INTRODUCTION

- Heat stress (HS) is an imbalance of thermal energy flowing in and out of an animal and is associated with seasonal infertility (SI).
- SI in swine manifests as spontaneous abortion, delayed puberty, and fewer piglets.
- SI costs the US and Iowa swine industries ~$420 million and ~$60 million annually, respectively.
- Elevated systemic insulin, despite reduced feed intake, is observed in swine experiencing HS.
- Compromised intestinal integrity is induced during HS due to hypoxia as a result of blood redirection to the periphery for heat dissipation, leading to increased lipopolysaccharide (LPS) in the blood (endotoxemia).
- Superoxide dismutase 1 (SOD1) is a marker of oxidative stress.
- We previously determined altered ovarian phosphatidylinositol-3-kinase (PI3K; Fig. 1) and steroidogenic (Fig. 2) signaling in pre-pubertal gilts undergoing HS.

![Diagram of cellular pathways active during HS.](image)

**Fig. 1.** Depiction of cellular pathways active during HS. The TLR4 pathway, activated by LPS, leads to phosphorylation of nuclear factor kappa B (pNFκB) which regulates the production of inflammatory cytokines. Insulin binds to insulin receptor (IR) which activates IRS1, leading to the PI3K which phosphorylates AKT. The TLR4 pathway can also activate the PI3K pathway, to phosphorylate AKT. Acylcoxyacyl hydrolase (ADAH) cleaves the lipid A moiety of LPS, rendering LPS unable to activate TLR4 pathway.

**Fig. 2.** Basic estradiol synthesis pathway. Cholesterol is brought into the mitochondria by steroid acyl regulatory protein (STAR) and converted to progesterone. This is the rate-limiting step to estradiol synthesis. CYP19A1 then converts testosterone to estradiol.

HYPOTHESIS

HS-induced metabolic endotoxemia alters ovarian signaling pathways and is at least partially culpable for seasonal infertility.

METHODS

Pre-pubertal gilts were used in both experiments. In the 35ºC HS study, HS gilts (35°C, 20-35% humidity, n = 3) had ad libitum feed intake while the TN group (20°C, 35-50% humidity, n = 3) were pair-fed (PF) to account for different plane of nutrition. In the LPS study, gilts (n = 6 per treatment) were jugular catheterized (Fig. 3) for blood sampling and the administration of LPS (5 µg/kg BW, Escherichia coli 055:B5). In both groups, gilts were humanely euthanized and tissues collected. All animal procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Protein was extracted from collected ovaries for western blotting. Images were quantified using ImageJ and data were analyzed in GraphPad Prism by unpaired t-tests. Statistical significance was set at P < 0.05, denoted by * and P < 0.01 denoted by **.

ACUTE LPS EXPOSURE DATA

**Fig. 4.** Relative ovarian AOAH protein expression did not differ between pre-pubertal gilts treated with LPS relative to control treated (CT) gilts.

**Fig. 5.** Relative ovarian TLR4 protein abundance is increased in pre-pubertal gilts treated with LPS compared to control treated (CT) gilts, P < 0.05.

**Fig. 6.** Acute LPS exposure did not affect ovarian IRS1 protein in pre-pubertal gilts treated with LPS relative to control (CT) gilts.

**Fig. 7.** Relative ovarian AKT protein in pre-pubertal gilts treated with LPS is unchanged compared to control treated (CT) gilts. However, pAKT protein is increased in LPS treated gilts, P < 0.05.

CONCLUSIONS

Acute LPS exposure increased TLR4 and pAKT protein abundance indicating responsiveness of the porcine ovary to endotoxemia.

Chronic HS increased SOD1, TLR4, pNFκB, and STAR protein abundance supporting induction of oxidative stress, inflammation, and altered steroidogenesis in the porcine ovary during HS.

Physiological alterations associated with HS and endotoxemia, including hyperinsulinaemia and compromised intestinal integrity, may impact steroidogenesis, activate ovarian TLR4 signaling, and thereby contribute to SI.

**Compromised Fertility = Compromised Production = Compromised Global Food Security**

REFERENCES

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