RESEARCH ABSTRACT

ANIMAL SCIENCE

Title: Investigating Biological Reasons for Seasonal Infertility **– NPB #12-115** revised

Investigator: Aileen F. Keating

Institution: Department of Animal Science, Iowa State University.

Co-Investigators: Jason W. Ross and Lance H. Baumgard

Date Submitted: 11-1-13

Scientific Abstract:

Effect of heat stress on phosphatidylinositol-3 kinase and steroidogenic signaling in gilt ovaries

Heat stress (HS) negatively affects reproductive performance in swine, but the biological reasons responsible for this impaired fecundity are poorly understood. Paradoxically HS decreases feed intake but unexplainably increases plasma insulin in a variety of animal models including pigs. Insulin can influence ovarian phosphatidylinositol-3 kinase (PI3K) signaling, which is important for follicle viability and regulating follicle activation and steroidogenesis. Two downstream mediators of PI3K action are Protein kinase B subunit 1 (*AKT1*), and the forkhead transcription factor subunit 3a (*FOXO3*). This study investigated the effects of HS on PI3K and steroidogenic signaling in the porcine ovary. In addition, pilot data on the impact of HS on ovarian glucose metabolism were collected through use of a PCR array. Crossbred gilts (35±4 kg) housed in constant climate controlled rooms in individual pens with *ad libitum* feed intake were exposed to thermal neutral (TN) conditions (20°C; 35-50% humidity; n = 3-6) or HS conditions (35°C; 20-35% humidity; n = 3-6) for 7 or 35 d to simulate acute and chronic HS, respectively. Additionally, a pair fed (PF) group were included in the 7d treatment. 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 *IR*, *IRS1*, *AKT1*, *FOXO3, LDLR*, *LHR*, *STAR*, and *CYP19a* mRNA were quantified by RT-PCR. Also, a PCR array to measure glucose metabolism was performed on the 7d TN, PF and HS samples. The IR and phosphorylated AKT (pAKT) proteins were localized by immunofluorescence staining. Western blotting was used to quantify the impact of HS on pIRS1, pAKT, STAR, and CYP19a protein levels. After 7 d of HS, increased ($P < 0.05$) levels of *IR*, *IRS1*, *AKT1*, *LDLR*, *LHR*, and *CYP19a* mRNA relative to TN and PF gilt ovaries. After 35d, all genes measured were increased ($P < 0.05$) by HS. Oocyte cytoplasm and cytoplasmic membrane of all stage follicles stained positive for the IR protein, while pAKT1 protein was located in the oocyte cytoplasm of all stage follicles, with apparent greater expression in larger stage follicles. Additionally, theca and granulosa cells of pre-ovulatory follicles were positive for pAKT protein. Western blotting revealed that IR, pIRS1, pAKT1, STAR and CYP19a were increased ($P < 0.05$) by HS after 7 or 35d.

These data suggest HS leads to altered expression of PI3K signaling pathway members, which could alter dynamics of follicle activation and affect follicle viability. Additionally, thermal stress acts as an endocrine disrupting environmental exposure. These findings have identified altered ovarian signaling that could be at least partially responsible for negative impacts of HS on fertility in swine.

Data from this project were presented at the American Society of Animal Science annual meeting in Phoenix. Nteeba, J., Ullerich, E.E., Pearce, S.C., Boddicker, R., Ross, J.W., Baumgard, L.H., and Keating, A.F. 2012. Effect of heat stress on phosphatidylinositol-3 kinase signaling in gilt ovaries.