

four trials. Pigs remained in their assigned pens until reaching market weight at approximately 114 kg. BarrierTM was added to the assigned treatment pit on a monthly basis (0.05% vol/vol) for each trial. A 5-phase, separate sex feeding program was used. Corn-soybean meal based diets were formulated to meet or exceed NRC (1998) nutrient requirements. Pig weights and feed disappearance were determined every two wks. Air samples were collected monthly at pit (30 cm above manure surface) and pig level, and from pit fans to measure concentrations of hydrogen sulfide (H₂S), ammonia (NH₃), and volatile organic compounds (VOC). Air samples were also collected at pig level and from pit fans at the beginning, middle, and end of each trial and evaluated for odor utilizing an olfactometer and trained human odor panel. Analysis of variance with repeated measures in time (within each trial) was used to analyze all data. Addition of BarrierTM to manure pits had no effect on ADG, ADFI, G/F, or days on test (P > 0.10). No differences in odor detection threshold, intensity, or persistence between treatments were observed (P > 0.10). H₂S and NH₃ concentrations were numerically reduced in pits treated with BarrierTM, but due to high variability in these measurements and minimal replication, differences were not significant (P > 0.10). Some VOC may have been affected by pit application of this product, but further refinements in procedures for collecting and analyzing these compounds is necessary. These results suggest that the addition of BarrierTM to anaerobic deep pits has no effect on odor or growth performance, but may provide some benefit for reducing H₂S and NH₃ emissions.

Key Words: Swine, Pit Additive, Odor

239 Mathematical evaluation of excess non-essential amino acid nitrogen and sulfur in feed ingredients used in swine diets. J.S. Knott* and G.C. Shurson, *University of Minnesota*.

Minimizing excess dietary nitrogen (N) and sulfur (S) is essential when formulating "environmentally friendly" swine diets. By calculating the amount of non-essential amino acid N and S in feed ingredients, it may be possible to select ingredients to minimize N and S excretion. The objective of this study was to develop a feed ingredient indexing system to rank ingredients based upon the amount of excess non-essential amino acid N and S. Chemical composition of essential amino acids (EAA) and cystine were used to calculate the contribution of N and S to the total molecular weight of each amino acid. Concentrations of EAA and cystine, crude protein, and total S for each ingredient (n = 79) were obtained from NRC (1998). Total N was calculated by dividing crude protein values by 6.25. The sum of N and S contained in EAA and cystine was compared to the sum of the total N and S in each ingredient. Ingredients were ranked on the basis of the amount excess non-essential N and S. The percentage of total N contributed from the EAA and cystine ranged from 26.11% (whey permeate) to 58.07% (blood meal). Based upon our calculations, 41 to 74% of N contained in feed ingredients is in the form of non-essential N and other N compounds. Corn ranked 47th of 79 ingredients and contained 39.82% of the total N

241 The use of haplotype information in the genetic dissection of genes affecting important traits. D. C. Ciobanu*, *Sygen International*.

Modern molecular biology and the science of genomics have opened up new and exciting possibilities to dissect complex phenotypic traits. With the human genome now nearly fully sequenced, there is an intense effort to increase the number of markers, in particular SNPs (Single Nucleotide Polymorphisms) to cover most of the genome. Using this genetic variation will substantially improve our ability to find genes/loci associated with specific diseases or quantitative traits. Multiple SNPs can be organized together in haplotypes. Haplotypes can cover a full gene, a set of genes or a part of a chromosome and carry more information than individual SNPs. In humans, haplotype variants have been shown to be associated with several diseases such as, Crohn disease, Saguenay-Lac-Saint-Jean cytochrome oxidase deficiency or type I diabetes. The construction of a comprehensive haplotype map for the human genome will greatly assist in the dissection of such complex genetic traits. SNP discovery programs are also building momentum in economically important livestock species. SNPs within coding sequences can be used to help map the transcriptome of each species and are particularly useful in

in EAA and cystine. Solvent extracted soybean meal ranked 15th and contained 47.59% of N in EAA and cystine. The percentage of total S contributed from methionine and cystine ranged from 55.4% in bakery waste to 5% in cassava meal. Theoretically, the methionine and cystine cannot contribute more than 100% of the total S in an ingredient. Calculated contributions of S from methionine and cystine exceeded 100% in 22 of the 79 ingredients. Total S values, and perhaps, methionine and cystine values of feed ingredients listed in NRC (1998) need to be re-evaluated and better defined. This information will become increasingly important to nutritionists when selecting ingredients to reduce gaseous emissions and nutrient excretion from swine facilities.

Key Words: Nutrients, Nitrogen, Sulfur

240 Effects of grazing management on sediment and phosphorus in pasture runoff. M.M. Haan*¹, J.R. Russell¹, W. Powers¹, R. Schultz¹, S. Mickelson¹, and J. Kovar², ¹*Iowa State University*, ²*National Soil Tilth Laboratory, Ames, IA*.

Three replications of five 0.4 ha smooth bromegrass paddocks with slopes up to 15° were utilized to determine the effects of grazing management on phosphorus (P) and sediment loss from pastures. Pasture management treatments included an ungrazed control (UG), summer hay harvest with winter stockpiled grazing (HS), continuous stocking to a residual sward height of 5 cm (CS5), and rotational stocking to a residual sward height of 5 cm (RS5) and 10 cm (RS10). Cattle in paddocks with the RS5 and RS10 treatments were removed to allow a 35-d rest period once desired sward heights were reached. Cattle in paddocks with the CS5 treatment were managed with a put and take method to maintain the desired sward height. Grazed paddocks were initially stocked with three mature Angus cows in May 2001. In June, August, and October of 2001 and April 2002, rainfall simulations were conducted at 6 sites within each paddock and 6 sites in a buffer zone down slope from each paddock. Rainfall simulators dripped at 7 cm/h over a 0.5 m² area for 1.5 h. Runoff was collected and analyzed for sediment and total and dissolved P. Simultaneous to each rainfall simulation, slope, ground cover, sward height and mass; and soil penetration resistance, surface roughness, and P and moisture concentrations were measured. Annual carrying capacities for paddocks with the UG, HS, CS5, RS5, and RS10 treatments were 0, 8, 81, 62, and 47 cow-days/ha (P < 0.01). Slope and pasture treatment in paddocks did not significantly affect the concentration of sediment in runoff. However, concentrations and amounts of total P in runoff from grazed paddocks were greater (P < 0.05) than UG and HS paddocks. Concentrations and amounts of total P in runoff from paddocks with the RS10 treatment were lower (P < 0.05) than paddocks with the RS5 treatment. The amounts of sediment and total P in runoff from paddocks that were grazed were greater (P < 0.05) than their respective buffers. Increasing the amounts of residual forage in pastures will reduce total P loss from pastures.

Key Words: Grazing, Water Quality, Phosphorus

Physiology

identifying the genes responsible for phenotypic variation in traits with important economic impact. Recently, new economically important alleles were found in the porcine PRKAG3 (protein kinase, AMP-activated, gamma 3 subunit) gene that affect glycogen content in the muscle and the resulting pork quality. In this study, haplotype analysis revealed the effects of PRKAG3 substitutions much more clearly than the analysis of individual SNPs. While single SNPs and haplotypes are both useful in the analysis of complex traits and the identification of associated genetic variation, the use of haplotypes is found to be more powerful.

Key Words: Genome, Haplotype, SNP

242 Protein identification using mass spectrometry. R. L. Cerny*, *University of Nebraska - Lincoln*.

Protein identification is a key component in the rapidly expanding area of proteomics. The mass spectrometry facility at the University of Nebraska has been involved with the identification of proteins for the past three years. Proteins isolated using both one and two dimensional gel

electrophoresis and visualized with Coomassie, Sypro Ruby (fluorescent dye), or silver staining have been analyzed. The identification protocol entails excision of the visualized protein, in-gel digestion, and peptide extraction. Two different mass spectrometric approaches have been used to characterize the proteins.

The first method is peptide mapping. This requires high resolution exact mass measurements of the tryptic peptides produced in the digestion; these data are normally obtained using MALDI-TOF mass spectrometry. The peptide masses are then searched against a database of theoretical masses of tryptic peptides of known proteins to make the identification. The second method is based on determining the partial sequence of the individual tryptic peptides using tandem mass spectrometry. This method combines nanoflow HPLC with electrospray ionization (ESI). In our facility, these experiments are performed on a hybrid mass spectrometer, the Micromass Q-TOF. This approach provides sequence information on a number of peptides originating from throughout the protein. The data produced can again be searched against known protein databases. This method has a major advantage in situations where the protein being analyzed does not have a corresponding entry in a database. Since actual sequence information is obtained, searches can be made against EST databases as well. If there is still no identification, de novo sequencing can provide a number of partial sequences that can be used to perform BLAST searches for homologies or to develop PCR primers.

These two approaches have been used to identify proteins from a wide variety of sources. These studies include the identification of proteins found in maize mitochondria, tomato viruses, human cancer cells, rat and mouse model systems, and bovine and porcine muscle tissue and reproductive systems. Examples will be presented that demonstrate how the two approaches have been used.

Key Words: Proteomics, Mass Spectrometry, Protein Identification

243 Transport, reservoirs, and fertile life of boar spermatozoa in the female tract. R.H.F. Hunter*, *Institute for Reproductive Medicine, Hannover School of Veterinary Medicine, Germany.*

Sperm transport in gilts and sows will be considered in terms of a uterine phase and then a more complex phase in the Fallopian tubes. An overriding requirement of successful transport in the uterus is that both utero-tubal junctions should be bathed by a sperm suspension for a time sufficient to permit formation of sperm reservoirs in the Fallopian tubes. The edematous processes of the utero-tubal junction present a formidable barrier to seminal plasma and spermatozoa. Spermatozoa negotiate this junction by swimming between the engorged papillae, thereby losing male secretions from their surface. Seminal plasma does not pass beyond the junction in bulk, nor can it be forced through under pressure. Sufficient spermatozoa to fertilize a proportion of the eggs may enter the Fallopian tubes within 15 mins of a pre-ovulatory mating, but 1-2 hours are required for enough spermatozoa to enter the tubes subsequently to fertilize 90-100% of the eggs. Thereafter, seminal contents and attendant polymorphs are largely voided from the uterine lumen. The extent of absorption of seminal plasma from the uterus still requires clarification. Fertilizing spermatozoa are arrested and stored in the caudal 1-2 cm of the isthmus before ovulation, binding to microvilli and/or cilia whilst under the influence of viscous glycoprotein secretions. Peri-ovulatory release of spermatozoa is tightly-controlled, such that initial sperm:egg ratios at the ampullary-isthmus junction are close to unity. However, this ratio changes within an hour of egg activation, but too late to compromise the block to polyspermy established in the inner region of the zona pellucida. Some 200-400 or more sperm heads may be found in the zona substance by the 4-cell stage. Completion of capacitation is suppressed during pre-ovulatory storage and only finally achieved in the fertilizing spermatozoa close to the time of ovulation. Ovarian follicular secretions, acting via local counter-current vascular transfer as well as systemically, act to regulate isthmus and sperm physiology.

Key Words: Spermatozoa, Uterus, Ovary

244 Deep uterine and uterine body insemination techniques in swine and the relationship of site of insemination to the required number of spermatozoa per inseminate. E. A. Martinez*, J. Roca, and J. M. Vazquez, *University of Murcia, Spain.*

Current procedures of pig artificial insemination (AI) usually employ 2.5-4 billion spermatozoa per insemination in a large volume of liquid

(70-100 ml), which are deposited through the cervix into the uterus at insemination. Important advances are being achieved in the development of new aspects of the AI technique (i.e., improvement of extenders, tests to evaluate the viability and fertilizing ability of spermatozoa, strategies to determine the time of ovulation, etc.). However, the current requirements of volume and sperm dosage to obtain optimal fertility after standard AI still are similar to those recommended since the inception of pig AI. It is now known that a 100-fold reduction of the standard pig AI dose can be used when spermatozoa are surgically deposited next to the uterotubal junction. From these results, technical solutions for non-surgical deep uterine insemination in pigs were claimed. However, the complex anatomy of the swine genital tract, mainly the cervical canal and the length and coiled nature of the uterine horns, was the principal obstacle to development of new procedures to inseminate non-surgically into the uterus. Recently, different devices have been developed to deposit non-surgically the semen in the uterine body (uterine body insemination) or anterior third of a uterine horn (deep uterine insemination, DUI). There is little information available about the relationship between the site of insemination and the required number of spermatozoa per inseminate. Published data show that in comparison with standard AI, a 3-fold reduction or a 20-fold reduction in the number of spermatozoa inseminated can be achieved without decreasing fertility when semen is deposited into the uterine body or into the upper first third of one uterine horn, respectively. In conclusion, uterine insemination technology might have a high impact on the fresh semen AI industry by decreasing the number of boars that are used. Selection of the boars could be more rigorous and only boars that have high genetic value would be included in the AI centers. In addition, DUI technology could assist in the adoption of other biotechnologies such as frozen semen, flow sorted sperm or non-surgical embryo transfer.

Key Words: Uterine Insemination, Fresh Semen, Fertility

245 Regulating the pig estrous cycle to utilize new insemination technologies. S.K. Webel*, *United Feeds, Inc.*

Researchers have focused on methods to control the estrous cycle and time of ovulation to facilitate scheduled breeding for several decades. This presentation reviews previous research and summarizes current available techniques. Natural methods such as boar exposure, gilt management and weaning management are well documented and routinely used by swine producers. Chemical or hormonal control has focused on controlling the luteal phase or stimulating follicular development. Progesterone or synthetic progestagens have been injected, fed and administered as subcutaneous or vaginal implants. Daily injections of progesterone inhibits estrus and with adequate dosing, results in normal fertility. Several synthetic progestagens administered orally or by injection inhibit follicular growth and estrus, but in many cases result in poor synchronization, increased incidence of cystic follicles and lower fertility. A unique progestagen, Altrenogest (Regumate™) has been shown to be very effective for suppressing estrus, and produces a high level of synchronization with resultant normal fertility. Shortening the luteal phase by injection of prostaglandin has limited practical value because corpora lutea (CL) are not susceptible to lysis prior to day 12 of the cycle. However, prostaglandins may have application for regressing accessory CL or those whose lifespan has been prolonged by estrogen treatment and for premature termination of pregnancy. Effective control of the estrous cycle has been achieved with a non-steroidal compound ICI 33828, but research was curtailed following identification of teratogenic properties. Follicular development and ovulation can be stimulated with pituitary gonadotrophic preparations, PMSG, hCG, GnRH or combinations of these hormones. Follicular development can be stimulated in prepubertal gilts, anestrous sows, lactating sows and following suppression of the estrous cycle with progestagens. Following follicular stimulation, ovulation can be induced by injection of HCG or GnRH. The most readily available and effective means for regulating the pig estrous cycle is to feed Altrenogest to suppress and regulate estrus and a combination of PMSG and hCG (PG-600) to promote follicular development.

Key Words: Pig Estrus Control, Follicle Stimulation

246 Anesthetic suppression of LH concentrations in barrows. J. A. Clapper*, *South Dakota State University.*

An anesthetic consisting of 50 mg/ml each of tiletamine, zolazepam, ketamine, and xylazine (TKX), has been used for short duration surgeries

in pigs, but its effects on LH concentrations are unknown. To determine if administration of TKX to pigs decreases LH concentrations and induces a stress response, as determined by an increase in serum concentrations of cortisol, the following experiment was performed. On d 1, 8 gilts and 6 barrows of similar weight (75 kg) were anesthetized with TKX (1 ml/22.5 kg BW i.m.) and indwelling jugular catheters were inserted. On d 6, blood samples were taken every 20 min for 4 h, then pigs were anesthetized with TKX and blood samples were taken every 20 min for 4 h. On d 8, blood samples were collected every 20 min for 4 h, then pigs were anesthetized with sodium thiopental (PEN; 8.9 mg/kg BW i.v.) and blood samples were collected every 20 min for 4 h. Serum concentrations of LH and cortisol were determined by RIA. Mean serum concentrations of LH and cortisol were each similar ($P > .05$) in barrows the 4 h before administration of TKX (pre-TKX). Mean serum concentrations of LH decreased ($P < .05$) by 40 min post administration of TKX and remained less compared to pre-TKX for 180 min. Mean serum concentrations of cortisol increased ($P < .05$) within 20 min of administration of TKX and remained elevated compared to pre-TKX concentrations for 180 min. Mean serum concentrations of LH did not differ ($P > .05$) in gilts before and after administration of TKX. Mean serum concentrations of cortisol increased ($P < .05$) within 20 min of administration of TKX in gilts and remained elevated for 140 min. Mean serum concentrations of LH and cortisol were each similar ($P > .05$) within barrows and gilts before and after administration of PEN on d 8. Administration of TKX to barrows and gilts increased serum concentrations of cortisol but transient decreases in serum concentrations of LH were observed only in barrows. These data indicate that gonadal status may influence the ability of TKX to alter LH concentrations.

Key Words: Pigs, LH, Cortisol

247 Increasing the holding time of boar spermatozoa at 15° C before freezing decreases fertility. H. David Guthrie* and G.R. Welch, *USDA, Beltsville Agricultural Research Center, Germplasm & Gamete Physiology Laboratory.*

An experiment was conducted to determine the effect of pre-freeze holding time and commercial extender type on boar spermatozoa viability and fertility. One ejaculate from each of six boars was held in two commercial boar semen extenders, Beltsville Thawing Solution and Androhep Plus, for either 3 or 24 h at 15° C to simulate overnight shipment, and then frozen in 0.5- and 5-mL straws. The spermatozoa thawed from the 5-mL straws were used for artificial insemination (AI) of 31 gilts and sows within 4 h before the expected time of ovulation induced by injection of 750 IU of hCG 130 h after the last feeding of Regu-Mate for estrus synchronization. The pregnancy rate 23 days after AI favored the 3 h compared to the 24 h holding time, 75 and 53%, respectively; but the difference was not significant. However, embryo number per animal and embryo survival percentage were decreased from 15 to 9 ($p = 0.0305$) and from 73 and 46% ($p = 0.0015$), respectively, as holding time at 15° C increased from 3 to 24 h. Pre-freeze and post-thaw sperm motility, plasma membrane integrity, and acrosome morphology were of little value in predicting the decrease in sperm fertility after increasing holding time from 3 to 24 h. These results are important because they indicate that fertility of thawed sperm will be reduced after overnight shipping of liquid semen to another location before cryopreservation, under conditions in which sperm would be expected to remain fertile in liquid form.

Key Words: Cryopreservation, Swine, Embryo Survival

248 Timed artificial insemination of beef heifers using a 7-11/Co-synch protocol. A. W. Thompson*¹, C. D. Holladay, and D. M. Grieger¹, *¹Kansas State University.*

Previous research has shown that an estrus synchronization program using a short period of feeding MGA in conjunction with a Co-synch protocol (7-11 Synch) was effective in synchronizing estrus in postpartum beef cows. The objective of this study was to test this synchronization protocol with yearling beef heifers in comparison to the Co-synch protocol using timed artificial insemination (A.I.). Fifty-eight commercial beef heifers from the Kansas State University Commercial Cow-Calf herd were used. Heifers were split into two groups; 28 heifers received the Co-synch protocol and 30 heifers were placed into the 7-11/Co-synch protocol. Beginning on day 1, heifers in the 7-11/Co-synch treatment were fed MGA (0.5mg/animal/d) individually for 7 days. On day 7, the last day of MGA feeding, heifers were injected with prostaglandin F₂α

(PGF). On day 11, all 58 heifers were started on the Co-synch protocol by receiving an injection of GnRH (100mg). One week later on day 18, all heifers received an injection of PGF. On day 20, all heifers were given a shot of GnRH (100mg) and artificially inseminated by a single technician. Ultrasonography was used to determine pregnancy status 29 days post-breeding. A greater ($p < .01$) percentage of heifers were pregnant in the 7-11/Co-synch treatment (66%) as compared to the Co-synch treatment alone (31%). This study demonstrates the potential of using a short MGA feeding period prior to Co-synch treatment to obtain acceptable pregnancy rates using timed A.I. in beef heifers.

Key Words: Heifer, Timed AI, Synchronization

249 Comparison of long-term and short-term MGA-Select for estrus synchronization in beef cows. J.D. Arseneau*¹, M.C. Claeys¹, J.M. DeJarnette², and R.P. Lemenager¹, *¹Purdue University, West Lafayette, IN, USA, ²Select Sires, Inc., Plain City, OH, USA.*

Multiparous ($n=100$; 42.2 ± 10.4 d postpartum) and primiparous ($n=44$; 90.8 ± 10.1 d postpartum) suckled beef cows were used in a study to compare the efficacy of a long-term MGA-Select to a short-term MGA-Select estrus synchronization program. Cows were randomly assigned on d 0 by age and days postpartum to two treatments: 1) 0.5 mg·hd⁻¹·d⁻¹ MGA d 1 to 14; 86 µg GnRH d 26; 25 mg PGF2α d 33 (Long-MGA), or 2) 86 µg GnRH d 26; 0.5 mg·hd⁻¹·d⁻¹ MGA d 27 to 32; 25 mg PGF2α d 33 (Short-MGA). Cows observed in estrus following PGF2α were inseminated approximately 12 h later. All cows not detected in estrus were fixed-time inseminated 72 to 75 h after PGF2α, and given a second injection of GnRH at the time of insemination. Pregnancy diagnosis was determined by ultrasonography 40 d after insemination. Data were analyzed separately for multiparous and primiparous cows. A greater percentage of multiparous cows tended ($P=0.07$) to exhibit estrus following Short-MGA (54.0%) compared to Long-MGA (36.0%). There was not a statistical difference between treatments for percentage of primiparous cows detected in estrus following synchronization (22.7% and 31.8% for Long-MGA and Short-MGA, respectively). Similar pregnancy rates were obtained between treatments from insemination following detected estrus. However, pregnancy rates from fixed time inseminations were greater for Long-MGA than Short-MGA (Multiparous; 65.6% and 34.8%, $P < 0.02$; Primiparous; 77.3% and 40.9%, $P < 0.01$). Overall pregnancy rates to insemination (estrus and time inseminations) for multiparous cows were similar ($P=0.30$) between Long-MGA (68.0%) and Short-MGA (58.0%). A greater ($P < 0.01$) percentage of primiparous cows conceived to insemination (estrus and time inseminations) following Long-MGA (77.3%) compared to Short-MGA (40.9%). In summary, although overall percentage of cows observed in estrus following synchronization was low in this study, a greater percentage of multiparous cows were detected in estrus following Short-term MGA-Select than Long-term MGA-Select. However, in a timed AI system, greater pregnancy rates were obtained from a 14 d MGA-Select regimen than a 6 d MGA-Select.

Key Words: Estrus Synchronization, MGA-Select, Beef Cows

250 Microarray analysis of gene expression in dominant ovarian follicles (DF) from heat stress (HS) and thermoneutral (TN) heifers. S.J. Kolath*¹, P.M. Coussens², S. Sipkovsky², S.J. Wilson¹, D.E. Spiers¹, J.N. Spain¹, and M.C. Lucy¹, *¹University of Missouri, ²Michigan State University.*

Heat stress decreases ovarian follicular growth and causes infertility in cattle. The objective was to compare gene expression in DF from heifers exposed to HS or TN environments. Estrous cycles were synchronized (estrus = d 0) in six Holstein heifers randomly assigned to HS ($n=3$) or TN ($n=3$) treatment. On d 5, heifers were moved into environmental chambers (19°C). Beginning on d 10, the ambient temperature for HS heifers was increased 3°C per day to 33°C on d 14. Ambient temperature for TN heifers remained at 19°C. Heat stress was coincident with the second follicular wave. On d 15, heifers were ovarioectomized and the second wave DF was collected (diameter = 12.0 ± 7 mm). Messenger RNA was isolated, reverse transcribed into cDNA, and labeled with either Cy3 or Cy5 for the purpose of hybridization to the BOTL microarray (1277 genes) from the Center for Animal Functional Genomics (<http://gowhite.ans.msu.edu>). A dye reversal design was employed in which HS and TN heifers were paired ($n=3$ pairs). A total of six microarrays were analyzed. Fluorescence was measured and normalized

using the GenePix 4000B scanner with GenePix Pro 4.0 software (Axon Instruments Inc., Union City, CA). Mean fluorescence intensity for each gene was analyzed for the effects of treatment, heifer (treatment), and replicate. Candidate genes had Cy5/Cy3 ratios that differed for HS and TN heifers ($P<.05$). Of the 35 candidate genes, 30 were increased in response to HS whereas 5 were decreased. Genes increased in HS heifers were transcription factors (TAF6, CEBPB, NFE2L2), DNA binding proteins (TOP3B, DZIP1), RNA binding proteins (GRSF1), and apoptosis-related factors (DAP, SIVA). Some transcription factors (LIM04, Tex27, KLF2) were decreased in HS heifers. DF respond to heat stress by down-regulating some transcription factors while up-regulating others. Up-regulation of transcription factors as well as DNA/RNA binding proteins may counteract apoptotic events caused by HS.

Key Words: Microarray, Ovary, Heat stress

251 Development of Pig Embryos in Culture Media Containing HEPES: Preliminary Results. J.N. Caamano*¹, G.M. Wu¹, T.C. McCauley¹, T.C. Cantley¹, A.R. Rieke¹, J. Mao¹, R. Farwell¹, B.A. Didion², C. Murphy¹, and B.N. Day¹, ¹University of Missouri, Columbia MO, ²Monsanto, St. Louis, Mo.

Recent advances in non-surgical embryo transfer in swine will require the development of a practical culture system that could be used under field conditions. The objective of this study was to assess the effect of culture medium containing HEPES in the development of pig embryos produced *in vitro* and *in vivo*. In Experiment 1, day 4 morulae produced *in vitro* (IVM/IVF/EC) were randomly allocated to 3 treatments and cultured for 48 hours. Embryos ($n=10$) were loaded into 0.25 ml straw (experimental unit) and placed in a portable incubator. Embryos were placed in TALP-HEPES-0.4% BSA (Group 1), modified NCSU-23 with reduced amount of NaHCO₃ [2mM] and the addition of 10 mM HEPES (Group 2). As a control (group 3), embryos were cultured in NCSU-23 under standard culture conditions (Nunc Dish, 5% CO₂ in air). In Experiment 2, embryos collected from donor gilts on day 4 after the onset of estrus (4-cell embryos) were cultured in modified NCSU-23 in 0.25 ml straws (mNCSU-straw; $n=15$) and in 1.5 ml microtubes (mNCSU-tubes; $n=15$). A group of control embryos were cultured in NCSU-23 under standard culture conditions ($n=11$). Blastocyst formation and embryonic cell number were assessed in each experiment. Blastocyst formation rates and embryonic cell numbers were analyzed using SAS-GLM procedure. In Experiment 1, blastocyst formation rate was higher ($P<0.01$) in embryos cultured as controls NCSU-23 ($69.0 \pm 5.3\%$) than in TALP-HEPES ($33.4 \pm 5.7\%$) or mNCSU-HEPES ($30.4 \pm 5.3\%$). Blastocyst cell numbers were also higher ($P<0.01$) in the control group than in the media containing HEPES. In Experiment 2, treatment did not ($P>0.64$) affect blastocyst formation rate, which was $90 \pm 10.0\%$, $90 \pm 10.0\%$ and $100 \pm 0.0\%$ for mNCSU-straw, mNCSU-tube and control, respectively. Embryonic cell numbers were higher ($P<0.01$) in embryos cultured in NCSU-23 (54.1 ± 2.1) than in the mNCSU in straws (32.9 ± 1.7) or microtubes (32.5 ± 1.7).

Key Words: Embryos, Culture Media, Swine

252 Endocrine responses to short-term feed deprivation in weanling pigs. B.E. Salfen* and J.A. Carroll, *Animal Physiology Research Unit, Agricultural Research Service-USDA, Columbia, Missouri.*

Our objective was to determine endocrine responses to a short-term (24 h) feed deprivation period in weaned pigs. Eight crossbred male pigs were weaned at 18 d of age and placed on a complex nursery diet. At 4 d post-weaning, pigs were non-surgically fitted with an indwelling jugular vein catheter. Pigs in the feed deprived group (FD; $n=4$) had feed removed 5 d post-weaning, and feed was returned 24 h later. Control pigs (Con; $n=4$) were allowed *ad libitum* access to feed. Blood samples were collected at 0 h, 6 h, 12 h, 24 h and 30 h for determination of ghrelin (GR) and IGF-I. Four sub-samples were collected at each time point at 15 min intervals. Body weights (BW) of pigs were determined at weaning, 21 d of age and after the 30 h time point (24 d of age). There was a time X treatment difference in BW ($P<0.05$). FD pigs gained less weight throughout the experiment than Con pigs (0.67 ± 0.34 vs. 0.32 ± 0.16 kg; $P<0.06$). There was no difference in serum GR or IGF-I among the four sub-samples ($P>0.90$), therefore the mean of the sub-samples were utilized for statistical analysis. Serum GR at T0 tended to be different between treatments ($P=0.06$), therefore GR concentrations of each pig at various time points were expressed relative to T0. There was

a time X treatment effect on GR concentrations ($P<0.01$). Serum GR in FD pigs tended to decrease 12 h compared to Con pigs ($P=0.08$). Relative concentrations of GR increased from 12 h to 24 h ($P<0.01$) and remained elevated through the 30 h time point in the FD treatment, but remained unchanged at all time points in Con pigs ($P=0.91$). There was a time X treatment effect on IGF-I ($P<0.001$). Serum IGF-I fell throughout the experiment and was lower in FD pigs at 12 h, 24 h and 30 h compared to Con pigs ($P<0.05$). Serum IGF-I increased following the return of feed at 30 h compared to 24 h ($P<0.06$). IGF-I concentrations rose throughout the study period in Con pigs ($P<0.01$). These results indicate that serum GR and IGF-I are influenced by short-term feed deprivation in weanling pigs.

Key Words: Piglet, Food Deprivation, Weaning

253 A model for long term evaluation of bioavailability and activity of value added dietary components using fistulated, multiple catheterized pigs. C.F. Shipley*¹, M.V. Eberhardt, K.M. Clements, M.C. Black, H.Y.M. Elshire, M.E. Tumbleson, and V.L. Jarrell, ¹University of Illinois, Urbana, Illinois.

Added values from agricultural products open markets to consumers interested in improved health. Health claims associated with bioactive components cite little evidence of bioavailability, bioactivity or safety and efficacy. Our objective was to develop an animal model for evaluating dietary components over 10 or more weeks of exposure. We modified techniques independently used to collect total feces and urine, sample digesta cranial to the cecum and prior to microbial metabolism in the lower gut (fistulated distal ileum), sample metabolites from blood prior to hepatic processing (catheterization of the hepatic portal vein) and sample metabolites in systemic blood following delivery to tissues (catheterization of the jugular vein). Eight crossbred Yorkshire barrows, body weight = 24.5 ± 2.7 kg, were used. Nonsurvival surgeries were conducted on 2 animals at 22 and 20 kg body weight. Catheters were inserted 20 cm into the jugular vein. Catheters were inserted 35 cm via the mesenteric vein into the hepatic portal vein. Catheters were recovered and placement confirmed post mortem. Catheter lengths were then adjusted to 13 and 15 cm, respectively, prior to conducting survival procedures on the remaining animals. Six animals were housed in metabolic crates such that feed intake, total feces and urine could be assessed. A 2 week post surgical period allowing acclimation to purified diet was followed by a 10 week trial. Vascular catheters were flushed 2X daily and more frequently during timed sampling periods. Three of 6 catheters placed in hepatic portal veins were removed from live pigs at 28, 33 and 38 days post-surgery. These animals remained in the study to completion. The loss of hepatic portal catheters was attributed to breakdown of adhesive at mesh anchors and peristaltic action of the small intestine. All 9 of 12 remaining vascular catheters were patent to the end of the study at 77 to 83 days. All 6 fistulas remained patent. Animals had attained an average body weight of 100.4 ± 5.4 kg with an average rate of gain of 0.93 ± 0.07 kg/d. We conclude that fistulated, multiple catheterized pigs can be used to evaluate long term effects of dietary components.

Key Words: Bioavailability, Pigs, Dietary Components

254 Comparison of gravid uterine parameters in naturally bred ewes and ewes after transfer of *in vitro* produced embryos, and in single, twin and triplet pregnancies. D. Pant*¹, J.T. Choi, J.S. Luther, P. Borowicz, R.M. Weigl, J.D. Kirsch, K.C. Kraft, D.A. Redmer, L.P. Reynolds, and A.T. Grazul-Bilska, *North Dakota State University.*

Large offspring syndrome may be initiated in ovine embryos after exposure to *in vitro* conditions. Various parameters of the gravid uterus on day 140 after fertilization were compared: (i) in ewes ($n=4$) bred naturally, and in ewes ($n=18$) after transfer of *in vitro* produced embryos, and (ii) in single, twin and triplet pregnancies after transfer of *in vitro* produced embryos. On day 5 after *in vitro* fertilization, two to three embryos with 16 or more cells were transferred to recipient ewes that were at day 5 after estrus. On day 140 of pregnancy, the reproductive tract was collected from all ewes and the following parameters were determined: the number, sex and weight of fetuses, weight of uterus and fetal membranes, weight and numbers of placentomes. Weight of fetuses was similar for naturally bred ewes and ewes after embryo transfer (ET), but the weight of individual placentomes was greater ($P<0.05$) for ewes after ET (8.5 ± 5 g) than for naturally bred ewes (5.4 ± 9 g) and the total

weight of placentomes/ewe tended to be greater ($P < 0.1$) for ewes after ET (0.66 ± 0.05 kg) than for the naturally bred ewes (0.48 ± 0.03 kg). The number of placentomes/ewe was less ($P < 0.01$) for single pregnancies (67.0 ± 2) than for twin (87.0 ± 1) or triplet (85.7 ± 7) pregnancies. The number of placentomes/fetus was greater ($P < 0.01$) for single (67.0 ± 2), less for twin (43.5 ± 0) and least for triplet (28.5 ± 2) pregnancies. However, the weight of each individual placentome was greater ($P < 0.06$) for triplet (11.2 ± 2 g) than for twin (8.3 ± 8 g) or single (7.7 ± 5 g) pregnancies. For single, twin and triplet pregnancies, the number of fetuses per ewe was negatively correlated with the weight of placentomes/fetus ($r^2 = -0.65$; $P < 0.003$), the number of placentomes/fetus ($r^2 = -0.86$; $P < 0.001$) and the weight of the fetus ($r^2 = -0.80$; $P < 0.001$), and positively correlated with the weight of individual placentome ($r^2 = +0.50$; $P < 0.03$). These data demonstrate the lack of LOS, indicating that culture conditions were optimal for production of ovine embryos. The low number of placentomes/fetus seen in multiple pregnancies appears to be compensated for by the increase in the total number of placentomes and the weight of each placentome.

Key Words: Large Offspring Syndrome, Ewe, In Vitro Fertilization

255 Impact of thermal environment and spray-dried plasma on the acute phase response of pigs challenged with lipopolysaccharide (LPS). J. W. Frank^{*1}, J. A. Carroll², G. L. Allee¹, and M. E. Zannelli³, ¹University of Missouri, ²Animal Physiology Research Unit, Agricultural Research Service, USDA, Columbia, MO, ³Simple Solutions, Attleboro, MA.

Forty barrows (TR4 × C22) were weaned at 17 d of age (6.27 ± 0.30 kg) and housed 2 pigs/pen in a thermal neutral environment (TN: constant 26.7°C) and fed diets with or without 7% spray-dried plasma (SDP). On d 7, one pig/pen was moved into a cold environment (CE: constant 15.6°C). Pigs were fitted with jugular catheters on d 11. The following day, 16 pigs/environment (8 pigs/dietary treatment) were challenged with $75 \mu\text{g}/\text{kg}$ of BW of LPS. Blood samples were collected over a 4.5-h period, then pigs were sacrificed for tissue sample collection. Pigs housed in the CE consumed more feed and had lower gain to feed ratios than pigs housed in TN from d 7 to 11 ($P < 0.001$). There were no environment by diet interactions from d 7 to 11 ($P > 0.78$). Basal serum ACTH and cortisol (CS) were lower in TN pigs compared to CE pigs ($P < 0.001$). Pigs fed diets without SDP had lower serum CS over the 4.5-h period compared to pigs fed SDP (time × diet, $P < 0.001$). Serum tumor necrosis factor- α were highest for pigs consuming SDP in the CE, while there were no differences among the other treatments (time × diet × environment, $P < 0.02$). Pigs housed in the CE had higher serum interleukin-1 β (IL-1) and interleukin-6 (IL-6) compared to TN pigs ($P < 0.001$). Furthermore, pigs consuming SDP also had slightly higher serum IL-1 ($P < 0.10$) and significantly higher IL-6 compared to the pigs fed no SDP. Overall there were no consistent interactions between feeding SDP and thermal environment. Pigs consuming diets containing SDP and subsequently challenged with LPS responded with elevated serum concentrations of CS and cytokines compared to pigs fed diets with no SDP. Housing pigs in a CE increased the basal ACTH and CS, and when challenged with LPS resulted in elevated serum cytokines. Pigs consuming SDP and exposed to an endotoxin challenge that circumvents mucosal immune protection become immunologically hyper-responsive.

Key Words: Pigs, Cytokines, Spray-dried Plasma

256 Immune response in *Escherichia coli* challenged pigs exhibiting a febrile response in absence of elevated TNF- α . T. A. Strauch^{*1}, J. A. Carroll¹, T. J. Fangman², C. E. Wiedmeyer², and A. K. Hambach², ¹Animal Physiology Research Unit, ARS-USDA, Columbia MO, ²College of Veterinary Medicine, University of Missouri, Columbia, MO.

The objective of this study was to determine the immune response in pigs challenged with *E. coli*. Twenty-two single sourced, 24-d old crossbred pigs (5.44 kg) were utilized. Pigs were individually housed, provided *ad libitum* feed and water, and assigned to blood collection ($n=10$) or rectal temperature measurement ($n=12$) groups. Blood collection pigs were non-surgically cannulated one day prior to blood collection. At 0 hr, all pigs received a 10 mL dose of 2.4×10^8 colony-forming units of *E. coli* K88 via a nasogastric tube. Blood collection and rectal temperature monitoring occurred hourly from -1 to 5 hr post-*E. coli*, every

30 minutes from 5 to 8 hrs, and at 24 hrs post-*E. coli*. Serum concentrations of cortisol (CS), interferon- γ (IFN), tumor necrosis factor- α (TNF), interleukin-1 β (IL-1), interleukin-6 (IL-6), serum amyloid A (SAA), C-reactive protein (CRP), haptoglobin (HG), and lipopolysaccharide (LPS) were measured. After *E. coli* administration, CS, IFN, IL-1, IL-6, SAA, CRP, HG ($P < 0.0001$), and LPS ($P < 0.002$) increased. Peak concentrations of CS and IL-6 occurred at 4 hr post-*E. coli*, IFN and rectal temperature peaked at 5.5 hr post-*E. coli*, and IL-1 peaked at 6 hr post-*E. coli*. Serum concentrations of TNF were undetectable throughout the sampling period. The greatest circulating concentrations of LPS were detected at 7.5 hr post-*E. coli*, and the acute phase proteins CRP, HG, and SAA were still increasing at 24 hr post-*E. coli*. While TNF was not associated with increased rectal temperature, positive correlations existed between rectal temperature and CS ($r = .49$; $P < 0.0001$), IFN ($r = .29$; $P < 0.0009$), IL-1 ($r = .45$; 0.0001), IL-6 ($r = .31$; $P < 0.0003$), and LPS ($r = .29$; $P < 0.0009$). These data indicate that circulating TNF does not play an integral role in initiating the febrile response in pigs challenged with *E. coli*; but that CS, IL-6, or IFN may act as endogenous pyrogens.

Key Words: *E. coli*, Cytokines, Acute Phase Proteins

257 Factors affecting temporal characteristics of estrus and ovulation on commercial sow farms. B. A. Belstra^{*}, W. L. Flowers, and M. T. See, North Carolina State University.

Elucidation of factors that affect estrus and ovulation characteristics may allow refinement of artificial insemination protocols and increased reproductive efficiency. Our objective was to examine the effects of season (spring, summer), genotype (A, B, C, D), parity (1, 2, 3, ≥ 4), lactation length (≤ 13 , 14 to 15, 16 to 17, 18 to 19, ≥ 20 d), and weaning-to-estrus interval (WEI; 3, 4, 5, 6, ≥ 7 d) on duration of estrus (DE) and onset of estrus-to-ovulation interval (EOI) in three sow farms. DE and EOI were estimated via boar exposure and transabdominal real-time ultrasonography, respectively, every 6 h from 2 to 10 d postweaning in 533 sows (89/season). Sows weaned in the spring (March, April) compared to the summer (July, August) had a shorter DE on farms 1 (52.7 vs. 60.5 h; $P < .001$) and 3 (47.3 vs. 55.8 h; $P < .001$) but only tended to have a shorter DE on farm 2 (52.1 vs. 54.5 h; $P < .10$). Similarly, EOI was shorter for sows weaned in the spring compared to the summer on farms 1 (37.9 vs. 48.1 h; $P < .001$) and 3 (35.4 vs. 42.7 h; $P < .001$) but not on farm 2 (38.1 vs. 40.4 h; $P > .11$). Multiple genotypes were only present on farms 1 (A, B, C) and 3 (C, D). DE was not different between genotypes on farms 1 or 3 ($P > .14$) but EOI was shorter for genotype B compared to either A or C on farm 1 (39.6 vs. 44.8 or 44.3 h; $P < .04$). Even after correction for differences in WEI between the parity classes, parity 1 sows tended to have a shorter DE and had a shorter EOI than sows ≥ 4 parities (52.3 vs. 54.6 h; $P < .07$ and 38.7 vs. 42.8 h; $P < .002$, respectively). As lactation length increased there tended to be a linear decrease of DE (56.2, 56.3, 54.0, 53.3, 52.9 h; $P < .08$) and EOI (43.7, 41.4, 40.4, 40.5, 39.4 h; $P < .09$). As WEI increased there was a linear decrease of DE (64.4, 57.5, 52.8, 49.7, 44.8 h; $P < .001$) and EOI (48.6, 44.1, 40.4, 37.1, 32.0 h; $P < .001$). These data suggest that in addition to weaning-to-estrus interval, season in particular can have a substantial affect on duration of estrus and time of ovulation within and between farms.

Key Words: Estrus, Ovulation, Sow

258 Ovarian follicular populations before weaning in sows are dependent on FSH. C.J. Bracken^{*}, B.L. McCormack, T.C. Cantley, R.P. Radcliff, and M.C. Lucy, University of Missouri.

The factors affecting follicular growth and the variation in weaning to estrus and weaning to ovulation intervals in sows are poorly understood. The objective was to determine if follicular populations before weaning in sows are dependent on FSH. The posterior vena cava anterior to the ovarian vein was cannulated in 20 sows at 12 3.0 d after farrowing. Sows were randomly assigned to receive either 30 mL of charcoal extracted follicular fluid (FF, $n=9$) or 30 mL saline ($n=11$) thrice daily (0700, 1500, and 2300 h) for 96 hours beginning at 14 \pm 3 d after farrowing. Sows were weaned 48 h after the last infusion. Blood samples were collected thrice daily beginning on the day of cannulation and continuing until ovulation. Serum FSH concentrations were measured by radioimmunoassay. Average follicular diameter was determined once daily by transrectal ultrasonography. Sows treated with FF had longer ($P < 0.05$) weaning to estrus (6.1 ± 0.4 d) and weaning to ovulation (8.6

± 0.5 d) intervals compared to saline treated sows (4.7 ± 0.4 d and 7.2 ± 0.4 d, respectively). A treatment by time effect was detected for serum FSH ($P < 0.001$) and follicular diameter ($P < 0.001$). Serum FSH concentrations and follicular diameter decreased in FF sows during the infusion period. After the infusion period serum FSH concentrations rebounded in FF sows and follicles resumed growth but failed to achieve equivalent diameters compared to control sows after weaning. We conclude that follicular growth in lactating sows is FSH-dependent before weaning and that suppressing follicular growth before weaning decreases size of follicles after weaning and increases intervals to estrus and ovulation.

Hour	FSH ng/mL*		Diameter (mm)*	
	FF	Saline	FF	Saline
-184	14.4 ± 2.1	13.8 ± 2.0		
-144 ^a	12.8 ± 2.1	13.0 ± 1.9	2.8 ± 0.2	2.5 ± 0.2
-48 ^b	3.1 ± 2.1	11.4 ± 1.9	1.0 ± 0.2	2.5 ± 0.2
0 ^c	23.7 ± 2.3	12.3 ± 1.9	1.2 ± 0.2	2.9 ± 0.2
48	27.0 ± 2.3	14.6 ± 1.9	3.0 ± 0.2	3.9 ± 0.2
72	8.2 ± 2.3	7.7 ± 1.9	3.6 ± 0.2	4.5 ± 0.2
168	8.1 ± 2.4	7.8 ± 2.6	5.8 ± 0.2	6.7 ± 0.2

*lsmeans SEM ^astart of infusion, ^bend of infusion, ^cday of weaning

Key Words: FSH, Follicle, Lactating Sows

259 Effect of PG600 and adjusted mating times on reproduction performance in weaned sows. S.M. Breen*, D.M. Hamilton, and R.V. Knox, *University of Illinois-Urbana, USA.*

Sows administered PG600 at weaning average > 91% return to estrus within a week, however farrowing rate is often not improved. Since weaning to estrus interval (WEI) influences estrus to ovulation interval (EOV) and PG600 alters WEI, lowered reproductive performance may result since standard mating times may be less than optimal. This study evaluated the effect of adjusted breeding times based on WEI and predicted EOV following PG600. All sows were given PG600 at weaning and allotted by genetics, parity, lactation length, and P2 backfat to adjusted (A, n = 43) or control (C, n = 43) mating times. Adjusted breeding involved: 1) 2-3 d WEI, AI at 36 and 48 h, 2) 4 d WEI, AI at 24 and 36 h, 3) 5 d WEI, AI at 12 and 24 h, and 4) 6-7 d WEI, AI at 0 and 12 h. Controls were AI at 0 and 24 h after onset of estrus. Estrous detection was performed twice daily for 7 d following PG600 and transrectal ultrasound was performed twice daily from onset of estrus until time of ovulation. There was no effect of treatment on return to estrus or ovulation (93.0 %) within 7 d of weaning. The EOV was influenced by WEI ($P < 0.001$) and tended ($P = 0.06$) to be longer for A (41.9 h) vs C (37.9 h). The percent of first AI occurring within 24 h before ovulation (73.3 vs 50.0%) was improved ($P < 0.05$) and closer to ovulation for A (-23 h) compared to C (-31h). Treatment did not affect the percent of second AI occurring within 24 h of ovulation but tended ($P < 0.06$) to influence the interval for the second AI to ovulation for A (-6.9 h) compared to C (-13.1 h). Treatment did not affect the overall percent of sows AI within 24 h before ovulation for A (98.9%) or C (91.3%). Treatment did not influence conception (87.8%) or farrowing (77.5%) rates or total pigs born (10.8). In conclusion, while AI times for C appear optimal for sows returning to estrus at 4-5 d, the A treatment tended ($P=0.1$) to have advantages over C at 2-3 d for first AI and at 6-7 d ($P < 0.05$) for second AI. However, farrowing data suggests a treatment x WEI interaction ($P = 0.13$) with AI times for C optimal at WEI 3-5 d, but not for 6-7 d. Control AI times may be optimal for early returning sows, but for late returning sows.

Key Words: Estrus, Ovulation, Breeding

260 Evaluation of pFSH and pLH on estrus induction and ovulation in prepubertal gilts. A.L. Jackson*¹ and R.V. Knox¹, ¹*University of Illinois Urbana-Champaign, IL.*

Increasing numbers of healthy embryos has important implications for efficiency of reproductive technologies. These studies investigated the ability of porcine FSH (pFSH) to induce estrus and ovulation in prepubertal gilts. In study 1, gilts (n=92) 176 d of age and 109 kg were randomly assigned in a 2 x 3 factorial treatment design to receive a total of 10 or 15 Armour units (AU) of pFSH containing 6, 10, or 15% pLH. Gilts received 2.5-cc injections of 1/6th the amount of pFSH, given s.c. at 8-h intervals for two days. Control gilts (n=15) received s.c. PG600.

In study 2, gilts received 15 AU pFSH, 10% LH in polyvinylpyrrolidone (PVP), s.c. either a single 10-cc injection (1P, n=16) or two 10-cc injections (2P, n=12). Control (n=12) received s.c. PG600. Estrus detection occurred twice daily with a mature boar. In study 1, estrus induction tended ($P = 0.01$) to be influenced by treatment, lowest for 15-15 (31%) and highest for 15-10 (77%) compared to PG600 (60%). Ovulation also tended ($P = 0.12$) to be influenced by treatment, lowest for 10-15 (24%) and highest for 15-10 (80%) compared to PG600 (76%). Number of corpora lutea (CL) was influenced ($P < 0.001$) by treatment, lowest for 10-15 (1.4) and highest for 15-10 (31) compared to PG600 (19). There was no treatment effect on cyst frequency compared to PG600. In study 2, estrus tended ($P < 0.02$) to be influenced by treatment, lowest for 1P (44%) and highest for 2P (54%) compared to PG600 (95%). Ovulation tended ($P < 0.0001$) to be influenced by treatment, lowest for 1P (33%) and highest for 2P (95%) compared to PG600 (100%). CL number was influenced ($P < 0.005$) by treatment, lowest for 1P (2.0) and highest for 2P (26.6) compared to PG600 (15.4). Treatment did not affect cysts. Results indicate that dose and percent LH content of pFSH tends to influence estrus and ovulation, but clearly affects CL number. Results also suggest that compared to PG600, the 15-10 regimen produced analogous estrus and ovulatory results. For single and double pFSH administration using PVP, the 2P treatment appears promising and comparable to PG600.

Key Words: FHS, Pigs, Estrus

261 A polymorphism in the pig erythropoietin receptor (EPOR) gene is associated with uterine capacity. J.L. Vallet*, B.A. Freking, K.A. Leymaster, and R.K. Christenson, *USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center.*

Selection for uterine capacity increased fetal hematocrits measured on d 105 of gestation. We hypothesized that this increase might be partially due to polymorphism(s) in the EPOR gene. The likely positions of the introns were predicted within the porcine EPOR gene by comparing the porcine EPOR cDNA to the human EPOR gene. Oligonucleotide primers based on the porcine cDNA were designed to amplify by PCR a region of the porcine EPOR gene that was likely to contain two moderately sized introns. The genomic DNA from 96 gilts from populations available at the Meat Animal Research Center (including 24 half Meishan, half white crossbred gilts, 24 gilts from lines selected either at random (CO), for ovulation rate (OR) or for uterine capacity (UC); and 48 pigs from other populations) were amplified by PCR using these primers, and the products were sequenced. The sequences were aligned and polymorphisms were identified. A genotyping assay for a C/T polymorphism in one of the introns was developed based on primer extension and mass spectrometry using Sequenom technology. This assay was used to genotype 212 gilts from the CO, OR and UC lines. The gilts had been unilaterally hysterectomized-ovariectomized, mated, and then slaughtered at 105 d of gestation. At slaughter, number of corpora lutea (CL) and litter size (a measure of uterine capacity) were recorded. Fetal and placental weights were also recorded, and a blood sample was collected from each fetus. Blood samples were measured for hematocrit and fetal plasma iron. Gilts were either homozygous CC or heterozygous CT, no TT gilts were observed. Litter size was greater ($P < 0.01$) in CT gilts (8.3 ± 0.5 , n=21) compared to CC gilts (6.9 ± 0.2 , n=191). CL number (15.1 ± 0.6 , 15.2 ± 0.2), fetal weight (778 ± 33 , 800 ± 11), placental weight (174 ± 11 , 189 ± 4), fetal hematocrit (36.9 ± 0.6 , 36.8 ± 0.2) and fetal plasma iron (1.17 ± 0.05 , 1.20 ± 0.02) did not differ between CT and CC genotypes, respectively. These results suggest that variation within the EPOR gene or another nearby gene is associated with differences in uterine capacity, but this effect is not mediated by changes in hematocrit, placental or fetal weights, CL number or fetal plasma iron.

Key Words: Swine, Erythropoietin, Pregnancy

262 Are ovariectomized heifers an appropriate model to study the fundamental nature of the estrus event in cattle? C.R. Burke*^{1,2}, M.L. Mussard¹, and M.L. Day¹, ¹*The Ohio State University, Columbus OH,* ²*Dexcel Research Ltd, Hamilton, New Zealand.*

The objectives were to determine: 1) if estradiol-induced estrus in ovariectomized (OVX) heifers is characteristically similar to that in ovarian-intact heifers, and: 2) if prior treatment with progesterone influences the nature of behavioral estrus in OVX heifers treated with estradiol. The rationale was that the OVX animal model would allow

greater systematic control and convenience in the study of fundamental aspects underlying the signal for behavioral estrus in cattle. The 23 OVX beef heifers used in the present study had not been treated with progesterone since ovariectomy (339 ± 2 d). These animals received an intravaginal progesterone insert (CIDR) for six days (P4; $n = 13$) or were not treated with progesterone (NoP4; $n = 12$), and all received 0.5 mg estradiol benzoate (EB) i.m. 24 h after removal of the CIDR insert (equivalent time in the NoP4 treatment). Additionally, estrus was synchronized in ovarian-intact (INT; $n = 6$) heifers to coincide with the time of estrus in the OVX animals. Estrus was monitored using an electronic surveillance system (HeatWatch, DDx Inc.) with an estrus event being defined as ≥ 4 mounts lasting ≥ 1 s within a 4 h period. All INT heifers had an estrus event characterized with 55.3 ± 24.7 mounts lasting 1.7 ± 0.1 s at intervals of 29.5 ± 5.4 min over a duration of 15.5 ± 2.4 h. None of these variables were different between the INT and P4 treatments. However, among the OVX heifers, P4 treatment increased the incidence ($13/13$ vs $9/12$; $P = 0.09$), number of mounts (47.8 ± 8.1 vs 20.4 ± 4.8 ; $P < 0.01$) and duration (13.3 ± 1.2 h vs 9.7 ± 1.3 h; $P = 0.07$), but not the interval from EB to onset of estrus (18.6 ± 0.6 h) as compared with the NoP4 treatment. The results show that behavioral characteristics of estrus in OVX heifers treated appropriately with progesterone and estradiol are not different than the spontaneous event in ovarian-intact heifers. In this regard, OVX heifers would appear to be a valid animal model to study the fundamental nature of behavioral estrus in cattle.

Key Words: Estrus Behavior, Progesterone, Ovariectomized Cattle

263 Recombinant leptin prevents fasting-mediated reductions in pulsatile LH release and stimulates GH secretion in peripubertal heifers. M. Maciel^{1,2,3}, D. Zieba^{2,3}, M. Amstalden^{2,3}, D. Keisler⁴, J. Neves¹, and G. Williams^{*2,3}, ¹Federal University of Santa Maria, Santa Maria, Brazil, ²Texas A&M University Agricultural Research Station, Beeville, TX, ³Texas A&M University, College Station, TX, ⁴University of Missouri, Columbia, MO.

Short-term fasting suppresses the pulsatile secretion of LH in several animal models, including the peripubertal heifer. Moreover, studies in monogastric species and in one ruminant model (E₂-implanted wether) have shown that leptin treatment prevents this effect. Under similar feeding conditions, leptin has also been shown to stimulate GH secretion. Studies reported herein utilized the intact, peripubertal heifer, fasted for 72 h, to determine if exogenous leptin could 1) prevent a fasting-induced reduction in pulsatile LH secretion, 2) modify GnRH-mediated LH release, and 3) stimulate GH secretion. Thirteen Brahman x Hereford heifers (13.5-16 mo; 280-350 kg) were used. Heifers were assigned randomly to two groups: 1) Control ($n=6$); sc injections of saline and 2) Leptin ($n=7$); sc injections of oleptin (19.2 ug/kg) at 12-h intervals. At the end of a 6-h blood sampling period on D 3, heifers received iv a physiological (0.0011 ug/kg) pulse, followed 90 min later by a pharmacological (0.22 ug/ml) dose of GnRH, with additional blood samples collected for 5.5 h. Plasma concentrations of leptin were greater ($P = 0.0003$) throughout the experiment in the Leptin compared to the Control group (28.5 ± 2.2 vs 4.5 ± 0.2 ng/mL). Fasting caused a marked decline ($P = 0.004$) between D 0 and 3 in the frequency of LH pulses in controls (4.7 ± 0.2 vs 2.8 ± 0.3 pulses/6h). However, this effect was attenuated by leptin, with pulse frequency in the Leptin group increasing ($P < 0.008$) from D 0 to 3 (3.1 ± 0.6 vs 4.4 ± 0.5 pulses/6h) and greater ($P < 0.005$) than controls on D 3. Leptin had no effect on GnRH-induced LH release. However, plasma concentrations of GH were greater ($P = 0.001$) in leptin-treated heifers compared to controls on day 3. Fasting-mediated declines ($P < 0.002$) in circulating insulin and IGF-1 were observed in both groups, and were unaffected by leptin treatment. Results indicate, for the first time, the ability of exogenous leptin to prevent fasting-mediated reductions in LH pulse frequency and to increase GH secretion in the intact heifer. (USDA-NRI00-35203-9132;CAPES)

Key Words: Leptin, LH, Heifer

264 Effects of chronic administration of recombinant leptin on LH secretion, metabolic hormones, and timing of puberty in growing beef heifers. M. Maciel^{1,2,3}, D. Zieba^{2,3}, M. Amstalden^{2,3}, D. Keisler⁴, J. Neves¹, and G. Williams^{*2,3}, ¹Federal University of Santa Maria, Santa Maria, Brazil, ²Texas A&M University Agricultural Research Station, Beeville, TX, ³Texas A&M University, College Station, TX, ⁴University of Missouri, Columbia, MO.

Circulating concentrations of leptin have been shown to increase during pubertal development. In beef heifers, serum concentrations of leptin increased linearly from 16 wk before until the wk of pubertal ovulation. Moreover, leptin treatment accelerates pubertal development in rodents. To test the hypothesis that exogenous leptin can hasten the onset of puberty in heifers, we examined the effects of recombinant leptin on timing of puberty, pulsatile and GnRH-mediated LH release, and plasma concentrations of GH, IGF-1, and insulin. Fourteen fall-born, prepubertal heifers (Brahman x Hereford, 12-13 mo; 290-310 kg) were used. Heifers were stratified by age and BW and assigned randomly to two groups (7 animals/group): 1) Control; heifers received sc injections of saline 2x daily at 12-h intervals for 40 d, and 2) Leptin; heifers received sc injections of oleptin (19.2 ug/kg) 2x daily at 12-h intervals for 40 d. Blood samples were collected at 10-min intervals for 5 h on days 0, 5, 10, 20, 30 and 40, and 2x daily throughout the study. On day 41, heifers received iv injections of GnRH at 0 (0.0011 ug/kg) and 90 min (0.22 ug/kg), with additional sampling for 5.5 h. Diets promoted a gain of 0.32 ± 0.09 kg/d and did not differ between groups ($P > 0.5$). Plasma concentrations of leptin increased markedly in leptin-treated heifers and were greater ($P < 0.0001$) than controls throughout (27.8 ± 0.8 vs 4.9 ± 0.12 ng/mL). None of the heifers reached puberty during the experiment. Mean concentrations of plasma LH, GH, IGF-1, and insulin were not affected by treatment ($P > 0.1$), nor was there an overall effect on the frequency of LH pulses ($P > 0.16$). However, a treatment x day interaction ($P = 0.02$) revealed that frequency of LH pulses (pulses/5h) was greater ($P = 0.03$) in controls (3.6 ± 0.36) than in leptin-treated heifers (1.7 ± 0.28) on day 10. Characteristics of GnRH-induced release of LH were not affected by treatment. In summary, leptin failed to alter the timing of puberty or endocrine characteristics in heifers under the conditions of this experiment. (USDA-NRI 00-35203-9132 and CAPES)

Key Words: Leptin, Puberty, Heifer

265 Effects of fat supplementation on reproductive parameters of beef heifers fed endophyte- infected fescue seed. J. D. Rhinehart*, E. S. Vanzant, J. D. Bailey, A. M. Arnett, E. Myers, K. K. Schillo, and L. H. Anderson, University of Kentucky.

This experiment was designed to characterize reproductive responses of beef heifers fed endophyte-infected fescue seed (E+) and to evaluate effects of fat supplementation on those responses. Sixteen pubertal crossbred heifers (BW = 414 kg) were randomly assigned to silage-based diets supplemented with endophyte-free fescue seed (E-, 60% of diet), endophyte-infected fescue seed (E+, 60% of diet), or E+ with soybean oil (E+/Fat, 60% and 4.5% of diet, respectively). Diets provided similar amounts of energy and protein. During a 27-d adjustment period on respective diets, estrus was synchronized using two injections of prostaglandin F_{2α}. Rectal temperature and jugular blood samples were collected daily throughout the study. Starting 12 hours prior to expected estrus, jugular blood samples were collected every two hours until ovulation. Heifers were observed for behavioral estrus four times daily. Beginning at the time of estrus, follicular dynamics were recorded every six hours, via transrectal ultrasonography, until ovulation. Rectal temperatures differed ($P < 0.08$) slightly early in the experiment but no differences occurred ($P > 0.20$) from day 7 (d0 = day of initial estrus) to completion (treatment x sampling day $P < 0.01$). Estrous cycle length was shorter ($P = 0.01$) in E+ (18.7 d) than in E- (21.6 d), but E+/Fat (20.0 d) was intermediate and similar to ($P > 0.16$) E+ and E-. Treatments did not affect the time from estrus to ovulation ($P = 0.20$) or from LH surge to ovulation ($P = 0.50$). Serum prolactin concentrations were greater ($P < 0.01$) for E- (238 ng/ml) than for E+ (158 ng/ml) or E+/Fat (151 ng/ml), which were similar ($P = 0.82$). Average daily gain was negative for all groups and was not affected by treatment ($P = 0.28$). We conclude that lower fertility in beef heifers grazing endophyte-infected fescue may be related to shortened estrous cycles. Fat supplementation did not appear to overcome the effects of endophyte.

Key Words: Endophyte, Fertility, Fat Supplementation

266 Effects of temperature-humidity index on pregnancy rate in beef cattle. J. L. Amundson*¹, T. L. Mader¹, and R. J. Rasby¹, ¹ *University of Nebraska-Lincoln*.

Cows exposed to adverse weather during the breeding season are assumed to have decreased fertility and pregnancy rates. The objective of this study was to quantify the effect of temperature-humidity index (THI) on pregnancy rate (PR) of cattle. Eleven years of breeding data were examined from a herd of commercial, *Bos taurus*, crossbred cows (n~180/y) from the University of Nebraska Dalbey-Hallack Research Farm in Virginia, Nebraska. Data included cow number, calf identification, calf sex, and calving date. All cows were bred by natural service, beginning in late-May, with a typical breeding season of 60 to 75 days. The bull to female ratio was approximately 1:25. Spring calving dates were recorded and used to calculate approximate breeding date. For standardization purposes, gestation length was 283 days in length. Relationships were determined between the proportion of cows bred in the first 30 days of the breeding season and mean temperature and THI during those 30 days. Weather data were compiled from the Great Plains Climate Center Weather Archives for a weather station located approximately 20 km from the research site. Average daily temperature and relative humidity were used to calculate daily THI for determining average THI for the first 30 days of the breeding period. The mean 30-day temperature and THI were 20.0 °C and 66.3, respectively. The change in PR, in the first 30 days of breeding, based on the entire breeding season or only the first 60 days were found to be -1.5% ($R^2=0.30$; $P<0.1$) and -1.6% ($R^2=0.47$; $P<0.05$), respectively, per °C increase in mean temperature, and -1.1% ($R^2=0.33$; $P<0.1$) and -1.1% ($R^2=0.49$; $P<0.05$), respectively, per unit increase in mean THI. Preliminary analysis indicated a PR threshold existed at THI=65. For the six years THI > 65, the change in 30-day PR, based on the entire breeding season or only the first 60 days were found to be -2.1% ($R^2=0.53$; $P<0.1$) and -1.8% ($R^2=0.72$; $P<0.05$), respectively, per °C increase in mean temperature, and -1.6% ($R^2=0.64$; $P<0.06$) and -1.4% ($R^2=0.81$; $P<0.05$), respectively, per unit increase in mean THI above 65. For the five years THI<65 slopes were not significant and R^2 were less than .12. Within a 30-day period, PR declines 1.4 to 1.6% for each unit increase in mean THI above 65.

Key Words: Beef Cattle, Pregnancy Rate, Heat Stress

267 Pregnancy status of beef heifers immunized against luteinizing hormone and luteinizing hormone-releasing hormone. C. D. Holladay*, A. W. Thompson, D.R. Eborn, and D.M. Grieger, *Kansas State University*.

Immunization against reproductive hormones is a promising method of preventing heifers from entering the feedlot pregnant due to accidental bull exposure. Previous studies have successfully vaccinated against luteinizing hormone (LH) and luteinizing hormone-releasing hormone (LHRH) by linking these hormones to carrier proteins. The objective of this study was to develop a vaccine against LHRH and/or the β subunit of bovine LH (LH β) using recombinant DNA methods. Glutathione-S-transferase (GST) coded for by the commercial expression vector pGEX-4T-3 (Pharmacia), served as the antigen of the control group, as well as the carrier protein for the three test groups. Three plasmids were constructed to encode for recombinant fusion proteins GST-LH β , GST-LHRH, and GST-LHRH-LH β . Protein was produced by a bacterial expression system and purified by GST affinity columns. Twenty yearling

beef heifers were immunized against GST (n=5), GST-LH β (n=5), GST-LHRH (n=5) or GST-LHRH-LH β (n=5). Three injections were given at two-week intervals with an equal volume of Freund's complete adjuvant for the primary immunization and Freund's incomplete adjuvant for both booster immunizations. A fertile bull was placed with the heifers one week after the final immunization. Pregnancy status was determined using rectal palpation and ultrasonography 15 weeks after first bull exposure. The pregnancy rate of heifers immunized against LHRH (20%) was reduced when compared to the other treatment groups ($p=0.01$). All 5 heifers in the GST and GST-LH β were pregnant compared to only 2 of 5 in the GST-LHRH-LH β and 1 of 5 in the GST-LHRH treatment groups. Of the four nonpregnant heifers in the LHRH immunized group, three possessed infantile reproductive tracts. This study suggests that the combination of immunizing against both LH and LHRH was not more effective than LHRH alone, however, the recombinant LHRH treatment could have potential as a sterilization vaccine.

Key Words: LHRH, Immunization, Heifer

268 A timed artificial insemination (TAI) protocol for synchronizing two inseminations within a 32-d period in dairy cows and heifers. J. P. Meyer*, S. J. Kolath, R. P. Radcliff, M. L. Rhoads, B. L. McCormack, and M. C. Lucy, *University of Missouri-Columbia*.

An estrous synchronization TAI protocol (PGPG with rapid resynchronization) that enables TAI of first and second (repeat service in cattle diagnosed nonpregnant) inseminations was tested in Holstein dairy cows (Exp. 1; n=80) and heifers (Exp. 2; n=51). The PGPG cattle were treated as follows: PGF_{2 α} (25 mg Lutalyse), 3 d, GnRH (100 μ g Cystorelin), 8 d, PGF_{2 α} , 2 d, GnRH, 4 h (heifers) or 8 h (cows), TAI. Control (PP) cattle were injected with PGF_{2 α} as above but were injected with saline (2 ml) in place of GnRH and were AI following estrus after the second PGF_{2 α} . Blood for progesterone analysis was collected immediately before each injection. Pregnancy was detected by ultrasonography 30 d after AI. Dairy cows (Exp. 1) treated with PGPG had greater insemination rates [IR; 36/36 (100%); $P<.001$], equivalent conception rates [CR; 14/36 (39%)], and greater pregnancy rates [PR; 14/36 (39%); $P<.01$] compared to PP cows [15/44 (34%), 4/15 (27%), and 4/44 (9%), respectively]. Heifers (Exp. 2) treated with PGPG had greater IR [26/26 (100%); $P<.05$], and equivalent CR [16/26 (62%)], and PR [16/26 (62%)] compared to PP heifers [21/25 (84%), 11/21 (52%), and 11/25 (44%), respectively]. Conception rates for cows and heifers with luteolysis after the second PGF_{2 α} were greater for PGPG compared to PP [27/41 (66%) and 9/25 (36%); PGPG and PP, respectively; $P<.05$]. The PGPG cows (n = 34) and heifers (n=8) diagnosed nonpregnant at 30-d pregnancy exam underwent rapid resynchronization TAI [PGF_{2 α} , 2 d, GnRH, 4 h (heifers) or 8 h (cows), TAI]. Pregnancy rates for cows and heifers after rapid resynchronization TAI [11/34 (32%) and 5/8 (63%), respectively] were similar to first TAI. We conclude that PGPG is a TAI protocol that can be used in dairy cows and heifers that achieves greater (cows) or equivalent (heifers) pregnancy rates compared to PP and AI at estrus. Cows and heifers treated with PGPG and diagnosed nonpregnant can be resynchronized and TAI within 2 d after a 30-d pregnancy exam.

Key Words: Estrous Synchronization, Dairy, AI

Ruminant Nutrition and Forages

271 Ruminant ammonia load does not impact histidine utilization by growing steers. K. C. Candler*, E. C. Titgemeyer, M. S. Awawdeh, and D. P. Gnad, *Kansas State University*.

Six ruminally cannulated Holstein steers (144 kg) housed in metabolism crates were used in a 6 x 6 Latin square to determine effects of rumen ammonia load on utilization of histidine (His). The basal diet (83% soybean hulls, 8% wheat straw, 0.4% urea, DM basis) was fed twice daily to provide 2.5 kg DM/d. Experimental periods were 6 d, with 2 d for adaptation to treatment and 4 d for total fecal and urinary collection for N balance. Basal abomasal infusions contained: 250 g/d amino acids, which supplied adequate amounts of all essential amino acids except His; 10 mg/d vitamin B-6, 10 mg/d folic acid, and 0.1 mg/d vitamin

B-12; and 300 g/d glucose. Basal ruminal infusions contained 180 g/d acetate, 180 g/d propionate, and 45 g/d butyrate to supply energy without increasing microbial protein supply. Treatments were continuously infused, arranged as a 3 x 2 factorial, and included: 0, 1.5, or 3 g/d L-His infused abomasally; and 0 or 80 g/d urea infused ruminally to supply a metabolic ammonia load. Total N intake increased from 89.5 g/d for steers receiving no urea to 126.6 g/d for steers receiving 80 g/d urea. Urea infusions increased ($P < 0.01$) rumen ammonia concentration from 8.6 to 19.7 mM and plasma urea from 2.7 to 5.1 mM. No change in N retention occurred in response to urea (35.1 and 37.1 g/d for 0 and 80 g/d urea, respectively, $P = 0.15$). There also was no His x urea interaction for N retention ($P = 0.64$). Retained N increased linearly ($P < 0.01$) with His (31.5, 37.8, and 39.0 g/d for 0, 1.5, and 3 g/d L-His, respectively). Fecal N was similar among all treatments, so change in