566 ASAS Centennial Presentation: Future research in physiology and endocrinology. G. E. Seidel*, *Colorado State University*, *Fort Collins*.

Over the next quarter century in North America, the following eventualities are likely for physiology/endocrinology research with agricultural animals.
1. Total funding adjusted for inflation will change little, but will continue to come less from public sources, and most of that will be in the context of human health. Much of the privately funded research will be herd-specific and remain proprietary.
2. Numbers of M.S., Ph.D. and postdoctoral students probably will decrease, but research in the context of credentialing will remain important.
3. Resources such as expanded data bases in genomics and proteomics, and remarkable new tools like small inhibitory RNA will continue to become available, likely at a faster rate than in the previous 25 years.
4. The huge amounts of data from production agriculture will make agricultural animals ideal models for some kinds of basic research such as studying fetal programming, resulting in synergy with more applied research. Most of these experimental animals will be in private, production herds and flocks, even when work is publicly funded.
5. The trend toward more interdisciplinary research will continue, especially considering interactions among reproduction, health, nutrition, selective breeding, management factors, and societal concerns; reductionist research probing deeper into cellular and molecular mechanisms will remain important as will whole animal approaches.
6. Agricultural animals are a product of evolution plus selective breeding. Insights of the former will aid progress in the latter. One focus of research in physiology and endocrinology will be understanding heterosis, inbreeding depression, and epigenetic effects, as it becomes possible to manipulate and identify the allelic structure of individual animals.
7. Additional insightful concepts will evolve that will simplify thinking in some respects, such as the maternal to embryonic shift in transcribed RNA in early embryos; however, animal biology will turn out to be even more complex than most of us currently imagine.

Key Words: Research, Physiology, Endocrinology

567 Effect of antioxidants on oxidative stress during maturation and in vitro culture of pig embryos. B. D. Whitaker* and J. W. Knight, *Virginia Tech*, *Blacksburg*.

This study evaluated the effects of different concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5, 5.0 mM) of N-acetyl-cysteine (NAC) supplemented to the oocyte maturation medium on embryo development. Embryo development was analyzed at 48 h and 144 h post-fertilization. There were no differences between cleavage rates for any of the treatment groups. Blastocyst formation in the 1.5 mM NAC (56.5 ± 9.2%) supplementation was higher ($P < 0.05$) than all other concentrations of NAC supplementation. Identical concentrations (1.5 mM) of NAC and N-acetyl-cysteine-amide (NACA) supplementation were compared by evaluating nuclear maturation, superoxide dismutase (SOD), glutathione (GSH) peroxidase, catalase and intracellular GSH concentrations, DNA fragmentation, fertilization success and embryo development. Supplementation of 1.5 mM NACA increased ($P < 0.05$) the levels of GSH peroxidase in the oocyte compared to the other treatment groups. The concentration of SOD was less ($P < 0.05$) with 1.5 mM NAC supplementation compared to the control but SOD was higher ($P < 0.05$) with 1.5 mM NACA supplementation compared to the control. The concentration of catalase was less ($P < 0.05$) with 1.5 mM NACA supplementation compared to the control but catalase was higher ($P < 0.05$) with 1.5 mM NAC supplementation compared to the control. There were no differences in the length of DNA migration, intracellular GSH concentration, nuclear maturation or fertilization when comparing 1.5 mM NAC and 1.5 mM NACA supplementation to the maturation media. There was no difference between cleavage rates of 1.5 NAC and 1.5 mM NACA supplementation to the maturation media. Blastocyst formation for 1.5 mM NAC (44.4 ± 4.7%) and 1.5 mM NACA (46.2 ± 3.4%) supplementation were higher ($P < 0.05$) than the control (32.1 ± 6.2%) oocytes. These results indicate that supplementing 1.5 mM of NAC or NACA to the oocyte maturation medium increased the percentage of viable embryos reaching the blastocyst stage of development and antioxidant supplementation may alleviate the free radicals associated with oxidative stress in the maturing porcine oocyte.

Key Words: Swine, N-Acetyl-Cysteine, Antioxidant

568 Glycomic analysis of saccharides that bind porcine sperm. E. D. Collins, C. Korneli, and D. J. Miller*, *University of Illinois*, *Urbana*.

The objective of this work was to identify glycans that bound different maturation states of porcine sperm. Interactions between glycans and lectins on the cell surface and extracellular matrix are proposed to mediate many cell-cell interactions, including those between sperm and the oviduct and sperm and the egg coat. Immature (uncapacitated) sperm bind to the oviduct, which forms a reservoir and maintains viability. After a final maturation (capacitation), sperm bind to the egg coat (zona pellucida) and undergo the acrosome reaction before penetrating the zona. Studies using competitive inhibitors have identified candidates but the specific glycans in the oviduct or zona pellucida that bind sperm remain controversial. The goal of this work was to use an alternate and unbiased approach to identify the glycans capable of binding sperm. A glycan array, developed to characterize the specificity of lectins and antibodies to glycans, was adapted for use with live cells. An array containing over 300 glycans was used in an adhesion assay to pinpoint glycans possibly involved in sperm-oviduct and sperm-egg interactions. Live porcine sperm from pooled ejaculates of 4-6 boars were either used immediately after collection and washing (uncapacitated), after incubation to capacitate sperm, or after subsequent incubation with A23187 to induce the acrosome reaction. Sperm were stained with DiI or SYTO-16, were incubated with the array and fluorescence of bound sperm was assessed. Sperm binding was specific and depended on structure and charge of the glycans. Uncapacitated sperm bound to only 4 glycans, all of which contained the same trisaccharide motif. Capacitated sperm bound 7 glycans, the 4 that uncapacitated sperm bound and 3 additional glycans, which also contained the same complete or partial trisaccharide motif. Acrosome-reacted sperm did not bind glycans. Identification of these glycans may lead to discovery of the molecules that are important in forming the oviduct storage site and in zona pellucida binding and may underpin the development of more accurate laboratory fertility tests.

Key Words: Sperm, Fertility, Fertilization

569 The relationship between sperm nuclear shape and boar fertility using Fourier Harmonics. K. L. Willenburg*[1], K. J. Rozeboom[2], and J. J. Parrish[1], [1]*University of Wisconsin*, *Madison*, [2]*ReproQuest, LLC*, *Fitchburg, WI*.

In this study, boar sperm nuclear shape was characterized using Fourier Harmonic analysis (FHA) and then related to boar fertility. Fertility was based on farrowing rates of 26 boars with at least 100 single sire matings and ranged from 88% to 37%. Boars were separated into two fertility groups, acceptable fertility (n=24, farrowing rate>60%) and unacceptable fertility (n=2, farrowing rate <60%). Harmonic amplitudes 0–5 (HA0–HA5), derived from FHA analysis, were previously shown to be an accurate, objective and repeatable measure of sperm nuclear shape. Ejaculates from each boar were evaluated for motility, viability, and FHA. Multivariate analysis of variance using HA0–HA5, found that fertility groups differed in sperm nuclear shape (P<0.05). Univariate analysis found only HA2 and HA4 were different between the groups (P<0.05). Fertility groups also did not differ in various measures of HA dispersion, motility or viability (P>0.05). Discriminate analysis was used to produce the best method to separate the two groups based on nuclear shape, shape dispersion, motility or viability. Diagnostic statistics (DS) were used to determine the best discriminate model for predicting the fertility group membership of a particular boar. The DS evaluated were: true positive (TP, classifying an acceptable boar correctly) and true negative (TN, classifying an unacceptable boar correctly). Various approaches can be used to evaluate diagnostic statistics. We chose the model with the fewest parameters and highest TP +TN value. The best model included viability, motility, HA 2 and 4, skewness, kurtosis and variance of HA 0 with a TP +TN value of 1.95. This model correctly classified 96% of the boars, misclassifying 1 of the acceptable boars. In comparison, a model containing viability and motility had a TP+TN=1.67, classifying 69% of the boars correctly. The data reveals that there are differences in sperm nuclear shape between boars with acceptable and unacceptable fertility. Further testing is needed on a new group of boars with known fertility to test the current diagnostic model.

Key Words: Boar, Fertility, Fourier Harmonics

570 Effect of number of motile, frozen-thawed boar sperm and number of inseminations on fertility in post-pubertal gilts. K. Spencer*[1], P. Purdy[2], H. Blackburn[2], S. Spiller[2], C. Welsh[2], T. Stewart[3], S. Breen[1], J. Taibl[1], B. Yantis[1], and R. Knox[1], [1]*University of Illinois*, *Urbana*, [2]*National Animal Germplasm Program, ARS, USDA*, *Fort Collins, CO*, [3]*Purdue University*, *West Lafayette, IN*.

The potential for use of frozen-thawed boar sperm (FTS) as a tool for genetic preservation and advancement has far reaching implications for breeding herd management. Numerous research studies have been carried out with FTS but fertility results are often less than the potential with liquid semen using multiple AI. We hypothesized that pregnancy rates (PR) and litter sizes (LS) would be influenced by both sperm numbers and numbers of inseminations when AI with FTS was performed based on numbers of motile cells. We tested the effects of number of motile FTS and one or two AI. The FTS used was from PIC crossbred boars cryopreserved in 0.5 mL straws at 500 million cells/mL. Each boar was represented across all treatments. Gilts (n = 20) were treated with PG600 then fed a synthetic progestagen (MATRIX), for 14 d to synchronize estrus. Following last feeding of MATRIX (LMF), gilts were checked once daily for estrus and upon expression of estrus on d 5-8 (n = 17) were inseminated with 1.0, 2.0, or 4.0 billion motile FTS once at 32 h, or twice at 24 h and 32 h. Ultrasound was performed at 12 h intervals to determine time of ovulation and pregnancy at d 24. Reproductive tracts were collected on d 32 following AI. In this first replicate, 90% of the gilts expressed estrus but only 17 were used for AI during d 6-9 following LMF. Estrus occurred at 153±24 h following LMF and ovulation at 37.4±8.4 h following onset of estrus. The interval from the 24 h AI to ovulation was -10.3 h and the interval from the 32 h AI to ovulation averaged -5.4±8.4 h. The PR (76.5%) was not affected by treatment (P>.10) as all treatments established pregnancies (range:50-100%). The LS at d 32 also was not affected by treatment (P>.10) and averaged 8.3±4.8 (range: 2-17) fetuses. Further replicates are in progress to clarify the effects of treatment on fertility. Even with few observations, it is clear that a single AI using 1 billion FTS can establish pregnancy using conventional AI. Still, increased sperm numbers and numbers of inseminations may influence pregnancy rate and litter size.

Key Words: Frozen-Thawed Boar Semen, Number of Motile Sperm, Litter Size

571 Comparison of exogenous porcine FSH/LH to PMSG/hCG for inducing follicular development and fertility in prepubertal gilts. S. M. Breen* and R. V. Knox, *University of Illinois*, *Urbana*.

PMSG/hCG is commonly used for induction of estrus and ovulation for production of oocytes and embryos. However, inconsistency in fertility responses has been a concern when using this combination. While in many species porcine FSH (pFSH) is used for improved efficacy, little data is available to indicate its potential value for use in pigs. Therefore, the objective of this study was to compare pFSH-LH (Bioniche) treatment with PG600 for induction of follicular development, estrus, ovulation, and embryo production. Terminal line prepubertal gilts were alloted to receive 25 mg of FSH (25F, n=31) and 50 mg of FSH (50F, n=33) each with 5 mg added LH, PMSG/hCG (PG600, n=32), and saline (n=31). The 25F, 50F, and saline were each divided into 6 injections given s.c. every 8 h for 2 d. In addition, the 25F and 50F each received an ovulatory dose of LH (5 mg, Bioniche) in a single injection on d 4. Gilts received PG600 in a single s.c. injection on d 0. Ultrasound was performed to assess follicles and ovulation. Gilts receiving 25F and 50F were AI at d 4.5 and d 5 while saline and PG600 were AI at onset of estrus and 24 h later. Reproductive tracts were collected 10 d following AI for determining ovulation and embryo numbers. The percentage of gilts with large follicles on d 4-5 was similar for 25F, 50F, and PG600 (82%) and greater (P<0.01) compared to saline (24%). A greater (P<0.01) proportion of the 25F and 50F gilts expressed estrus (81%) compared to PG600 (62%) and saline (20%). The percentage of gilts that ovulated was similar for 25F, 50F (80%) and PG600 (68%) but greater (P<0.01) than saline (26%). FSH treated gilts had increased (P<0.05) ovulation rate (20 CL) compared to PG600 (10 CL) and saline (8 CL) but FSH increased (P<0.01) the percentage of gilts with cysts (26-48%) compared to PG600 (15%) and saline (3%). The percentage of gilts pregnant (64%) was similar (P>0.1) between treatments but 25F (8.4) and 50F (10.4) both increased (P<0.01) the number of embryos compared to PG600 (5.4) and saline (4.2). These data suggest that with improved delivery methods, pFSH could be useful as an alternative exogenous gonadotropin for use in swine.

Key Words: Gilts, FSH, Ovulation