RESEARCH REPORT
ANIMAL SCIENCE

Title: Understanding the biology of seasonal infertility to develop mitigation strategies for swine – **NPB #13-129**

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Industry Summary:

Mitigating seasonal infertility is critically important to increased production efficiency for American pork producers. Elevated ambient temperature is a significant factor contributing to reduced litter size and farrowing rates. Despite the reproducibility and predictability of seasonal infertility, knowledge of the underlying biological mechanisms contributing to the reduced fertility during the summer months is extraordinarily lacking. Defining the biological underpinnings contributing to reduced reproductive ability during seasonal infertility is the first and urgently needed step in developing mitigation strategies designed to reduce the nearly $1 billion annual revenue losses to the swine industry. Swine producers are subject to numerous variables that are difficult to predict (i.e. fluctuations in input costs and disease outbreaks) and that can dramatically impact profitability. For example, although PRRS is not nearly as economically devastating as heat stress (HS), extensive mitigation strategies are employed to combat the PRRS virus, despite the fact that PRRS is relatively unpredictable. On the contrary, HS is a predicable event (particularly seasonal infertility) for which mitigation strategies are dramatically lacking.

This project significantly improved our understanding of the interaction between HS and reproduction in sows and gilts and the potential relationship with seasonal infertility.

Specifically this project was enabled the following impacts/discoveries that we are using to move the pork industry forward:

1) In Objective 1, we demonstrated that seasonal infertility in large commercial pork production system was not strongly correlated with HS during the wean-to-estrus interval (WEI).
    a. We are now pursuing strategies and funding to determine the impact that the lactation environment has on P1 weaned sow reproductive performance in the summer.
2) In Objective 1, we did not see differences in circulating LPS binding protein (LBP) or insulin during the WEI of sows weaned during the summer or spring.
    a. This is important in strengthening our understanding of the biological consequences of the lactation to gestation transition during different seasons.

3) In Objective 2, we demonstrated that thermoregulatory response to HS is repeatable in gilts exposed to HS more than 4 months between exposures.
    a. This was a critically important finding for the pork industry. Subsequently, our group has leveraged this project and samples collected during it to secure additional dollars to pursue the identification of specific genomic regions in pigs associated with contributing to production efficiency during HS.
4) In Objective 2, we demonstrated a lack of impact on HS during a Matrix synchronized follicular phase.
    a. Based on this project we are now hypothesizing that seasonal infertility manifests itself through the combination of seasonal, environmental, metabolic conditions, and genetics of the sows.
5) In Objective 2, we determined that HS during the follicular phase did not affect 17β-estradiol production in the follicle but altered proteins involved in ovarian physiology. However, gilts susceptible to HS, as determined by their thermoregulatory response, did have reduced corpora lutea size, which could have implications for pregnancy maintenance and litter-size.

The execution of this important research has advanced our understanding of the seasonality of swine reproduction. These meaningful results will have both an immediate and long-term value to U.S. Pork Producers. Data produced from these experiments is providing a foundation for future experiments that will identify valuable markers for reproductive success during seasonal infertility enabling the development of mitigation strategies. In addition to generating a much needed understanding of the immediate effects of HS during the WEI, we also expect to enhance basic understanding of the detrimental effects that HS has on porcine oocyte development which is foundational for future applied swine management and production practices.

Take home message for U.S. pork producers regarding this proposal:

Approximately 300,000 P1 sows are weaned during the period of seasonal infertility in the US, and based on this project, seasonal infertility causes a 17% reduction in litters produced due to a combination of both lower breeding rates and farrowing rates. This represents a tremendous opportunity to improve the efficiency of the US swine herd. Moderately reducing these losses from 17% to 12% during seasonal infertility represents nearly 400,000 piglets produced while requiring fewer replacement females, creating opportunities for more rapid genetic advancement. We are continuing to pursue mitigation strategies through current projects and project proposals submitted to the National Pork Board. This research project has made an extremely valuable impact on our research efforts leading to the development of mitigation strategies.

Keywords: Seasonal infertility, heat stress, reproduction

Scientific Abstract:

Four abstracts summarizing the body of work completed:

A. Understanding the biological mechanisms contributing to seasonal infertility in swine is essential for developing mitigation strategies to improve reproductive efficiency. Study objectives were to retrospectively analyze the relationship between physiological responses to HS during the wean-to-estrus interval (WEI) and the phenotypes associated with seasonal infertility, such as increased WEI and reduced farrowing rates. Rectal temperature (Tr), skin temperature (Ts), and respiratory rate (RR) were collected five times daily for seven days following weaning, during two 4-week periods of heat detection and insemination resulting in peak reproductive performance (Spring; n=424 P1 sows) and the lowest period of reproductive performance (Summer; n=445 P1 sows). Plasma was collected on day 1 and 3 during the WEI and used to measure circulating insulin and lipopolysaccharide binding protein (LBP) levels on a subset of 80 sows representing three reproductive outcomes for each season: farrowed (serviced within six days of weaning), not farrowed (serviced within six days of weaning), and sows with a WEI greater

than 15d (>15WEI). Reproductive parameters were analyzed utilizing PROC MIXED, whereas farrowing rate was evaluated using PROC GLIMMIX. Compared to Spring, a substantial reduction in the percentage of sows demonstrating estrus by 7d post weaning was observed in the Summer (89.1 vs. 79.5, ± 1.7%, $P < 0.01$), and among these sows the farrowing rate was decreased in the Summer (91.1% vs. 82.3%, ±1.8%, $P<0.01$). However, of litters produced, total born, born alive, still-born, and mummies per litter were not different between seasons ($P > 0.1$), although Summer-weaned sows tended to have an increased WEI ($P = 0.064$). The relationship between reproductive, physiological, and environmental parameters were analyzed using PROC CORR. While no effect of season was observed for Tr and RR, Ts was observed to be greater in Spring-weaned sows ($P < 0.01$); correlations across season were observed between WEI and Tr ($R = 0.07$, $P = 0.03$), Ts ($R= -0.12$, $P < 0.01$), and RR ($R = -0.12$, $P < 0.01$). Insulin and LBP were similar across seasons and were not different by reproductive status or day of WEI. These data indicate that thermal indices of HS during the WEI do not explain decreased reproductive efficiency observed during seasonal infertility in P1 sows.

B. Identifying factors associated with susceptibility or resistance to HS is likely a prerequisite to developing mitigation strategies to improve pig reproductive efficiency. Study objectives were to determine if the HS response early in life predicts future reproductive success during HS. During phase I of the study, pre-pubertal gilts (n=235; 78±1.2 kg BW) were exposed to a TN period (24 h; 22±0.5°C, 62±13% RH; fed *ad libitum*) followed by a HS period (24 h; 30±1°C, 49±8% RH; fed *ad libitum*). Respiration rate (RR), skin temperature (T_S), and rectal temperature (T_R) were recorded at 16 regularly scheduled time points within each experimental period. Body weights (BW) and daily feed intake (FI) were also recorded during the experiment. Interestingly, HS T_R between gilts did not explain the variation in the decrease in FI during the acute phase of HS ($R^2<0.01$, $P<0.05$). Also, a low proportion of variability in the severity of BW loss during HS could be explained by T_R ($R^2=0.03$, $P<0.05$) or FI ($R^2=0.09$, $P<0.01$). Gilts deemed the most tolerant (T; n=48) and susceptible (S; n=48), as defined by their ability to maintain a minimal T_R during HS, were subjected to phase II after puberty. During phase II, gilts were fed Matrix® for 14 d in TN conditions (18°C; limit fed 2.7 kg/d). Following synchronization, estrus detection and artificial insemination were conducted over a period of 9 d during cyclical and progressive HS conditions (21 to 35°C for 9 d). Gilts were slaughtered after 43-48 d of gestation in TN conditions (21°C). Fetal weight and crown-rump length were increased by 7.4 and 2.8%, respectively, in gilts classified as S compared to T ($P<0.01$). Fetal count, corpus luteum count and size, and embryo survivability were not correlated with post-pubertal HS T_R whereas fetal weight ($R^2=0.07$) and crown-rump length ($R^2=0.07$) were positively correlated with HS T_R ($P<0.05$). Positive correlations existed between pre-pubertal and post-pubertal HS T_R ($R^2=0.40$, $P<0.05$). Interestingly, pre-pubertal TN T_R was also correlated with post-pubertal HS T_R ($R^2=0.30$, $P<0.01$) suggesting that pre-pubertal thermoregulatory responses to HS, despite variable between animals, were predictive of future responses to HS. Importantly, the thermoregulatory response (T_R, T_S, RR) and production response (decreased FI and BW) to HS appear to be only marginally related, indicating that production losses during HS are independent from the thermoregulatory response during HS.

C. Mitigating HS effects in swine breeding stock is crucial as it negatively impacts reproductive performance. The objectives of the study were to determine if a gilt characterized as tolerant or susceptible to a pre-pubertal HS challenge can maintain their tolerance or susceptibility post-pubertal and to identify the relationship between a gilt's thermal regulatory response to HS following Matrix® synchronization and reproductive performance. Individual gilts identified as tolerant (n=50) or susceptible (n=50) to pre-pubertal HS were selected based on their ability or inability, respectively, to remain euthermic during the peak HS period. Gilts were placed in individual stalls and underwent estrus synchronization in a thermal neutral environment (20°C). Rectal temperature (Tr), skin temperature and respiration rate were recorded seven times per day. Rectal temperature during a two-day thermal neutral period and the average of the

last three time points (MaxTr) on each day of a HS period (9 days) were used to create a thermal rectal delta (TrDelta) value for each gilt. The average TrDelta, MaxTr, and thermal neutral Tr of all gilts were 0.6 ± 0.03°C, 38.9 ± 0.02°C, and 38.3 ± 0.02°C, respectively. The time from Matrix® withdrawal to standing estrus averaged 5.8 ± 0.1 days with 84.7% of gilts receiving 2-3 artificial insemination services. For all pregnant gilts the average uterine wet weight, ovary weight, corpora lutea (CL) numbers, and CL diameter was 5.6 ± 0.14 kg, 21.6 ± 0.32 g, 17.8 ± 0.3, and 10.2 ± 0.06 mm, respectively. Fetal measurements of total number, weight, and crown-rump length (CRL) averaged 13.9 ± 0.3 fetuses, 24.5 ± 0.33 g, and 73.8 ± 0.34 mm, respectively. HS tolerant gilts had a significantly longer return to estrus following Matrix® withdrawal and slightly larger CL diameter. Fetal weight and fetal CRL were significantly greater in gilts previously classified as susceptible to HS.

D. Heat stress (HS) is caused by the sustained elevation of core body temperature due to high ambient temperatures. HS is associated with seasonal infertility, which results in economic losses for the swine industry. Hyperinsulinemia and metabolic endotoxemia are physiological hallmarks of HS, both of which potentially modulate ovarian function via the toll-like receptor 4 (TLR4), the receptor for LPS, and/or the phosphotidylinositol-3 kinase (PI3K) pathways. Our previous findings demonstrated that HS enhanced phosphorylation of ovarian AKT (pAKT), increased TLR4, steroidogenic acute regulatory protein (StAR), and aromatase (CYP19A) protein abundance in pre pubertal gilts exposed to 7 or 35 d of HS. The current study investigated whether HS also altered TLR4, PI3K and enzymes involved in steroid hormone production in heat-stressed, post-pubertal gilts. The estrous cycles of 12 post-pubertal gilts were synchronized using Matrix®, administered orally for 14 d, followed by exposure to thermal neutral conditions (TN; 20.3°C ± 0.1°C) or cyclical HS conditions (26-32°C) during follicular development (5 d) preceding ovulation. Both TN and HS gilts were limit-fed 2.7 kg/d for the duration of the study. HS gilts had increased ($P = 0.01$) average rectal temperatures (39.8°C ± 0.2°) compared to the TN controls (38.8°C ± 0.2°) demonstrating hyperthermia in response to elevated ambient temperatures. Gilts were euthanized and ovaries collected for protein isolation and analysis. The abundance of ovarian pAKT, StAR, CYP19A and TLR4 were determined using western blotting. No impact ($P > 0.05$) of HS on protein abundance of CYP19A or StAR was observed. TLR4 was increased ($P < 0.05$) in ovaries from HS gilts relative to the TN controls. Additionally, HS decreased ($P < 0.01$) phosphorylation of ovarian AKT, relative to TN gilts. These findings demonstrate that ovarian signaling is altered by HS: activation of TLR4 indicates an ovarian response to elevated, systemic LPS, while decreased pAKT may reflect reduced altered PI3K activity. These data provide mechanistic insight into ovarian physiological alterations that could contribute to seasonal infertility in post-pubertal swine.

Introduction:

Heat stress (HS) has a tremendous negative economic impact on the swine industry with the financial burden projected to increase with predicted climate change (St-Pierre *et al.* 2003, IPCC 2007). HS-induced decreased revenue is driven by both compromised production and reproduction. Pigs are particularly sensitive to HS because they lack functional sweat glands and have a thick layer of subcutaneous adipose tissue that acts as an effective insulation layer (Curtis, 1983). The impact of HS on reproduction is quite apparent with day-28 pregnancy rates reaching their lowest levels in August into October and reduced farrowing rates in November and December (based on matings conducted in June-September). While seasonal infertility could arguably be related to factors such as photoperiod, seasonal infertility in pigs is associated with periods of excessive heat, and HS has been repeatedly demonstrated to negatively impact reproductive efficiency, particularly due to lost embryonic development (Tompkins *et al.* 1967, Omtvedt *et al.* 1971). Further, a recent meta-analysis of publications (1970-2009) revealed the effect of HS on feed intake and growth to be more pronounced in recent years suggesting a possible indirect and negative impact of genetic selection for growth and carcass traits on pig thermal sensitivity (Renaudeau *et al.* 2011). This reduced thermal tolerance may be partly mediated by altered

body composition (i.e. increased lean tissue), as synthesizing and maintaining skeletal muscle generates metabolic heat (Brown-Brandl *et al.* 2001).

With respect to reproduction, tolerance to HS and HS-induced exacerbation of infertility appear to occur differentially in different genetic lines. For example, Bloemhof and colleagues (2008) demonstrated that lines selected for increased farrowing rate (FR) were more sensitive to HS (i.e. reduced litter size and total number born) and that FR per first insemination was compromised compared to those lines not selected for increased FR. We hypothesize that animals capable of conceiving and producing a litter during HS are capable of enlisting physiological adaptations (increased heat dissipation or hormonal changes) enabling them to be more HS tolerant. An inability to maintain a healthy body temperature (BT) has significant implications for the production of gametes capable of yielding developmentally competent embryos. *In vitro* oocyte maturation mimics the *in vivo* oocyte development that occurs during late proestrus and during the first part of behavioral estrus prior to ovulation, concomitant with the first service, since ovulation occurs at approximately 55-60% of the way through the behavioral estrus (Soede & Kemp 1997). We have developed an *in vitro* oocyte maturation model to investigate the effects of HS on oocyte development. Oocytes subjected to HS during *in vitro* maturation have an impaired ability to survive beyond the 4-cell stage of development, despite being fertilized and cultured in thermal-neutral (TN) conditions.

Following weaning, sows undergo a dramatic metabolic reorganization during the WEI. Adequate production of gonadotropins, particularly follicle stimulating hormone, is essential for the recruitment and stimulation of developmentally competent oocytes. Gonadotropin secretion from the anterior pituitary is a result of gonadotropin releasing hormone release from the arcuate nucleus of the hypothalamus. Emerging literature has demonstrated the role of the peptide hormone, kisspeptin as being a key component for activating the hypothalamic pituitary gonadal axis and links the metabolic status/energy balance of an animal to the regulation of reproduction (Lents *et al.* 2008, Roa *et al.* 2011). Collectively, we hypothesize that sows and gilts exposed to elevated ambient temperatures during the WEI, which are least capable of controlling core BT, are the most likely to contribute to the reduced FR observed during the period of seasonal infertility.

We have recently demonstrated increased basal insulin in a variety of HS models (Baumgard & Rhoads 2012), including gilts (Pearce *et al.* 2013). The insulin increase (a potent anabolic signal) occurs despite a marked reduction in feed intake and hyper-catabolic hormonal milieu that dominates during HS. While the reason for enhanced circulating insulin during HS response remains unclear, it likely includes insulin's role in activating the cellular stress response (Li *et al.* 2006). Insulin activates the phosphatidylinsoitol-3 kinase (PI3K) pathways (Kasuga, 1996), which critically regulate oocyte activation, viability and ovarian estradiol production. In many physiological states, including polycystic ovary syndrome (PCOS) and obesity, where insulin levels are abnormally high, there are associated reproductive problems including reduced fecundity and increased pregnancy loss. Our preliminary data demonstrate increased expression of the gene encoding the insulin receptor (INSR) in ovaries of gilts exposed to HS, indicating ovarian sensitivity to increased insulin levels that predominate during HS conditions. Additionally, HS increases genes encoding the ovarian steroidogenic enzymes, suggesting that altered 17β-estradiol (E2) levels result during HS conditions. In addition, follicular fluid (FF) provides an important microenvironment for maintaining oocyte competency (Mc Natty, 1978) and FF composition can be altered by environmental conditions (Gosden et al., 1988; Fortune, 1994). It is known that the local E2 levels in the FF differ from those in plasma (Renaville et al., 2008), and that changes to FF fatty acid (FA) profile in favor of palmitic and/or stearic acid can be particularly detrimental for oocyte development (Leroy et al., 2005; Vanholder et al., 2005).

Objectives:

Objective 1: To retrospectively identify physiological parameters occurring prior to and during estrus which are associated with increased WEI, reduced conception rates, and reduced farrowing rate. Our working hypothesis is that the inability to regulate core body temperature, specifically during the WEI, occurring concomitant with the recruitment and development of ovulatory follicles negatively impacts reproductive ability.

Testing this hypothesis was accomplished by monitoring rectal temperature and respiration rate during the WEI in addition to blood obtained on day 1 and 3 of the WEI.

Objective 2: To determine if HS tolerance early in life is predictive of reproductive success during HS. Our hypothesis was that gilts demonstrating a high susceptibility to HS (as demonstrated by their inability to maintain a safe body temperature) are most likely to demonstrate reduced pregnancy success during future HS. To accomplish this, gilt response (based upon change in body temperature) to HS was determined prior to puberty. HS susceptible and HS tolerant gilts demonstrating normal estrous cycles were synchronized at approximately 210 days of age using Matrix. During Matrix withdrawal and follicle development, gilts will be subjected to HS and artificially inseminated during estrus.

Materials & Methods:

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals.

Objective 1:

Animal Work:

Objective one was conducted in collaboration with two 5000 sow farms located in the Western US during calendar year weeks 29 to 32 (Summer; high incidence of infertility in P1 sows) and weeks 12-15 (Spring; low incidence of infertility in P1 sows). Briefly, rectal temperatures, respiration rate and skin temperature were collected approximately five times per day during the seven days of the WEI on 450 and 419 P1 weaned sows during the summer and spring, respectively. In addition, serum and plasma were isolated for retrospective analysis on days 1 and 3 post wean for each sow. All rectal temperatures, skin temperatures, respiration rates, and production data were entered into our electronic database for retrospective analysis.

Insulin and LBP binding protein analysis

Blood plasma drawn on days 1 and day 3 was selected retrospectively for 80 sows based on reproductive performance classifications. Sows were classified based on WEI and farrowing status. Groupings consisted of those sows that had a WEI of 4 or 5 days, were inseminated resulting in farrowing (Farrowed; n = 13 Spring, n= 15 Summer), sows that had a WEI of 4-5 days, were inseminated and did not farrow (Not Farrowed; n = 15 Spring, n = 14 Summer), and sows that had a WEI greater than 15 days (WEI >15; n = 10 Spring, n = 10 Summer).

For each assay, a standard curve was established on each plate and two pooled samples were assessed on all plates to quantify variation across the plate and for normalization. Insulin and LBP concentrations were measured in blood plasma utilizing commercially available assays (Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden and Hycult Biotech, Uden, Netherlands, respectively), following the manufacturer's instructions. All samples were quantified using a microplate reader (Synergy 4 Bioteck, Winooski VT).

Statistical Analysis:

Data collected were analyzed utilizing the Mixed and Glimmix procedures in SAS (9.3, Cary, NC). Season and barn were utilized as the fixed effects, with week nested within season considered as the random effect. Reproductive performance information was provided by our producer collaboration and were analyzed using the Mixed procedure. Physiological parameters measured included rectal temperature, skin temperature, and respiratory rate. These items were measured for correlation to production measurements such as: total born, born alive, still born and mummies, and WEI were all evaluated in respect to changes over season. Farrowing rate was evaluated using the Glimmix procedure, evaluating the percentage in which a positive or negative result occurred in relation to farrowing. WEI was evaluated further by percentage of pigs to reach estrus at 3 time points

post weaning. Data evaluated represent a proportion of the population sampled. Reproductive performance measurements were evaluated for correlation to physiological measurements. The CORR procedure of SAS was utilized to evaluate the relationship of respiratory rate, rectal temperature, and skin temperature as they are related to WEI, farrowing rate, total born, and live born. Reproduction parameters were also evaluated for relationship to each other in this analysis. Data were reported as R2 values and Pearson correlation coefficients. Insulin values were measured in blood plasma utilizing a Porcine Insulin ELISA kit (Mercodia, Sweden). Blood plasma was drawn on days 1 and day 7 for 80 sows based on reproductive performance classifications: farrowed (F), not farrowed (NF), WEI of greater than 15 days (WEI15). Sows that produced a litter were limited to a litter size of 10-14 pigs and were drawn evenly across season, barn, and week during the time that the blood was collected. Samples were randomly assigned to plate and grouped by day of draw. Standard curve was established for each plate, and 2 pooled samples were assessed on each plate to quantify variation across the plate. Relative absorbance of insulin was quantified using a microplate reader (Synergy 4 Bioteck, Winooski VT), at 450 nm. Concentrations were reported as µg/L. Insulin values were compared across season, day, and reproductive classification using the mixed procedure of SAS. Individual and 3 way interaction of season, day, and reproductive class were utilized as the fixed effects to compare relative absorbance values of insulin; week nested within season was utilized as a random effect.

Objective 2-Phase 1:

Animals and Experimental Design

Two hundred thirty-six crossbred gilts (44-48 per replication) were utilized for the experiment. Body weights were 59±16 kg, 64±24 kg, 77±17 kg, 88±15 kg, and 103±26 kg, respectively, for replications 1-5. Gilts were randomly assigned and housed in individual pens, for each of the five replications, in one of two experimental rooms (22-24 pigs per room per replication) and fed *ad libitum* during the entire length of the experiment. Each replication, gilts were acclimated for at least 72 h in thermal neutral (TN) conditions (Room A: 21.4°C, 59.7% relative humidity (RH) Rep 1; 21.6°C, 63.6% RH Rep 2; 21.6°C, 71.4% RH Rep 3; 21.8°C, 72.5% RH Rep 4; 22.1°C, 46.0% RH Rep 5; Room B: 22.2°C, 47.3% RH Rep 1; 22.6°C, 54.0% RH Rep 2; 22.1°C, 75.8% RH Rep 3; 21.9°C, 73.1% RH Rep 4; 21.4°C, 47.6% RH Rep 5). After acclimation, all pigs were maintained in TN conditions for 24h TN period followed by exposure to HS conditions (Room A: 30.0°C, 44.0% RH Rep 1; 30.1°C, 53.2% RH Rep 2; 30.0°C, 57.2% RH Rep 3; 30.1°C, 46.1% RH Rep 4; 30.4°C, 36.7% RH Rep 5; Room B: 28.9°C, 45.6% RH Rep 1; 29.2°C, 57.0% RH Rep 2; 29.1°C, 59.4% RH Rep 3; 28.7°C, 50.8% RH Rep 4; 30.2°C, 40.2% RH Rep 5) for 24h.

All gilts were fed a standard industry diet of primarily corn and soybean meal formulated to meet or exceed nutrient requirements. Pigs were exposed to a 12h:12h light-dark cycle during the acclimation period and continuous light during the TN and HS days. Ambient temperature was controlled but humidity was not governed and both parameters were recorded every 30 min by four data loggers (Lascar EL-USB-2-LCD, Erie, PA) in each room and later condensed into averages for each time point. Rectal temperature (°C; T_R) was measured with a digital thermometer (Welch Allyn SureTemp® Plus 690, Skaneateles Falls, NY, USA), skin temperature (°C; T_S) was measured using a calibrated infrared thermometer (ST 380A Infrared Thermometer, HDE, Allentown, PA), and respiration rate (RR) was determined by counting flank movements in 15 seconds and multiplying by four. Feed intake (FI) was measured daily and vital parameters were recorded for each pig hourly from 0800-2400 during both 24 h TN and HS phases. Body weights (BW) were collected at the beginning of the acclimation period and at the end of the TN and HS periods.

Determination of HS tolerance and susceptibility based on the thermoregulatory response

Each rectal temperature taken during the TN period was condensed into an average to represent each individual pig's basal core temperature (TN T_R). Only the T_R's recorded 4h after the start of HS were factored into the average to represent core body temperature during the 24h HS period (HS T_R). The difference in core body temperature (ΔT_R) was calculated by subtracting TN T_R from the HS T_R. The calculated ΔT_R was then

plotted against the HS T_R in order to determine the relationship between each pig's average HS T_R and difference in core body temperature between the two 24h periods. Since the greater the ΔT_R was associated with a higher HS T_R, each pig's resilience to the heat load was classified on the basis of HS T_R (i.e. higher and lower HS T_R were considered markers of HS susceptibility or tolerance, respectively). For each repetition, the top 10 most tolerant and susceptible (as determined by HS T_R) were identified and used for the second part of the experiment.

Statistical Analysis

All data were statistically analyzed using SAS University Edition software, version 9.4 (SAS Institute Inc., Cary, NC). Daily temperature indices and production data were analyzed using PROC TTEST using the paired option and PROC CORR to generate *t* critical values and Pearson's correlation coefficients. Data are reported as means (PROC MEANS was used to generate mean and SEM estimates) and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Objective 2-Phase 2

Animals

One hundred gilts were selected from a previous study identifying gilts as being tolerant (n= 50) or susceptible (n=50) to HS based on their ability or inability, respectively, to maintain a minimal Tr during a 24-hour pre-pubertal HS period (Phase I above). All gilts from the previous study underwent estrus detection beginning at 160 days of age and continued until 220 days of age to ensure the selected gilts had demonstrated at least two estrous cycles. At approximately 220 days of age, the gilts were transported to a facility that enabled individual housing and were limit fed six pounds of feed per day. One gilt was removed from the study due to illness.

Acclimation and Synchronization Period

Each gilt was placed in an individual stall in a controlled-environment research facility at Iowa State University. Animals were assigned to individual pens so that each classification (i.e. tolerant and susceptible) were equally represented and evenly spaced in each of two rooms (50 animals each) throughout the entire facility. The acclimation period began 16 days prior to starting the HS period; room temperature was maintained at approximately 20° Celsius (C). Fans were placed throughout the rooms to ensure equal distribution of heat, which was monitored multiple times per day by placing five equidistantly spaced data loggers throughout each of the two rooms in the barn. Fourteen days prior to HS, all gilts were placed on an estrus synchronization program utilizing Matrix®. Gilts were fed once daily (6 pounds) in the morning at 0700 and 6.8 mL of Matrix® (15 mg altrenogest) was administered by top dressing the gilts feed in each individual feeder, per manufactures guidelines. Feed consumption was monitored on all animals to ensure the complete dose was effectively consumed in all gilts.

Heat Stress Period

The HS period began on the last day of Matrix® feeding treatment and continued for nine consecutive days as the average time for females to return to estrus following Matrix® administration is 5-9 days (Flowers, 1999). Temperature was controlled in a diurnal pattern with the daily HS period beginning at 1000 and turned off at 2200 to simulate natural temperature patterns. The temperature during the 12 hours of HS was increased incrementally during the first three days (28.9°C, 31.1°C, and 33.3°C on day one, two and three, respectively) and then was held to 35°C for the remaining 6 days. The low temperature for the resting 12 hours was 21.1°C for the entire period.

Temperature Measurements

Rectal temperature, respiration rate and skin temperature were measured on the two days before HS to establish a thermal neutral average baseline for each gilt. The same measurements were taken at seven time points each day during the HS period at 0800, 1400, 1500, 1600, 1900, 2000 and 2100.

Estrus Detection, Artificial Insemination and Pregnancy Check

During the Matrix® withdrawal period each gilt underwent estrus detection for breeding. Four boars were utilized via fence line exposure to enable estrus detection each morning after feeding and prior to HS (i.e. between 0700 and 1000). Gilts were bred with a single dose of pooled semen (terminal Duroc) on the first day of standing estrus and received additional insemination each day they continued to exhibit behavioral estrus. By the tenth day all but one of the 99 gilts had demonstrated a standing estrus and had received at least one dose of semen. The remaining 98 gilts inseminated underwent estrus detection 18-22 days later as well as ultrasound checked at ~36 days of pregnancy to identify those returning to estrus or to confirm pregnancy, respectively.

Harvesting and Fetal Analysis

Gilts were harvested at 42-47 days of pregnancy (with day of first service considered day zero) at a sow slaughter facility in a single group. The reproductive tract from each gilt was collected and immediately refrigerated until analysis, which occurred within 48 hours for all reproductive tracts. The reproductive tract from each gilt was weighed using a digital scale for a total tract measurement and then the fetuses and ovaries were removed. Fetuses were counted to determine litter size for each gilt and then individually weighed using a digital scale and measured with Ultra Tech digital calipers (General Tools, Secaucus, NJ) to determine crown-rump length. Ovarian weight of each gilt was measured and the corpora lutea (CL) on each ovary were counted and the diameter of each CL was measured with Ultra Tech digital calipers (General Tools, Secaucus, NJ). Embryo survival was calculated for each gilt by dividing the number of fetuses in the litter by the number of CL on the gilt's ovaries. Eight gilts were confirmed to be non-pregnant by the absence of fetuses and CL.

Temperature and Statistical Analysis

Average Tr was measured during three-hour periods (twice a day) for each gilt two days prior to HS and each day during HS beginning four hours after HS induction (1400, 1500, and 1600) and during the last three hours of HS (1900, 2000, 2100). The Tr change (TrDelta) was determined using each gilts average thermal neutral temperature subtracted from the average Tr collected during the three hour period beginning nine hours after HS induction. For each gilt, the thermal neutral Tr, HS average Tr and the Tr difference between thermal neutral and HS values from the initial study (Seibert, Baumgard, Ross; unpublished) were used to determine if a correlation existed between the pre-pubertal thermoregulatory response and the post-pubertal thermoregulatory response. The Proc Corr function of SAS was used for this Pearson correlation as well as to determine if any correlation exists between the post-pubertal TrDelta values with each of the reproductive performance measures collected from each gilt. Additionally, gilts were assigned to their initial tolerant and susceptible classifications and t-tests were conducted to determine statistical differences of the reproductive performance measures between each classification.

Results:
Objective 1.

Incidence of seasonal infertility was detected in P1 sows by comparison of Summer and Spring

Data and blood samples were collected on a total of 419 (Spring) and 450 (Summer) P1 weaned sows. Infertility was markedly increased during the Summer compared to the Spring. Compared to the Spring, sows weaning their first litter during the Summer had an approximate 11% reduction in their ability to achieve estrus within 7 days, and of those that did and were inseminated, a 9% reduction in farrowing rates was observed (Table 1). Additionally, we observed a greater number of sows culled in the Summer due to their inabltiy to demonstrate behavioral estrus compared to the Spring.

Based on the data observed in Table 1, 224 piglets per 100 P1 sows weaned are not produced from matings during the first 7 days of the WEI during seasonal infertility, that are produced during other parts of the year. When including the recovered pregnancy from sows moved to the opportunity area and eventually serviced, the actual lost piglet production is reduced to 140 piglets per 100 P1 sows weaned, but this comes at an expense of 210 extra non-productive sow days experienced during seasonal infertility; which is in addition to an increase of 3% of P1 sows never experiencing estrus after weaned during seasonal infertility.

Table 1. Reproductive summary of P1 gilts during Summer and Spring

Season[1]	P1 Weaned[2]	Wean-to-Estrus Inverval (WEI) </=7 days[3]				Wean-to-Estrus Inverval (WEI) >7 days[4]				No Heat[5]
	N	N	Average WEI (d)	Farrowing Rate	Number Born Alive	N	Average WEI (d)	Farrowing Rate	Number Born Alive	N
Spring	419	370	4.14	91%	12.73	45	24.4	75.6%	11.47	4
		88.3%				10.7%				1.0%
Summer	450	345	4.24	82%	12.68	89	26.2	70.8%	12.57	16
		76.7%				19.8%				3.6%

[1]Spring; calendar weeks 12-15 and Summer; calendar weeks 28-32.
[2]The number of sows used for analysis following weaning their first litter.
[3]Production metrics for sows achieving estrus in 7 days or less following weaning.
[4]Production metrics for sows achieving estrus but not with 7 days following weaning.
[5]Number of sows that never demonstrated estrus and were culled due to lack of estrus.

Thermoregulation during the WEI is not strongly correlated to reproductive performance.

As demonstrated in Table 2, correlation analysis was conducted and demonstrated only weak relationships between rectal temperature of P1 weaned sows during the WEI and the length of the WEI, or the reproductive outcome of those serviced.

Table 2. Retrospective of thermal response of P1 weaned sows during the WEI and subsequent reproductive performance.

Measured Reproductive parameters, correlation[a]							
	WEI[4]	**Rectal Temp[5]**	**Skin Temp[6]**	**Resp. Rate[7]**	**Total Born[8]**	**Live Born[9]**	
WEI		0.0731	-0.1233	-0.1222	-0.0758	-0.0685	Corr. Coeff.[1]
		0.034	<0.001	<0.001	0.044	0.067	P value[2]
		846	846	846	718	718	n[3]
Rectal Temp			-0.0309	0.3891	0.0124	-0.0056	Corr. Coeff.[1]
			0.3644	<.0001	0.7405	0.8802	P value[2]
			866	866	715	715	n[3]
Skin Temp				0.0001	-0.0440	-0.0299	Corr. Coeff.[1]
				0.9995	0.2405	0.4254	P value[2]
				866	715	715	n[3]
Resp. Rate					0.1023	0.1191	Corr. Coeff.[1]
					0.006	0.001	P value[2]
					715	715	n[3]
Total Born						0.9001	Corr. Coeff.[1]
						<0.001	P value[2]
						718	n[3]

[a] Correlation of production parameters to biological responses to heat.

[1] Correlation coefficient.

[2] Significance value for measured correlation.

[3] Number of sows eligible for each correlation.

[4] WEI: based on number of days from weaning to date of first service.

[5] Rectal temperature, average for day 1 through 7 following weaning.

[6] Skin temperature: average for day 1 through 7 following weaning.

[7] Respiratory rate: average for day 1 through 7 following weaning.

[8] Total Born: number of pigs born to sows for the P2 litter.

[9] Live Born: number of total pigs born live to sows for the P2 litter.

By comparison, the length of the WEI was significantly greater for P1 weaned sows during the Summer than in the Spring (Figure 1) however, we did not observe a statistical difference in in rectal temperature or respiration rate in sows during the WEI between the summer and spring (Figures 2 and 3). Counterintuitively, and likely the result of the use of cool-cells in the summer, skin temperature was lower in the summer in comparison to the spring (Figure 4).

The relationship between the rectal temperature of P1 weaned sows during the WEI and the length of the WEI was not affected by season (Figure 1). Furthermore, we also conducted a retrospective analyis of three physiological indicators of HS (rectal temperature, skin temperature, and respiration rate) and did not identify a relationship with the length of the WEI during either the Summer or Spring (Figure 5 and Table 3).

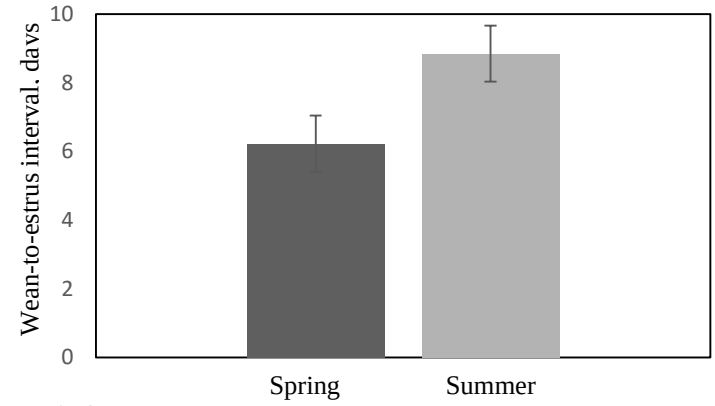


Figure 1. WEI, determined as the number of days from the date of weaning to the date of service, as averaged within season. WEI tended to be longer during the summer season, as compared to spring (P = 0.064).

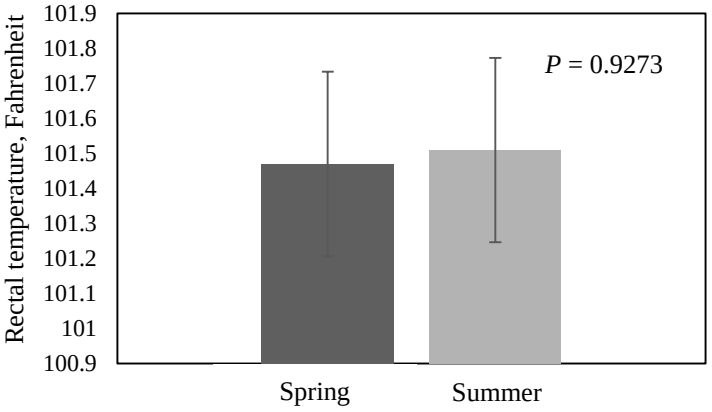


Figure 2. Rectal temperature was collected over 7 days post weaning during 4 consecutive weeks of the Spring (calendar weeks 12-15) and Summer (calendar weeks 28-32). No differences in rectal temperature were observed during the time measured, for Summer (101.51 ± 0.26) as compared to Spring (101.47 ± 0.26).

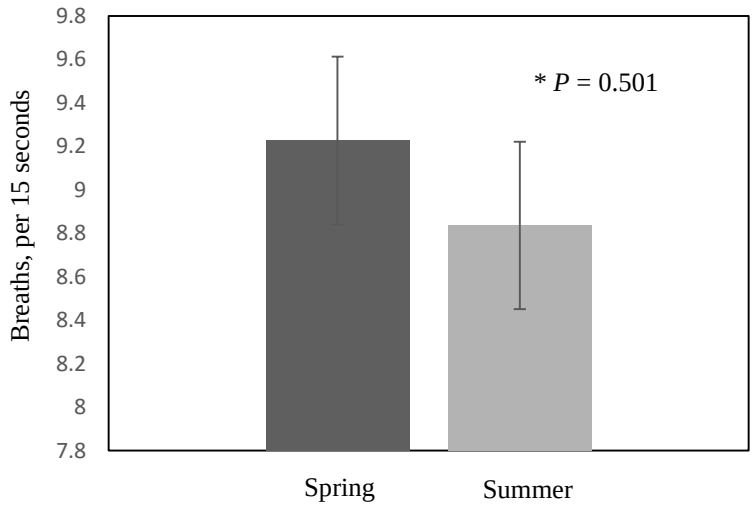


Figure 3. Respiratory rate was measured on sows at rest over 7 days post weaning during 4 consecutive weeks of the Spring (calendar weeks 12-15) and Summer (calendar weeks 29-32). No difference in average respiratory rate was observed across seasons (Spring, 9.23 ± 0.39; Summer 8.84 ± 0.39).

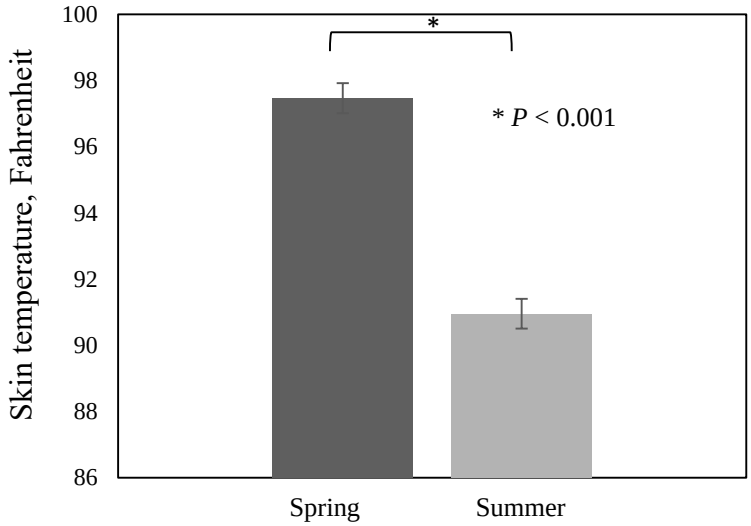


Figure 4. Skin temperature was collected over 7 days post weaning during 4 consecutive weeks of the Spring (calendar weeks 12-15) and Summer (calendar weeks 28-32). Average skin temperature was significantly lower for sows in the Summer (97.47 ± 0.45), as compare to skin temperature of sows in the Spring (90.95 ± 0.45).

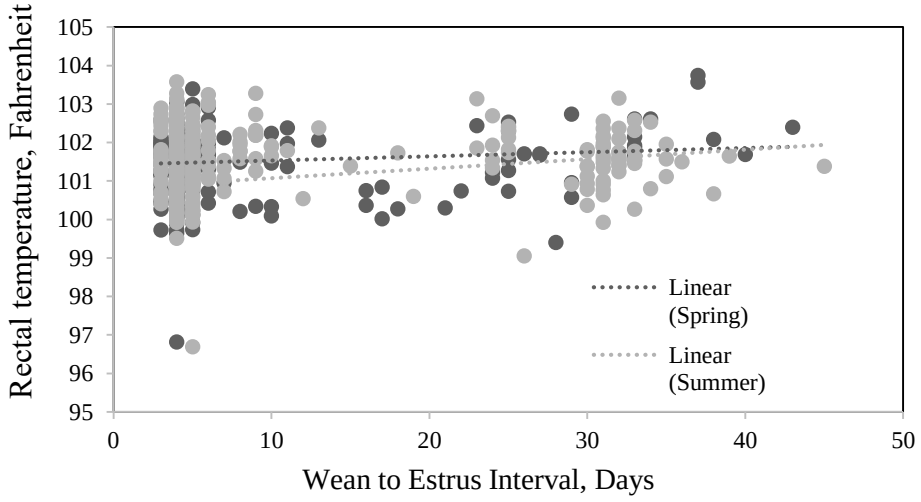


Figure 5. No significant relationships were observed in relation to rectal temperature of P1 weanded sows and the length of the WEI during spring or summer. Rectal temperautre was recorded 5 times daily for six days following weaning and the day of service less the day of weaning was used to calculate the WEI. Light colored dots represent data collected during the Spring (calendar weeks 12-15) and and dark colored dots represent Summer (calendar weeks 29-32).

Table 3. Correlation of physiological indicators of heat stress and WEI by season.

	Rectal Temperature[5]	Skin Temperature[6]	Respiratory Rate[7]	
Spring WEI[4]	0.10214	0.03394	-0.03535	Corr. Coeff.[1]
	0.0375	0.4905	0.4726	P value[2]
	415	415	415	n[3]
Summer WEI[4]	0.05381	-0.03916	-0.16987	Corr. Coeff.[1]
	0.265	0.4174	0.0004	P value[2]
	431	431	431	n[3]

[1] Correlation Coefficient

[2] Significance value for measured correlation

[3] Number of sows eligible for comparison, total sows enrolled for analysis: 869

[4] Wean to estrus interval: based on number of days from weaning to date of first service

[5] Rectal temperature: Calibrated, collected over 7 days post weaning, based on averages of temperatures recorded at 8 separate times during daylight

[6] Skin temperature: Calibrated, collected over 7 days post weaning, based on averages of temperatures recorded at 8 separate times during daylight

[7] Respiratory rate: collected over 7 days post weaning, based on averages of temperatures recorded at 8 separate times during daylight. Recorded only when sows were resting, breaths per minute

Circulating insulin and LPS binding protein during the WEI is not strongly correlated to reproductive performance.

Increased LBP and insulin have both been demonstrated to be useful markers of HS. HS compromises intestinal integrity, enabling increased LPS exposure and as a result, increased circulation of LBP. Addtitionally, altered insulin circulation has been repeatedly observed in chronically heat-stressed animals. Retrospectively, we compared cirulating LBP and insulin in gilts that farrowed a litter after having an average (4-5 days) WEI (Farrowed), P1 weaned sows that had an extended (> 15 days) WEI (WEI>15), and P1 weaned sows having an average WEI and not farrowing a litter from that insemination (Not Farrowed) for both the Spring and Summer. For both circulating insulin (Figure 6) and LBP (Figure 7) we did not observe a significant effect of gilt classification or season.

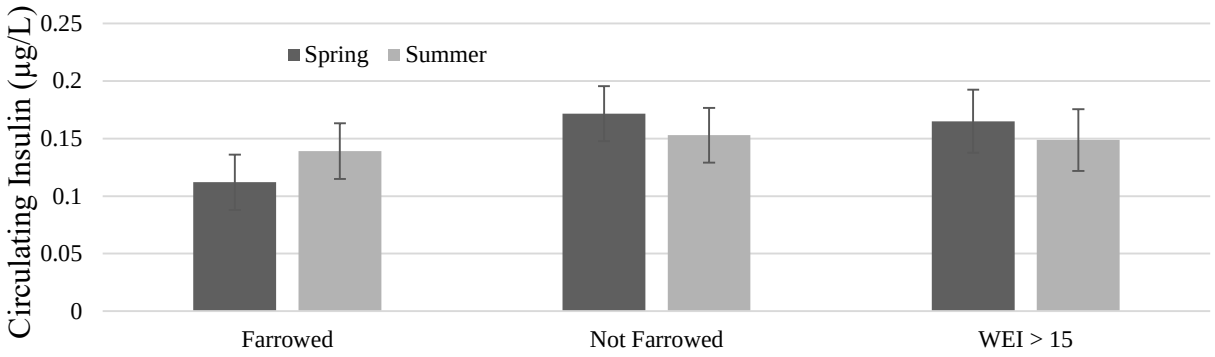


Figure 6. Circulating concentration of insulin, averaged from samples collected on days 1 and 3 of the WEI during the Spring (weeks 12-15) and Summer (weeks 29-32). Blood samples were grouped by reproductive outcome. Farrowed represents P1 weaned sows producing a litter after having an average (4-5 days) WEI, Not Farrowed represents P1 weaned sows having an average WEI and not farrowing a litter from that insemination. WEI >15 includes P1 weaned sows that had an extended (> 15 days) WEI.

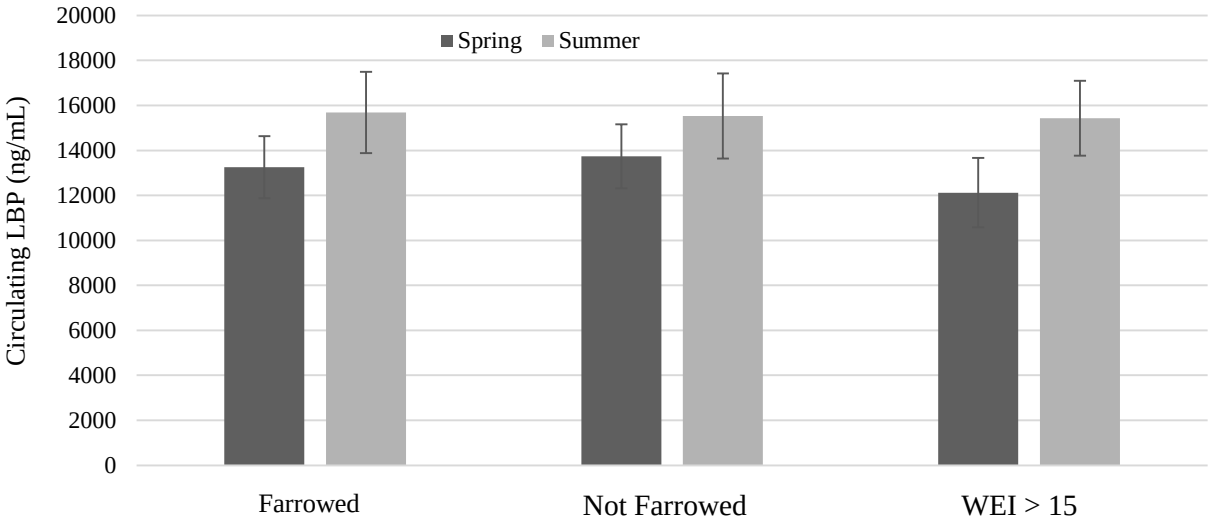


Figure 7. Circulating concentration of LBP, averaged from samples collected on days 1 and 3 of the WEI during the Spring (weeks 12-15) and Summer (weeks 29-32). Blood samples were grouped by reproductive outcome. Farrowed represents P1 weaned sows producing a litter after having an average (4-5 days) WEI, Not Farrowed represents P1 weaned sows having an average WEI and not farrowing a litter from that insemination. WEI >15 includes P1 weaned sows that had an extended (> 15 days) WEI. No differences in circulating LBP were observed across season or breeding outcome.

Objective 2.

The thermoregulatory HS response is highly variable but repeatable for individual pigs.

We have preliminarily characterized the pig's ability to regulate core body temperature in response to an acute HS load. Skin temperature, respiration rate, and rectal temperature were recorded hourly on 235 gilts (PIC maternal by Duroc terminal sire line cross) during a 24-hour thermal neutral phase and again during a 24-hour bout of HS (Figure 8). We categorized a subset of animals as "tolerant" (those with the lowest rectal temperatures during HS; n =50) or "susceptible" (those with the highest rectal temperatures during HS; n =50). Interestingly, those categorized as susceptible had a greater rectal temperature in both HS and thermal neutral conditions (Figure 9).

Figure 8. Considerable variation exists between animals with respect to their rectal temperature during thermal neutral conditions (top panel) and their thermoregulatory response during HS (bottom panel). Colored lines are individual pigs; black line is mean.

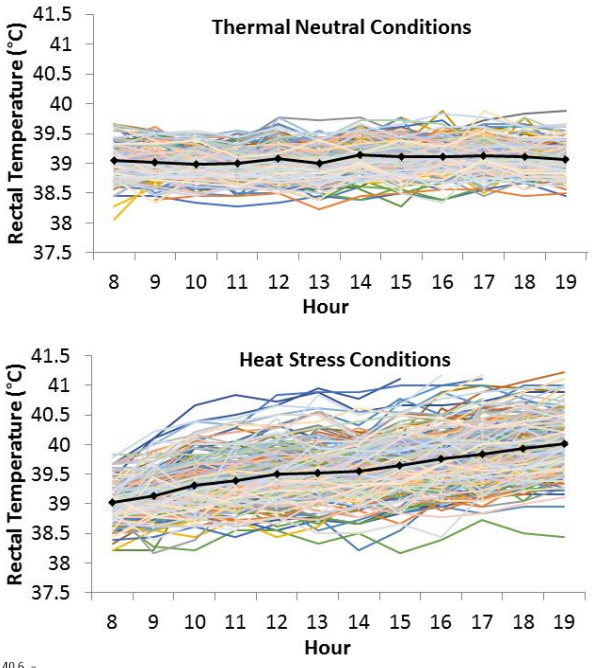
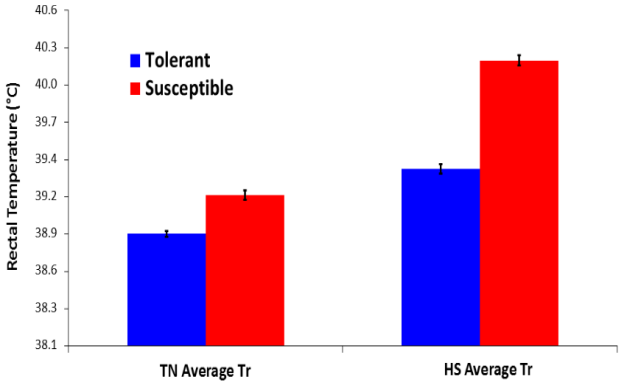


Figure 9. Rectal temperature for pigs designated tolerant and susceptible were different during the 24-hour thermal neutral (TN) phase. The difference in maximum rectal temperature (Tr) during HS was exacerbated to a greater degree in susceptible than tolerant pigs.



After achieving sexual maturity, susceptible and tolerant gilts were then subjected to nine days of cyclical heat during Matrix® withdrawal to create a synchronized estrus during HS conditions. The rectal temperature response to HS of tolerant and susceptible gilts was repeatable (Figures 10 and 11; Table 4).

Figure 10. Fifty gilts previously identified as "susceptible" (S) or "tolerant" (T) during thermal neutral conditions were monitored for 10 days. Vertical bar indicates onset of cyclical HS during a Matrix synchronized follicular phase. Animals classified as T or S based on their response to an initial HS challenge (four months earlier) remained different in their ability to thermoregulate in response to HS.

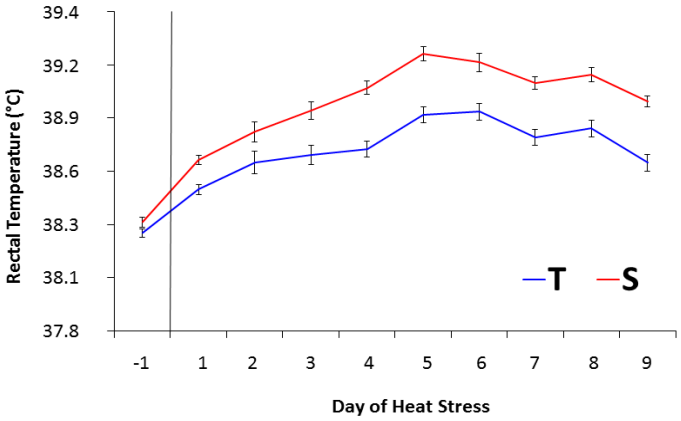
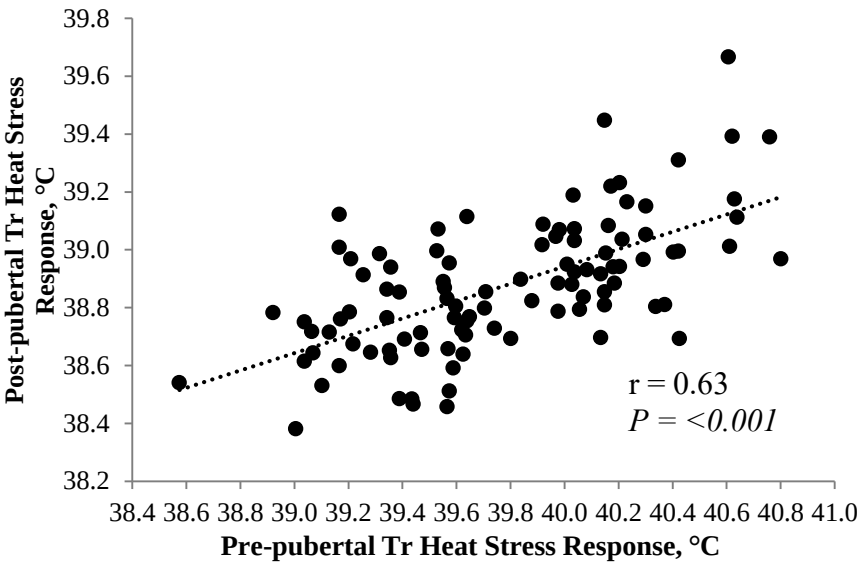


Table 4. Thermoregulatory response of gilts classified as tolerant or susceptible following Matrix synchronization.

Measurement[1]	Classification[2]	N[3]	Temperature ± SEM[4]	*P*-Value[5]
TN Tr[6]	Tolerant	50	38.28°C ± 0.03	0.12
TN Tr[6]	Susceptible	49	38.34°C ± 0.02	
MaxTr[7]	Tolerant	50	38.74°C ± 0.02	< 0.001
MaxTr[7]	Susceptible	49	39.01°C ± 0.03	
Tr Delta[8]	Tolerant	50	0.452°C ± 0.03	< 0.001
Tr Delta[8]	Susceptible	49	0.663°C ± 0.04	
bTr[9]	Tolerant	50	38.91°C ± 0.04	< 0.001
bTr[9]	Susceptible	49	39.29°C ± 0.05	

[1]Temperature measurement.
[2]Classification following pre-pubertal heat stress response.
[3]Number of crossbred (Yorkshire x Landrace x Duroc) animals used for analysis.
[4]Temperature ± Standard Error of Mean.
[5]*P*-Value: Pearson correlation of the heat stress classifications with temperature measurements.
[6]Thermal neutral rectal temperature.
[7]Heat stress rectal temperature response during last three hours of heat stress period.
[8]Change in temperature response (heat stress-thermal neutral rectal temperature).
[9]Max heat stress rectal temperature on day of first breeding.

Figure 11. There is a positive correlation between the average post-pubertal and pre-pubertal HS temperature response of each gilt (r = 0.63, *P < 0.001*). The pre-pubertal HS response was assessed by rectal temperature and correlated to the corresponding rectal temperature response during the post-pubertal HS period for each gilt.



Susceptibility or tolerance to HS classification did not affect reproductive outcome when subjected to heat stress for 9 days following Matrix® supplementation

Following estrus synchronization using Matrix® during the acclimation period, the average time until behavioral estrus for the 98 gilts demonstrating estrus was 5.8 ± 0.1 days. Gilts classified as tolerant had a slightly (P = 0.006) greater average time until behavioral estrus (6.1 ± 0.1 days) compared to gilts classified as susceptible (5.5 ± 0.1 days). All gilts that exhibited a standing estrus received one artificial insemination on each day in which they exhibited a standing response with the average number of breeding services for all gilts being 2.4 ± 0.1.

Reproductive Efficiency

The reproductive efficiency of the group of gilts was determined through analyzing the litter and reproductive tract of each individual animal after harvest (42-47 days of gestation). Of the 98 gilts that were artificially inseminated, eight were determined to be non-pregnant as evident by the absence of fetuses at harvest. Entire whole uterine tracts were weighed to record a total tract weight with the average tract weight of all bred gilts being 5.6 ± 0.14 kg. The tolerant gilts had an average tract weight of 5.5 ± 0.22 kg and lacked significant difference (P > 0.05) from the susceptible gilts that had an average tract weight of 5.7 ± 0.18 kg.

Fetal Measurements

After total reproductive tract weight was recorded, the fetuses were removed and individually analyzed. The average number of fetuses for all gilts was 13.91 ± 0.34 and was not different (*P >0.05*) between the tolerant (13.6 ± 0.48 fetuses) and susceptible (14.2 ± 0.48 fetuses) gilts. Each individual fetus was weighed and the average fetal weight for all gilts was 24.5 ± 0.33 g with an average fetal weight of 23.6 ± 0.45 g for the tolerant gilts being significantly lower (P = 0.007) than the average of 25.4 ± 0.45 g for the susceptible gilts (**Figure 12**). The crown-rump length (CRL) of each individual fetus was recorded and the average for all gilts was 73.8 ± 0.34

mm. The average CRL was 72.8 ± 0.46 mm for the tolerant gilts and was significantly lower (P = 0.002) than the average CRL of 74.8 ± 0.46 mm for the susceptible gilts (**Figure 13**).

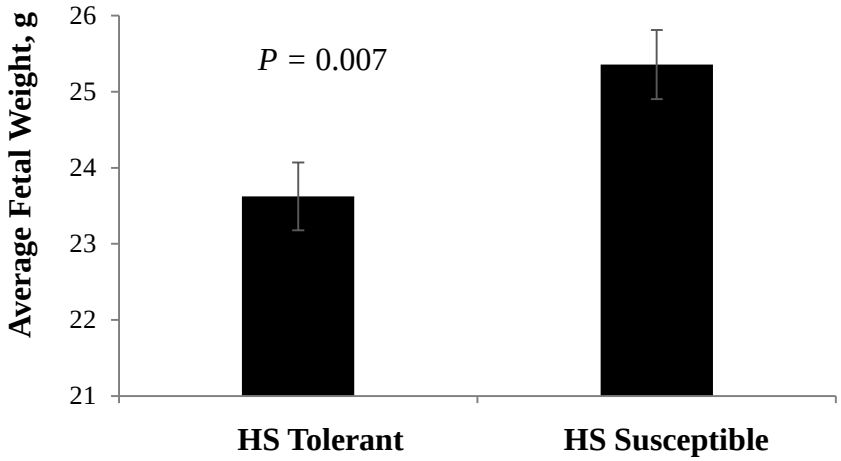


Figure 12. Average fetal weight of pregnant gilts conceived during HS that were classified as tolerant or susceptible to HS prior to puberty. Tolerant gilts had an average fetal weight of 23.6 ± 0.45 g while the HS susceptible gilts had a significantly higher average fetal weight of 25.4 ± 0.45 g.

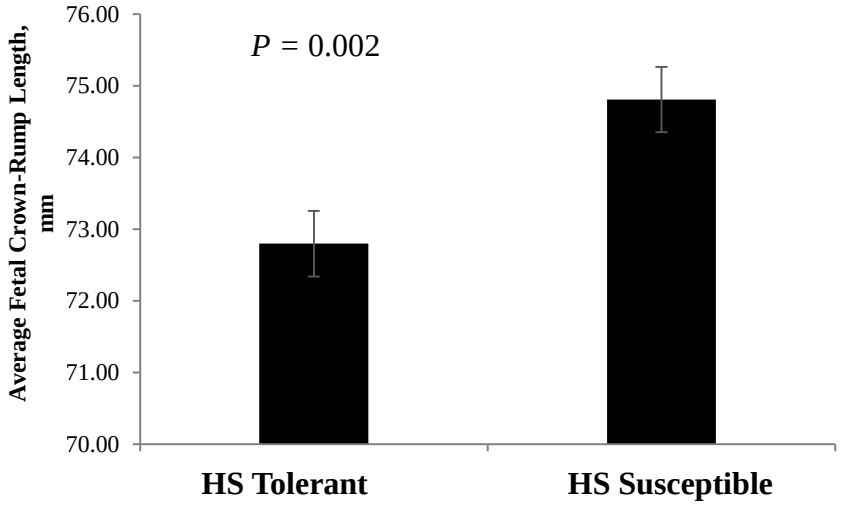


Figure 13. Average fetal crown-rump length (CRL) of pregnant gilts conceived during HS that were classified as tolerant or susceptible to HS prior to puberty. The HS tolerant gilts had an average CRL of 72.8 ± 0.46 mm while the HS susceptible gilts had a significantly higher average CRL of 74.8 ± 0.46 mm.

Ovarian Measurements

Ovaries were excised from each tract and weighed individually to get a total ovarian weight. The average ovarian weight for all bred gilts was 21.6 ± 0.3 g with the tolerant gilts averaging 21.7 ± 0.4 g and having no significant difference (P > 0.05) from the susceptible gilts averaging 21.6 ± 0.6 g of total ovarian weight. The CL on the ovaries from each gilt were counted to determine total CL number and measured to record an average CL diameter. The average total number of CL for all bred gilts was 17.8 ± 0.34. The tolerant gilts averaged 17.6 ± 0.34 total CL and showed no significant difference (P > 0.05) from the susceptible gilts that averaged 18.1 ± 0.62 total CL. The average CL diameter of all bred gilts was 10.2 ± 0.06 mm and there was a small difference (P = 0.056) between the tolerant gilts averaging 10.3 ± 0.08 mm and the susceptible gilts averaging 10.1 ± 0.08 mm per CL (**Figure 14**). After total fetus and CL numbers were recorded a percentage was calculated to determine the survivability of embryos, assuming all

oocytes were fertilized. The average embryo survivability of all the bred gilts was 78.9 ± 1.8% and was not different between classifications.

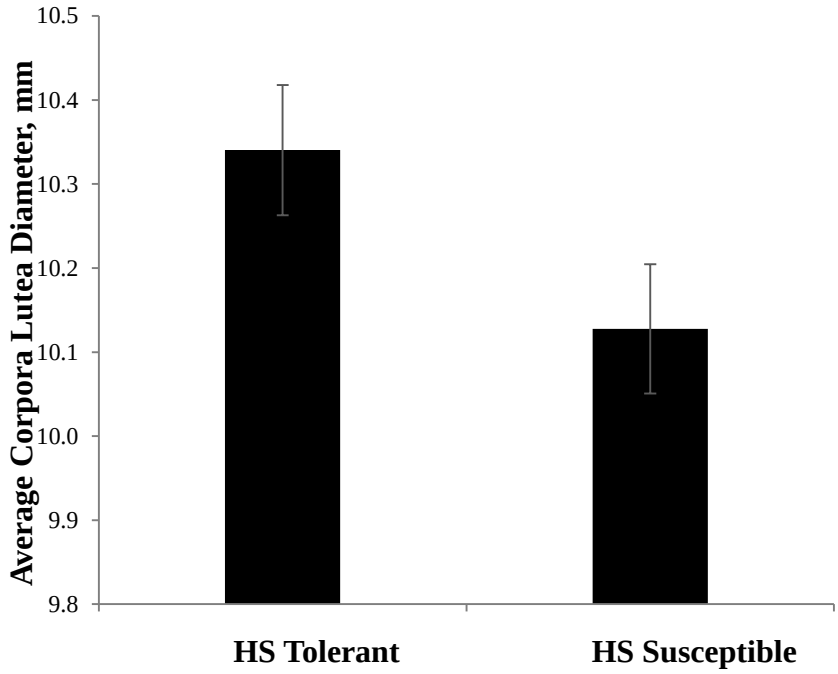


Figure 14. Average CL diameter of pregnant gilts conceived during HS that were classified as tolerant or susceptible to HS prior to puberty. The HS tolerant gilts average a CL diameter of 10.3 ± 0.08 mm while the HS susceptible gilts had a slightly significantly smaller average CL diameter of 10.1 ± 0.08 mm.

Ovarian follicle steroid synthesis was not affected by HS

An additional group of sexually mature gilts were subjected to cyclical HS (n=6) or thermal neutral conditions (TN; n=6) following a Matrix synchronized estrus. At 120 h following Matrix withdrawal, gilts were sacrificed, ovarian tissue collected and dominant follicles of each gilt aspirated of follicular fluid which was centrifuged to remove cellular debris and stored for analysis of 17β-estradiol and LBP. We had previously demonstrated altered ovarian signaling in ovaries from pre-pubertal gilts (Nteeba et al., 2015), thus we evaluated endpoints observed to be sensitive to HS in the current study.

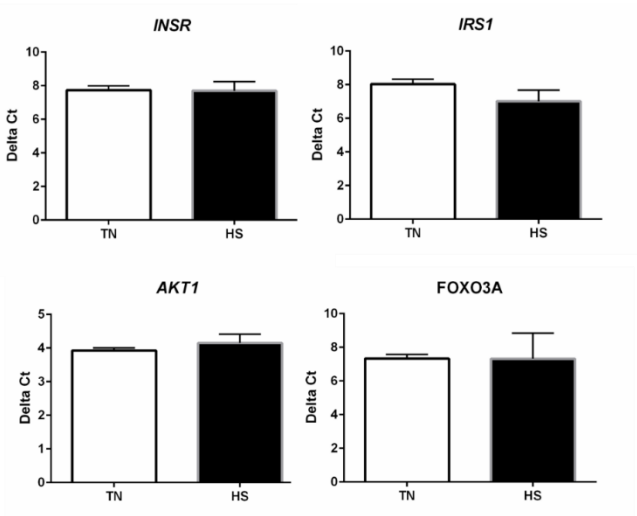


Figure 15. Impact of HS on mRNA encoding proteins involved in insulin and PI3K signaling. There was no impact of HS on these signaling molecules ($P > 0.05$).

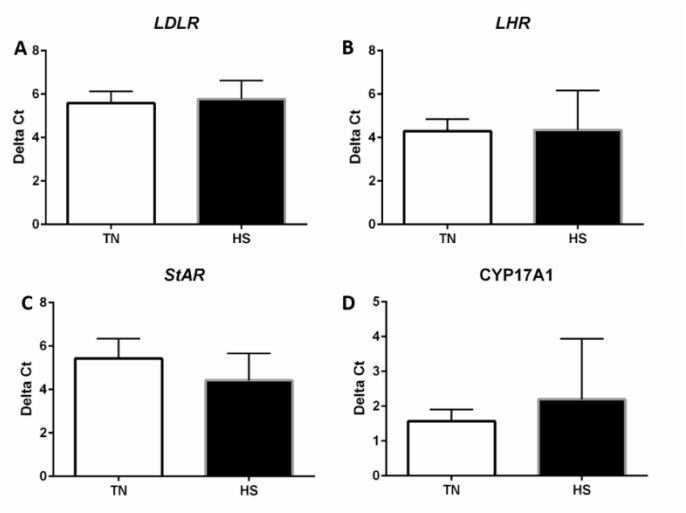


Figure 16. Impact of HS on mRNA encoding proteins involved in steroid synthesis. There was no impact of HS on these signaling molecules ($P > 0.05$).

We discovered that contrary to our previous data, that HS did not impact mRNA encoding *INSR*, *IRS1*, *AKT1*, or *FOXO3A* in cycling gilts (**Figure 15**). In addition, HS did not affect mRNA encoding the steroidogenic genes LDLR, LHR, StAR or CYP17A1 (**Figure 16**). These data may indicate that there are differential effects of HS on ovarian mRNA dependent on the cyclical status of the gilt.

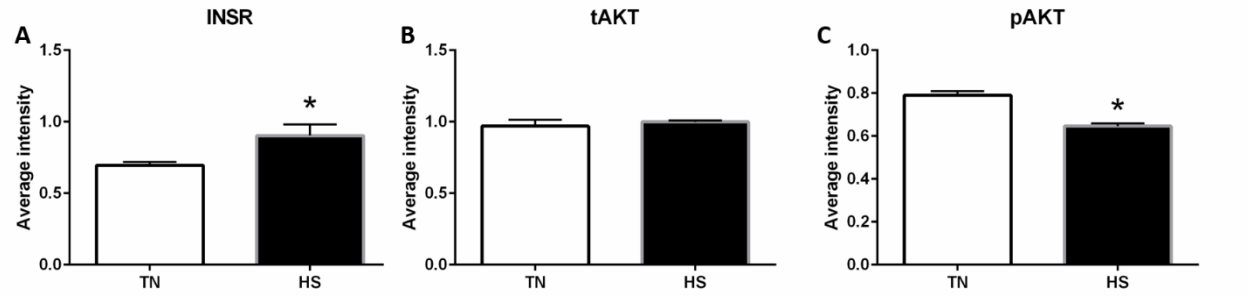


Figure 17. HS increased ($P < 0.05$) protein abundance of the (A) INSR, had no effect on (B) total AKT, but decreased PI3K signaling as evidenced by a reduction ($P < 0.05$) in (C) pAKT, the major PI3K signaling mediator.

We next evaluated the impacts of HS during the follicular phase on ovarian proteins, INSR, AKT (total and phosphorylated (pAKT). Whereas we had previously discovered that 35 d of HS in pre-pubertal gilts increased both insulin and PI3K signaling, in synchronized post-pubertal gilt ovaries insulin signaling was increased ($P < 0.05$) but PI3K was decreased ($P < 0.05$) (**Figure 17**).

Furthermore, we determined impacts of HS during the follicular phase in synchronized gilts on StAR and CYP19A1, proteins that are critical for 17β-estradiol, the major ovarian produced steroid hormone during this phase of the estrous cycle and we did not observe any impact of HS thereon (**Figure 18**).

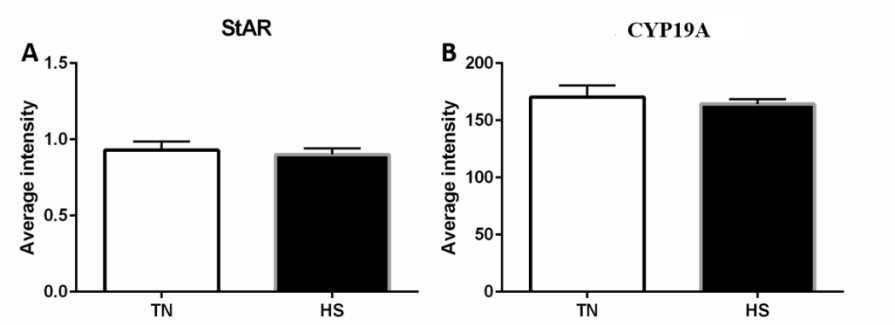
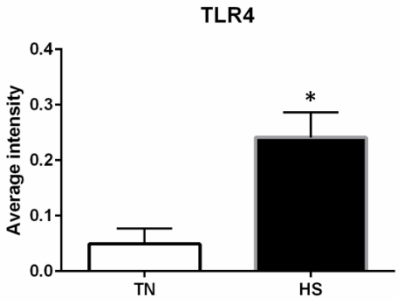


Figure 18. HS did not impact protein abundance of (A) StAR or (B) CYP19A1 ($P > 0.05$), proteins that are required for 17β-estradiol synthesis.

We have become interested in the impact of circulating lipopolysaccharide (LPS) which arises due to compromised intestinal integrity and which has been demonstrated to negatively impact reproduction in mice and cows (1, 2). Therefore, we evaluated protein levels of Toll-like receptor 4 (TLR4), the classical receptor for LPS, in ovarian tissue and discovered that TLR4 is dramatically increased in ovaries from HS gilts, relative to their non-HS counterparts (**Figure 19**).

Figure 19. TLR4 is increased during HS ($P < 0.05$), indicating that the ovary is responding to increased circulating LPS that is present during HS in gilts.



Based upon these observations, we measured both 17β-estradiol and LPS binding protein (LBP) in the aspirated follicular fluid collected from the ovaries of TN and HS gilts. We observed no change in 17β-estradiol concentration, nor was there an increase in LBP levels due to HS (**Figure 20**). However, there is likely a temporal pattern in the increase of 17β-estradiol and LBP due to HS, this we cannot completely rule out HS-induced effects on these endpoints based upon this single snapshot in time.

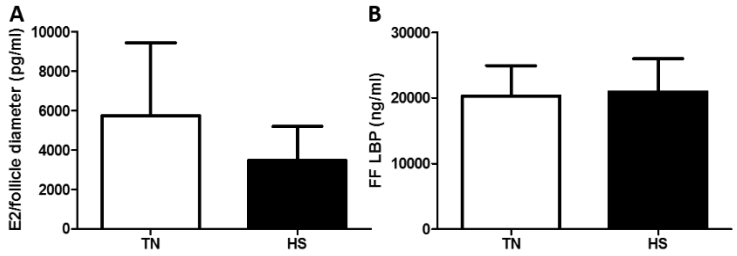


Figure 20. HS did not impact (A) 17β-estradiol concentration nor LBP in follicular fluid aspirated after HS during the follicular phase.

Finally, in order to evaluate the potential for increased degradation of 17β-estradiol due to HS, we investigated 17β-estradiol sulfonation by sulfotransferase 1E1 (SULT1E1). Sulfonated 17β-estradiol (E-S) represents a substrate for multidrug resistance protein 1 (ABCC1). We found that both ovarian SULT1E1 and ABCC1 protein were elevated by HS (**Figure 21).** What this translates to is that 17β-estradiol may be degraded too quickly in the ovaries of heat-stressed pigs. Whether the increase in 17β-estradiol production proceeds increased 17β-estradiol degradation or vice versa remains unclear but this is a hugely novel finding, and opens an avenue for future investigation as regards ovarian causes of seasonal infertility.

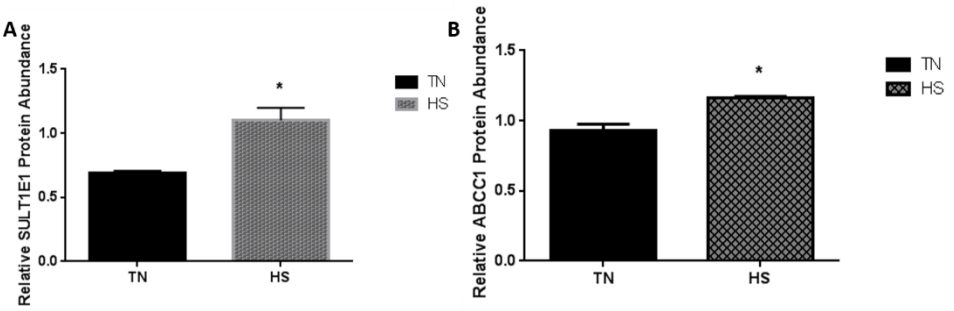


Figure 21. HS increases ($P < 0.05$) (A) SULT1E1 and (B) ABCC1, indicating increased degradation of 17β-estradiol during HS.

IX. Discussion

In the northern hemisphere, many species demonstrate seasonal breeding and will breed during the winter months so that, post-parturition, food availability and weather will be favorable to offspring. Domestic swine, being polyestrous unlike other domestic livestock species (sheep and horses), do not exhibit seasonal anestrus and demonstrate normal length estrous cycles year round. However, one problem plaguing the modern production pig is bouts of seasonal infertility during the late summer and early fall months (Love, 1978). Many questions with respect to both cause and mitigation surround this phenomenon and its potential contribution to a national reduction in reproductive performance. While seasonal infertility affects all ranges of production systems (size, environment) in many countries, there remains lack of consensus on the factors causing seasonal infertility and the importance of each factor (Hurtgen and Leman, 1980, Peltoniemi et al., 1999). The specific factors influencing seasonal infertility are debatable, as there are many influencers from genetics to environment. The most studied factors include photoperiod, heat stress, genetics, and management (Love et al., 1993; Prunier et al., 1994; Auvigne et al., 2010).

Photoperiod may be an important influencer due to its importance in regulation of normal seasonal breeding (Love et al., 1993). The synthesis and secretion of melatonin levels, produced in the pineal gland, fluctuate based on the amount of available sunlight within a day, ultimately affecting the start and stop of anestrous periods in animals (Revel et al., 2009). Since domestic swine are polyestrous, the effects of photoperiod are debatable. However, the bout of seasonal infertility in the summer months has led researchers to study this mechanism in domestic swine to identify a relationship between photoperiod and seasonal infertility. Melatonin levels have been shown to mimic the circadian rhythm found in wild boars (seasonal breeders) leading to the conclusion that photoperiod could be an important factor of seasonal infertility. However, many studies have concluded that seasonal infertility is not a consistent problem across animals and years (Auvigne et al., 2010). This leads to the question, are there shifting factors that magnify photoperiods bringing about seasonal infertility? As wild boar are seasonal breeders, genetic parameters are quickly indicted as an influencer due to the evolutionary relationship of wild boars and domestic swine. However, the inconsistency of seasonal infertility amongst specific animals and the high variability in reproductive performance suggests otherwise (Martinat-Botté et al., 1984).

Mitigating seasonal infertility is important since it drastically decreases the economic potential of swine enterprises. Based on a 2012 feed cost analysis, annual economic losses to the swine industry due to lost reproductive performance during seasonal infertility was estimated to be approximately $55 per sow (Pollmann, personal communication with Keating and Ross). Seasonal infertility is a reoccurring problem and while it could arguably be related to other factors such as photoperiod, seasonal infertility in pigs is often associated with periods of excessive heat. HS has been identified as a primary contributing cause of seasonal infertility due to the associated decrease in reproductive performance occurring during the hotter months of the year (June-September). Due to their lack of functional sweat glands, pigs are poor at dissipating body heat and must rely on other strategies to control body heat such as panting and regulating metabolic heat production through altering their feed intake (Whittow, 1971) which can lead to decreased production performance. Renaudeau et al. (2011) conducted a meta-analysis of data (1970-2009) and reported that increased ambient temperature had a negative impact on average daily feed intake and average daily gain. The same study reported that the effect of temperature was greater in the more recently published data suggesting that as the swine industry selects for an increased efficiency in lean muscle accretion pigs are becoming less tolerant to periods of excessive HS. This intolerance may in part be due to increased metabolic heat production from increased synthesis in skeletal muscle and growth (Hocquette et al., 1998). The effects of HS vary depending on production stage during exposure to increased ambient temperatures. Since reproductive efficiency is the combined performance of several production stages, mitigating HS across all stages is crucial in maintaining production efficiency in the pork industry. The effects that HS has on reproductive efficiency range from decreases in litter size, farrowing rate, fetal performance, in addition to increased instances of early embryonic death (Xue et al., 1994, Hurtgen et al. 1980, and Peltoniemi et al., 1999). It can also affect long term performance through an increase in WEI length and interrupting ovarian follicular development (Prunier et al., 1996).

In objective one of this study, we were unable to demonstrate a strong direct effect of HS during the WEI to have a significant impact on P1 weaned sow's subsequent reproductive performance despite that compromised reproduction was significantly greater in summer, as expected. It is quite possible that HS could still be impacting reproduction during periods of seasonal infertility, albeit less impactful during the WEI. High ambient temperatures have commonly been implicated as an influencer of SI, largely due to the correlation of SI occurring during the hottest parts of the year (Prunier et al., 1994). Auvigne et al. (2010) showed, across a five year period, the year with the highest number of hot days showed the largest statistical difference in decreased reproductive performance. Collectively, these observations lead to a conclusion that increased ambient temperatures do not alone influence seasonal infertility, but could exacerbate other factors contributing to reduced fertility during seasonal infertility. One factor that has been shown to have a larger effect on seasonal infertility when accompanied with HS is nutrition and nutritional management. When a female reaches her critical temperature limit (27-30°C), feed intake decreases (Prunier et al., 1997). If HS occurs during lactation and feed intake decreases, the female's ability to rebreed could be hindered (Prunier et al., 1996).

A decrease in ovarian activity can cause early loss of pregnancy and decreased FR which are direct manifestations of seasonal infertility (Love et al., 1993). Lopes et al. (2014) demonstrated reduced ovarian activity during the months associated with seasonal infertility by showing that during ovulation there were less follicles on the ovaries in females ovulating during the summer and fall months compared to females ovulating in the winter and spring months (12.4 ± 0.3 and 13.5 ± 0.3 follicles, respectively). Similar to these findings, Bertoldo et al. (2011)

reported that in a group of sows culled for early pregnancy loss during the summer and spring, the summer culls had fewer CL present on their ovaries compared to the spring culls (11.6 ± 3.3 vs. 9.3 ± 0.99). This suggests that CL number appears to be influenced by environmental factors associated with seasonal infertility.

Summary and Conclusions

Collectively, this project advanced our understanding of seasonal infertility and the role of HS on several specific areas. In Objective 1, it was demonstrated that seasonal infertility in large commercial pork production system was not strongly correlated with HS during the WEI, suggesting that HS during other phases of production, such as late gestation and/or lactation could influence reproductive ability. Differences in circulating LBP or insulin during the WEI of sows weaned during the summer or spring were not observed and enabled us to improve our understanding of the biological consequences of the lactation to gestation transition during different seasons. In objective 2, it was demonstrated that thermoregulatory response to HS is repeatable in gilts exposed to HS more than 4 months between exposures. This was a critically important finding for the pork industry as subsequently our group leveraged this project and samples collected during it to secure additional dollars to pursue the identification of specific genomic regions in pigs associated with contributing to production efficiency during HS. Despite demonstrating the repeatability of the thermoregulatory response to HS in gilts in objective 2, we demonstrated a lack of impact on HS during a Matrix synchronized follicular phase to negatively influence reproductive performance. It is possible that the severity of the HS was not great enough to compromise fertility or that seasonal infertility as observed in the industry is a more complex trait representing the combination of seasonal, environmental, metabolic conditions, and genetics of the sows. Despite HS not directly influencing pregnancy to day 42-47 of gestation in objective 2, a variety of ovarian pathways were affected by HS having the potential to compromise reproduction such as altered folliculogenesis, reduced CL size, which could have implications for pregnancy maintenance and litter-size. In addition, our demonstration that 17β-estradiol degradation could be increased during HS opens a novel avenue for investigation and for understanding the biological changes induced in cyclical gilts during HS. Additionally, comparison of pre-pubertal gilt effects of HS with those observed in cyclical gilts implies that increased circulating gonadotrophins may alter the hormonal milieu in a manner that in turns impacts the ovarian HS response.

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