



Effects of increased ambient temperature and supplemental altrenogest before pregnancy establishment in gilts

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Abstract

Heat stress (HS) mitigation strategies are critically needed to combat the substantial economic effects on animal agriculture. The manifestations of seasonal infertility include delayed puberty onset, reduced conception rates, decreased litter size, and increased wean to estrus interval. To assess the effects of HS during early gestation and evaluate the benefit of supplemental altrenogest (ALT) as a mitigation strategy, 30 crossbred postpubertal gilts (157 ± 11 kg body weight) were subjected to estrous synchronization via 14 d oral administration of ALT. Artificial insemination during estrus was performed, and gilts were then placed into one of four treatment groups: HS (35 ± 1 °C for 12 h/31.60 ± 1 °C for 12 h) with (HSALT, *n* = 7) or without (HSCON, *n* = 7) 15 mg/d ALT supplementation or thermal neutral (TN; 20 ± 1 °C) conditions with (TNALT, *n* = 8) or without (TNCON, *n* = 8) 15 mg/d ALT supplementation until 12 d post-estrus (dpe). Administering ALT occurred at 0600 hours from 3 to 12 dpe, and rectal temperatures (T_{re}) and respiration rates (RR) were recorded. Blood was collected via jugular venipuncture on 0, 4, 8, and 12 dpe. Gilts were euthanized humanely at 12 dpe followed by the collection of ovarian tissue, and uterine flushing for conceptus collection. In HS compared with TN gilts, RR and T_{re} were increased ($P < 0.01$) but unaffected by ALT supplementation. Feed intake was reduced ($P < 0.01$) by HS but unaltered by the ALT treatment. Corpora lutea (CL) weight was reduced ($P < 0.01$) in HSCON gilts when compared with TNCON and HSALT gilts despite progesterone concentrations in serum and luteal tissue not being affected by treatment ($P \geq 0.10$). CL diameter was reduced ($P \leq 0.05$) in HSALT gilts compared with other treatments. Interleukin-1 β (IL1B) uterine flush concentration was not affected ($P > 0.20$) by environment or ALT supplementation, although moderate ($P = 0.06$) interaction between environment and ALT existed, as IL1B concentration in TNALT was increased ($P = 0.03$) compared with TNCON gilts. While environment did not affect conceptus development ($P = 0.90$), ALT supplementation advanced conceptus elongation ($P < 0.01$). Collectively, these data demonstrate that HS may affect luteal development before pregnancy establishment, and ALT increases conceptus elongation by 12 dpe.

Key words: altrenogest, conceptus, heat stress, pregnancy

Abbreviations: ALT, altrenogest; bpm, breaths per minute; CL, corpora lutea; dpe, days post estrus; FI, feed intake; HS, heat stress; IL1B, interleukin-1 β ; P_4 , progesterone; RR, respiration rate; TN, thermal neutral; T_{re} , rectal temperature

Introduction

The seasonal reduction in gilt and sow productivity observed in the swine industry imposes a significant economic strain on pork production (St-Pierre et al., 2003; Pollmann, 2010). Occurring in conjunction with summer months, increased ambient temperatures and the overall thermal load in livestock are significant contributors to the observed seasonal depression on swine fertility (Love, 1978; Auvigne et al., 2010). Delayed puberty onset (Paterson et al., 1991) and prolonged wean to estrus intervals (Omtvedt et al., 1971; Love, 1978) are characteristic phenotypic indicators of season infertility. Sows experiencing irregular inter-estrus intervals (25 to 35 d) after mating coupled with reduced farrowing rates and litter sizes is also associated with seasonal infertility (Omtvedt et al., 1971; Love, 1981; Xue et al., 1994; Ross et al., 2017).

Effective communication between the developing conceptus and dam is a prerequisite to pregnancy establishment (Bazer et al., 1982) and, if disrupted, can result in failure to

carry progeny to term. Exposure to stressors before implantation can be detrimental to conceptus survival and pregnancy maintenance. Increased ambient temperatures during the first 15 d of gestation negatively impact conceptus viability (Edwards et al., 1968; Wettemann et al., 1988). Additionally, progesterone (P_4), while essential for pregnancy maintenance, is also vital for the induction of endometrial secretions important for conceptus development and implantation (Ka et al., 2007). Furthermore, reduced serum P_4 levels have been associated with late summer to early autumn months in pregnant sows (Wrathall et al., 1986). Separate from environmental and nutritional effects, exogenous P_4 from the time of corpus luteum formation to day 30 of pregnancy has beneficial effects on conceptus survivability (Ashworth, 1991). This study investigated the hypothesis that increased ambient temperatures during early gestation would negatively impact corpora lutea (CL) function and conceptus development during the pre-implantation

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window; supplementation with a P₄ analog could alter the peri-implantation environment and conceptus development during heat stress (HS).

Materials and Methods

Animals

All animal procedures in this study were conducted with approval by the Iowa State University Institutional Animal Care and Use Committee. This experiment utilized 30 crossbred gilts (157 ± 11 kg body weight) housed in individual stalls (57 × 221 cm) at the Iowa State University Swine Nutrition Farm research facility (Ames, IA); each stall equipped with a stainless-steel feeder and nipple drinker. Gilts were provided ad libitum water throughout the duration of the experiment and fed 2.7 kg of a standard diet once daily. Feed intake (FI) was measured daily by weighing feeders to account for feed disappearance from the previous feeding. Any residual feed remaining in the feeder from the previous feeding was removed and discarded.

Estrous synchronization, acclimation, and artificial insemination

Before synchronization, all gilts were intramuscularly injected with 5 mL of commercially available gonadotropins (P.G. 600, Merck Animal Health, Summit, NJ 07901) to induce estrus. Behavioral estrus was monitored using an intact boar. Additionally, at approximately 18 d following P.G. 600 injection, blood samples were collected to quantify P₄ levels in a subset of gilts to validate CL function and cyclicity. After cyclicity was confirmed, estrus was synchronized by orally administering 6.8 mL of 15 mg altrenogest (ALT, Matrix, Merck Animal Health, Summit, NJ 07901) at the time of feeding (0700 hours) for 14 d. Animals were moved into stalls during estrus synchronization to permit acclimation before starting the experiment.

Gilts were subjected to fence line boar exposure for estrus detection and breeding, 24 h following the last ALT administration. Boar exposure occurred twice daily (0600 and 1800 hours) for 7 d lasting approximately 60 s per gilt during the ALT withdrawal period. During the period of boar exposure, gilts were observed for behavioral signs of estrus including vulva swelling, increased attention to boar presence, and reduced FI. Gilts were declared in estrus upon exhibiting standing behavior in response to back pressure. Gilts were artificially inseminated 12 h following the onset of estrus behavior using pooled terminal Duroc semen (Swine Genetics International, Cambridge, IA 50046), and they received additional inseminations every 12 h, up to 36 h after the onset of estrus or until estrus behavior ceased. All inseminations were performed by the same technician to minimize variation.

Treatment period

Following estrus, gilts were randomly assigned to one of the four treatment groups: diurnal HS (35 ± 1 °C for 12 h/31.6 ± 1 °C for 12 h) supplemented with (HSALT, *n* = 7) or without (HSCON, *n* = 8) 15 mg/d ALT or thermal neutral (TN; 21 ± 1 °C) also supplemented with (TNALT, *n* = 8) or without (TNCON, *n* = 7) 15 mg/d ALT supplementation until 12 d post-estrus (dpe). Once placed into environmental treatment, gestational ALT supplementation began, with ALT gilts being orally administered 6.8 mL Matrix (15 mg ALT) once daily at the time of feeding (0600 hours) from day 3 to 12 and

CON gilts receiving no ALT supplementation. Gilts assigned to TN conditions remained in the same stall they were housed in during estrus synchronization, detection, and insemination. Gilts assigned to HS treatment were moved into an adjacent room with stalls identical to the TN room that they were previously acclimated to, where elevated temperature treatments were implemented.

All gilts were introduced to assigned environmental conditions at 1900 hours, to normalize the initial thermal load experienced for those assigned to HS conditions. Thermal conditions began at a minimum of 48 h after the beginning of estrus onset to ensure that ovulation was not confounded by ambient temperature. In the case of gilts detected in standing heat in the morning (a.m.), introduction to their respective thermal environment occurred 60 h after estrus was detected. Gilts experienced estrus onset in the evening (p.m.) were introduced to their thermal environment 48 h after estrus was detected.

Response to thermal conditions

Beginning on day 2 of the ALT withdrawal period, rectal temperature (T_R), respiration rate (RR), and FI were recorded to establish baseline measurements before the onset of treatment conditions, signs of estrus behavior, and independent of ALT used for synchronization.

Baseline data were recorded until early signs of estrus were observed. This time frame between the last ALT administration and estrus onset was denoted as period 1 (P1). T_R was measured using a digital thermometer (Welch Allyn Sure Temp Plus 690, Skaneateles Falls, NY, USA) three times hourly in the a.m. (0700 to 0900 hours) and p.m. (1700 to 1900 hours). The three a.m. and three p.m. readings were subsequently averaged for experiment analysis. RR was measured at the same times as T_R, by counting flank movements in a 15-s time span and multiplying by four to obtain breaths per minute (bpm). FI was determined by providing 2.7 kg of feed in the a.m. and recording the remaining feed weight during both p.m. and a.m. time points. Both ambient temperature and humidity were recorded every 15 min by four data loggers (Lascar EL-USB-2-LCD, Erie, PA) placed equidistantly apart in the room and later condensed into averages for each time point.

Blood sampling

Blood was obtained via jugular venipuncture (10 mL; BD vacutainers) on 0, 4, 8, and 12 dpe. Serum samples were collected by centrifugation at 1500 × *g* for 10 min, aliquoted, and stored at -80 °C until further analysis.

Harvesting and tissue collection

At approximately 288 h (12 dpe) following detection in standing estrus, gilts were humanely euthanized via captive bolt followed by exsanguination. Timing of euthanasia from the onset of estrus was performed to target the narrow window of conceptus elongation where differences in conceptus growth could be discernable by classifying conceptuses as spherical or filamentous. Following confirmation of insensibility, reproductive tracts were collected, and ovaries were assessed for CL number, diameter, and weight. CL were excised and flash frozen from one ovary per animal. Following ovary removal, each uterine horn was flushed for the retrieval of luminal contents. Briefly, uterine flushing was performed by positioning hemostats at the proximal end of

each horn next to the uterine bifurcation and injecting 20 mL sterile saline at the distal end of the uterine horn through the oviduct. The saline injection was manipulated through the uterine horn, and uterine flush contents were collected. Following flushing, the conceptuses recovered were classified by stage of development, counted, and measured if the conceptuses could be individually distinguished. Conceptuses still exhibiting a spherical or tubular morphology were classified as spherical. Conversely, conceptuses that had elongated beyond tubular morphology were classified as filamentous. Following initial measurements, total conceptus weight was recorded and flash-frozen in liquid nitrogen and stored at -80°C . Individual conceptus weights were not recorded as separation was not feasible after flushing. Uterine flush contents were placed on ice before being centrifuged at $600 \times g$ for 10 min for the removal of cell debris. Uterine flush samples were then stored at -80°C until further analysis.

Hormone and protein assays

Lysed CL and serum P_4 levels were determined using a solid phase enzyme-linked immunosorbent assay (ELISA; Cat # EIA-1561, DRG Instruments GmbH, Germany) that utilized competitive binding principle to detect P_4 . All assays were performed per the manufacturer's guidelines. For CL P_4 analysis, tissue was first extracted from flash-frozen tissue in 5% trichloroacetic acid, homogenized, and diluted at 1:100 for detectability by the assay. Serum collected from 12 dpe was diluted at 1:3 for detectability. Samples were run in triplicate, with samples from the same estrous cycle day being constant across assigned plates, and treatments being randomized for representation within plates. Diluted and undiluted samples of ALT were also analyzed to determine assay sensitivity to synthetic progestin. All samples were within the detectable range of the assay.

Interleukin- 1β (IL1B) levels in uterine flushings were assessed using a solid-phase ELISA that employed a quantitative sandwich enzyme immunoassay technique (Cat # DLB50, R&D Systems, Minneapolis, MN, USA). All assays were conducted in accordance with the manufacturer's guidelines. Uterine flush samples from each horn were first pooled for representation of whole uterine IL1B and to account for inter-horn variation in conceptus spacing at the time of flushing. Samples were run in duplicate and randomized within the plate. IL1B was detectable in all flush samples for the assay.

Statistical analysis

Statistical Analysis SAS University Edition (Cary, NC 27513) was used for all statistical analyses. PROC MIXED was used for the analysis of T_R , RR, and FI with time point of the experiment as the repeated measure. The model tested the effect of treatment, environment, and the treatment \times environment interaction. Hormone and protein assays were analyzed using PROC MIXED examining the effect of treatment, environment, and the treatment \times environment interaction. Progesterone analysis included CL count as a covariate. For IL1B analysis, the length of environmental assignment was included in the model, and the total conceptus weight was included as a covariate. CL size (weight and diameter) analysis included treatment, environment, and the treatment \times environment interaction, and CL count as a covariate. Chi-Square (χ^2) analysis was performed using the PROC FREQ function to determine the association between environment and treatment on conceptus development.

Results

HS reduced FI and increased T_R and RR. All were unaffected by ALT supplementation

FI was reduced (18%; $P < 0.01$) in HS gilts compared with TN controls but did not differ between ALT treatments ($P = 0.58$; Figure 1). Thermal conditions also affected RR, with HS gilts having increased ($P < 0.01$) RR compared with TN counterparts (102 vs. 27 bpm; Figure 2). No difference was observed for RR between ALT and CON groups ($P = 0.99$; Figure 2). In HS gilts, T_R was increased ($P < 0.01$) compared with TN gilts, and T_R was not different between ALT and CON treatments ($P = 0.92$; Figure 3).

HS conditions did not affect serum or luteal progesterone production

Serum P_4 concentrations did not differ between treatment groups at 0, 4, 8, or 12 dpe (Figure 4A). Luteal P_4 was not affected by thermal conditions ($P = 0.90$) or ALT treatment ($P = 0.52$; Figure 4B). No detectable signal from ALT was observed on the P_4 ELISA.

CL weight and diameter were altered by treatments

CL weight was reduced ($P < 0.01$) in HSCON (0.45 g) compared with TNCON (0.51 g) and HSALT (0.52 g; Figure 5A). A tendency for reduced CL weight ($P = 0.09$) existed for TNALT (0.48 g) compared with HSALT but did not differ compared with HSCON ($P > 0.1$). TNCON did not differ from TNALT or HSALT ($P \geq 0.2$). CL diameter tended to be decreased ($0.07 \leq P \leq 0.05$) in the HSALT group compared

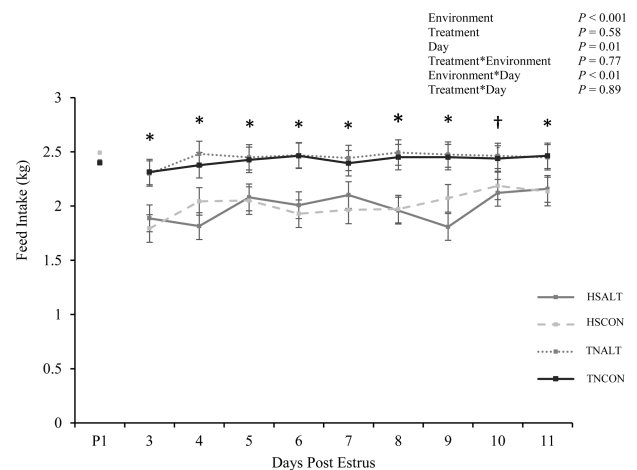


Figure 1. Impact of cyclical heat stress on feed intake (FI). Postpubertal gilts were assigned to one of four different treatments: thermal neutral (TN) conditions ($20 \pm 1^{\circ}\text{C}$, 36% to 57% relative humidity) with (TNALT) and without (TNCON) 15 mg/d of orally administered altrenogest (ALT) or heat stress (HS) conditions ($35 \pm 1^{\circ}\text{C}$ for 12 h, $31 \pm 6^{\circ}\text{C}$ for 12 h, 21% to 31% relative humidity) with (HSALT) or without (HSCON) ALT during day 3 to 12 post estrus. P1 describes baseline FI measurements taken before the treatment onset. Environment assignment and ALT administration lasted from day 3 to 12 post estrus. Animals were limit fed 2.7 kg at 0600 hours. FI was measured daily, both in the morning (a.m.) and in the evening (p.m.). Line graphs denote FI each day post estrus for each group \pm SEM. * indicates the difference between environment at specified time points ($P < 0.01$); † indicates difference between environment ($P < 0.05$). No differences were detected in response to the ALT treatment.

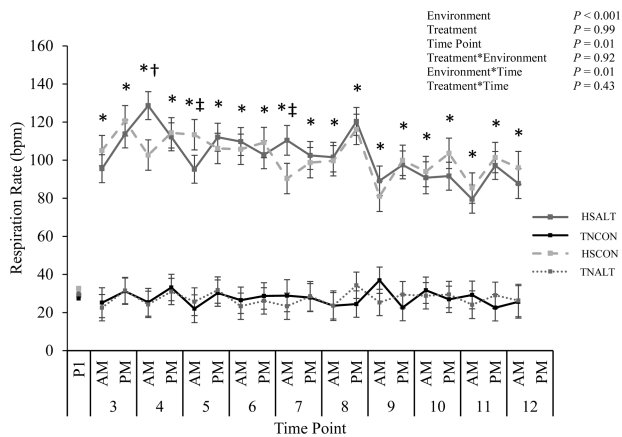


Figure 2. Impact of cyclical heat stress on respiration rate (RR). Postpubertal gilts were assigned to one of four different treatments: thermal neutral (TN) conditions ($20 \pm 1^\circ\text{C}$, 36% to 57% relative humidity) with (TNALT) and without (TNCON) 15 mg/d of orally administered altrenogest (ALT) or heat stress (HS) conditions ($35 \pm 1^\circ\text{C}$ for 12 h, $31 \pm 6^\circ\text{C}$ for 12 h, 21% to 31% relative humidity) with (HSALT) or without (HSCON) ALT during day 3 to 12 post estrus. P1 describes baseline RR measurements taken before the treatment onset. RR was recorded by observing flank movements for 15 s and multiplied to determine beats per min (bpm). RR was assessed three times in the morning (a.m.) and evening (p.m.) and averaged for each time point per treatment and environment \pm SEM. Line graphs denote bpm for each time point post estrus for each group \pm SEM. * indicates the difference between environment at specified time points ($P < 0.01$); † denotes the difference between ALT treatments in the HS group ($P < 0.05$); ‡ denotes a tendency for the difference between ALT treatments in the HS group ($P < 0.10$).

with TNCON, HSCON, and TNALT (10.22 vs. 10.59 , 10.59 , 10.56 ± 0.13 mm, respectively; [Figure 5B](#)).

Conceptus development was advanced by ALT supplementation although unaffected by HS

On all litters evaluated on conceptus morphology, all conceptuses were either fully elongated or still in the spherical morphology, excluding one gilt that had one conceptus still in the spherical stage and the remaining were elongated; thus, the litter was classified to be filamentous in morphology. Environment did not affect the stage of conceptus elongation rate (71.4%, HS vs. 69.2%, TN; $P = 0.90$; [Figure 6A](#)). For gilts administered ALT, 12 of 13 (92.5%) yielded conceptuses that had begun rapid elongation and were filamentous in morphology, compared with gilts not supplemented with ALT, where only 6 of 14 (42.9%) yielded filamentous conceptuses ($P < 0.01$; [Figure 6B](#)).

Effect of increased ambient temperature and ALT supplementation on conceptus IL1B production

Conceptus IL1B concentration was increased in TNALT ($P = 0.02$) and HSCON ($P = 0.03$) compared with TNCON (792.2, 728.7, respectively, vs. 148.81 pg/mL). No difference was detected in HSALT when compared with the TNALT or TNCON or HSCON ($P \geq 0.10$; [Figure 7A](#)). No environmental effects on IL1B levels were detected ($P = 0.57$; [Figure 7B](#)). Similarly, treatment ALT differences on IL1B were observed ($P = 0.37$; [Figure 7C](#)). IL1B concentration was increased in conceptus classified as filamentous compared with conceptuses classified as spherical (730.1 vs. 78.1 pg/mL; $P < 0.01$).

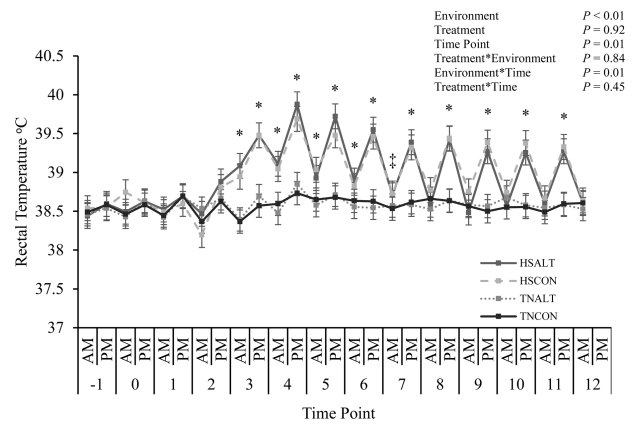


Figure 3. Impact of cyclical heat stress on rectal temperature (T_R). Postpubertal gilts were assigned to one of four different treatments: thermal neutral (TN) conditions ($20 \pm 1^\circ\text{C}$, 36% to 57% relative humidity) with (TNALT) and without (TNCON) altrenogest (ALT) supplementation or heat stress (HS) conditions ($35 \pm 1^\circ\text{C}$ for 12 h, $31 \pm 6^\circ\text{C}$ for 12 h, 21% to 31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered ALT during day 3 to 12 post estrus. Environment assignment and ALT administration lasted from day 3 to 12 post estrus. T_R was recorded three times each morning (a.m.) and evening (p.m.). The line graph represents average morning and evening T_R per treatment \pm SEM. There was an increase ($P < 0.01$) in average T_R in the HS environment compared with TN. * and † indicate a difference of ($P < 0.01$) and ($P < 0.1$), respectively.

Discussion

The predictable reduction in gilt and sow reproductive performance associated with seasonal increases in ambient temperature results in marked economic losses to the pork industry ([St-Pierre et al., 2003](#); [Pollmann, 2010](#)). This continual challenge to swine production is characterized by numerous factors, including delay in puberty achievement ([Paterson et al., 1991](#)), prolonged or irregular returns to estrus ([Sterning et al., 1990](#); [Peltoniemi et al., 1999](#)), and the reduction in farrowing rate and litter size ([Omtvedt et al., 1971](#); [Xue et al., 1994](#)). Though the decrease in performance is likely the result of several contributing factors, including photoperiod ([Love et al., 1993](#)), the effects of increased temperature also impede optimal reproductive performance in pigs ([Auvigne et al., 2010](#); [Ross et al., 2017](#)).

The establishment of pregnancy is a highly coordinated process in which sufficient communication between conceptus and dam must occur ([Bazer et al., 1982](#); [Geisert et al., 1982a, 1982b](#)). This peri-implantation window is thought to be sensitive to the negative effects of HS, specifically on conceptus viability ([Edwards et al., 1968](#); [Wettemann et al., 1988](#)). Additionally, early pregnancy disruption has a greater incidence during the summer/autumn months compared with winter/spring months ([Tast et al., 2002](#)). It is unclear whether the increased thermal load itself causes conceptus death, or if conceptus death is an indirect effect resulting from compromised CL function and subsequent P4 production ([Wrathall et al., 1986](#)). This prompted the current investigation, in which the effects of HS from the time of luteinization to pregnancy recognition were assessed in gilts. Simultaneously, half of the gilts were supplemented with a P₄ analog to establish the utility of this mitigation approach to rescue any potential negative effects that HS exerted on luteal P₄ production.

Inherently, pigs struggle to cope with HS largely due to their lack of functional sweat glands coupled with selection for

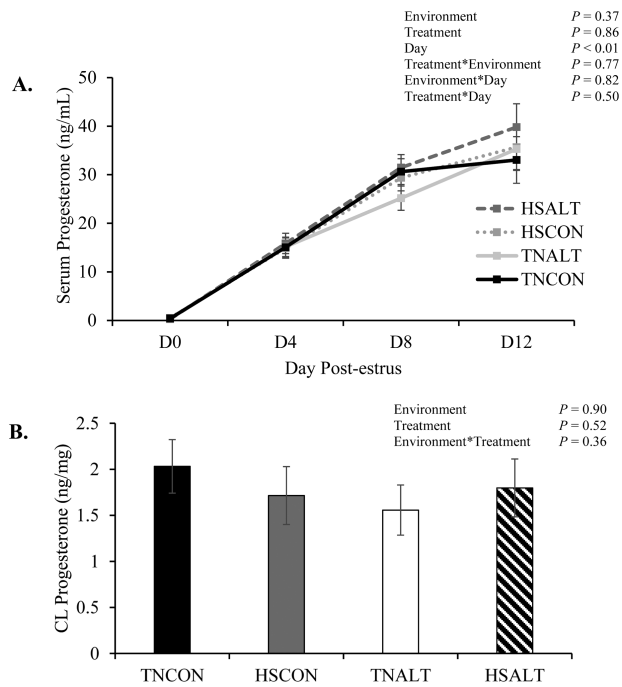


Figure 4. Impact of increased ambient temperature and altrenogest (ALT) supplementation on serum and luteal progesterone concentrations. Bred gilts were subject to either thermal neutral (TN) conditions (20 ± 1 °C, 36% to 57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation or heat stress (HS) conditions (35 ± 1 °C for 12 h, 31 ± 6 °C for 12 h, 21% to 31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered ALT during day 3 to 12 post estrus. Graphs represent LS means for serum and luteal progesterone (P_4) concentrations in each group \pm SEM. (A) Blood was collected to assess P_4 concentrations on 0, 4, 8, and 12 d post estrus (dpe) and analyzed using colorimetric ELISA. Serum P_4 concentrations did not differ at 0, 4, 8, and 12 dpe. (B) Corpora lutea (CL) were collected from each animal, powdered, and homogenized in 5% trichloroacetic acid. Homogenates were diluted and analyzed to quantify P_4 concentrations. No differences were detected across the environment ($P = 0.90$) or ALT treatment ($P = 0.52$).

increased lean tissue accretion, resulting in greater metabolic activity (Brown-Brandl et al., 2001; Baumgard and Rhoads, 2013). In the current study, HS gilts exhibited notable behavioral signs (increased T_R and RR and decreased FI) of stress recapitulating previous studies and confirming that the gilts were experiencing a substantial heat load.

The CL weight tended to be reduced in HSCON gilts compared with TNCON counterparts, an observation corroborating our previous results (Bidne et al., 2019). Conversely, CL weight in HSALT gilts was increased compared with HSCON but was not different from TN counterparts, indicating that ALT supplementation rescued CL weight from being reduced by HS. This is interesting as supplemental P_4 has previously been reported to have negative effects on CL size and health (Spies et al., 1960), although the administration of P_4 was from day 10 to 25, in gilts of whom not all were pregnant, and at a greater dose than investigated in this study. Despite being heavier in weight, HSALT CLs were decreased in diameter in comparison to other treatment groups.

The variation in the aforementioned CL weight and diameter was not reflected in treatment differences in luteal or circulating P_4 concentration. Thus, supplementation with ALT did not result in any endocrine disruption in supplemented

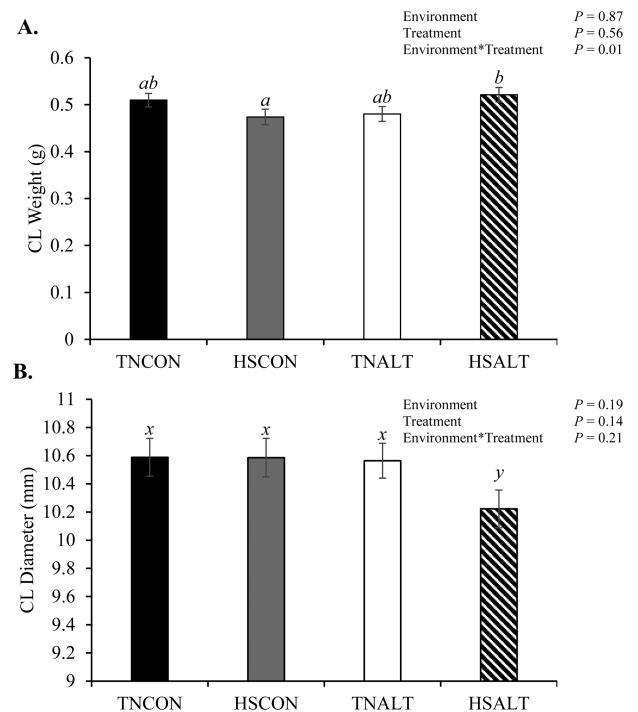


Figure 5. Impact of increased ambient temperatures and altrenogest (ALT) supplementation on corpora lutea (CL) size. Bred gilts were subjected to either thermal neutral (TN) conditions (20 ± 1 °C, 36% to 57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation or heat stress (HS) conditions (35 ± 1 °C for 12 h, 31 ± 6 °C for 12 h, 21% to 31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered ALT during day 3 to 12 post estrus. Bar charts depict LS means for CL weight (g) and diameter (mm) for each group \pm SEM. (A) Average CL weight for each group was recorded. An environment by ALT treatment interaction was observed ($P = 0.01$). CL weight was decreased ($P < 0.01$) in HSCON compared with HSALT (0.47 vs. 0.52 g, respectively). CL of HSALT gilts tended to be heavier ($P = 0.08$) compared with CL of TNALT gilts. (B) CL diameter was measured and averaged within treatments. A tendency ($P \leq 0.07$) for decreased CL diameter was observed in HSALT compared with other groups. Differences in letters denote statistical difference (a,b: $P < 0.05$; x,y: $P < 0.01$).

gilts. The relationship between CL size and P_4 production has shown to be dissimilar in cattle as well (Sartori et al., 2002). It is worth noting, however, that at timepoints when differences would have been expected to be largest for serum P_4 between treatments (8 and 12 dpe), HS gilts had become partially acclimated to the environment, as demonstrated by the gilt's ability to resume eutherma at night. Further work is needed to test the effect of initial thermal load response on circulating P_4 when production is near or at its highest levels (8 to 12 dpe).

The rate at which the porcine CL develops is rapid, with the luteal mass initially growing by cellular hypertrophy then dramatically increasing the mitotic activity and cell proliferation by approximately day 15 (Ricke et al., 1999). During luteinization, angiogenic factors increase the vascularity of the rapidly growing structure (Murphy et al., 2001). After ovulation, the early luteal structure experiences hypoxic conditions due to reduced blood flow around ovulation (Niswender et al., 1976). Hypoxia is considered an initial stimulus of angiogenic factors to promote new vascular development (Reynolds et al., 2000). Additionally, hypoxic

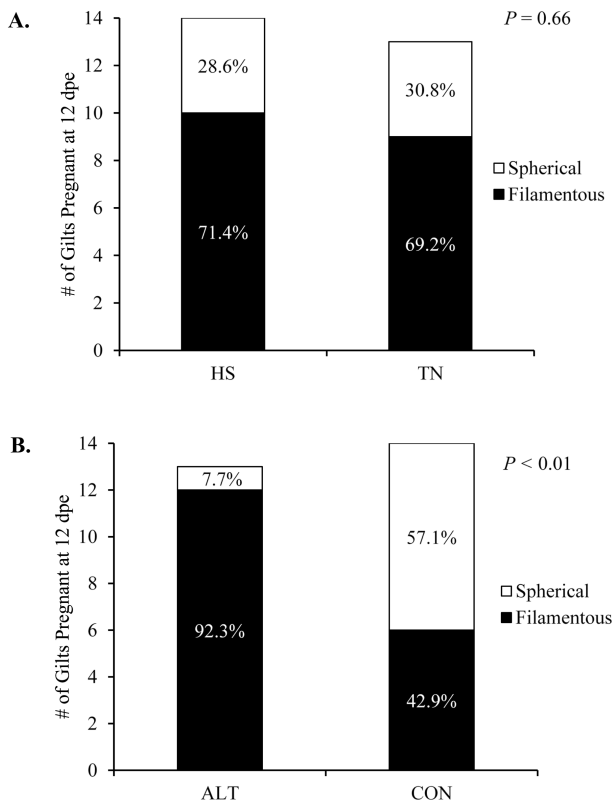


Figure 6. Effect of environmental conditions and altrenogest (ALT) treatment on conceptus development. Bred gilts were subjected to either thermal neutral (TN) conditions (20 ± 1 °C, 36% to 57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation or heat stress (HS) conditions (35 ± 1 °C for 12 h, 31 ± 6 °C for 12 h, 21% to 31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered ALT during day 3 to 12 post estrus. Conceptuses were recovered from 27 of 30 gilts inseminated. Bars represent the number of gilts under a specified environment or treatment that yielded conceptuses. The black portion indicates conceptuses filamentous in morphology, and the white portion represents gilts with spherical conceptuses at the time of flushing. (A) No effect was observed between environment on conceptus development ($P = 0.90$). (B) An increase ($P < 0.01$) in the percentage of gilts with filamentous conceptuses was observed for those treated with ALT compared with CON gilts (92.3% vs. 42.9%).

conditions increase vascular endothelial growth factor in pig granulosa cells (Basini et al., 2004). Thus, during early luteal formation, hypoxic conditions are supported to be beneficial in prompting the development of the necessary vasculature. In pigs, one mechanism of dissipating heat is to increase circulation to the periphery, creating a hypoxic environment for internal organs (Lambert et al., 2002). In the current study where gilts were subjected to an intense heat load for most of the luteal phase, it is possible that the initial internal hypoxic environment did not influence luteal formation. Further investigation is needed to determine the duration of hypoxic conditions on CL function, and if the prolonged duration of intense heat challenge affects the viability of the luteal tissue due to deprived blood flow.

Around day 12 of pregnancy, pig conceptus undergoes morphological changes, transitioning from a spherical to a filamentous conformation (Heuser and Streeter, 1929). The elongation process occurs rapidly, transitioning to the filamentous shape in a matter of hours (Geisert et al., 1982a).

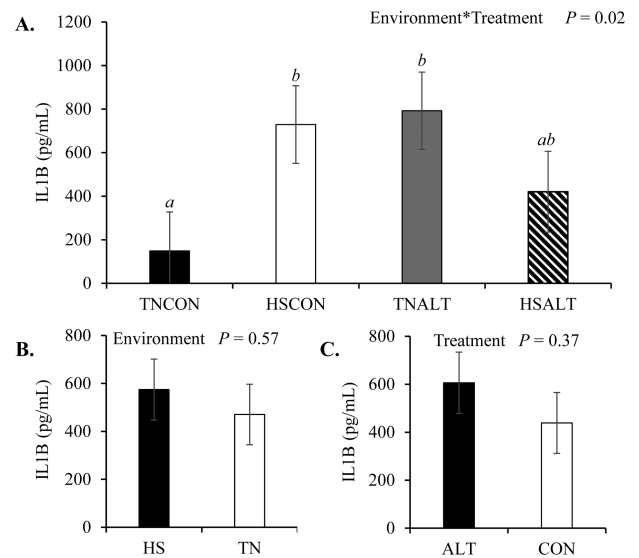


Figure 7. Effect of increased ambient temperatures and altrenogest (ALT) supplementation on conceptus interleukin-1 β (IL1B) production. Bred gilts were subjected to either thermal neutral (TN) conditions (20 ± 1 °C, 36% to 57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation or heat stress (HS) conditions (35 ± 1 °C for 12 h, 31 ± 6 °C for 12 h, 21% to 31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered ALT during day 3 to 12 post estrus. Uterine flushes were collected by injection of 20 mL sterile saline into the uterine lumen and manipulating flush volume proximally and distally to the uterine bifurcation. IL1B was measured in the uterine flush using colorimetric ELISA. Data represent IL1B concentration (pg/mL) LS means \pm SEM. (A) An increase was observed between TNCON and TNALT (148.8 vs. 728.7 pg/mL, $P = 0.02$) and between TNCON and HSCON (148.8 vs. 792.17, $P = 0.03$). (B) No difference was observed between gilts in HS or TN conditions on conceptus IL1B production ($P = 0.57$). (C) No difference was observed between gilts in ALT or CON treatment groups on conceptus IL1B production ($P = 0.37$). Differences in letters denote statistical difference ($P < 0.05$).

This process of elongation is critical for conceptus survival to ensure adequate surface area at the time of attachment. Concomitant with elongation, the conceptus increases the synthesis and release of IL1B (Ross et al., 2003) to activate numerous cell signaling pathways facilitating implantation (Ross et al., 2010; Jeong et al., 2016). The window of expression for IL1B is narrow, only being required during the short period as the conceptus completes the spherical to filamentous morphological change. To target this narrow window of conceptus elongation herein, tissues were collected at a precise time relative to estrus onset. In the uterine flushings, differences in IL1B concentration were not noted across environmental or ALT treatments. An interacting effect of environment and ALT treatment was observed, wherein TNALT and HSCON gilts had increased IL1B concentration in uterine flush compared with TNCON. We suspect that ALT could have influenced this difference as a result of increased elongation in the ALT-treated gilts compared with CON. It is worth noting, however, that since IL1B is produced exclusively by the conceptus, concentration is dependent not only on that stage of development but also on the number of conceptuses present. For gilts that yielded filamentous conceptuses, accurate distinction and count of individual conceptuses were not possible due to entanglement during the collection process. To best account for the number of conceptuses, the total conceptus weight for each pregnancy was included in

the model. Although IL1B in the uterine flush was not different across treatments, IL1B was increased in the uterine lumen containing conceptuses classified as filamentous compared with those classified as spherical, aligning with previous research results (Ross et al., 2003). Further work is warranted to examine the direct effects of HS on conceptus viability at the time of elongation. On day 16, conceptus viability was compromised by HS, evident by increased fragmentation of the trophectoderm (Wettemann et al., 1988), illustrating the sensitivity of the conceptuses to the direct effects of environment during the peri-implantation window, in which we propose that IL1B could be a valuable marker for conceptus health during this critical time of development.

A greater percentage of gilts treated with ALT yielded filamentous conceptuses compared with CON counterparts. The regulation of this trophoblastic elongation is not well defined, as it is unclear whether it is conceptus mediated or initiated through maternal signaling. It has been reported that conceptus IL1B is necessary for trophoblastic elongation to occur, where IL1B2-deleted embryos fail to elongate by day 12 (Whyte et al., 2017). These findings suggest that P₄, or in this case a P₄ analog, may play a critical role in facilitating events leading up to maternal recognition and implantation. The ability of P₄ to mediate uterine secretion was validated by Vallet et al. (1998), where exogenous P₄ on days 2 and 3 of gestation increased endometrial secretions and conceptus production of estrogen on day 11. The evidence of supplemental P₄ benefits during early pregnancy is somewhat inconclusive in the literature. Ashworth (1991) demonstrated an improvement in fetal viability when the dam was supplemented with P₄ from day 4 to 30 of pregnancy. Conversely, Mao and Foxcroft (1998) determined that conceptus survival rate decreased following exogenous P₄ treatment which began 36 h after estrus onset. The timing of P₄ supplementation appears to be critical, as an improvement in pregnancy rate when ALT was provided on day 2 to 4 (83%) compared with day 1 to 4 where pregnancy rate was reduced to 38% (Soede et al., 2012). Considering the increased rate of conceptus elongation observed in ALT-treated gilts in this study, coupled with the lack of reduction in CL weight, suggests the need for further investigation on possible luteotropic factors produced by the elongating conceptus before maternal recognition.

For pregnancy to be established, P₄ first suppresses its receptor in the uterine endometrium. This downregulation of P₄ receptor allows for conceptus-derived estrogens to interact with its receptor, ERS1, subsequently promoting the upregulation of growth factors and IL1B pathways important for trophoblastic elongation (Ka et al., 2007; Geisert et al., 2017). Considering the current knowledge of the role P₄ has during the peri-implantation window, the production of various growth factors in response to ALT acting on the uterine endometrium is a possible explanation as to why an increased number of conceptus reached the filamentous stage in the ALT-supplemented gilts. Further work is needed to fully comprehend the effect of supplemental P₄ on the gilt endometrium at day 12 of pregnancy.

Conclusion

Developing and implementing HS mitigation strategies are needed to combat the economic burden associated with seasonal infertility. HS impacts CL weight but its ability to produce P₄ during early pregnancy appeared unscathed. The

supplementation of a P₄ analog accelerated conceptus elongation and, during HS, improved CL weight compared with HSCON counterparts. These findings provide useful implications for improving conceptus viability during HS as well as possibly mitigating the negative effects of HS on CL development, both of which require further investigation to confirm application efficacy.

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Conflict of interest statement

This project was supported by the Iowa Pork Producers Association. Any opinion, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the Iowa Pork Producers Association. No conflicts of interest, financial, or otherwise are declared by the authors.

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