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

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Date Submitted: 2/8/16

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; 20.3ºC ± 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.