Heat Stress during Pig Oocyte In Vitro Maturation Impacts Embryonic Development and Gene Expression

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Summary and Implications

Gene expression of heat stressed oocytes matured *in vitro* was evaluated for potential markers which could be used to characterize the effects of heat stress on developing oocytes in the pig. Three heat stress scenarios were administered during *in vitro* oocyte maturation. Heat stressed oocytes had reduced maturation rates, decreased developmental competency, and altered expression of heat stress and developmental competency markers.

Introduction

Seasonal infertility associated with elevated temperatures has a detrimental impact on reproductive efficiency of sows throughout the U.S. Evidence from other in vitro studies shows heat stress may impact not only early embryo development but also oocyte development. Objective of this study was to identify molecular markers of heat stress in metaphase II (MII) oocyte and 4-cell stage embryos that could be used to characterize mitigation strategies utilized to reduce detrimental effects of elevated temperatures during oocyte maturation.

Materials and Methods

Sow ovaries were obtained from an abattoir and transported to the laboratory in a thermos maintained at 30-35°. Antral follicles (2-5mm) were aspirated and cumulus oocyte complexes (COC) were collected for maturation. During *in vitro* maturation, COCs were subjected to four environmental conditions: *in vitro* maturation at 39°C for 42 h (control), *in vitro* maturation for 42 hours at 41.0°C, (HS1); *in vitro* maturation for 21 hours at 39.0°C followed by 21 hours at 41.0°C, (HS2); *in vitro* maturation for 21 hours at 41.0°C followed by 21 hours at 39.0°C (HS3). Twenty-five MII oocytes from each treatment were collected for analysis. The remaining MII oocytes were transferred to modified Tris-buffered medium (mTBM) for fertilization (35 oocytes per 50 µL droplet). Following fertilization oocytes were washed and placed in PZM3 media for maturation. Embryos were collected for evaluation and analysis at the 60 hrs (4 to 8 cell stage) and day 6 (blastocyst). Quantitative RT-PCR was used to analyze the expression of MIR21, PDCD4 and HSP90A in oocytes and embryos response to HS.

Results and Discussion

Control and HS2 oocytes demonstrated the highest maturation rate (71.2 ± 3.7%, and 70.2 ± 0.7 %) compared to HS1 (55.1 ± 6.3 %) and HS3 (54.0 ± 6.2 %) suggesting that oocytes experiencing HS during the later stages of oocyte maturation are more tolerant of heat stress than those exposed to heat stress during early stages of oocyte maturation or for the duration of *in vitro* maturation. The percentage of MII arrested oocytes producing embryos capable of development to the four cell stage within 60 hrs was not statistically different between treatments (Control, 51.3 ± 6.2%; HS1, 38.3 ± 4.4%; HS2, 48.2 ± 6.3%; HS3, 52.0 ± 8.6%). However blastocyst formation rate was significantly affected by treatment. Control embryos produced the greatest number of blastocysts on Day 6 as a percentage of 4-cell embryos at 60 hours post fertilization (29.4 ± 4.5%) compared to other treatments (HS1, 1.6 ± 1.1%, HS2, 13.3 ± 1.0%; HS3, 21.6 ± 3.8%). HSP90A expression was down regulated in addition to MIR21 (Figure 1) in the 4-cell embryos of group HS1 while PDCD4 was significantly up regulated. These markers, particularly MIR21 and PDCD4 may be useful markers to characterize the effects of heat stress on maturing swine oocytes.

Acknowledgements

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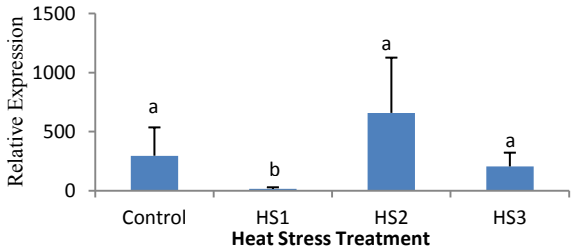

Figure 1. MIR21 expression in 4-cell embryos produced from oocytes following various heat stress conditions.