

## ANIMAL SCIENCE

**Title:** Investigating hyperinsulinemia and increased circulating lipopolysaccharide involvement in seasonal infertility - **NPB 14-230**

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**Date Submitted:** 01-25-16

### Scientific Abstract:

Heat stress (HS) during hot summer months causes seasonal infertility in swine, characterized by longer wean to estrus interval, increased numbers of rebreeds and spontaneous abortion. We previously discovered alterations to ovarian molecular signaling pathways that regulate follicle and oocyte viability as well as steroid hormone production in pre-pubertal gilts that were heat-stressed (35°C) for 7 or 35 d. We have demonstrated that heat-stressed pigs have increased systemic insulin as well as lipopolysaccharide, thus this project investigated the impacts of both of these physiological perturbations on ovarian function. Ovaries were collected from pre-pubertal gilts who underwent one of the following experimental paradigms: 1) pre-pubertal gilts (n = 6/treatment) were exposed to HS or thermoneutral (TN) conditions for 5 d, in the presence of hyperinsulinemia (0.1 U.kg/BW) 15 min prior to euthanasia; 2) pre-pubertal gilts (n = 6/treatment) were exposed to a bolus of LPS (10 µg/kg) and euthanasia performed 8 h later. Ovaries from both groups of animals were processed for total RNA or protein isolation, followed by quantitative reverse transcriptase PCR (qRT-PCR) or western blotting to measure alterations to RNA and protein abundance, respectively, due to experimental treatment (HS or LPS). Hyperinsulinemia during HS increased ( $P < 0.05$ ) mRNA encoding *LHR*, *STAR*, *CYP11A1*, *HSD3B1*, *CYP17A1* and *CYP19A1* and also increased ( $P < 0.05$ ) protein abundance of pAKT, CYP19A1 and tended ( $P = 0.07$ ) to increase CYP11A1 protein. No impact on mRNA encoding *INSR*, *IRS1*, *cKIT*, *AKT1*, *FOXO3* or *LDLR* or protein abundance of IRS1 or AKT was observed. Increased ( $P < 0.05$ ) ovarian SULT1E1 and ABCC1 protein was induced by HS, proteins that degrade 17β-estradiol were observed, a novel finding that could explain deranged estrous cyclicity during HS. A bolus of LPS induced ( $P < 0.05$ ) mRNA encoding *FOXO3*, *LDLR*, *STAR*, and *CYP11A1* but reduced ( $P < 0.05$ ) *LHR*, *CYP17A1* and *CYP19A1*. Increased ( $P < 0.05$ ) protein abundance of TLR4 and pAKT were observed. Additionally TLR4, the ligand for LPS, was demonstrated to be increased ( $P < 0.05$ ) in ovaries from pre-pubertal gilts who experienced cyclical HS for 5 d. Taken together, these data support that the ovary is responsive to increased LPS, which could partially explain altered reproduction due to HS in swine. Also, hyperinsulinemia during HS abrogated some of the previously observed ovarian changes due to HS alone, thus new avenues for investigation have been opened and these data further our biological understanding of ovarian influences culpable for seasonal infertility.

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These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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