplementation (0 or 0.3 mg/kg BW) equaling four treatment groups: pasture (P), pasture + grain (PG), pasture + grain + Se (PGS), or pasture + Se (PS). P and PS mares received approximately 100% of calculated NRC DE requirements, while PG and PGS received 120%. Selenium supplementation began 110 d prior to estimated foaling (d 0) and all dietary treatments were terminated at parturition. Plasma and muscle were collected on d 0 with plasma continuing every 14 d and muscle every 28 d until parturition. Additionally, BW, BCS, and rump fat measurements were observed every 14 d. At parturition colostrum, foal plasma and muscle samples were collected and continued every 14 d for plasma and 28 d for muscle until day 56. All data were analyzed using PROC MIXED of SAS. Mare BW, BCS, and rump fat was affected by nutrition ($P < 0.02$) but not Se ($P > 0.40$). Mares fed grain (PG and PGS) had greater BW, BCS, and rump fat measurements throughout the trial. Mare plasma, muscle, and colostrum Se concentrations were greater ($P < 0.01$) in mares fed Se (PS and PGS). Mares fed grain (PG and PGS) also had greater plasma Se ($P < 0.01$) than mares fed pasture. Mare and foal plasma Gsh-Px concentrations were unaffected by treatment ($P \geq 0.25$). Foal plasma and muscle Se concentrations were greater when dams were fed supplemental grain ($P = 0.03$, $P = 0.01$ respectively) and supplemental Se ($P = 0.0002$, $P < 0.0001$ respectively). Data indicate maternal plane of nutrition and Se supplementation influences mare and foal plasma, muscle, and colostrum Se concentrations but not Gsh-Px activity.

Key Words: Se, Maternal Nutrition

287 Effect of selenium supplementation and dietary energy manipulation on mares and their foals: Equine colostrum quality and passive transfer of IgG. J. F. Thorson*[1], B. J. Karren[2], M. L. Bauer[1], C. A. Cavinder[2], J. A. Coverdale[2], and C. J. Hammer[1], [1]*North Dakota State University*, *Fargo*, [2]*Texas A&M University*, *College Station*.

To investigate maternal plane of nutrition and role of Se yeast on colostrum quality and passive transfer of Immunoglobulin G (IgG), 28 quarter horse mares were used in a randomized complete block design. Mares were blocked by expected foaling date and assigned randomly within block to dietary treatments. Dietary treatments were arranged as a 2x2 factorial with two levels of nutrition, pasture or pasture + grain (fed at 0.75% BW) and two levels of Se supplementation (0 or 0.3 mg/kg BW). This resulted in four treatments: pasture (P), pasture + Se (PS), pasture + grain (PG), and pasture + grain + Se (PGS). P and PS mares received approximately 100% of calculated NRC DE requirements, while PG and PGS received 120%. Selenium supplementation began 110 d prior to the estimated foaling (d 0) and all dietary treatments were terminated at parturition. Colostrum samples were obtained at parturition for fat, protein, milk urea nitrogen (MUN), somatic cell count (SCC) and IgG analysis. Foal serum samples were collected at 0, 6, 12, 18, and 24 h after parturition for IgG analysis. All data were analyzed using a mixed model of SAS. There was no effect ($P \geq 0.77$) of Se or level of nutrition on foal birth weight. There was also no effect ($P \geq 0.18$) of Se or level of nutrition on colostrum fat, protein, MUN, or SCC. Mares fed grain had lower ($P = 0.03$) colostrum IgG levels (76.46 mg/ml) compared to mares on pasture (126.64 mg/ml). Foals from mares fed grain tended ($P = 0.07$) to have lower serum IgG (13.66 mg/ml) compared to foals from mares on pasture (15.26 mg/ml). In summary, grain supplementation to mares during the last trimester of pregnancy resulted in decreased IgG concentrations in colostrum and tended to lower foal serum IgG. However, volume of colostrum was not measured and thus further studies are needed to determine if changes in colostral IgG are due to alterations in colostral volume.

Key Words: Equine, IgG, Passive Immunity

288 Differential mRNA expression of amino acid transporters in the equine small and large intestine. A. D. Woodward*, S. J. Holcombe, C. Colvin, J. Liesman, and N. L. Trottier, *Michigan State University*, *East Lansing*.

The role of cationic and neutral amino acid absorption in the large intestine of equids is unknown. The objective of this study was to determine the relative abundance of amino acid transporter mRNA in the proximal jejunal mucosa (JM) and left ventral colon musoca (LVM) in the adult horse. It was hypothesized that transporters capable of cationic and neutral amino acid trans-membrane uptake are equally expressed in the JM and LVM of the adult horse. Tissue from the proximal jejunum and large ventral colon was obtained from three adult horses shortly following euthanasia. The mucosa was gently separated from the seromuscular layer, flash-frozen in liquid N, and stored at -80 °C for RNA isolation. Concentration and quality of RNA was determined, and Relative Quantitative Real Time-PCR (Q-RT-PCR) was conducted using amino acid transporter specific primers and the standard curve method. Primers were designed and complementary DNA was synthesized for amino acid transporters $B^{0,+}$ (GenBank, XM001489968), CAT-1 (GenBank, XM001492839), and LAT-2 (GenBank, XM001493818); GAPDH (GenBank, XM001496020) was used as a housekeeping gene. Analysis of variance was done on cycles for the gene of interest adjusted by the cycles for the housekeeping gene. Abundance of the amino acid transporter $B^{0,+}$ mRNA was similarly expressed in the JM compared to the LVM ($P = 0.38$). Compared to the LVM, amino transporter LAT-2 relative abundance was higher ($P < 0.05$) in the JM. The amino acid transporter CAT-1 was expressed in the JM but showed little expression in the LVM ($P < 0.001$). The data indicate both the JM and the LVM may be equally capable of sodium-dependent uptake of neutral amino acids through the $B^{0,+}$ amino acid transporter. Sodium-independent uptake of neutral amino acids through the LAT-2 transporter occurs predominantly in the JM. However, uptake of cationic amino acids via the CAT-1 transporter may occur within the JM but not in the LVM. In conclusion, amino acid transporter mRNA expression will vary depending on location within the intestinal tract of adult horses.

Key Words: Horse, Amino Acid Transporter, PCR

289 Differential gene expression in two segments of the equine intestinal tract using a bovine long oligo microarray. A. D. Woodward*, S. S. Sipkovsky, S. J. Holcombe, J. Liesman, and N. L. Trottier, *Michigan State University*, *East Lansing*.

Gene expression profiling along the equine intestine is critical in order to identify genes related to nutrient digestion and gastrointestinal dysfunction. This study was conducted to 1) determine whether or not equine intestinal tissue hybridizes to a bovine long oligo microarray (BLO), and 2) determine whether or not genes are differentially expressed between the small and large intestinal tract. We hypothesized mRNA expression will be shown on the BLO, and genes of interest will differ in relative abundance between the small and the large intestine. Tissue from the proximal jejunum and large ventral colon was obtained from three adult horses shortly following euthanasia. The mucosa was gently separated from the seromuscular layer, flash-frozen in liquid N, and stored at -80 °C for RNA isolation. Dye-labeled cDNA was synthesized for hybridization to the BLO, which was comprised of 8400 oligos. Expression of mRNA was detected on all three microarrays, indicating equine mRNA hybridized to the BLO. Differences in expression intensity ratios of the jejunal mucosa (JM) compared to the left ventral colon mucosa (LVM) were found for 750 genes ($P \leq 0.05$). Of those, 60 genes showed greater than a three-fold difference in intensity of mRNA expression in the JM compared to the LVM. In regards to genes known to be involved in amino acid transport, mRNA abundance for CAT-3 was higher in the JM compared to the LVM ($P < 0.05$), and tended to be higher for the excitatory amino acid transporter 1 (EAA1, $P = 0.08$). Several amino acid transporters showed similar mRNA expression in both the JM and the LVM. Among these were CAT-7 ($P = 0.41$), LAT-1 ($P = 0.44$), LAT-3 ($P = 0.26$), amino acid transport system N2 ($P = 0.50$) and solute carrier family 3 ($P = 0.70$). These results indicate cationic amino acid uptake may occur predominately in the JM compared to the LVM, while uptake of other amino acids may occur similarly in both the JM and LVM in the adult horse.

Key Words: Horse, Microarray, Amino Acid Transporter