RESEARCH REPORT
ANIMAL SCIENCE

Title: Trans-generational effects of *in utero* heat stress on reproduction in the gilt and sow, **NPB15-043**

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Industry Summary:
High temperatures and humidity have been shown to have direct, detrimental effects on pregnant gilts and their developing litters. In 1968, Teague et al. determined that heat stressed females had lower ovulation rather than those housed in cooler temperatures. Similarly, Warnick et al. (1965) determined that females housed at high ambient temperatures from conception through d 25 of gestation had fewer embryos than those housed in thermoneutral environments. Late gestational heat stress has been shown to decrease the number of live pigs per litter and increase the number of still born piglets (Omtvedt et al., 1971) Heat stress during late gestation may also cause lower piglet birth weights (Omtvedt et. al, 1971). Heat stress has also been shown to affect feed intake during lactation (Quiniou and Noblet, 1999: Williams et al., 2013).

These negative effects, coupled with effects on growth and carcass composition, have been estimated to cost the swine industry one billion dollars annually (Pollman, 2010). In order to determine the full magnitude of the effect of gestational heat stress, it is important to understand if gestational heat stress can affect future generations. Black and Erickson (1968) determined that the ovary undergoes rapid development from days 30 to 60 of gestation. Therefore, by collecting reproductive tracts of pregnant gilts that have been housed in heat stress environments during gestation, we can determine whether the fetal ovary has been damaged and if gestational heat stress affects future generations.

Gestational heat stress may lead to effects on the fetuses (i.e. transgenerational changes) in the reproductive capacity of boars and gilts. The objective of this study was to assess fetal and placental development and the development of gonads in conceptuses whose mother was subjected to gestational heat stress (GHS; 28 to 38^{o} C; 65 to 88% relative humidity; n=16) or thermoneutral (GTN; 17 to 22^{o}C; 56 to 65% relative humidity; n=14) conditions during weeks 4-8 of pregnancy. High temperatures increased rectal temperature (38.5±.04 vs. 38.0±.04 ^{o}C; P<.001), skin temperature (35.5±.2 vs. 28.7±.2 ^{o}C; P<.001), and respiration rate (44.3±2.6 vs. 19.5±2.7 breaths per min; P<.001) in pregnant gilts. Surprisingly, weight of the pregnant tract (12.0±1.2 vs. 12.5±1.3 kg), number of viable conceptuses (13.8±.8 vs. 15.3±.9), number of non-viable conceptuses (.3±.2 vs. .1±.2), the number of mummies (.2±.1 vs. .3±.1), and the %survival (number of viable conceptuses/number corpora lutea; 89±4 vs. 90±5%) did not differ (P>.10) for GHS vs. GTN, respectively. Upon dissection, neither did weight of the fetus (82.3±3.6 vs. 84.9±3.8 g), placenta (155.5±14.7 vs. 170.1±15.6 g), or fetal fluid (80.4±10.0 vs. 90.4±10.6 g), (P>.10) for GHS vs. GTN, respectively. The ratio of male to female fetuses was similar (P>.10). The weight of male fetuses (86.2±3.8 vs. 86.4±4.0 g), combined

testis weight (34.2±1.4 vs. 32.8±1.5 mg), and combined testis weight as a % of fetal weight (.040±.001 vs. .038 ±.001) did not differ (P>.10) for GHS vs. GTN, respectively. The weight of female fetuses (81.2±3.6 vs. 83.5±3.8 g), combined ovarian weight (25.2±1.0 vs. 26.1±1.1 mg), and combined ovarian weight as a % of fetal weight (.031±.001 vs. .031 ±.001) did not differ (P>.10) for GHS vs. GTN (respectively). While the numerical differences between treatment groups generally were in favor of the GTN environment, lack of statistically significant difference leads to the conclusion that heat stress from wk 4 to 8 of gestation in gilts did not change the growth of the fetus, placenta, ovary or testis at mid-gestation.

This project was specifically designed to go beyond direct effects of heat stress on pregnant sows and to evaluate the effect of being heat stressed as a fetus; i.e. transgenerational effects of *in utero* heat stress. Gestational heat stress has previously been shown to impact subsequent growth and carcass composition at slaughter. Boddicker et al. (2014) found that pigs exposed to heat stress *in utero* exhibited an increase in subcutaneous fat thickness compared to pigs who were exposed to thermoneutral conditions *in utero*. Pigs heat stressed *in utero* may also have heavier hot carcass weights at slaughter compared to pigs that developed under thermoneutral conditions *in utero* (Cruzen et al. 2015). Johnson et al. (2015) also determined that gestationally heat stressed pigs experience a reduction in protein accretion rate and feed efficiency. Although research has been done to determine the transgenerational effects of *in utero* heat stress on carcass composition and growth, little work has been done on the transgenerational effects of *in utero* heat stress on the reproductive capacity of gestationally heat stressed gilts. The studies conducted by Boddicker et al. (2014), Cruzen et al. (2015), and Johnson et al. (2015) suggest that because growth traits are affected by gestational heat stress, it is possible that reproductive characteristics may also be affected in gilts heat stressed *in utero*. By further understanding how gestational heat stress affects the developing fetus, producers will have more accurate estimates on the total costs of heat stress.

Gestational heat stress may lead to transgenerational changes in the reproductive capacity of boars and gilts. The objective was to assess pregnancy development in gilts whose mothers were subjected to heat stress (GHS; n=23; 28 to 38 ^{o}C; 65 to 88% relative humidity) or thermoneutral (GTN; n=25; 17 to 22 ^{o}C; 56 to 65% relative humidity) conditions as a developing fetus (*in utero*) from wk 4 to 8 of pregnancy. Female progeny (generation 1; G1) from both GTN and GHS mothers remained on farm under commercial conditions and were artificially inseminated at second estrus. During the 8th wk of gestation, gilts (GTN-G1; n=55 and GHS-G1; n=50) were sacrificed for the collection of the reproductive tracts and fetal tissues. Data were obtained from four replicates, and replicates differed for weight of the pregnant tract (9.9±1.0 vs. 11.7±0.7 vs. 15.0±1.0 vs. 13.6±0.7 kg; P<0.003). Two possible explanations are offered for these replicate effects: different Duroc boars were used for two replicates due to a PRRS outbreak; and environmental conditions at the farm for the portion of gestation of the grand-dams could have differed as the replicates were all within one year's time. Thus effect of temperature at other points in gestation or adaptation to temperature extremes due to previous environments are possible. The weight of the pregnant tract (12.7±0.6 vs. 12.4±0.6 kg), number of viable conceptuses (12.3±0.6 vs. 12.7±0.5), and the %survival (number of viable conceptuses/number corpora lutea; 77±4 vs. 75±3%) did not differ (P>.10) for GHS-G1 and GTN-G1 (respectively). A sex-specific transgenerational effect on fetal weight was observed, because male fetuses from GHS-G1 had increased weight (129.0±4.8 vs. 119.5±4.5 g) but female fetuses were similar (117.4±4.7 vs. 115.8±4.5 g) (GHS-G1 vs. GTN-G1; Treatment by sex, P<0.012). The conclusion was that *in utero* heat stress from wk 4 to 8 of gestation had gender-specific transgenerational (first generation) effects. The fact that these effects were observed despite essentially no measurable direct effects points to the likelihood that the impact of heat stress on swine production has been underestimated.

Key Findings:

- physiological coping mechanisms to heat stress were observed (higher respiration rate and skin temperature), but were incapable of preventing the rise in rectal temperature.
- physical reproductive parameters were the same for gilts heat stressed as those housed under thermoneutral conditions.
- -GHS and GTN piglets did not differ for anogenital distance or size of fetal ovaries or testes at 60d of gestation.
- -GHS and GTN piglets taken to term also did not differ.
- -GHS and GTN gilts mated and slaughtered at d60 of pregnancy were strikingly similar.
- While many numerical differences favored the GTN gilts, very few were statistically different.
- -male fetuses of GHS gilts were heavier than GTN male fetuses, though female fetuses did not differ between treatments.

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Scientific Abstract:

Gestational heat stress may lead to transgenerational changes in the reproductive capacity of boars and gilts. The objective of this project was to assess fetal and placental development and the development of gonads in conceptuses whose mother was subjected to either gestational heat stress (GHS; 28 to 38° C; 65 to 88% relative humidity; n=12) or gestational thermoneutral (GTN; 17 to 22°C; 56 to 65% relative humidity; n=11) conditions during pregnancy or *in utero* as a developing fetus. Gilts were housed in the Brody Environmental Chambers from week (wk) 4 to 8 of pregnancy, then sacrificed during wk 8 of gestation for collection of reproductive tracts and fetal tissues, and a subset of gilts (GHS n=23; GTN n=25) were moved to the University of Missouri Swine Teaching Farm and allowed to farrow. During pregnancy, GHS gilts had greater rectal temperature (38.5±.04 vs. 38.0±.04 °C; P<.001), skin temperature (35.5±.2 vs. 28.7±.2 °C; P<.001), and respiration rate (44.3±2.6 vs. 19.5±2.7 breaths per min; P<.001) than GTN. Sow was the experimental unit for analyses of fetal development. Weight of the pregnant tract (12.0±1.2 vs. 12.5±1.3 kg), number of viable conceptuses (13.8±.8 vs. 15.3±.9), number of non-viable conceptuses (.3±.2 vs. .1±.2), number of mummies (.2±.1 vs. .3±.1), and the %survival (number of viable conceptuses/number corpora lutea; 89±4 vs. 90±5%) did not differ (P>.10) for GHS vs. GTN, respectively. Upon dissection, the weight of the fetus (82.3±3.6 vs. 84.9±3.8 g), placenta (155.5±14.7 vs. 170.1±15.6 g), fetal fluid (80.4±10.0 vs. 90.4±10.6 g), and placental efficiency (fetal weight/placental weight; 0.60±.04 vs. 0.55±.05) did not differ (P>.10) for GHS vs. GTN, respectively. The ratio of male to female fetuses was similar (P>.10) for GHS (1.3±.3) and GTN (1.6±.3). Weight of male fetuses (86.2±3.8 vs. 86.4±4.0 g), combined testis weight (34.2±1.4 vs. 32.8±1.5 mg), and combined testis weight as a % of fetal weight (.040±.001 vs. .038 ±.001) did not differ (P>.10) for GHS vs. GTN, respectively. Weight of female fetuses (81.2±3.6 vs. 83.5±3.8 g), combined ovarian weight (25.2±1.0 vs. 26.1±1.1 mg), and combined ovarian weight as a % of fetal weight (.031±.001 vs. .031 ±.001) did not differ (P>.10) for GHS vs. GTN, respectively. After treated females farrowed, it was determined that litter size (13.6±0.7 vs. 13.6±0.6), piglet birth weight (1.26±0.03 vs. 1.28±0.03 kg), and weaning weight (3.61±0.10 vs. 3.71±0.10 kg) did not differ (P>0.10) between GHS and GTN females, respectively. Female progeny (generation 1; G1) from both GTN and GHS mothers remained on farm and were AI at second estrus. During the 8th wk of gestation, gilts that came from the GTN (GTN-G1; n=55) and GHS (GHS-G1; n=50) were

sacrificed for the collection of the reproductive tracts and fetal tissues (grandprogeny). Sow was the experimental unit for analyses of fetal development. An effect of replicate between replicates 1, 2, 3, and 4 was observed for the weight of the pregnant tract (9.9±1.0 vs. 11.7±0.7 vs. 15.0±1.0 vs. 13.6±0.7 kg; P<0.003). The weight of the pregnant tract (12.7±0.6 vs. 12.4±0.6 kg), number of viable conceptuses (12.3±0.6 vs. 12.7±0.5), and the %survival (number of viable conceptuses/number corpora lutea; 77±4 vs. 75±3%) did not differ (P>.10) for GHS-G1 and GTN-G1 (respectively). A sex-specific transgenerational effect on fetal weight was observed, because male fetuses from GHS-G1 had increased weight (129.0±4.8 vs. 119.5±4.5 g) but female fetuses were similar (117.4±4.7 vs. 115.8±4.5 g) (GHS-G1 vs. GTN-G1; Treatment by sex, P<0.012). Placental weight was lesser in females vs. male (155.5±5.7 vs. 170.1±5.7 g; P<0.001) but placental efficiency (fetal weight/placental weight) did not differ between females and males (82.9±2.3 vs. 80.5±2.3; P>.10) or GHS-G1 vs. GTN-G1 (P>.10). The conclusion was that heat stress from wk 4 to 8 of gestation in gilts did not change the growth of the fetus, placenta, ovary or testis at mid-gestation, and *in utero* heat stress from wk 4 to 8 of gestation had gender-specific transgenerational (first generation) effects.

Introduction:

Seasonal infertility caused by high ambient temperatures and humidity during the summer months can have negative effects on the reproduction of female swine. These conditions may cause heat stress leading to large economic losses for swine producers. In a 2003 study (St. Pierre et al.), it was estimated that the swine industry loses approximately $299 million annually to heat stress. These losses can be attributed to both a decrease in reproductive capabilities of gilts and sows, as well as decreased growth and performance of pigs located at grow-finish facilities.

Heat stress may affect the reproductive capabilities of gilts and sows, as well as the growth and carcass composition of their offspring. Effects on the reproductive performance of female swine include delayed time to puberty (Flowers et al., 1989; Paterson et al., 1991), fewer piglets born per litter (Bloemhof et al., 2013), lower ovulation rates (d'Arce et al., 1970; Flowers et al., 1989), and lower birth weights (Omtvedt et al., 1971). Sows are extremely susceptible to heat stress during lactation, due to the high level of metabolic heat produced during lactation. Sows experiencing heat stress will decrease feed intake which may in turn lead to decreased milk production and a subsequent decrease in piglet growth (Quiniou and Noblet, 1999; Williams et al., 2013).

Developing fetuses subjected to heat stress *in utero* may also show detrimental effects of heat stress later in life. Carcasses from animals exposed to heat stress during the first half of gestation have been shown to have less lean content and greater fat content (Boddicker et al., 2014). Similarly, gestationally heat stressed pigs are slower to accrete protein than they are to accrete lipids (Johnson et al., 2015b). Gestationally heat stressed pigs are also less efficient at converting feed to lean body gain (Johnson et al., 2015). Thus, it has been demonstrated that maternal environment during gestation has the ability to affect later growth performance of market hogs, although little work to determine if maternal environment during gestation affects the reproductive ability of future generations has been done.

Currently, producers mainly work to cool sows during lactation with the goal to maintain sufficient feed intake by the lactating sow. The aim of this study was to determine if females heat stressed *in utero* experienced a decrease in reproductive ability attributed to damage of the ovary. With our results, we hope to aid producers in determining whether cooling females in gestation would be of economic benefit, thereby alleviating the effects of gestational heat stress throughout later generations of female breeding stock.

Objectives:

1) Define the consequences of gestational heat stress (GHS) during the period of fetal ovarian development (d 30 to 60 of gestation) on:
    a. Development of the uterus, placenta, fetus, and fetal reproductive tissues (ovary and uterus)
    b. Age at puberty, and reproductive performance of gilts that were subjected to GHS *in utero*
    c. Litter size of first parity sows that were subjected to GHS *in utero*

Materials & Methods:
Objective 1a):
Animals and Facilities

All animal procedures were reviewed and approved by the University of Missouri Animal Care and Use Committee. Choice Genetics F1 Landrace x Large White gilts (n=80) were synchronized using Matrix® (Merck Animal Health, De Soto, KS) at the University of Missouri Swine Research Complex (SRC). After estrus was detected, gilts were mated to an unrelated maternal line. Gilts were diagnosed pregnant at the SRC at approximately 24 days after insemination. After pregnancy diagnosis, gilts were transported to the Brody Environmental Chambers at the University of Missouri. Of the four chambers, two were used for this experiment. Each chamber measured 9.3 x 5.2 m. One chamber housed animals at heat stress (GHS) conditions. The other chamber housed animals at thermoneutral (GTN) conditions. Gilts were housed in stalls (2.4 x 0.6m) during gestation. The front portion of each stall's floor was solid and the back portion was grated metal that allowed for fecal material and urine to fall into the gutter below. Each stall contained an individual nipple waterer.

Experimental Design

Once gilts were moved to the Brody Environmental Chambers at approximately 24 days of gestation, they were allotted to either the heat stress (GHS) (n=39) or thermoneutral (GTN) (n=39) chamber based on body weight (GHS vs. GTN; 152.9±2.8 vs. 152.0±3.0 kg; $P<0.83$) and relatedness. Ambient temperatures of the chambers remained the same if GTN (17 to 22°C; RH 56 to 65%) or were increased to GHS (28 to 38°C; RH 65 to 88%) conditions over a period of five days. The HS chambers reached maximal cyclical HS at day 30 of gestation and remained at maximum temperature until gilt removal. Gilts were housed in the environmental chambers until day 60 of gestation when they were weighed and sent to the University of Missouri Meat Lab for slaughter (GHS n=16; GTN n=14) or transported to the University of Missouri Swine Teaching Farm for use in Experiment Two. Once gilts were slaughtered, the pregnant reproductive tracts were collected and taken to the University of Missouri Animal Science Research Center for dissection and further data collection.

Thermal Measurements

The response to the thermal environment was measured at 0700 and 1600 h daily. Respiration rate was measured by counting breaths per minute, skin temperature was measured on the shoulder using a Raynger ST infrared gun (Raytek, Santa Cruz, CA), and rectal temperature was measured using a Thermistor rectal thermometer (Cole Parmer North America, Vernon Hills, IL).

Feeding of Gilts

Gilts were fed a standard corn-soybean meal gestation diet at 0615 h using rubber feed tubs. Gilts were fed 2.2 kg of feed and were given 30 minutes to eat. Any refused feed was measured and recorded at 0645 h. Gilts were given a 15 minute period to rest before thermal response data was collected.

Slaughter Data Collection Procedure

Gilts were removed from the Brody Environmental Chambers at 60±3 d of gestation and were weighed using a livestock scale (Mosdal Scale Systems, Lanesboro, MN). Gilts were then transported to the University of Missouri Meat Lab where they were killed by electrocution and exsanguination. Reproductive tracts were removed and placed in plastic bags labeled with gilt ID and time of slaughter. Immediately following, tracts were placed on ice and transported to the University of Missouri Animal Sciences Research Center for further dissection and data collection.

Upon arrival, the entire reproductive tract was weighed and recorded. Ovaries were then removed and weighed. The number of CL were counted and the diameter of five follicles were measured. Ovaries were then placed in liquid nitrogen for further analyses of gene expression. After removal of the ovaries, the broad

ligament was dissected from the uterine horns and the length of each uterine horn was measured. The right uterine horn was labeled as horn 1, and the left uterine horn was labeled as horn 2.

Each uterine horn was opened, and the contents were exposed. The number of viable, non-viable, and mummified conceptuses was counted. Fetuses were labeled as 1-1, 1-2, 1-3,… if located on horn 1 (right horn) or 2-1, 2-2, 2-3, … if located on horn 2 (left horn). Counting started at the outside end of each horn and worked inward towards the uterine body. The entire conceptus (fetus, placenta, and fluid) was removed and weighed. The fluid was then drained from the placenta, and the fetus and placenta were weighed separately. Small samples (< 1 g) of two placentas were taken (one from each horn), combined, and frozen in liquid nitrogen. Fetuses were sexed and crown-rump length was measured. Anogenital distance was also measured. Male and female fetuses were later dissected using a dissecting microscope. The fetal testes and ovaries were removed and weighed (Denver Instrument, Denver, CO). The fetal testes and ovaries were then placed in 10% buffered formalin phosphate (Fisher Scientific, Fair Lawn, NJ).

Once the uterus was empty, the vascular implantation site lengths were measured for each fetus, and the empty uterus was weighed. Each implantation site was then cut out, spread on heavy paper, and traced in order to measure the area of placental attachment. Placental area was then analyzed using the tracing function of the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Analysis of Fetal Testes and Ovaries

Fetal testes and ovaries were placed in tissue processing cassettes (Fisher Scientific, St. Louis, MO). The cassettes were sent to the University of Missouri School of Veterinary Medicine where the ovaries and testes were fixed on microscope slides.

Fetal testes analysis. Pictures were taken of the fixed fetal testes at 40x magnification using a Leica DM400 B microscope (Leica Microsystems, Wetzlar, Germany). Pictures of the fetal testes were then analyzed using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Two seminiferous tubules per animal were analyzed. The number of sertoli cells and germ cells were counted on both tubules. The tracing function of the public domain NIH Image program was used to measure the area of each tubule.

Fetal ovarian analysis. Pictures were taken of the fixed fetal ovaries at 40x magnification using a Leica DM400 B microscope (Leica Microsystems, Wetzlar, Germany). Pictures of the fetal ovaries were then analyzed using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Diagonal lines were drawn from the top-left to the bottom-right corner and from the top-right to the bottom-left corner using the straight line selection tool. The number of germ cells touching the two lines was counted to estimate the number of germ cells present in the fetal ovary.

Statistical Analysis

Data were analyzed using the MIXED procedure (PROC MIXED) of SAS (SAS Institute Inc., Cary, NC). The experiment was conducted in four groups of gilts that were exposed to one of two treatments (GTN and GHS). Data with one measurement per gilt were analyzed with a model that included main effects of treatment, group, and treatment by group interaction. Data with multiple measurements per gilt were analyzed with a model that included main effects of treatment, group, fetal sex, treatment by group interaction, and treatment by fetal sex interaction. Data means are expressed as least square means ± SEM. Means were considered significant at $P < 0.05$.

Objective 1b):

Animals and Facilities

Animals and facilities of GHS and GTN females. All animal procedures were reviewed and approved by the University of Missouri Animal Care and Use Committee. Choice Genetics F1 Landrace x Large White gilts (n=80) were synchronized using Matrix® (Merck Animal Health, De Soto, KS) at the University of Missouri Swine Research Complex (SRC). After estrus was detected, gilts were mated to an unrelated maternal line. Gilts

were diagnosed pregnant at the SRC at approximately 24 days after insemination. After pregnancy diagnosis, gilts were transported to the Brody Environmental Chambers at the University of Missouri. Of the four chambers, two were used for this experiment. Each chamber measured 9.3 x 5.2 m. One chamber housed animals at heat stress (GHS) conditions. The other chamber housed animals at thermoneutral (GTN) conditions. Gilts were housed in stalls (2.4 x 0.6m) during gestation. The front portion of each stall's floor was solid and the back portion was grated metal that allowed for fecal material and urine to fall into the gutter below. Each stall contained an individual nipple waterer. At 60 days of gestation, gilts were transported to the University of Missouri Teaching Farm where they were housed in environmentally controlled gestation rooms, until moving to farrowing crates prior to parturition. Gilts remained in the farrowing room until weaning at 21 days post-parturition.

Animals and facilities of GHS-G1 and GTN-G1 females. At weaning, female offspring (GHS-G1; GTN-G1) were moved to the nursery where they remained until movement into the finishing room. Prior to the initiation of heat checking, GHS-G1 and GTN-G1 females were moved from the finishing room to the modified open front (MOF) building at the University of Missouri Teaching Farm where they stayed for the remainder of the study.

Experimental Design

Environmental chambers experimental design. Once F0 generation gilts were moved to the Brody Environmental Chambers from the Swine Research Center (Columbia, MO) at approximately 24 days of gestation, they were allotted to either the heat stress (HS) (n=39) or thermoneutral (TN) (n=39) chamber based on body weight (GHS vs. GTN; 152.9±2.8 vs. 152.0±3.0 kg; $P<0.83$) and relatedness. Ambient temperatures of the chambers remained the same if TN (17 to 22°C; RH 56 to 65%) or were increased to HS (28 to 38°C; RH 65 to 88%) conditions over a period of five days. The HS chambers reached maximal cyclical HS at day 30 of gestation and remained there until gilt removal. Gilts were housed in the environmental chambers until day 60 of gestation when they were sent to the University of Missouri Swine Teaching Farm for farrowing at approximately day 114 of gestation.

Farm trial experimental design. Pregnant gilts (GHS n=23; GTN n=25) were housed in an environmentally controlled gestation facility until movement into farrowing crates prior to parturition. After eight gilts had farrowed naturally, the remaining four gilts were induced with an injection of Lutalyse® (Zoetis, Parsippany, NJ) followed by an injection of Oxytocin 24 hours later in order to keep offspring ages in a tight range.

Farrowing Data Collection Procedure

Farrowings were attended, and at birth, each piglet was caught, dried, and weighed. Each piglet was given an ear tag and was subsequently placed back with its dam to nurse. Gestation length, the number of live born, the number of stillborn, the number of mummies, and the number of weaned pigs were also recorded. At three days of age, piglets had their tails docked, teeth clipped, ears notched for identification, and iron administered to prevent anemia. GHS-G1 and GTN-G1 males were castrated and the testes were sent to the University of Missouri Animal Science Research Center. The neonatal testes were weighed and placed in 10% buffered formalin phosphate (Fisher Scientific, Fair Lawn, NJ). GHS-G1 and GTN-G1 piglets were further weighed at processing, one week, two weeks, and at weaning and weights were recorded. At weaning, GHS-G1 and GTN-G1 females were moved to the nursery where they remained until being moved into the finishing room for further growth. Around 160 days of age, GHS-G1 and GTN-G1 gilts were moved to the modified open front (MOF) building where they stayed for the remainder of the study.

Feeding of Gilts

Feeding in the farrowing room. Sows were fed a standard corn-soybean meal gestation diet. Sows were given *ad libitum* access to feed in order to maximize feed intake. Access to water was *ad libitum*, as well. Feed intake was not recorded.

Feeding of GHS-G1 and GTN-G1 gilts during gestation. Gilts were floor fed a standard corn-soybean meal gestation diet before boar exposure. Gilts were fed 2.2 kg of feed per head in pens of eight G1-gilts per pen. Feed intake was not recorded.

Heat Check and Breeding Data Collection

Heat check procedure. At 160 days of age, GHS-G1 and GTN-G1 females began exposure to heat check boars for 10 minutes daily. Gilt behavior was observed and vulva scores were recorded. Gilts were scored on a 3-point scale. Females that exhibited no signs of heat were assigned a "0" score. Those that showed some signs of heat (i.e. swollen vulva, increased interest in the boar, vulvar discharge) were assigned a "1" score. Females that were believed to be close to standing heat were assigned a "2" score. When females exhibited standing heat they were assigned a "3" score. Females in groups one and two were exposed to the boar for 58 and 86 days, respectively. Females in groups three and four were both exposed to the boar for 60 days. All groups underwent heat detection for extended lengths of time, because few females reached puberty during the allotted 30 days.
Blood sample collection procedure. Blood samples were taken on days 58 and 65 of heat checking from GHS-G1 and GTN-G1 females in group one (GHS-G1 n=3; GTN-G1 n=1) that did not reach puberty by 58 days after the onset of heat checking. Blood samples were taken from females in group two (GHS-G1 n=5; GTN-G1 n=3) on days 86 and 93 days of heat checking that did not reach puberty by day 86 of heat checking. Groups three (GHS-G1 n=15; GTN-G1 n=18) and four (GHS-G1 n=18; GTN-G1 n=23) GHS-G1 and GTN-G1gilts that had not reached puberty 30 days after the onset of heat checking had blood samples taken at 30 and 37 days of heat checking. Blood samples were collected from the jugular vein. Blood samples were spun 24 hours after their collection in order to collect serum for analysis (progesterone assay) to confirm lack of ovulation.
Breeding procedure. Gilts that reached puberty were mated on their first post-pubertal estrus after having reached 210 days of age. GHS-G1 and GTN-G1 gilts were artificially inseminated at detection of standing estrus and 24 hours later using commercial Duroc semen (International Boar Semen, Eldora, IA). Pregnancy was diagnosed by ultrasound approximately 24 days post-insemination.

Slaughter Data Collection Procedure

Gilts were removed from the University of Missouri's Swine Teaching Farm at 60±3 d of gestation and were weighed using a livestock scale. Gilts were then transported to the University of Missouri Meat Lab where they were killed by electrocution and exsanguination (GHS-G1 n=50; GTN-G1 n=55). Reproductive tracts were removed and placed in plastic bags labeled with gilt ID and time of slaughter. Immediately following, tracts were placed on ice and transported to the University of Missouri Animal Sciences Research Center for further dissection and data collection.

Upon arrival, the entire reproductive tract was weighed and recorded. Ovaries were then removed and weighed. The number of CL were counted and the diameter of five follicles were measured. After removal of the ovaries, the broad ligament was dissected from the uterine horns and the length of each uterine horn was measured. The right uterine horn was labeled as horn 1, and the left uterine horn was labeled as horn 2.

Each uterine horn was opened and the contents were exposed. The number of viable, non-viable, and mummified conceptuses was counted. Fetuses were labeled as 1-1, 1-2, 1-3,… if located on horn 1 (right horn) or 2-1, 2-2, 2-3, … if located on horn 2 (left horn). Counting started at the outside end of each horn and worked inward towards the uterine body. The entire conceptus (fetus, placenta, and fluid) was removed and weighed. The fluid was then drained from the placenta, and the fetus and placenta were weighed separately. Fetuses were sexed and crown-rump length was measured. Anogenital distance was also measured.

Once the uterus was empty, the vascular implantation site lengths were measured for each fetus, and the empty uterus was weighed. Each implantation site was then cut out, spread on heavy paper, and traced in order to measure the area of placental attachment. Placental area was then analyzed using the tracing function of the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Analysis of Neonatal Testes

Neonatal testes were placed in tissue processing cassettes (Fisher Scientific, St. Louis, MO). The cassettes were sent to the University of Missouri School of Veterinary Medicine where the testes were fixed on microscope slides. Pictures were taken of the fixed neonatal testes at 40x magnification using a Leica DM400 B microscope (Leica Microsystems, Wetzlar, Germany). Pictures of the neonatal testes were then analyzed using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Two seminiferous tubules per animal were analyzed. The number of sertoli cells and germ cells were counted on both tubules. The tracing function of the public domain NIH Image program was used to measure the area of each tubule.

Statistical Analysis

Data were analyzed using the MIXED procedure (PROC MIXED) of SAS (SAS Institute Inc., Cary, NC). The experiment was conducted in four groups of first generation gilts that were exposed to one of two treatments *in utero* (GTN and GHS). Data with one measurement per gilt were analyzed with a model that included main effects of treatment, group, and treatment by group interaction. Data with multiple measurements per gilt were analyzed with a model that included main effects of treatment, group, fetal sex, treatment by group interaction, and treatment by fetal sex interaction. Data means are expressed as least square means ± SEM. Means were considered significant at $P < 0.05$, and gilts with progesterone concentrations of 0.5 ng/mL or greater were determined to have ovulated (Magness and Ford, 1983).

Results: Report your research results by objective.

Temperature data are presented as evidence that 'heat stress' was achieved in the experiment. Gilts subjected to heat stress from days 30 to 60 of gestation had greater rectal temperature (38.5±.04 vs. 38.0±.04 ^{o}C; P<0.001) compared with GTN. GHS gilts had greater skin temperature (35.5±.2 vs. 28.7±.2 ^{o}C; P<.001) than GTN. Heat stressed gilts also had greater respiration rate (44.3±2.6 vs. 19.5±2.7 breaths per min; P<.001) compared with GTN.

Objective 1a: Slaughter Data Results

Effects of heat stress on uterine and ovarian measures of the gilt. Total uterine weight (12.40±0.92 vs.13.38±1.00 kg), empty uterine weight (3.06±0.18 vs. 3.42±0.20 kg), and uterine length (345.25±20.06 vs. 377.75±21.66 cm) did not differ (P>0.10) for GHS vs. GTN (respectively). Ovarian weight (17.21±1.22 vs. 17.35±1.31 g), the number of corpora lutea (CL) (15.38±0.73 vs. 17.15±0.79), and CL weight (1.15±0.06 vs. 1.00±0.07 g) did not differ (P>0.10) for GHS vs. GTN (respectively).

Effects of heat stress on litter and placental measures of the gilt. No treatment differences were detected (P>0.10) between GHS and GTN for total number of fetuses per litter (14.4±0.8 vs. 16.3±0.8), number of viable fetuses per litter (13.8±0.7 vs. 15.3±0.8), number of nonviable fetuses per litter (0.3±0.7 vs. 0.4±0.2), and the number of mummies per litter (0.3±0.2 vs. 0.6±0.2). Survival (number of fetuses/number of CL) also did not differ (P>0.10) between GHS (91.6±3.7%) and GTN (89.1±4.0%) (respectively). Implantation length was similar (P>0.10) between GHS (19.52±1.08) and GTN (20.81±1.10) (respectively). The combined weight of the fetus, placenta, and placental fluids (334.24±20.26 vs. 378.64±20.56 g) did not differ (P>0.10) for GHS vs. GTN (respectively). Similarly, the weight of the placenta (154.2±13.4 vs. 180.1±13.5 g) and fluid (82.46±8.51 vs. 100.68±8.68 g) also did not differ (P>0.10) for GHS vs. GTN (respectively). Placental efficiency (fetal weight/placental weight) was similar (P>0.10) between GHS (69.93±4.55) and GTN (62.71±4.60) (respectively).

Fetal weight did not differ (P>0.10) between GHS (97.80±3.87 g) and GTN (102.08±3.91 g) (respectively). The weight of male fetuses (100.49±4.08 vs. 106.91±4.02 g) and female fetuses (96.24±4.37 vs.

96.36±4.49 g) also did not differ (P>0.10) for GHS vs. GTN (respectively). Similarly, fetal length (15.43±0.21 vs. 15.17±0.21 cm) was similar (P>0.10) for GHS vs. GTN (respectively).
Effects of heat stress on fetal ovarian and testes development. Fetal ovarian weight (27.819±0.867 vs. 26.721±0.949 mg) and fetal testes weight (37.419±1.770 vs. 36.994±1.717 mg) did not differ (P>0.10) for GHS vs. GTN (respectively). Ovarian weight as a percentage of body weight (2.99±0.09 vs. 2.94±0.10) and testes weight as a percentage of body weight (3.82±0.13 vs. 3.60±0.12) were similar (P>0.10) for GHS vs. GTN (respectively). Female anogenital distance (2.461±0.190 vs. 2.187±0.198 mm) did not differ (P>0.10) for GHS vs. GTN (respectively). Similarly, male anogenital distance (31.803±0.508 vs. 32.241±0.490 mm) also did not differ (P>0.10) for GHS vs. GTN (respectively).

Discussion

In summary, gilts housed in heat stress conditions exhibited a physiological response to their environment. Heat stressed animals had increased respiration rates, skin temperatures, and rectal temperatures. This increase in respiration rate, skin temperature, and rectal temperature indicate a failed attempt of the gilt to cool itself during periods of high temperature and humidity, further indicating that the treatment conditions elicited a heat stress response. Overall, no other treatment effects were found between litters developed under heat stressed conditions or thermoneutral conditions. Therefore, we conclude that gestational heat stress from weeks four to eight of gestation had no significant effect on the pregnancy of the dam.

Objective 1b) and c):
Farrowing Data Results

Litter size did not differ (P>0.10) between GHS (13.6±0.7) and GTN (13.6±0.6) females that were housed in the Brody Environmental Chambers at the University of Missouri from days 30-60 of gestation. Birth weight (1.26±0.03 vs. 1.28±0.03 kg) was not affected by treatment (P>0.10) for piglets born to GHS or GTN dams, although male piglets (1.28±0.02 kg) tended to be heavier (P<0.09) than female piglets (1.25±0.02 kg). Similarly, day three piglet body weights (1.58±0.04 vs. 1.59±0.04 kg) did not differ (P>0.10), while male piglets (1.60±0.03 kg) tended (P<0.10) to weigh more than female piglets (1.57±0.03 kg). Week one piglet body weights (2.29±0.06 vs. 2.30±0.06 kg), week two piglet body weights (3.61±0.10 vs. 3.71±0.10 kg), and week three piglet body weights (weaning weight) (5.37±0.16 vs. 5.63±0.15 kg) did not differ (P>0.10) for GHS vs. GTN. Female piglet anogenital distance (3.14±0.69 vs. 3.03±0.66 mm) did not differ (P>0.10) for offspring from GHS vs. GTN dams, and male piglet anogenital distance (95.66±0.68 vs. 94.26±0.65 mm) also did not differ (P>0.10) for offspring from GHS vs. GTN dams.

Slaughter Data Results

Effects of in utero heat stress on uterine and ovarian measures of G1 females. No effect of treatment was observed for total uterine weight, weight of uterine contents, or uterine length, although an effect of replicate was observed for these measures. Ovarian weight did not differ (P>0.10) between GHS-G1 (17.08±0.48) and GTN-G1(16.77±0.44) females (respectively). The number of CL (16.29±0.45 vs. 17.06±0.39) and the number of follicles (34.58±1.60 vs. 35.32±1.31) did not differ for GHS-G1 vs. GTN-G1, but an effect of replicate was observed for both CL and follicle number.

Effects of in utero heat stress on litter and placental measures of GHS-G1 and GTN-G1 gilts. The number of viable fetuses (12.3±0.6 vs. 12.7±0.5) and the number of nonviable fetuses (0.3±0.1 vs. 0.2±0.1) were similar (P>0.10) between GHS-G1 and GTN-G1 females (respectively). An effect of replicate was observed for the number of viable fetuses and the number of nonviable fetuses. Survival (number of fetuses/number of CL) (76.8±3.7 vs. 74.5±3.3) did not differ (P>0.10) for GHS-G1 vs. GTN-G1, but an effect of replicate was observed.

Implantation length was similar (P>0.10) between GHS-G1 (21.46±0.74 cm) and GTN-G1 (21.12±0.70 cm) (respectively). An effect of replicate was observed for implantation length. Combined fluid, placental, and fetal weight did not differ (P>0.10) between GHS-G1 (386.05±15.46 g) and GTN-G1 (370.44±14.67 g), but a

replicate and sex effect was observed. Placental weight (168.3±8.0 vs. 157.3±7.6 g) also did not differ (P>0.10) for GHS-G1 vs. GTN-G1 females (respectively), although replicate and sex effects were observed. Male GHS-G1 fetuses tended (P<0.09) to have heavier placentas (178.3±8.4 g) than male GTN-G1 fetuses (161.9±7.8 g), female GHS-G1 fetuses (158.3±8.3), and female GTN-G1 fetuses (152.8±7.9) (respectively). Placental fluid weight was similar (P>0.10) between GHS-G1 (94.09±6.29 g) and GTN-G1 (96.65±5.89 g) (respectively). Placental fluid weight differed between replicates. Placental efficiency (fetal weight/placental weight) did not differ (P>0.10) for GHS-G1 (81.1±3.1) vs. GTN-G1 (82.3±2.9) (respectively). Placental efficiency was observed to differ between replicates.

Fetal weight was similar (P>0.10) between GHS-G1 (123.21±4.62 g) and GTN-G1 (117.64±4.39 g) (respectively), although an effect of replicate was observed. Overall, male fetuses (124.24±3.27 g) were larger (P<0.001) than female fetuses (116.62±3.28 g), and male GHS-G1 fetuses (129.00±4.77 g) were larger (P<0.012) than male GTN-G1 fetuses (119.47±4.47 g), female GHS-G1 fetuses (117.42±4.74), and female GTN-G1 fetuses (115.82±4.52 g) (respectively). Similarly, male fetuses (16.49±0.14 cm) were longer (P<0.001) than female fetuses (16.26±0.14 cm), while male GHS-G1fetuses (16.72±0.21 cm) were longer (P<0.018) than male GTN-G1fetuses (16.25±0.19 cm), female GHS-G1 fetuses (16.34±0.20 cm), and female GTN-G1 fetuses (16.18±0.19 cm) (respectively). GHS-G1 (16.53±0.20 cm) and GTN-G1 (16.22±0.19 cm) did not differ (P>0.10) in length (respectively). GHS-G1 females (49.8) tended (P<0.09) to have a lower percent of male fetuses than GTN-G1 females (54.5) (respectively).

Progesterone Analysis Results

When progesterone was assayed, it was found that all group 1 females from which blood samples were collected (GHS-G1 n=0/3; GTN-G1 n=0/1) did not ovulate within 65 days of the onset of heat checking. Conversely, heat was not detected on farm in eight group 2 females, but three of the eight females were found to have ovulated by progesterone analysis (GHS-G1 n=2/5; GTN-G1 n=1/3). All group 3 females from which blood samples were collected (GHS-G1 n=0/15; GTN-G1 n=0/18) did not ovulate within 30 days of the onset of heat checking. Heat was not detected on farm in 41 group 4 females, but 18 of the 41 females were found to have ovulated after progesterone analysis (GHS-G1 n=6/18; GTN-G1 n=12/23). Pubertal age did not differ between treatments.

Discussion:

Most of the data on impacts of heat stress on pregnant sows measures the direct impact on the sow. This work was unique in that piglets born to dams kept in either heat stress or thermoneutral conditions (GHS-G1; GTN-G1) were kept on farm in order to determine if *in utero* heat stress affected ovarian development and reproductive development. The lack of significant effects of *in utero* treatment on litter, placental, ovarian, fetal, and uterine characteristics after slaughter data collection were surprising. This lack of significance can be potentially explained by a variety of factors. One possible explanation may be an inability to heat stress dams to a level at which the developing pregnancy was affected. It is also possible that there was no effect of heat stress from days 30-60 of gestation on litter, placental, ovarian, fetal, and uterine characteristics.

Although significant differences between GHS-G1 and GTN-G1 pregnancies were not detected, many effects of replicate were observed. Replicate effects were observed for such characteristics as total uterine weight, weight of uterine contents, uterine length, number of CL, and number of follicles. The number of viable fetuses, number of non-viable fetuses, survival (number fetuses/number CL), implantation length, combined placental, fluid, and fetal weight, placental weight, placental fluid, placental efficiency, and fetal weight also showed effects of replicate. These replicate effects may be attributed to one of two protocol deviations: differing sires and the temperature during gestation for GHS-G1 and GTN-G1 females. All GHS-G1 and GTN-G1 females were bred with IBS commercial Duroc semen. The genetics used to breed GHS-G1 and GTN-G1 females changed halfway through the study due to a PRRS outbreak at the original boar stud used. Therefore, it is possible that the replicate effects observed in this study can be attributed to genetic differences transmitted via sire. Similarly, these replicate effects may be caused by exposure to differing environmental temperatures during gestation. GHS-G1 and GTN-G1 females were housed in a naturally ventilated building that lacked the

capacity to compltely cool or warm the building based on environmental conditions. As such, GHS-G1 and GTN-G1 females that were bred and pregnant during replicates 1 and 2 were exposed to high heat and humidity (June-September). Conversely, GHS-G1 and GTN-G1 females that were pregnant during replicates 3 and 4 were exposed to cooler conditions (October-January). In a sense, these females may have experienced naturally occurring heat stress or thermoneutral conditions.

Fetal weight, placental weight, and combined placental, fluid, and fetal weight measurements all exhibited effects of sex during this study. For example, male fetuses were significantly heavier than female fetuses. Similarly, male placentas tended to weigh more than female placentas. This observation is given credence by the observation that male piglets are heavier at birth, as well. Therefore, it can be concluded that this difference in weight can be traced back to mid-gestation, as well. The difference in combined fetal, placental, and fluid weight can be attributed to singular differences in fetal and placental weight, because fluid weight did not differ for male and female fetuses.

Although overall male fetuses were heavier than female fetuses, male fetuses whose dam had been heat stressed *in utero* were heavier than males from *in utero* thermoneutral dams and heavier than all females (HS and TN). This specific transgenerational effect on male fetal weight suggests damage to the ovary of gilts exposed to heat stress *in utero*. Rance et al. (1997) determined that a quantitative trait locus (QTL) for body weight is located on the X-chromosome. Therefore, body weight can be described as an X-link trait, whereby, any mutation of the X-chromosome and the body weight QTL may manifest as a difference in male body weight, compared to female body weight. Males have a higher probability to phenotypically express an X-linked mutation, because males are hemizygous for the X-chromosome. Females may not show a phenotypic change due to the homozygous nature of their chromosomal structure. This homozygosity for the X-chromosome allows the F_1 female to compensate for any damage that may have occurred to the maternal X-chromosome during development of the F_0 ovary *in utero*. Conversely, the hemizygosity for the X-chromosome in the F_1 male, or the lack of a secondary, paternal X-chromosome, causes the male to phenotypically express chromosomal changes that may have occurred to the maternal X-chromosome during development of the F_0 ovary *in utero*.

Once GHS-G1 and GTN-G1 females had been exposed to a heat check boar daily with no detection of heat, blood samples were collected in order to determine progesterone concentration and ovulatory status. Females in groups 1 and 2 were exposed to the heat check boar for longer than groups 3 and 4 because few females had expressed standing heat by 30 days post boar exposure. This inability to detect heat may have been due to the extreme heat and humidity, human error, or the expression of silent heat. After analyzing the blood samples of females in groups 1 and 2, it is likely that the hot conditions caused females to not cycle, because few samples contained high levels of progesterone. Many blood samples from group 4 contained high levels of progesterone; therefore, it is more likely that GHS-G1 and GTN-G1 gilts experienced silent heat or the individuals detecting heat did not observe standing estrus when it occurred.

In summary, direct effects of gestational heat stress on litter size and piglet weight at farrowing were not detected. Similarly, no effects of GHS were detected on subsequent piglet growth. GHS-G1 and GTN-G1 females showed no differences on litter characteristics such as uterine weight, uterine horn length, number CL, or number of follicles at 60 days of gestation. Conversely, many replicate effects were observed on such parameters as the number of viable fetuses, number of non-viable fetuses, survival, fetal weight and implantation length. These effects may be attributed to either a difference in sire genetics or environmental temperature during gestation. Male and female fetal weight also differed significantly, in that male fetuses were heavier than female fetuses. This discovery corresponds with the finding that female piglets typically weigh less than male piglets at birth. Male GHS-G1 fetuses weighed more than male GTN-G1 and all female fetuses (GHS-G1 and GTN-G1). The previous conclusion by Rance et al. (1997) that a quantitative trait locus (QTL) for body weight is located on the X-chromosome may lead us to conclude that the ovary was affected at a molecular level during gestational development and that body weight may be an X-linked trait that is more frequently expressed in males than females. In order to understand the mechanism behind the sex-specific transgenerational effects detected, further investigation is needed.

The results and conclusions from this study suggest a quantifiable effect of *in utero* heat stress on male offspring development. Overall, all other measures of reproductive performance were similar between treatments. Although not significantly different between treatments, GHS-G1 females had 0.4 fewer fetuses per litter compared to GTN-G1 females. On large commercial farms a loss of 0.4 pigs per litter may be a cause of large monetary loss during the summer months. Furthermore, male fetuses developing within dams that were heat stressed *in utero* were heavier than all other fetuses. Although this does not suggest a negative effect of gestational heat stress, it is likely that these offspring will grow similarly to other pigs within the same litter, thereby negating part of the potential benefit of heavier piglets at birth. It is also possible that the female ovary was affected in other ways that were not quantified during this study. Therefore, cooling females during gestation may be of value on farms farrowing large quantities of pigs throughout the hot summer months.

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