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Effects of Chronic Heat Stress on Ovarian Steroidogenesis Pathway Members in Gilts

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Effects of Chronic Heat Stress on Ovarian Steroidogenesis Pathway Members in Gilts

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Summary and Implications
The molecular effects of chronic heat stress on expression profiles of insulin- and phosphatidylinositol-3 kinase (PI3K)-mediated steroidogenesis signaling pathway components were evaluated in gilt ovaries. Comprehending the molecular mechanism by which heat stress compromises pig reproductive performance is of substantial relevance and such knowledge will be indispensable in developing strategies and therapeutics to mitigate these negative effects. Our results indicate that after 35 days of thermal stress, expression of PI3K signaling pathway members is significantly altered, which could impact follicle activation, affect follicle viability and potentially modify ovarian steroid synthesis, thus leading to negative impacts on fertility in swine.

Introduction
Climate change is likely to increase the frequency of extreme heat events in tropical and subtropical regions, and during the summer months in temperate regions resulting in exacerbated heat-induced economic loss in the livestock industry. In pigs, high ambient temperatures above the zone of thermoneutrality cause heat stress (HS) which negatively affects growth performance and efficient reproduction. For instance, temperatures higher than 27°C (80°F) have been associated with decreased feed conversion, increased weight loss, dysregulated estrous cycle, reduced conception rate, reduced embryonic survival, higher frequency of stillbirths leading to increased mortality rates and impaired fecundity. Despite chronic seasonal infertility and compromised sow performance during and immediately following the hot annual seasons, the biological reasons responsible for impaired fecundity are poorly understood. Additionally, for yet unknown biological reasons, HS paradoxically decreases feed intake but increases plasma insulin in a variety of animal models including pigs. It is known that insulin influences ovarian phosphatidylinositol-3 kinase (PI3K) signaling, which is important for follicle viability and for regulating follicle activation and steroidogenesis. Therefore, in order to begin understanding the molecular mechanism(s) underlying the effects of heat stress on reproductive performance in pigs, we hypothesized that heat stress results in impaired reproductive performance through aberrant expression of genes involved in insulin mediated PI3K-induced ovarian steroidogenesis.

Materials and Methods
Crossbred gilts (35±4 kg) were housed in constant climate controlled rooms in individual pens and provided with ad libitum access to feed. Intake were exposed to thermal neutral (TN) conditions (20°C; 35-50% humidity; n = 3) or HS conditions (35°C; 20-35% humidity; n = 3) for 35 d to simulate chronic HS. Gilts were euthanized, one ovary was stored at -80°C and the other ovary was fixed in 4% paraformaldehyde. Total RNA was isolated and levels of Insr, Akt1, Foxo 3a, Star, Lhr and Cyp19a mRNA were quantified by RT-PCR and normalized to either β-Actin or Gapdh as a housekeeping gene. Statistical analysis was performed using the unpaired t-test function of GraphPad Prism software.

Results and Discussion
After 35 d, HS increased (P < 0.05) Insr, Akt1 and Foxo3a mRNA expression, supporting hyper-activation of PI3K signaling. There was also elevated (P < 0.05) Star, Lhr and Cyp19a mRNA levels in HS compared to TN ovaries. The pituitary gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) drive cyclic recruitment of follicles to ovulation and play critical roles in regulating ovarian steroid production. In PI3K/Akt–regulated steroidogenesis, through increased Akt phosphorylation, LH stimulates thecal cells in antral follicles to produce androstenedione and testosterone which is converted to estrogen in the granulosa cells under the influence of FSH. Since LH promotes follicular growth, maturation and ovulation, increased Lhr mRNA could indicate higher levels of estradiol production with a concomitant reduction in follicular recruitment and higher tendencies for pseudo-pregnancies.

Taken together, these results indicate that the negative effects of chronic HS on reproduction performance in pigs could be due to malproduction of ovarian hormones that are required to promote and or sustain pregnancy. Additionally, aberrant action of PI3K signaling caused by HS could affect the oocyte viability.
Figure 1. Effect of chronic HS on ovarian Insr, Akt1 and Foxo3a mRNA expression in the ovary. Total RNA was isolated from TN or HS gilt ovaries (n = 3 per treatment) and RT-qPCR used to compare (A) Insr, (B) Akt1 and Foxo3a mRNA levels. The unpaired t-test function of GraphPad Prism software was used to compare differences in cycle numbers, relative to β-actin as a house-keeping gene. Results are presented as fold-change in HS ovaries relative to TN controls. * indicates a statistical significant difference from the control at P < 0.05.

Figure 2. Effect of HS on mRNA expression of ovarian estrogen synthesis pathway members. Total RNA was isolated from TN or HS gilt ovaries (n = 3 per treatment) and RT-qPCR used to compare Star, Lhr and Cyp19a mRNA levels. The unpaired t-test function of GraphPad Prism software was used to compare differences in cycle number, relative to Gapdh as a house-keeping gene. Results presented as fold-change in HS ovaries relative to TN controls. * indicates a statistical significant difference from the control at P < 0.05, † indicates different from control; P < 0.1.

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