RESEARCH ABSTRACT

ANIMAL SCIENCE

Title: Advancing Investigations in Swine Reproduction Efficiency at Iowa State – Phase 2 – **#16-265 IPPA**

Investigator: Aileen F. Keating, PhD.

Institution: Iowa State University

Date Submitted: October 15th, 2018

Scientific Abstract: Heat stress (HS) during hot summer months causes seasonal infertility in swine, characterized by longer wean to estrus interval, increased numbers of rebreeds and spontaneous abortion. We previously discovered alterations to ovarian molecular signaling pathways that regulate follicle and oocyte viability as well as steroid hormone production in heat stressed pre- or post-pubertal gilts. We have demonstrated that heat-stressed pigs have increased systemic insulin as well as lipopolysaccharide, thus this project investigated the impacts of both of these physiological perturbations on ovarian function. Our previous studies focused on the follicular phase of the estrous cycle, however, two projects described herein determine the impact of HS on the function of the corpus luteum (CL) and blastocyst development in post-pubertal gilts who were synchronized in estrus. Experiment 1: Post-pubertal gilts (n = 8) were synchronized to undergo estrus at a similar time point through feeding altrenogest (Matrix™) for 14 days. Gilts underwent thermal neutral (TN) conditions until estrus behavior was successfully detected through monitoring vaginal mucus, swollen vulva and boar exposure. At the time, gilts were randomly assigned to a TN or heat stress (HS) room two days after ovulation and treatment continued for 12 days (to the time of peak corpus luteum function). HS pigs were exposed to cyclical heat over a 24 h period with 12 h at 95 ± 1°F 21-31% relative humidity and cooling to 89 ± 1°F, 21-31% relative humidity for 12 h in a diurnal pattern. Experiment 2: Post-pubertal gilts (n = 8) were synchronized to undergo estrus at a similar time point through feeding altrenogest (Matrix™) for 14 days. Gilts underwent thermal neutral (TN) conditions until estrus behavior was successfully detected through monitoring vaginal mucus, swollen vulva and boar exposure. At the time, gilts were randomly assigned to a TN or HS room two days after ovulation and treatment continued for 12 days (to the time of peak corpus luteum function). Gilts were artificially inseminated 2-3 times beginning at 2 day post-estrus (dpe) at 1900h and assigned to one of four experimental treatments (n = 8/treatment): TN no altrenogest (TN); TN plus altrenogest (TNA); HS no altrenogest (HS); HS plus altrenogest (HAS). HS pigs were exposed to cyclical heat over a 24 h period with 12 h at 95 ± 1°F 21-31% relative humidity and cooling to 89 ± 1°F, 21-31% relative humidity for 12 h in a diurnal pattern. For both experiments: Rectal temperatures, respiration rate and feed intake were monitored. Blood samples were collected to monitor insulin and steroid hormones. At the end of the experimental period, gilts were humanely euthanized, ovaries collected, the corpora lutea excised, measured and weighed. Tissue was frozen for subsequent laboratory analyses. Uteri were flushed with sterile saline and flushings collected for laboratory analyses. We determined in experiment 1 a reduction in corpora lutea weight and diameter due to HS. Feed intake was reduced by HS and insulin:feed intake was

increased, thus as demonstrated in multiple HS studies by our group at ISU, the gilts were hyperinsulinemic. Progesterone per unit of CL tissue was actually increased during HS, which we theorize may be a compensatory effect in response to reduced tissue mass, in order to maintain a constant progesterone level in circulation, what we do not know is whether this compensatory response eventually becomes overwhelmed if HS continues. This is a future area of interest for the group. We have not demonstrated an effect of HS on any of the enzymes measured to date in this tissue. However, the availability of more sensitive tools that have become available at ISU will be fully utilized to further assess effects of HS on the CL (ongoing). Our preliminary data to date from experiment 2 indicate that altrenogest increased IL1 beta in TN but not HS gilts. Interestingly, altrenogest supplementation appeared to increase conceptus development.

Introduction: Annual economic losses to global animal agriculture due to thermal (heat) stress (**HS**) surpass billions of dollars. In the United States, summer-induced decreased production is well-documented in every aspect of animal agriculture. This heat-induced economic burden is due to poor sow reproductive performance, increased morbidity, mortality, suboptimal growth, inefficient nutrient utilization, decreased carcass value and carcass processing problems (1). We have recently estimated (in discussion with Dr. Steve Pollman) seasonal infertility in swine costs the US economy $420 million; and the Iowa economy $60 million, based on the number of sows and the 2013 price of grain. The fiscal losses occur despite recent advances in cooling systems, barn management, and aggressive implementation of other heat abatement strategies.

The US swine industry experiences seasonal infertility (conception rates) and impaired reproductive performance (embryonic death), particular during July, August and September (2, 3). For example, 28 day pregnancy rates reach their lowest levels from August to October, and consequently reduced farrowing rates occur in November and December. The deleterious effects of HS on pig reproduction variables are likely to increase in the future if climate change continues as some predict and as genetic selection for lean tissue accretion enhances the animal's sensitivity to HS. Consequently, climate change threatens the global protein food supply chain and compromises the competiveness of the US hog industry.

Heat-stressed animals redistribute blood to the periphery in an attempt to maximize radiant heat dissipation ultimately resulting in loss of intestinal barrier function. We have repeatedly demonstrated this in acutely and chronically heat-stressed pigs and have recently shown that HS increases (>2 fold) circulating LPS within 6 hours. LPS is detrimental for reproductive parameters, as it is thought to inducing preterm labor and contributing to premature embryonic loss: both phenomena observed in heat-stressed pigs. LPS decreases expression of the progesterone and luteinizing hormone (LH) receptors, and since progesterone is essential for maintenance of pregnancy and LH is required for ovulation, these support our hypothesis that LPS contributes to seasonal infertility. LPS I.V. infusion to cows also reduced corpus luteum function and size, reduced circulating progesterone and accelerated activation of the primordial follicle pool. Despite this knowledge in non-swine species, there is little information on the effects of LPS in swine.

Objectives: Our objective with this funding is to facilitate our long-term commitment in ***disseminating decision-impacting research in the swine industry*** through building collaborative research efforts linking foundational investigations, creating new knowledge to inspire and enable applied research experiments that will impact swine industry management decisions to improve reproductive efficiency and performance in pigs. To accomplish this objective, we will leverage existing and prior funding to scientifically expand and explore

opportunities that have been generated, and will continue arise from current funding. The funding in this project will be utilized for projects that will extend our current and completed research projects towards developing industry impact.

Specific Phase II objectives:

Objective 2.1. To investigate if the luteal phase of the estrous cycle is sensitive to heat stress, thereby contributing to spontaneous abortion during heat stress

Objective 2.2. To assess if progesterone supplementation during gestation impacts offspring survival during heat stress

Materials & Methods:

Animal live phase experiment 1. Post-pubertal gilts (n = 8) were synchronized to undergo estrus at a similar time point through feeding altrenogest (Matrix™) for 14 days. Gilts underwent thermal neutral (TN) conditions until estrus behavior was successfully detected through monitoring vaginal mucus, swollen vulva and boar exposure. At the time, gilts were randomly assigned to a TN or heat stress (HS) room two days after ovulation and treatment continued for 12 days (to the time of peak corpus luteum function). HS pigs were exposed to cyclical heat over a 24 h period with 12 h at 95 ± 1°F 21-31% relative humidity and cooling to 89 ± 1°F, 21-31% relative humidity for 12 h in a diurnal pattern. Rectal temperatures, respiration rate and feed intake were monitored. Blood samples were collected to monitor insulin and steroid hormones. At the end of the experimental period, gilts were humanely euthanized, ovaries collected, the corpora lutea excised, measured and weighed. Tissue was frozen for subsequent laboratory analyses. Uteri were flushed with sterile saline and flushings collected for laboratory analyses.

Animal live phase experiment 2. Post-pubertal gilts (n = 8) were synchronized to undergo estrus at a similar time point through feeding altrenogest (Matrix™) for 14 days. Gilts underwent thermal neutral (TN) conditions until estrus behavior was successfully detected through monitoring vaginal mucus, swollen vulva and boar exposure. At the time, gilts were randomly assigned to a TN or HS room two days after ovulation and treatment continued for 12 days (to the time of peak corpus luteum function). Gilts were artificially inseminated 2-3 times beginning at 2 day post-estrus (dpe) at 1900h and assigned to one of four experimental treatments (n = 8/treatment): TN no altrenogest (TN); TN plus altrenogest (TNA); HS no altrenogest (HS); HS plus altrenogest (HAS). HS pigs were exposed to cyclical heat over a 24 h period with 12 h at 95 ± 1°F 21-31% relative humidity and cooling to 89 ± 1°F, 21-31% relative humidity for 12 h in a diurnal pattern. Rectal temperatures, respiration rate and feed intake were monitored. Blood samples were collected to monitor insulin and steroid hormones. At the end of the experimental period, gilts were humanely euthanized, ovaries collected, the corpora lutea excised, measured and weighed. Tissue was frozen for subsequent laboratory analyses. Uteri were flushed with sterile saline and flushings collected for laboratory analyses.

RNA isolation: Ovaries were stored in RNA*later* solution at -80°C. Total RNA was isolated using an RNeasy Mini kit and concentrated using an RNeasy MinElute kit. RNA was eluted using 14 μL of RNase-free water. RNA concentration was determined using an ND-1000 Spectrophotometer (λ = 260/280nm; NanoDrop technologies, Inc., Wilmington, DE).

First strand cDNA synthesis and real-time polymerase chain reaction (PCR): Total RNA was reverse transcribed into cDNA utilizing the Superscript III One-Step RT-PCR System. Two microliters of diluted cDNA (1:50) was amplified using an Eppendorf Mastercycler using a Quantitect™ SYBR Green PCR kit. A typical cycling program consisted of a 15 min hold at

95°C and 45 cycles of: denaturing at 95°C for 15 sec, annealing at 58°C for 15 sec, and extension at 72°C for 20 sec at which point data will be acquired. Product melt conditions were determined using a temperature gradient from 72°C to 99°C with a 1°C increase at each step.

Protein Isolation: Pools of whole ovarian protein homogenates were prepared from cultured ovaries via homogenization in tissue lysis buffer. Briefly, homogenized samples were placed on ice for 30 min, followed by two rounds of centrifugation at 10,000 rpm for 15 min. Supernatant was collected and sample stored at -80°C until further use. Protein was quantified using a standard BCA protocol on a 96-well assay plate. Emission absorbance values were detected with a λ= 540nm excitation on a Synergy™ HT Multi-Detection Microplate Reader using KC4™ software (Bio-Tek® Instruments Inc., Winooski, VT).

Western Blot Analysis: SDS-PAGE was used to separate protein homogenates which were subsequently transferred to nitrocellulose membranes. Briefly, membranes were blocked for 1-4 h with shaking at 4°C in 5% milk in Tris-buffered saline (TBS) with Tween-20 (TTBS). Membranes were incubated with primary antibody in 5% milk in TTBS for 1 h at 4°C. Membranes were washed with TTBS three times for 10 min. HRP-conjugated secondary antibody was added for 1h at room temperature. Membranes were again washed in TTBS, followed by a single wash for 10 min in TBS. Western blots were detected using chemiluminescence (ECL plus reagent) and exposed to X-ray film. Densitometry of the appropriate bands was performed using NCBI Image J software.

Corpus Luteum Progesterone Extraction
From each animal, 2-4 CLs were powdered with a mortar and pestle. Approximately 100 mg of powdered tissue per animal was weighed into a new tube, 1 mL of 5% trichloroacetic acid added and homogenized. These tubes were centrifuged at 10,621 x g at 4°C for 5 minutes to clarify the supernatant, which was then removed and placed into a new tube. The extractions were stored at -80°C until further analysis.

Progesterone quantification: Concentrations of serum and CL P_4 were obtained using a colorimetric competitive binding ELISA specific to P_4, according to manufacturer's protocol. Serum collected at 0, 4, and 8 dpe was run undiluted. Serum collected at 12 dpe was diluted 1:3 and CL extractions were diluted 1:100 to bring them into the detectible range of the assay as per manufacturer's protocol.

Insulin quantification: A porcine Insulin enzyme linked immunosorbent assay (ELISA, 10-1200-01) was obtained from Mercodia (Uppsala, Sweden) and the assay performed as per manufacturer's instructions.

Statistical Analysis: All data were statistically analyzed using GraphPad Prism software. Comparison of two treatments was performed using T-test; comparison of more than two treatments was performed by ANOVA. A *P*-value < 0.05 was considered significantly different.

Results:

Objective 2.1. To investigate if the luteal phase of the estrous cycle is sensitive to heat stress, thereby contributing to spontaneous abortion during heat stress: We have determined a reduction in corpora lutea weight and diameter due to HS. Feed intake was reduced by HS and insulin:feed intake was increased, thus as demonstrated in multiple HS studies by our group at ISU, the gilts were hyperinsulinemic. Progesterone per unit of CL tissue was actually increased during HS, which we theorize may be a compensatory effect in response to reduced tissue mass, in order to maintain a constant progesterone level in

circulation, what we do not know is whether this compensatory response eventually becomes overwhelmed if HS continues. This is a future area of interest for the group. We have not demonstrated an effect of HS on any of the enzymes measured to date in this tissue. However, the availability of more sensitive tools that have become available at ISU will be fully utilized to further assess effects of HS on the CL (ongoing).

Katie Bidne who spearheaded this work was awarded her MS degree and is now pursuing a PhD program at the University of Nebraska. This work was presented at the Society for the Study of Reproduction annual meeting and a manuscript is being prepared for submission in 2018 to the Journal of Animal Science. Bidne, K.L., Romoser, M.R., Kvidera, S.K., Baumgard, L.H., Ross, J.W., Keating, A.F. Heat Stress in the luteal phase decreases luteal size but not progesterone production in gilts.

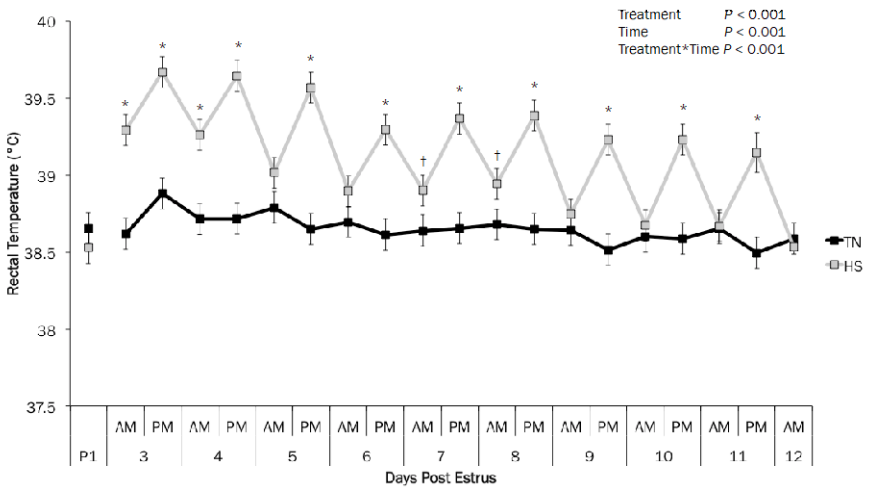


Figure 1. Impact of chronic heat stress on rectal temperature. Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C, 36-57% relative humidity; TN) conditions from 3 – 12 dpe. Rectal temperature was recorded three times each morning and evening. Line graph represents average morning (AM) and evening (PM) rectal temperature per treatment ± SEM. There was an increase (P < 0.001) in average rectal temperature in the HS treatment compared to TN. * indicates difference between treatments at individual time points (P < 0.05); † indicates a tendency for difference between time points (P < 0.1).

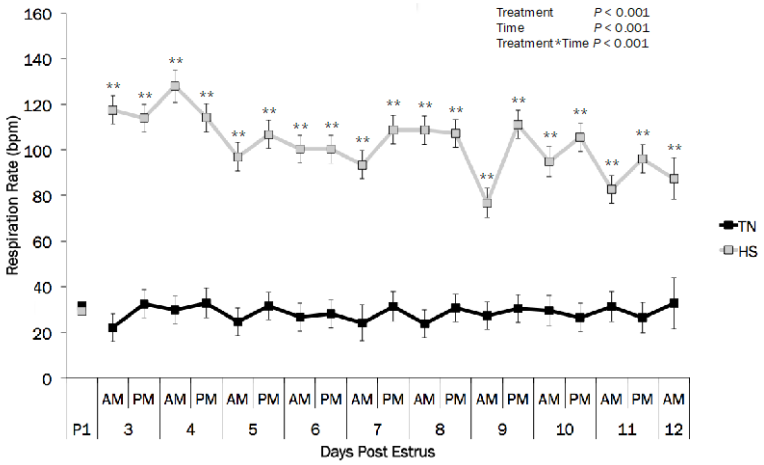


Figure 2. Impact of chronic heat stress on respiration rate. Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C , 36-57% relative humidity; TN) conditions from 3 – 12 dpe. Respiration rate was recorded three times each morning and evening. Line graph represents average morning (AM) and evening (PM) respiration rate per treatment ± SEM. There was an increase (P < 0.001) in average respiration rate in the HS treatment compared to TN. ** indicates difference between treatments at individual time points (P < 0.001).

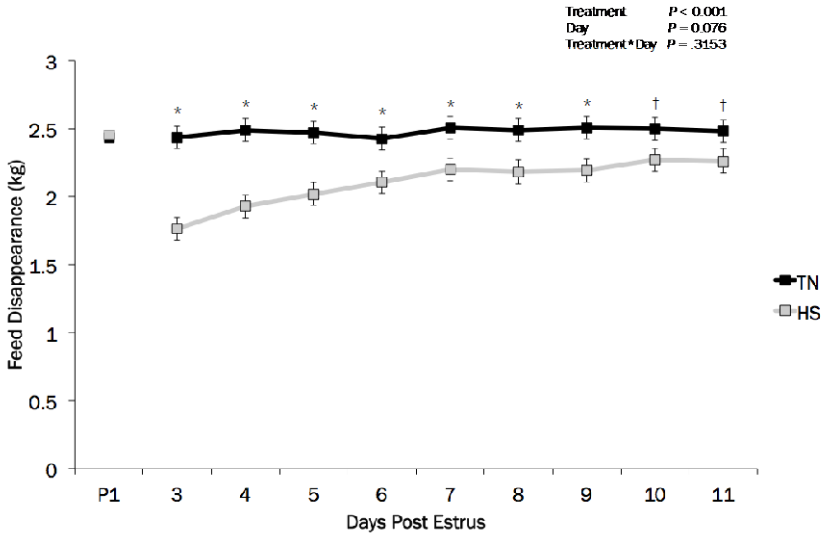


Figure 3. Impact of chronic heat stress on feed disappearance. Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C , 36-57% relative humidity; TN) conditions from 3 – 12 dpe. Animals were limit fed 2.7 kg daily at 0600h. Feed disappearance was recorded daily. Line graph represents feed disappearance each day in each group ± SEM. * indicates difference between treatments at individual time points ($P < 0.05$); † indicates a tendency for difference between time points ($P < 0.1$).

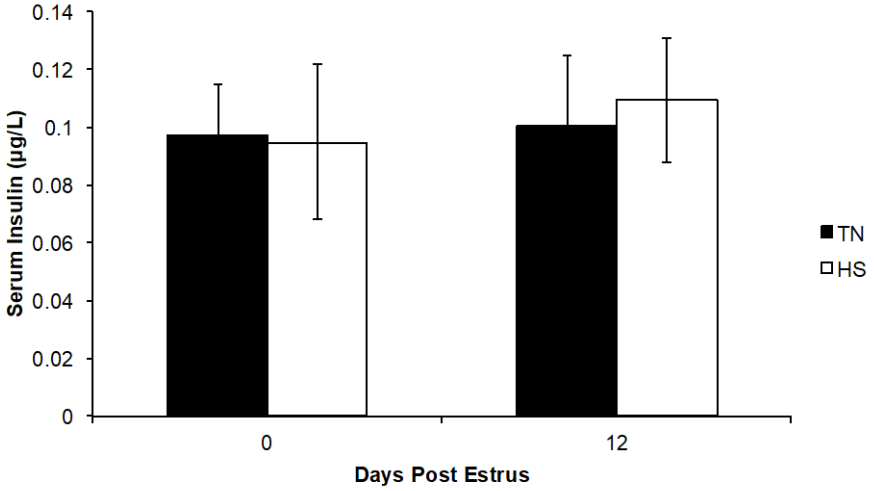


Figure 4. Impact of chronic heat stress on serum insulin concentrations. Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C , 36-57% relative humidity; TN) conditions from 3 – 12 dpe. A fasting blood sample was collected on 0 and 12 dpe and analyzed for insulin concentrations via colorimetric ELISA. Bar graph represents the average serum insulin concentration in each group ± SEM. $P > 0.05$ between groups at each time point.

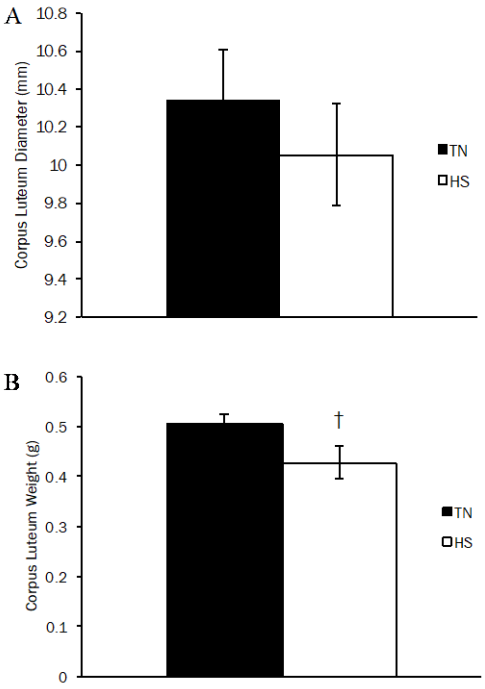


Figure 5. Impact of chronic heat stress on corpus luteum diameter and weight. Post-pubertal gilts were exposed to diurnal heat stress conditions from 3 – 12 dpe. After euthanasia, ovaries were removed and the diameter of each corpus luteum measured using digital calipers. Bar charts represent the average (A) diameter and (B) weight in each group ± SEM. † indicates a tendency for difference between time points $P < 0.1$.

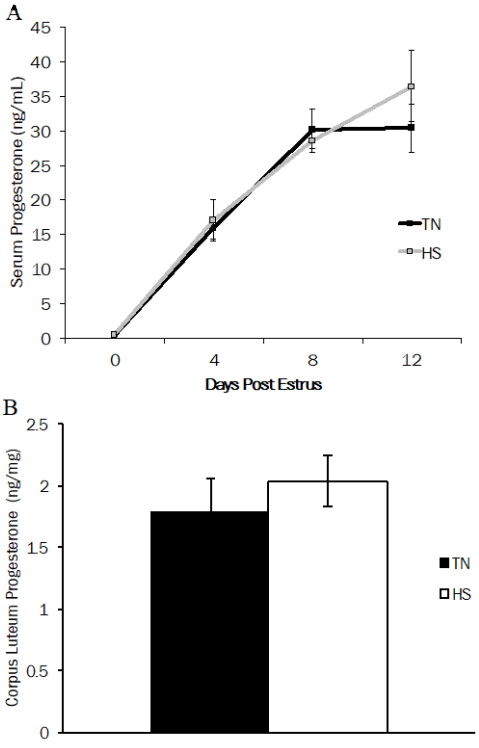


Figure 6 (right). Impact of chronic heat stress on serum and luteal progesterone concentrations. Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C, 36-57% relative humidity; TN) conditions from 3 – 12 dpe. (A) Blood was collected on 0, 4, 8, and 12 dpe and analyzed for progesterone concentrations via colorimetric ELISA. Line graph represents the average serum progesterone concentration in each group ± SEM. P_4 levels were different between time points, but within each time point no differences were noted, $P > 0.05$. (B) Corpora lutea from each animal were powdered and homogenized in 5% trichloroacetic acid and diluted samples run on a colorimetric ELISA to quantify P_4 content. Bar graph represents the average luteal P_4 concentration per mg of tissue in each group ± SEM; $P = 0.48$.

Figure 7 (right). Impact of chronic heat stress on serum progesterone relative to total CL weight. Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C , 36-57% relative humidity; TN) conditions from 3 – 12 dpe. Blood was collected on 0, 4, 8, and 12 dpe and analyzed for progesterone concentrations via colorimetric ELISA. After euthanasia, ovaries were removed and the diameter of each corpus luteum measured using digital calipers. Bar graph represents the average serum progesterone concentration per total CL tissue weight in each group ± SEM; $P = 0.07$.

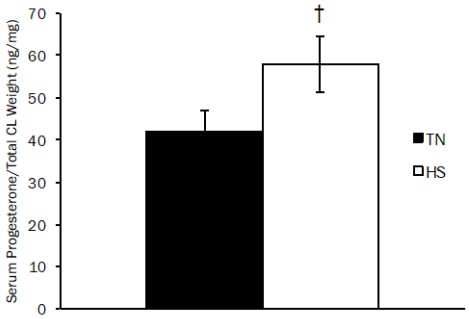


Figure 8 (right). Impact of chronic heat stress on luteal STAR and 3BHSD protein abundance.
Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C, 36-57% relative humidity; TN) conditions from 3 – 12 dpe. Bar charts represent relative luteal (A) STAR and (B) 3BHSD protein abundance for TN and HS animals as densitometric mean ± SEM, $P > 0.05$. Representative western blots are presented for STAR and 3BHSD proteins. Equal protein loading is confirmed by Ponceau S staining.

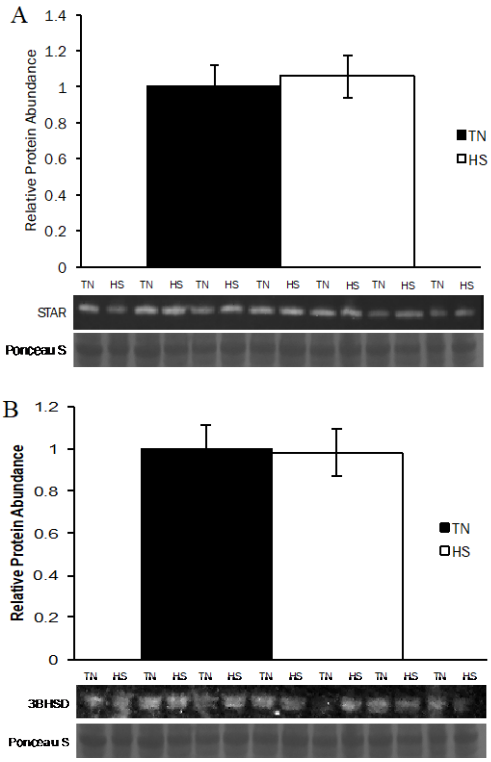
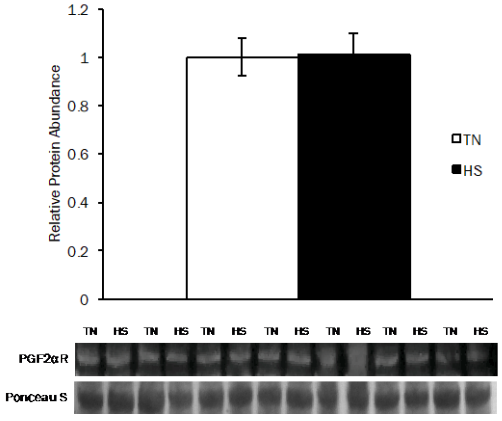


Figure 9 (right). Impact of chronic heat stress on luteal PGF2αR abundance.
Post-pubertal gilts were exposed to diurnal heat stress (35±1°C for 12h/31.6°C for 12h, 21-31% relative humidity; HS) or thermal neutral (20±1°C, 36-57% relative humidity; TN) conditions from 3 – 12 dpe. Bar chart represent relative luteal PGF2αR protein abundance for TN and HS animals as densitometric mean ± SEM, $P = 0.94$. Representative western blots are presented for PGF2αR protein. Equal protein loading is confirmed by Ponceau S staining.



Objective 2.2. To assess if progesterone supplementation during gestation impacts offspring survival during heat stress

This project complements the work in Objective 1. In many species, it is known that progesterone supplementation (intramuscularly in humans; via CIDR in cows) can maintain a pregnancy if that pregnancy is susceptible to demise (demise due to a variety of factors). Thus, we completed a project to investigate if progesterone supplementation would be a strategy to improve pregnancy outcome in swine during heat stress (HS). Post-pubertal gilts (n = 8) were synchronized to undergo estrus at a similar time point through feeding altrenogest (Matrix™) for 14 days. Gilts underwent thermal neutral (TN) conditions until estrus behavior was successfully detected through monitoring vaginal mucus, swollen vulva and boar exposure. At the time, gilts were randomly assigned to a TN or HS room two days after ovulation and treatment continued for 12 days (to the time of peak corpus luteum function). Gilts were artificially inseminated 2-3 times beginning at 2 day post-estrus (dpe) at 1900h and assigned to one of four experimental treatments (n = 8/treatment): TN no altrenogest (TN); TN plus altrenogest (TNA); HS no altrenogest (HS); HS plus altrenogest (HAS). HS pigs were exposed to cyclical heat over a 24 h period with 12 h at 95 ± 1°F 21-31% relative humidity and cooling to 89 ± 1°F, 21-31% relative humidity for 12 h in a diurnal pattern. Rectal temperatures, respiration rate and feed intake were monitored. Blood samples were collected to monitor insulin and steroid hormones. At the end of the experimental period, gilts were humanely euthanized, ovaries collected, the corpora lutea excised, measured and weighed. Ovarian and corpora lutea tissue was frozen for subsequent laboratory analyses. Uteri were flushed with sterile saline and conceptus plus flushings collected for laboratory analyses. Our preliminary data to date indicate that altrenogest increased IL1 beta in TN but not HS gilts. Interestingly, altrenogest appears to increase conceptus development but statistical analysis is continuing.

Matthew Romoser spearheaded this work and was awarded his MS degree. He is now working in the pork industry through Fast Genetics. This paper is in preparation for submission to the Journal of Animal Science. Romoser, M.R., Bidne, K.L., Baumgard, L.H., Keating, A.F., Ross, J.W. Effects of increased ambient temperatures and supplemental altrenogest prior to pregnancy establishment in gilts

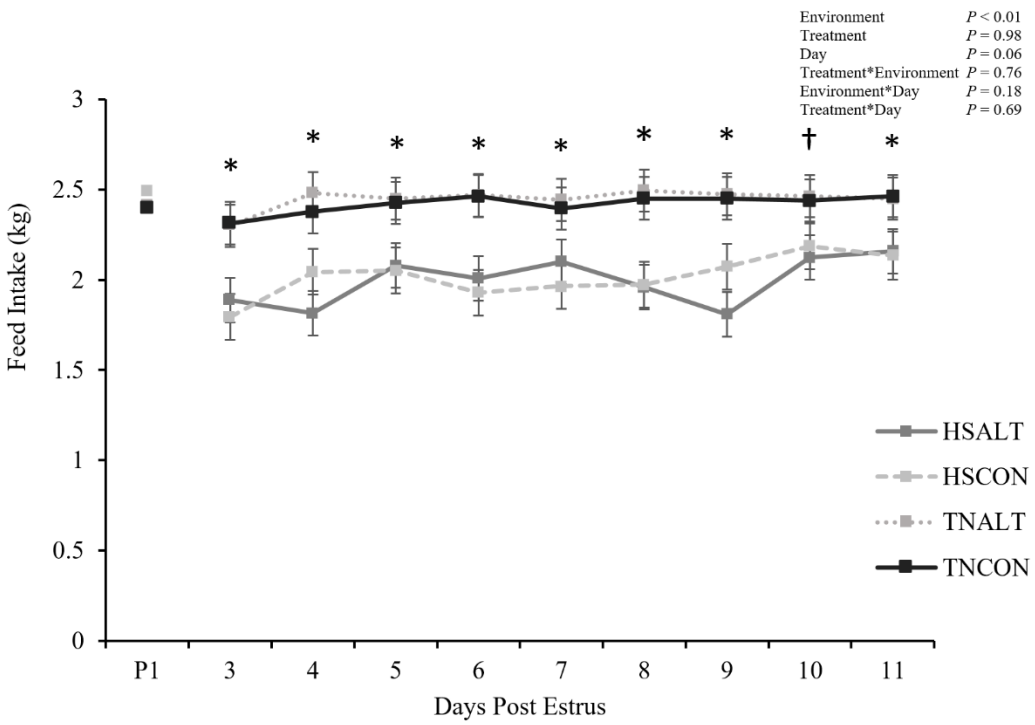


Figure 1. Impact of cyclical heat stress on feed intake. Post-pubertal gilts were assigned to one of four different treatments, heat stress (HS) conditions (35 ± 1°C for 12h, 31 ± 6°C for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions (20 ± 1°C, 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Environment assignment and ALT administration lasted from d 3-12 post estrus. Animals were limit fed 2.7 kg at 0600h. Feed intake (FI) was tracked daily, both A.M. and P.M. Line graphs denote FI each d post estrus for each group ± SEM. * indicates difference between environment at specified time points ($P < 0.01$); † indicated difference between environment ($P < 0.05$). No Differences were detected across ALT treatments.

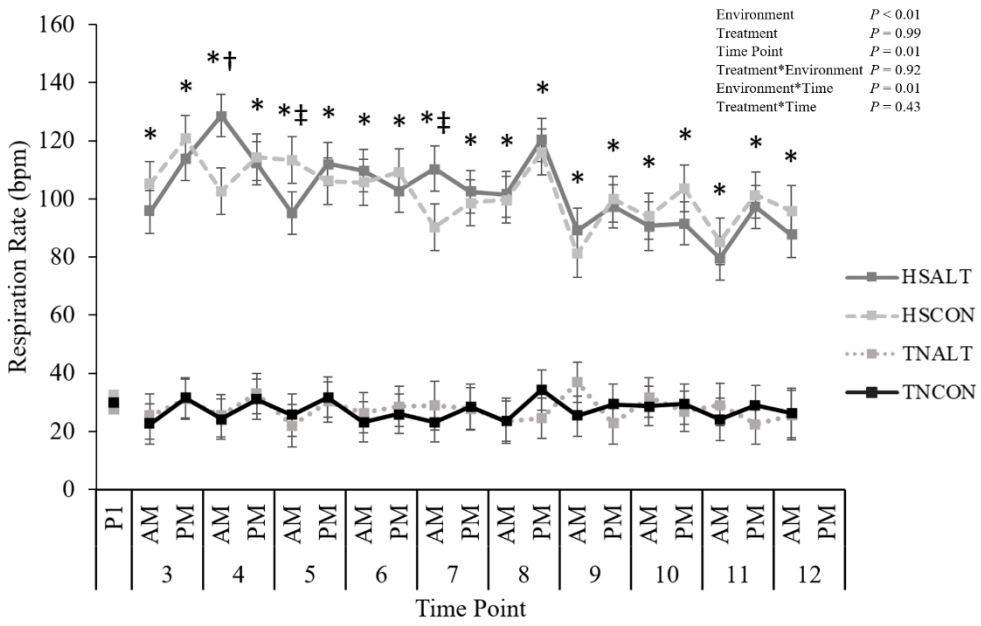


Figure 2 Impact of cyclical heat stress on respiration rate. Post-pubertal gilts were assigned to one of four different treatments, heat stress (HS) conditions (35 ± 1°C for 12h, 31 ± 6°C for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions (20 ± 1°C, 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Respiration rate (RR) was recorded by observing flank movements for 15 seconds and multiplied to determine beats per minute (bpm). RR was assessed three times in morning (AM) and evening (PM) and averaged together for each time point per treatment and environment ± SEM. Line graphs denote bpm for each time point post estrus for each group ± SEM. * indicates difference between environment at specified time points ($P < 0.01$); † denotes difference between ALT treatments in HS group ($P < 0.05$); ‡ denotes a tendency for difference between ALT treatments in HS group ($P < 0.10$).

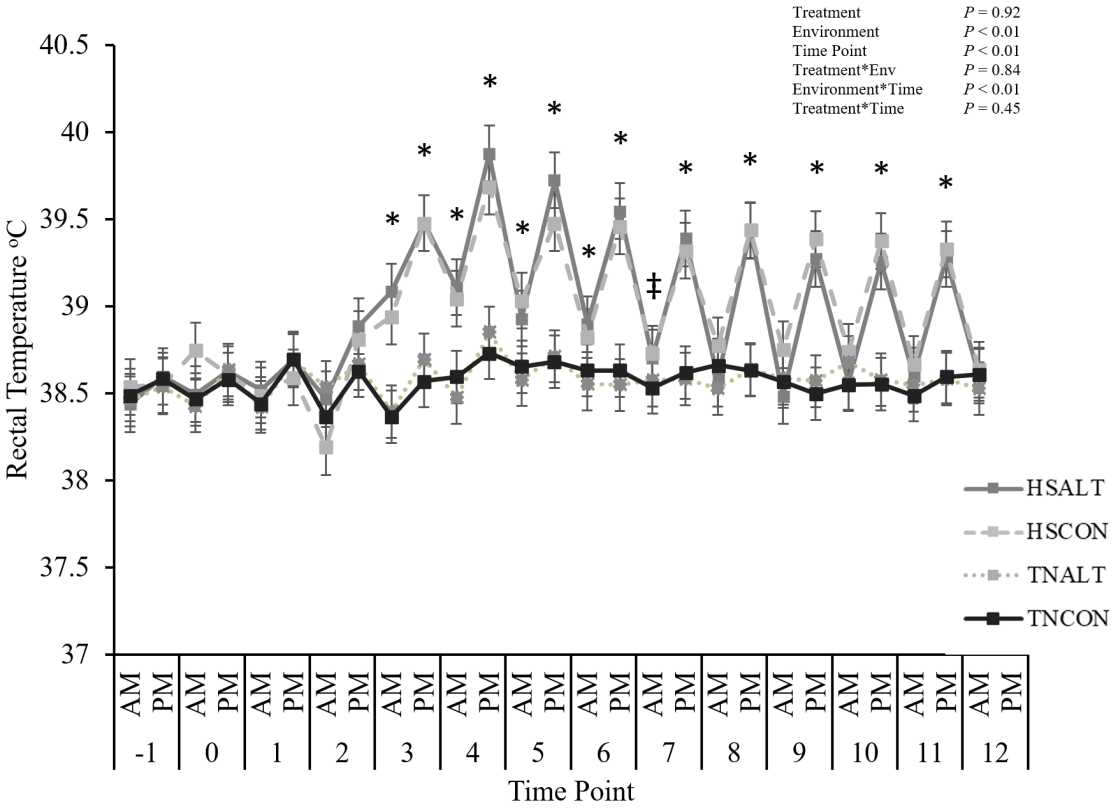


Figure 3. Impact of cyclical heat stress on rectal temperature. Post-pubertal gilts were assigned to one of four different treatments, heat stress (HS) conditions (35 ± 1°C for 12h, 31 ± 6°C for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions (20 ± 1°C, 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Environment assignment and ALT administration lasted from d 3-12 post estrus. Rectal temperatures (T_R) was recorded three times each morning (AM) and evening (PM). Line graph represents average morning and evening T_R per treatment ± SEM. There was an increase ($P < 0.01$) in average T_R in the HS environment compared to TN. * and ‡ indicate a difference of ($P < 0.01$) and ($P < 0.1$) respectively.

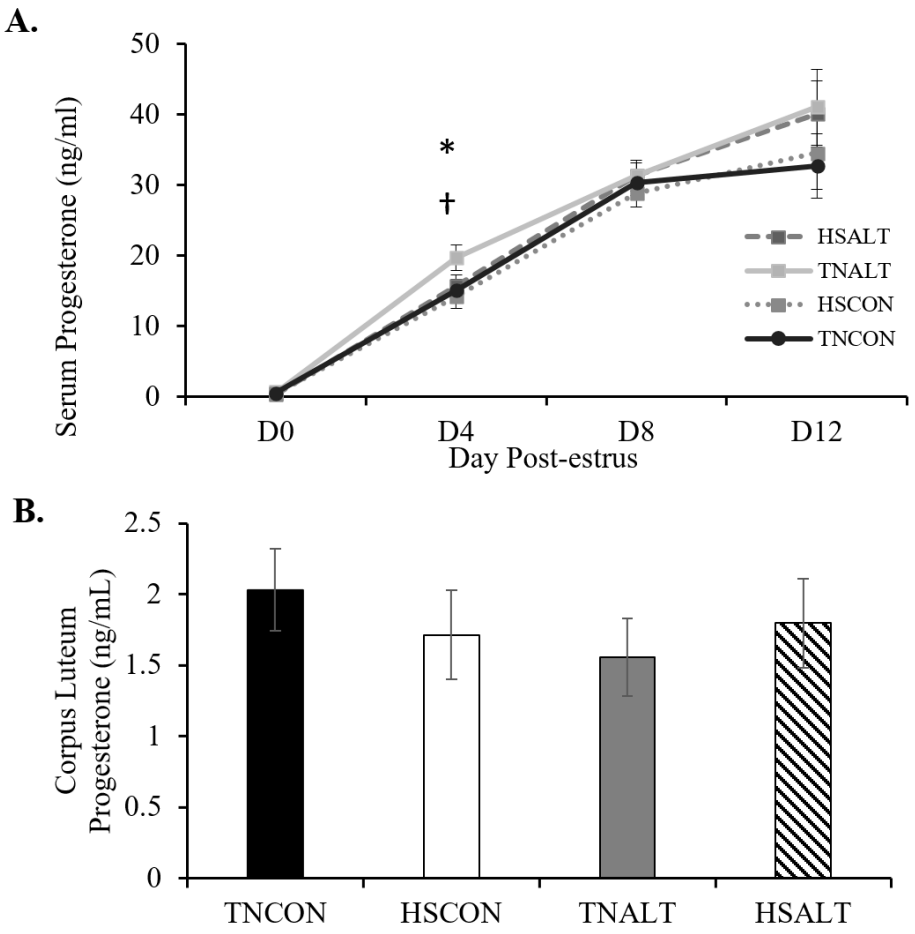


Figure 4. Impact of increased ambient temperature and altrenogest supplementation on serum and luteal progesterone concentrations. Bred gilts were subject to either heat stress (HS) conditions (35 ± 1°C for 12h, 31 ± 6°C for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions (20 ± 1°C, 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Graphs represent LS means for serum and luteal P4 concentrations in each group ± SEM. A. Blood was collected to assess progesterone (P4) concentrations on 0, 4, 8, 12 d post estrus (dpe) and analyzed using colorimetric ELISA. Serum P4 concentrations did not differ at 0, 8 and 12 dpe. Serum P4 concentrations at 4 dpe were increased ($P < 0.05$; *) for TNALT compared to HSCON (19.73 vs. 14.18 ng/ml). A tendency for increased serum P4 concentrations exists at 4 dpe between TNALT and TNCON ($P < 0.1$; †). B. Corpora Lutea (CL) were collected from each animal, powdered and homogenized in 5% trichloracetic acid. Homogenates were diluted and analyzed to quantify P4 concentrations. No differences were detected across environment ($P = 0.90$) or ALT treatment ($P = 0.52$).

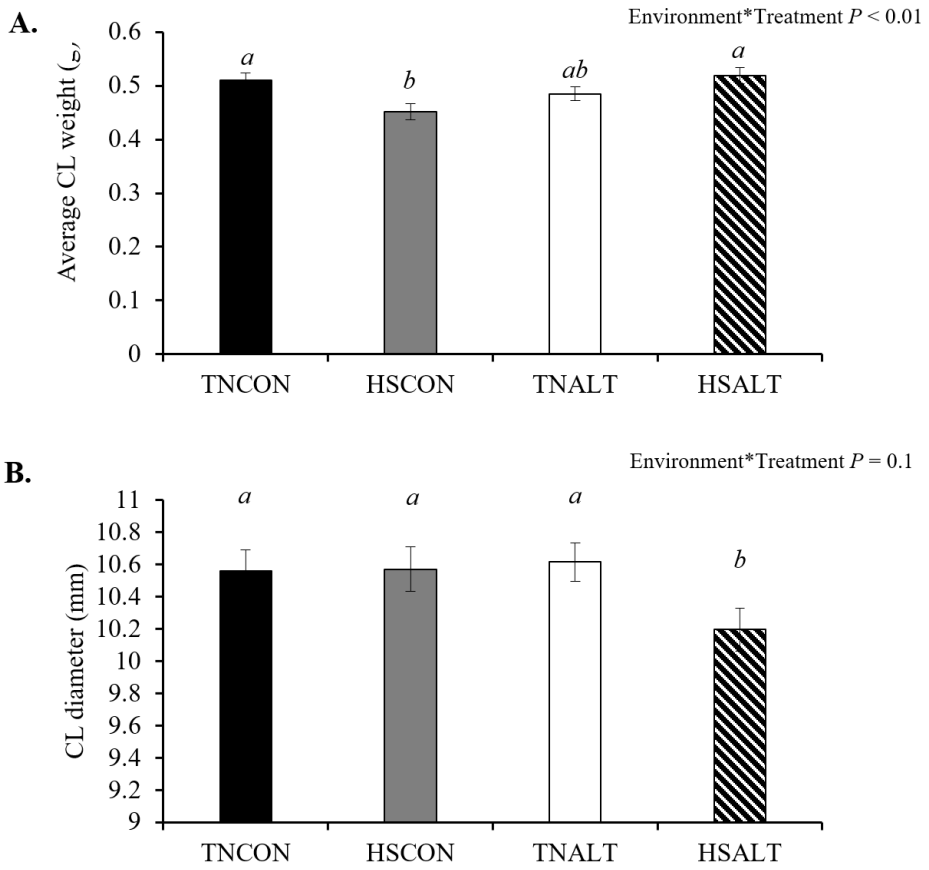


Figure 5. Impact of increased ambient temperatures and altrenogest supplementation on corpus luteum size. Bred gilts were subject to either heat stress (HS) conditions (35 ± 1°C for 12h, 31 ± 6°C for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions (20 ± 1°C, 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Bar charts depict LS means for CL weight (g) and diameter (mm) for each group ± SEM. **A.** Average corpora lutea (CL) weight for each group were recorded. An environment by altrenogest treatment interaction was observed ($P < 0.01$). CL weight was decreased ($P < 0.01$) in HSCON compared to TNALT and HSALT (0.45 vs. 0.51 and 0.52 g respectively). CL of TNALT gilts tended to be heavier ($P < 0.1$) compared to CL of HSCON gilts. Differenced in letters denote statistical difference ($P < 0.01$). CL diameter was measured and averaged within treatments. **B.** A tendency for environment by altrenogest treatment interaction was observed ($P = 0.1$) CL diameter was decreased ($P \leq 0.05$) in HSALT compared to remaining group. Differenced in letters denote statistical difference ($P < 0.05$). Ovulation rates did not differ across individuals (data not shown).

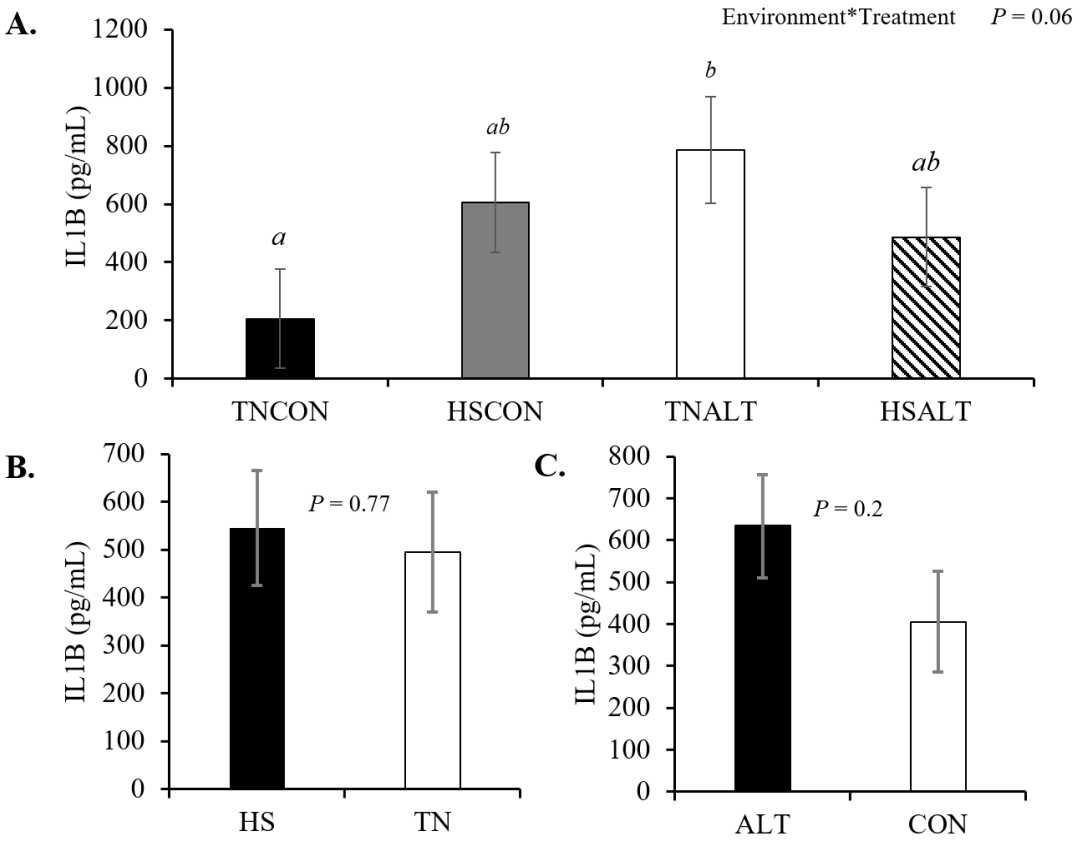


Figure 6. Effect of increased ambient temperatures and altrenogest supplementation on conceptus Interleukin-1β production. Uterine flushes were collected by injection of 20 mL sterile saline into the uterine lumen and collected after manipulating flush volume proximally and distally to the uterine bifurcation. Interleukin-1β (IL1B) was measured in the uterine flush using colorimetric ELISA. Data presented represent IL1B concentration (pg/mL) LSMEANS ± SEM. **A.** An increase (P = 0.03) was observed between TNCON and TNALT (205.28 vs. 785.38 pg/ml). **B.** No difference was observed between gilts in HS or TN conditions on conceptus IL1B production (P = 0.77). **C.** No difference was observed between gilts in ALT or CON treatment groups on conceptus IL1B production (P = 0.2).

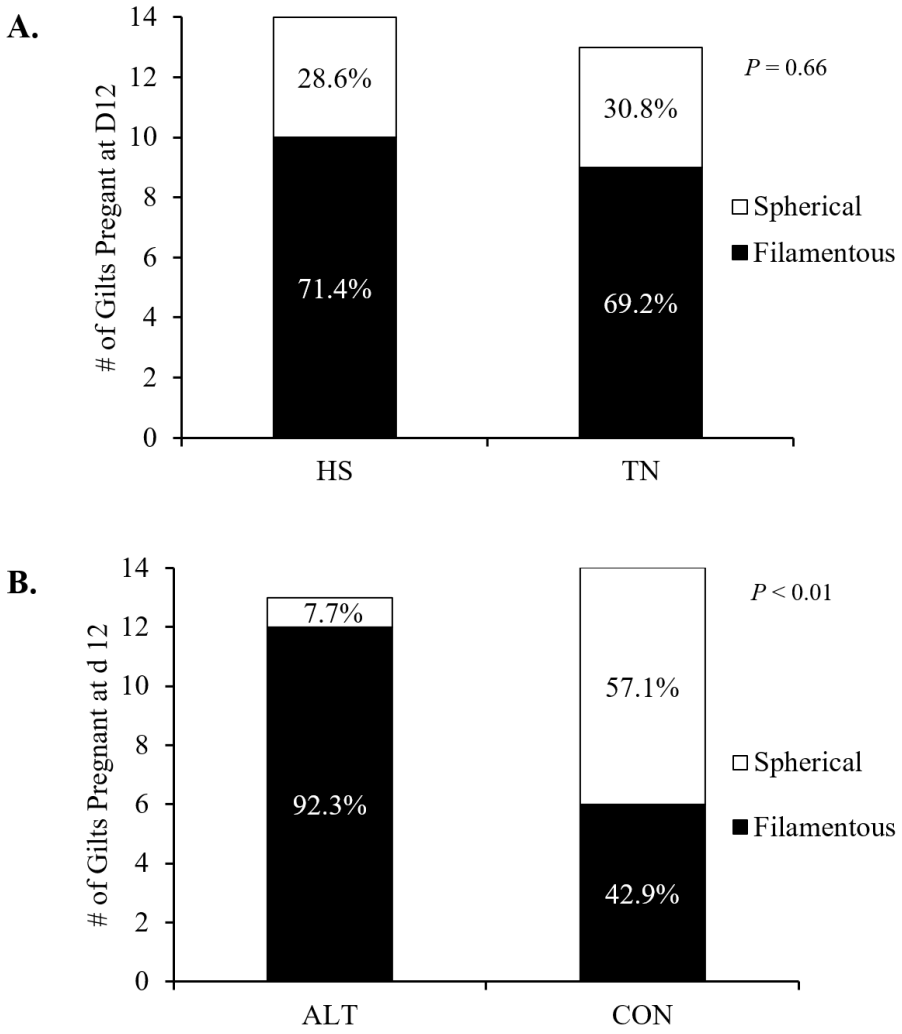


Figure 7. Effect of environmental conditions and altrenogest treatment on conceptus development. Conceptuses were recovered from 27 of 30 gilts inseminated. Bars represent number of gilts under specified environment or treatment that yielded conceptuses. Black portion indicates conceptuses filamentous in morphology, white portion represents gilts with spherical conceptuses at the time of flushing. **A.** No effect was observed between environment on conceptus development ($P = 0.66$). **B.** An increase ($P < 0.01$) in the percentage of gilts with filamentous conceptuses was observed for those treated with ALT compared to CON gilts (92.3 vs 42.9%).

Completion of Projects initiated in Phase I.

Objective 1.1: To determine the beneficial effects of L-arginine supplementation during gestation on litter size and weight, in addition to progeny performance. The objective of this study was to evaluate the effects of feeding supplemental arginine to gestating gilts through evaluation of reproductive performance, and subsequent postnatal growth and development. Dr. Beth Hines was awarded her doctoral degree and this study was included in her dissertation. Two manuscripts are on the verge of submission to the Journal of Animal Science based upon this work. Dr. Hines began a faculty position at Pennsylvania State University in 2018.

Hines, E.A., Romoser, M.R., Kiefer, Z.E., Keating, A.F., Baumgard, L.H., Niemi, J., Gabler, N.K., Patience, J.F., Haber, B., Williams, N.H., Kerr, B.J., Touchette, K.J., Ross, J.W. The impact of dietary supplementation of arginine during gestation in a commercial swine herd I: Gilt reproductive performance. In preparation for submission to Journal of Animal Science.

Hines, E.A., Romoser, M.R., Kiefer, Z.E., Keating, A.F., Baumgard, L.H., Niemi, J., Haber, B., Williams, N.H., Kerr, B.J., Touchette, K.J., Ross, J.W. The impact of dietary supplementation of arginine during gestation in a commercial herd: II. Offspring performance. In preparation for submission to Journal of Animal Science.

Objective 1.2: To understand the molecular pathways affected in the ovary in response to heat stress. The rationale for this study was that more knowledge is needed to improve our understanding of how the ovary and oocyte mitigate stress, and this knowledge will allow for the development of techniques to alleviate the detrimental effects of HS on reproductive efficiency. Dr. Ben Hale defended his doctoral degree his findings were published in Biology of Reproduction (a leading Reproduction Journal). Briefly, he discovered that autophagy induction is protective during HS on oocyte viability.

Hale, B.J., Hager, C.J., Siebert, J.T., Selsby, J.T., Baumgard, L.H., Keating, A.F., Ross, J.W. 2017. Heat stress induces autophagy in pig ovaries during follicular development. Biology of Reproduction. 97(3):426-437.

Objective 1.3: To develop and test novel selection approaches to optimize replacement gilt selection. This objective presents value to the swine industry as a method that producers could use to identify potential replacements with an increased probability of achieving first estrus by 180 and 200 days of age. This is important as currently the age that a gilt reaches puberty is a useful predictor of sow lifetime productivity. Currently, data has been collected on a large number of gilts and analysis is progressing.

Discussion: The work completed during this phase of funding has been valuable in determining data that explain the biological mechanisms that occur during HS and which are lightly likely to contribute to seasonal infertility associated with HS.

Although there is ample evidence in non-swine species to support that LPS causes regression/destruction of the corpus luteum to result in embryonic loss, surprisingly, whether this occurs in swine remains unknown. Since we know that the gut becomes leaky during HS and that the pig becomes endotoxic (chronic and low level), it was logical that HS might affect the corpus luteum to impact offspring survivability. Thus, our projects described herein devised a strategy to exposure the sow to HS only during the luteal phase. The animals experienced thermal neutral conditions during the follicular phase and 48h after behavioral estrus was observed (thus assuming ovulation happened in this window), gilts experienced a diurnal pattern of thermal neutral or heat stress conditions. Rectal temperatures and respiration rates confirmed

that the animals were heat stressed. The experiment continued until day 12 post-ovultion since this is the time at which progesterone production is at maximum in swine. Blood samples were collected at days 4 and 8 post-ovulation to determine if there was a temporal effect of HS on progesterone production from the corpus luteum. The weight and diameter of the corpora lutea were also captured to determine effects of HS exposure.

In a second paradigm, we introduced pregnancy and the potential for progesterone supplementation to be used as a strategy to improved gestational outcomes during HS. Thus, this second project compared the effects of HS during pregnancy as well as then whether during pregnancy that progesterone supplementation alone was beneficial and finally the dual effect of HS in the presence of progesterone supplementation was assessed. We have discovered that indeed HS reduces the size of the corpora lutea in swine which could contribute to seasonal infertility. We also discovered that despite not apparent overall effect of HS on progesterone production, that given the smaller size of the corpora lutea during HS, that more progesterone is actually being produced by that tissue. What remains unclear is whether these corpora lutea can sustain this heightened progesterone production during chronic HS events. In the gestation project, we have discovered a reduced corpora lutea weight during pregnancy and HS, consistent with the effects noted in open gilts. We also noted that progesterone supplementation during HS returns the corpora lutea weight to thermal neutral levels – indicating a potential therapeutic effect of progesterone supplementation on corpora lutea size. We also however discovered that despite a return to thermal neutral weight during HS due to progesterone supplementation, that he corpora lutea were smaller. This discovery along with that in the open animals, indicates that there may be a shift in cellular composition of the corpora lutea. We also noted that there was an increased in IL1beta, during progesterone supplementation which may be an area of interest in the future for this area of research. Interestingly, there was also an increase in the number of filamentous (more developed) blastocysts due to altrenogest (progesterone) supplementation. These findings to date indicate that the corpus luteum in swine is sensitive to HS and that this could contribute to seasonal infertility. Additionally, the potential for progesterone supplementation to improve fertility outcomes in swine in the absence and during HS is promising and could be a logical intervention for use in production systems.

Additional manuscripts and abstracts that have resulted directly or indirectly from Phase I or Phase II of this funded project.

Graves, K.L., Seibert, J.T., Keating, A.F., Baumgard, L.H., Ross, J.W. 2018. Characterizing the acute heat stress response in gilts: II. Assessing repeatability and association with fertility. Journal of Animal Science. 96(6):2419-2426.

Seibert, J.T., Graves, K.L., Johnson, T.P., Keating, A.F., Baumgard, L.H., Ross, J.W. 2018. Characterizing the acute heat stress response in gilts: I. Thermoregulatory and production variables. Journal of Animal Science. 96(3):941-949.

Dickson, M.J., Hager, C.L., Al-Shaibi, A., Thomas, P.Q., Baumgard, L.H., Ross, J.W., Keating, A.F. 2018. Impact of heat stress during the follicular phase on porcine ovarian steroidogenic and phosphatidylinositol-3 signaling. Journal of Animal Science. 96(6):2162-2174.

Kim, K.S., Seibert, J.T., Edea, A., Graves, K.L., Kim, E.S., Keating, A.F., Baumgard, L.H., Ross, J.W., and Rothschild, M.F. 2018. Characterizing the acute heat stress response in gilts: III. Identification of underlying genomic control. Journal of Animal Science. 96(6):2074-2085.

Bidne, K.L., Dickson, M.J., Ross, J.W., Baumgard, L.H., Keating, A.F. 2018. Disruption of female reproductive function by endotoxins. Reproduction. 155(4):R169-R181.

Abstracts presented:

Adur, M.K., Romoser, M.R., Bidne, K.L., Seibert, J.T., Hines, E.A., Keating, A.F., Baumgard, L.H., Ross, J.W. 2018. Effect of increased ambient temperature on porcine endometrial heat

stress response during the peri-implantation phase. Society for the Study of Reproduction annual meeting, New Orleans.
Seibert, J.T., Adur, M.K., Thomas, P.Q., Keating, A.F., Baumgard, L.H., Ross, J.W. 2018. The effects of heat stress of lipopolysaccharide exposure on ovarian heat shock protein expression in pigs. Society for the Study of Reproduction annual meeting, New Orleans.

Schulz, R.B., Bidne, K.L., Romoser, M.R., Adur, M.K., Seibert, J.T., Baumgard, L.H., Keating, A.F., Ross, J.W. Effect of heat stress on corpus luteum function and expression of microRNA during early pregnancy in pigs. Society for the Study of Reproduction annual meeting, New Orleans.

Corredor, F.A., Leach, R., Ross, J.W., Keating, A.F., Serão, N.V.L. 2018. Genetic and genomic analysis of vulva size in Landrace and Yorkshire gilts. American Society of Animal Science annual meeting.