

**TRANSGENERATIONAL EFFECTS OF *IN UTERO* HEAT STRESS ON  
REPRODUCTION IN PIGS**

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Master of Science

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by  
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REPRODUCTION IN PIGS

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## **DEDICATION**

This thesis is dedicated to the late Leo Bernhard, my grandfather, who took a chance on raising pigs many years ago. Your passion for pigs was infectious, and I would never have discovered this amazing industry if it wasn't for you. I hope to make you proud one day, Grandpa.

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# TRANSGENERATIONAL EFFECTS OF *IN UTERO* HEAT STRESS ON REPRODUCTION IN PIGS

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## 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 either subjected to gestational heat stress (GHS; 28 to 38°C; 65 to 88% relative humidity; n=16) or gestational thermoneutral (GTN; 17 to 22°C; 56 to 65% relative humidity; n=14) conditions during pregnancy or *in utero* as a developing fetus. Gilts were housed in the Brody Environmental Chambers from weeks 4 to 8 of pregnancy before sacrifice during the 8<sup>th</sup> week of gestation for the collection of the 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 to farrow at approximately day 114 of gestation. During pregnancy, GHS gilts had greater rectal temperature (38.5±0.04 vs. 38.0±0.04 °C; P<0.001), skin temperature (35.5±0.2 vs. 28.7±0.2 °C; P<0.001), and respiration rate (44.3±2.6 vs. 19.5±2.7 breaths per min; P<0.001) compared with GTN. Sow was the experimental unit for analyses of fetal development. The weight of the pregnant tract (12.0±1.2 vs. 12.5±1.3 kg), number of viable conceptuses (13.8±0.8 vs. 15.3±0.9), the

number of non-viable conceptuses ( $0.3 \pm 0.2$  vs.  $0.1 \pm 0.2$ ), the number of mummies ( $0.2 \pm 0.1$  vs.  $0.3 \pm 0.1$ ), and the % survival (number of viable conceptuses/number corpora lutea;  $89 \pm 4$  vs.  $90 \pm 5\%$ ) did not differ ( $P > 0.10$ ) for GHS vs. GTN. Upon dissection, the weight of the fetus ( $82.3 \pm 3.6$  vs.  $84.9 \pm 3.8$  g), placenta ( $155.5 \pm 14.7$  vs.  $170.1 \pm 15.6$  g), fetal fluid ( $80.4 \pm 10.0$  vs.  $90.4 \pm 10.6$  g), and placental efficiency (fetal weight/placental weight;  $0.60 \pm 0.04$  vs.  $0.55 \pm 0.05$ ) did not differ ( $P > 0.10$ ) for GHS vs. GTN. The ratio of male to female fetuses was similar ( $P > 0.10$ ) for GHS ( $1.3 \pm 0.3$ ) and GTN ( $1.6 \pm 0.3$ ). The weight of male fetuses ( $86.2 \pm 3.8$  vs.  $86.4 \pm 4.0$  g), combined testis weight ( $34.2 \pm 1.4$  vs.  $32.8 \pm 1.5$  mg), and combined testis weight as a % of fetal weight ( $0.040 \pm 0.001$  vs.  $0.038 \pm 0.001$ ) did not differ ( $P > 0.10$ ) for GHS vs. GTN. The weight of female fetuses ( $81.2 \pm 3.6$  vs.  $83.5 \pm 3.8$  g), combined ovarian weight ( $25.2 \pm 1.0$  vs.  $26.1 \pm 1.1$  mg), and combined ovarian weight as a % of fetal weight ( $0.031 \pm 0.001$  vs.  $0.031 \pm 0.001$ ) did not differ ( $P > 0.10$ ) for GHS vs. GTN. After treated females farrowed, it was determined that litter size ( $13.6 \pm 0.7$  vs.  $13.6 \pm 0.6$ ), piglet birth weight ( $1.26 \pm 0.03$  vs.  $1.28 \pm 0.03$  kg), and weaning weight ( $3.61 \pm 0.10$  vs.  $3.71 \pm 0.10$  kg) did not differ ( $P > 0.10$ ) between GHS and GTN females. Female progeny (generation 1; G1) from both GTN and GHS mothers remained on farm and were artificially inseminated at second estrus. During the 8<sup>th</sup> week of gestation, gilts that came from GTN (GTN-G1; n=55) and GHS (GHS-G1; n=50) dams were sacrificed for the collection of the reproductive tracts and fetal tissues. 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 ( $9.9 \pm 1.0$  vs.  $11.7 \pm 0.7$  vs.  $15.0 \pm 1.0$  vs.  $13.6 \pm 0.7$  kg;  $P < 0.003$ ), respectively. The weight of the pregnant tract ( $12.7 \pm 0.6$  vs.  $12.4 \pm 0.6$  kg), number of viable conceptuses ( $12.3 \pm 0.6$  vs.

12.7±0.5), and the %survival (number of viable conceptuses/number corpora lutea; 77±4 vs. 75±3%) did not differ (P>0.10) for GHS-G1 and GTN-G1. 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. males (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>0.10) or GHS-G1 vs. GTN-G1. The conclusion was that heat stress from weeks 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 weeks 4 to 8 of gestation had gender-specific transgenerational (first generation) effects.

## CHAPTER 1

### 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., 2015b). 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 these results, producers will be better equipped to make decisions about the economic value of cooling females during gestation.

## CHAPTER 2

### LITERATURE REVIEW

#### Introduction

It is well established that heat stress directly affects reproductive traits in female pigs, but it is unclear if heat stress can cause transgenerational effects on reproduction. The greatest detriments to reproductive ability are observed when pregnant female pigs are heat stressed during the first 30 days of gestation and late in gestation. This chapter will review literature on reproductive traits of gilts and sows and the effects heat stress has been shown to have on reproduction and fetal development.

#### **Basic aspects of female swine reproduction and reproductive management**

##### The estrous cycle of the gilt

The pig is categorized as a polytocous animal that ovulates multiple follicles during the estrous cycle. The estrous cycle of the gilt ranges from 18-24 days, with the average cycle lasting a total of 21 days (Soede et al., 2011). Most female pigs exhibit their first estrous cycle between 180-220 days of age. A gilt's first estrous cycle can be influenced by factors such as body condition and exposure to an intact male (Soede et al., 2011). The estrous cycle can be broken down into the follicular phase and the luteal phase. The period of sexual receptivity, referred to as standing estrus, follows the release of oocytes. The estrous cycle is regulated by reproductive hormones released from the

hypothalamus, pituitary, ovaries, and uterus (Soede et al., 2011). These hormones work together to control the follicular phase, luteal phase, and standing estrus.

#### Hormonal control of the estrous cycle

Once a gilt reaches puberty, she will exhibit sexual receptivity and ovulate her first cohort of follicles. Follicular growth and ovulation are controlled by a complex relationship between follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol ( $E_2$ ), progesterone ( $P_4$ ), and inhibin (Senger, 2012).

*Estrus: the period of sexual receptivity.* Day 0 of the estrous cycle is defined as the time at which a sow or gilt is sexually receptive to the male (Senger, 2012). During this period of receptivity, the female will stand for the male in order for him to initiate copulation. This estrus behavior is induced in part by a surge of  $E_2$  released prior to ovulation (Senger, 2012). A series of experiments was performed in 1970 that led researchers to conclude that sexual receptivity is induced by interactions between both sensory and hormonal signals (Signoret). This period of sexual receptivity may last anywhere from 24 to 96 hours (Soede and Kemp, 1997). During this period of sexual receptivity, the female will ovulate anywhere from 10 hours to 85 hours after the onset of estrus (Soede and Kemp, 1997). Ovulation itself may last for a duration of approximately 1-3 hours under normal conditions (Soede et al., 1992).

*Prevalent hormones during follicular growth.* During the period of corpora luteal dominance, follicles range from 3-4mm in diameter (Hazeleger et al., 2005). In order to reach ovulatory status, these follicles must continue to grow. Follicular growth begins following luteolysis or lactation (Soede et al., 2011; Senger, 2012). Prior to luteolysis, corpora lutea (CL) steadily secrete  $P_4$ . During the period of high  $P_4$ ,  $P_4$  acts as a negative

control to block gonadotropin release. Therefore, once the CL regress, P<sub>4</sub> drops rapidly, allowing for the release of LH and FSH from the anterior pituitary. Follicles are enclosed by layers of both granulosa and theca cells. These two cell layers work together to synthesize E<sub>2</sub> by what is known as the two-cell-two-gonadotropin theory (Evans et al., 1981). Theca interna cells express LH receptors that, when activated by the binding of LH, create androgens. These androgens then diffuse into the granulosa cell layer. When FSH binds to its receptors on the granulosa cell, the androgens are then made into E<sub>2</sub> via aromatization (Hansel and Convey, 1983). As this process continues, more and more E<sub>2</sub> is produced until a threshold is reached and LH surges. This LH surge causes the ovulation of a group of follicles. The selection of these ovulated follicles occurs between days 14-16 of the estrous cycle (Morbeck et al., 1992). These follicles will have grown to a diameter of greater than 4mm and gained the competence to respond to the LH surge via an increase in LH receptors (Knox, 2005). During this time, follicles also secrete inhibin, which acts as a negative feedback on FSH production.

*Hormones of the luteal phase.* This period of high E<sub>2</sub> and inhibin prior to ovulation leads to a low reserve of antral follicles after the LH surge. Conversely, after ovulation E<sub>2</sub> and inhibin begin to decrease, allowing for another wave of follicular development. These developing follicles again begin to secrete inhibin driving FSH down. During this time, CL are developing and secreting P<sub>4</sub>. Progesterone reaches peak concentration between days 8 to 9 of the estrous cycle. High levels of P<sub>4</sub> block the secretion of gonadotropins and keep follicles from further development until luteolysis (Soede et al., 2011).

### Reproduction on farm

Gilts reach puberty between six to seven months of age, on average. Producers will begin boar exposure around this time until a gilt exhibits her first standing estrus. Females are not bred at their first heat, but rather will be bred, either naturally or artificially inseminated, at the second heat. Females are inseminated in gestation stalls that are 0.61 meters wide and 2.13 meters long (Estienne and Harper, 2010). These crates allow the producer easy access to the animals and allow for greater welfare for each individual gilt. The top ten pork producers in the USA housed 90% of their female breeding stock in gestation crates as of 2005 (USDA). It is expected that this number has decreased since the compilation of the 2005 data because of the political campaign aimed at the removal of gestation crates from farms.

Before a gilt is due to farrow, she will be moved to a farrowing crate where she will deliver her piglets. The average length of gestation is 114 days, approximately. She will nurse these piglets for around 21 days until they are weaned and put into a nursery. Sows will be moved from farrowing crates into a breeding facility where they will either be sent to the cull market or be re-bred after returning to heat around four to five days after weaning.

### **Development of the pig *in utero***

During embryonic and fetal development, many structures and organ systems develop from three primary cell layers (germ layers) known as the endoderm, the mesoderm, and the ectoderm (Patten, 1948). The endoderm gives rise to such structures as the liver and parts of the digestive and respiratory tracts (Marrable, 1971). The oviducts, uterus, heart, muscle, and bones all originate from the mesoderm layer. Such

structures as the spinal cord and brain develop from the ectoderm (Patten, 1948; Marrable, 1971). The gestation period for the developing pig can be divided into the embryonic (12 to 36 days) and fetal periods (36 to 114 days) (Marrable, 1971).

#### Development prior to the embryonic stage

After fertilization of the female oocyte, the developing zygote will undergo progressive cleavage and cavitation until the commencement of its rapid elongation on day 10 of pregnancy. This elongation will continue until day 12. Blastocysts elongating around days 10 and 11 will increase in diameter at a rate of 0.25 mm/h (Geisert et al., 1982a). During this time the conceptus will grow from 3mm to 10mm. Once these blastocysts have developed to a diameter of 10mm, they will begin to elongate into a filamentous form. To attain this filamentous form, the blastocysts will elongate at a rate of 30-45 mm/h (Geisert et al., 1982a). Porcine conceptuses will elongate to a length of approximately 800 to 1000 mm (Bazer and Johnson, 2014).

Around this time of elongation, the estrogen secreted by the trophoctoderm of the conceptus will act as the pregnancy recognition signal in pigs. The trophoctoderm must secrete the estrogenic signal for pregnancy recognition between days 11 and 15 of pregnancy (Bazer, 2013). During the female pig's estrous cycle, she will experience luteal regression around day 15 if she is not pregnant (Bazer et al., 1984). Therefore, the timing of the release of trophoctodermal estrogen to signal pregnancy is of great importance. Estrogen secretion during pregnancy from the trophoctoderm cells acts to redirect the flow of prostaglandin  $F_{2\alpha}$  toward the uterine lumen (Bazer et al., 1984). This redirection blocks prostaglandin  $F_{2\alpha}$  from reaching the corpus luteum and acting as a luteolytic factor. Once the prostaglandin  $F_{2\alpha}$  is secreted into the uterine lumen, it will be

metabolized to prevent luteolysis (Spencer et al., 2004). If estrogen is not released by the developing conceptus, prostaglandin  $F_{2\alpha}$  will continue to flow towards the uterine venous drainage and cause regression of the corpora lutea. If the CL is degraded, the secretion of progesterone will cease, causing a loss of pregnancy (Bazer et al., 1982). Progesterone concentrations must stay high in order to induce the endometrium to secrete factors necessary for the development of the conceptus and implantation (Ziecik et al., 2011).

#### Development during the embryonic stage

By 14 days of gestation, the embryonic neural tube has further enlarged itself into a primitive brain, and small openings on either side of the head begin to develop that will later become part of the inner ear (Marrable, 1971). Around this same time, a tubular heart starts to develop that will begin to contract at approximately 15 days of gestation. The coelom, or the cavity that separates the two mesodermal layers, also develops at this time. This cavity will later be divided into the pericardial, pleural, and peritoneal cavities (Marrable, 1971).

By day 18 of gestation, many of the biological systems that the piglet will depend upon for a healthy life have begun to develop. For example, the neural tube is swollen into the form of a primitive brain and continues to form into what will become the spinal cord. Several nerves have also begun their formation by this time (Marrable, 1971). At 18 days of gestation, the liver has begun to form and is present in the form of a bud. Although the lungs have not developed by this time, there are small outgrowths present that will later become the lungs (Marrable, 1971). Blood is delivered to the embryo via the developing system of arteries and veins. The heart continues to grow in relation to

embryonic weight until between 19 and 24 days of gestation. By day 26, the heart begins to decline in weight as a percentage of the total body weight (Lowrey, 1911).

Around 28 days of gestation, the systems that were present at day 18 have continued to develop. Both the upper and lower jaws are distinguishable at this time and the digestive tract has further developed in which the duodenum, ileum, cecum, colon, and rectum are now easily identifiable (Marrable, 1971). During this time, underdeveloped skeletal elements have developed and bones such as the skull, vertebrae, and limbs are present in the form of cartilage that will later develop into mature bone (Marrable, 1971).

#### Development during the fetal stage

Growth during the fetal stage of development can be split into two types of growth: allometric and isometric. The porcine fetus developing from days 36 to 55 of gestation is classified as growing allometrically. Allometric growth is defined as growth that occurs disproportionately throughout the body. As the fetus develops from 55 to 114 days of gestation, or until birth, it grows isometrically. Isometric growth occurs when an organism grows proportionally.

By 55 days of gestation, many of the organs and structures of the body have developed to their final shapes, although they still require further growth. For example, the eyes, ears, and snout are in their final location and are shaped as they will be at birth, although tissue differentiation will continue until further into development (Marrable, 1971). The stomach is also in its mature shape by this time. The circulatory system is highly similar to that of the neonate, but some differences exist that allow the fetal pig to exist *in utero* (Marrable, 1971). The musculoskeletal system has reached a point in

development in which the bones of the limbs, skull, and ribs have begun to ossify, and muscles of the limbs, neck, and trunk have developed in relation to their final location near the bones they support (Marrable, 1971).

Fifty-five days through gestation marks the point at which the developing fetus has the appearance of a neonatal pig. From 55 days onward, the fetal pig grows primarily in an isometric pattern. Both upper and lower teeth in the fetal pig erupt from the gums between days 70 and 80 of gestation, and the pig develops hair by 90 days. This hair is covered by an epidermal layer that will dissipate by farrowing (Marrable, 1971).

#### Sexual differentiation in the developing porcine fetus

Fetal sex is determined at conception, but the development of the gonad does not begin until around 18 days of gestation. Although the gonad is not clearly identifiable at this time, a thickening of the epithelium on the mesonephros indicates the site of each developing gonad, whether it be testes or ovaries. Large cells present in the gut and yolk sac can be identified as primitive germ cells (Marrable, 1971). A swelling also appears that will later become the external genitalia (Marrable, 1971).

Before 28 days of gestation, the developing pig is considered sexually indifferent, due to the inability to detect obvious sexual differences between males and females (Marrable, 1971). Although the immature ovaries and testes are indifferent when observed by the naked eye, they can be differentiated with the use of microscopy (Marrable, 1971). Primordial mammary glands can be identified by day 26, but are present in both male and female fetuses (Marrable, 1971). Therefore, these small mammary glands cannot be used to determine the sex of the developing fetus.

At approximately 41 days of gestation, the mesonephric tubules degenerate in female fetuses and are incorporated into the reproductive system of the developing male. The male mesonephric ducts are later called the vas deferens. These tubules will work to carry sperm throughout the reproductive tract as the animal ages and becomes sexually mature. During the days between days 36 to 55 of gestation, the external genitalia also form in the porcine fetus, allowing for the ability to determine fetal sex without the use of microscopy (Marrable, 1971). By 80 days of gestation, the testes have moved to the edge of the abdominal cavity close to the groin. Before farrowing, the testes will later migrate to the scrotal swellings between the legs (Marrable, 1971).

#### Ovarian development

A reserve of primordial follicles is established *in utero* during fetal development of the female pig (Hansel and Convey, 1983). At 18 days *post coitum*, primordial germ cells have already begun to migrate to the future location of the germinal ridge. This germinal ridge, or underdeveloped gonad, is not established until approximately 24 to 25 days *post coitum* (Black and Erickson, 1968). By 30 to 40 days, a large majority of the cellular population within the developing ovary consists of oogonia, with a few germ cells in the first stage of meiotic prophase around 40 days (Black and Erickson, 1968). Oocytes of the prenatal ovary begin to reach the diplotene stage, at which point they will rest and stay until further development at puberty (Black and Erickson, 1968). The number of germ cells present in the porcine fetal ovary peaks at 50 days around 1,100,000 germ cells. Cellular mitosis also peaks at 50 days post insemination and experiences a subsequent decline in germ cell numbers following this peak (Black and Erickson, 1968). After birth, the ovary continues to develop oocytes until around 35 days

of age (Fulka et al., 1972). During this period of continued oogenesis and up until 10 weeks of age, the ovary also continues to increase linearly in weight. After 10 weeks of age, the growth of the ovary is correlated with continued follicular growth until a decrease in follicular number at puberty, at which point ovarian size remains constant (Dyck and Swierstra, 1983).

### **Effects of heat stress on female pigs**

A multitude of trials have been conducted to study the effects of gestational and lactational heat stress on female pigs. These studies have indicated that sows and gilts kept under heat stress conditions when in a phase of reproduction are at greater risk for negative effects on a variety of litter characteristics.

#### Physiological responses to heat stress

When a pregnant gilt or sow is heat stressed, she will react both behaviorally and physiologically in an attempt to cool herself. Pigs are unable to successfully overcome severe thermal stress due to a decreased capacity to evaporatively cool themselves due to an inability to sweat (Bloemhof et al., 2013; Boddicker et al., 2014). A 1978 study recorded the rectal temperature and respiratory rates of pregnant sows and gilts (Kelley and Curtis, 1978). They observed that females housed in rooms with high ambient temperatures exhibited significantly higher rectal temperatures and respiration rates. An increase in respiration rate demonstrates the animal's attempt to elicit heat exchange via the water vapor found in the lungs of the animal (Willmer et al., 2000). Animals exposed to high ambient temperatures may also exhibit an increase in skin temperature. This is due to a shunting of blood which acts to move the heated blood to the surface where it can be cooled via conduction or convection (Willmer et al., 2000). Another mechanism

large animals will use in order to cool themselves during periods of high ambient temperatures is decreasing feed intake (Heitman and Hughes, 1949). This decrease in feed intake will lower the amount of metabolic heat produced by the animal. Heat stressed animals also increase their water intake (Flowers et al., 1989). Brown-Brandl et al. (1998) conducted a study in which they sought to understand how acute heat stress affects heat production and respiration rate in pigs. Their results confirmed the results obtained by Kelley and Curtis in 1978. It was found that acutely heat stressed pigs undergo a period of acclimation to the higher temperatures until a threshold is met at which they can no longer successfully acclimate, and therefore, their respiration rates increase in an attempt to dissipate heat (Brown-Brandl et al., 1998).

#### The effects of heat stress on breeding characteristics

Several studies have been published regarding the effects of heat stress on reproduction during the period prior to and at breeding. Heat stress during this time can cause such problems as a delay in puberty and a lower conception rate. The results of studies showing the effects of heat stress on ovulation rate are conflicting.

*Effect of heat stress and photoperiod on time to puberty and estrous cycle length.* Flowers et al. (1989) conducted a study in which gilts at 140 days of age were assigned to one of two treatments: control (15.6°C, 35% RH) or heat stress conditions (33.3°C, 35% RH). Estrus detection was initiated at 180 d of age with the use of a mature boar. Termination of estrus detection occurred at 230 d of age. Puberty was defined as the presence of elevated progesterone (> 2.0 ng/ml) for two consecutive weeks and the exhibition of a standing response. Flowers et al. determined that fewer heat stressed gilts reached puberty compared to those kept under thermoneutral conditions (C=18/20; HS=4/20).

Heat stressed gilts that did reach puberty displayed a decreased ovulation rate at puberty ( $C=12.1\pm 2.5$ ;  $HS=9.3\pm 5.1$ ). Gilts housed in HS conditions ( $213.3\pm 12.1$  d) also reached puberty at an older age than those kept in control conditions ( $204.5\pm 6.1$  d) (Flowers et al., 1989).

A study conducted in 1991 also reported differences in the percentage of females that reached puberty during either winter or summer months (Paterson et al.). This study housed gilts in a grower shed in pens of 7 to 9 pigs in either winter (short-day) or summer months (long-day). A subset of these females was exposed to a boar starting at approximately  $164.8\pm 0.18$  d of age until 225 d of age in order to detect estrus and stimulate puberty. It was determined that only  $74.0\pm 3.37\%$  of gilts exposed to the boar during cold winter months reached puberty while  $89.4\pm 4.13\%$  of gilts exposed to the boar during hot summer months reached puberty (Paterson et al., 1991). Therefore, photoperiod, along with ambient temperature, may also affect an animal's ability to attain puberty.

Edwards et al. (1968) also demonstrated that gilts exposed to high ambient temperatures may exhibit longer estrous cycles. Gilts confined in chambers with high ambient temperatures ( $38.9^{\circ}\text{C}$ ) for 17 hours daily experienced longer estrous cycles ( $22.0\pm 0.43$  d) than the same gilts that cycled before their confinement in heat stress conditions ( $20.0\pm 0.41$  d); whereas, females kept in thermoneutral ( $23.4^{\circ}\text{C}$ ) conditions before ( $21.4\pm 0.41$  d) and during ( $21.8\pm 0.43$  d) the treatment period showed no difference in cycle length. In contrast, females kept at dry-bulb temperatures of  $33.3^{\circ}\text{C}$  and dew-point temperatures of either  $15.6^{\circ}\text{C}$  or  $28.9^{\circ}\text{C}$  showed no difference in estrous cycle lengths before and after treatment exposure (d'Arce et al., 1970).

*Effect of heat stress on conception rate and ovulation.* In 1965, Warnick et al. began a two-year trial studying the effects of temperature on early embryo survival in gilts. Researchers housed gilts in rooms kept at either 32.2°C or 15.6°C for up to 3 days post-breeding or from 3 to 25 days post-breeding. When conception rates were analyzed, Warnick et al. determined that females housed at 32.2°C continuously through 25 d post-breeding averaged 10.9 embryos, while gilts housed at 15.6°C for the same period of time averaged 13.5 embryos per gilt (Warnick et al., 1965). Ovulation rates between animals kept at 32.2°C and 15.6°C did not differ significantly, although gilts kept in high ambient temperatures were found to have 14.9 CL versus 15.5 CL in those kept in a cooler environment (Warnick et al., 1965).

Flowers et al. (1989) also observed that heat-stressed gilts (33.3°C, 35% RH) had ovulation rates of 2.8 fewer CL than those kept in control conditions (15.6°C, 35% RH; 9.3±5.1; 12.1±2.5 CL). Gilts kept for longer periods of time in high ambient temperatures have lower ovulation rates than those exposed to high ambient temperatures for shorter lengths of time, as well (d'Arce et al., 1970). Higher dew-point temperatures can also affect the ovulation rate of gilts. Teague et al. (1968) determined when gilts were kept at dry-bulb temperatures of 33.3°C and dew-point temperatures of 28.9°C the average number of CL was 12.3±0.5 compared to gilts kept at dry-bulb temperatures of 26.7°C and dew-point temperatures of 11.1°C that averaged 14±0.5 CL. Although insignificant, gilts housed in low temperature and low humidity environments averaged 1.1 embryos per gilt more than females housed in high temperature and high humidity environments (Teague et al., 1968).

## The effects of heat stress during gestation and farrowing

As well as causing such problems as a decrease in ovulation rate, conception rate, and a delay in puberty, heat stress occurring during gestation can cause further issues for swine producers. Females exposed to heat stress during gestation may experience a change in fetal and piglet birth weights, litter sizes, and the rates of females returning to estrus.

*Effect of heat stress on return to estrus.* In a study conducted by Omtvedt et al. (1971), pregnant gilts were subjected to either heat stress conditions (37.8°C for 17hr; 32.2°C for 7hr) or thermoneutral conditions (23.3°C) during early, mid, and late gestation. Fourteen gilts were allotted to each treatment during the following stages of gestation: pre-implantation (0-8 d), implantation (8-16 d), mid-pregnancy (53-61 d), and late pregnancy (102-110 d). Six of the 14 gilts heat stressed from days 0-8 post-breeding returned to estrus, and three of the 14 gilts housed under heat stress conditions from days 8-16 post-breeding returned to estrus 17-21 days post-breeding (Omtvedt et al., 1971). All females kept under control conditions during this same time remained pregnant. Edwards et al. reported similar findings in which six of the 22 gilts exposed to high ambient temperatures (38.9C for 17hr; 32.2C for 7hr) from days 1-15 post-breeding did not successfully conceive, whereas only one gilt of the 19 housed in a cool environment from days 1-30 post-breeding did not conceive (Edwards et al., 1968). Teague et al. also reported that as dry-bulb temperature increased, the proportion of gilts that were pregnant decreased (Teague et al., 1968).

*Effect of heat stress on litter size and fetal/piglet birth weight.* Heat stress during late gestation (102 to 110 d) has been shown to cause a decrease in the number of live pigs

farrowed per litter ( $6.0 \pm 0.76$  vs.  $10.4 \pm 0.76$ ) and increase the number of stillborn piglets per litter ( $5.2 \pm 0.62$  vs.  $0.4 \pm 0.62$ ) (Omtvedt et al., 1971). These same piglets whose mothers were heat stressed from days 102 to 110 of gestation tended to have lower birth weights ( $1.2 \pm 0.05$  kg) compared to those whose mothers were kept in control conditions ( $1.4 \pm 0.05$ ) (Omtvedt et al., 1971). Sows heat stressed late in gestation also weaned fewer piglets at 21-days ( $4.3 \pm 0.43$  vs.  $9.2 \pm 0.43$ ) (Omtvedt et al., 1971). Bloemhof et al. analyzed reproduction records against meteorological data from multiple farms in Spain and Portugal. When total number of piglets per litter was analyzed, it was determined that as the maximum temperature on the day of successful insemination increased, the total number of piglets born decreased (Bloemhof et al., 2013).

In contrast, Williams et al. found no difference in litter size or piglet birth weight between gilts kept in heat stress or thermoneutral conditions during gestation. Gilts heat stressed during the last 20 days of gestation and kept in thermoneutral conditions during farrowing/lactation had  $11.6 \pm 0.7$  piglets per litter, and those housed in thermoneutral conditions prior to farrowing had  $11.5 \pm 0.7$  piglets per litter (Williams et al., 2013). Piglets whose mothers were heat stressed during the last 20 days of gestation had similar birth weights ( $1.40 \pm 0.11$  kg) to those who developed in thermoneutral conditions ( $1.47 \pm 0.13$  kg) (Williams et al., 2013).

#### The effects of heat stress during lactation

Many of the negative effects associated with heat stress during lactation are caused by a decrease in feed intake. Multiple studies have demonstrated a decrease in feed intake in sows exposed to high ambient temperatures during lactation. Feed intake decreased from 5,666 g/d to 3,079 g/d when ambient temperatures were increased from

18 to 29°C during lactation in a study conducted by Quiniou and Noblet (1999). Williams et al. also found a significant decrease in feed intake in heat stressed sows during lactation compared to those in thermoneutral conditions during this same period (2013). Lactating sows must meet high energy demands to maintain their own body condition, as well as in order to produce enough milk to nurse a litter of fast-growing piglets (Black et al., 1993). It is thought that the inability to intake enough energy through the diet will cause body condition loss and a subsequent decrease in milk production.

*Effect of heat stress on milk production and piglet growth.* In order to study the response of lactating sows to increasing ambient temperatures, lactating females were housed at temperatures of 18°C, 22°C, 25°C, 27°C, or 29°C for 21 d post-farrowing (Quiniou and Noblet, 1999). As previously stated, females housed in cool conditions ate significantly more than females housed in high ambient temperatures (5,666 g/d vs. 3,079 g/d). Females housed at temperatures of 29°C experienced a significant increase in body weight loss (-36 kg) that can be attributed to a decrease in feed intake (Quiniou and Noblet, 1999). Results from this study suggested that as ambient temperature increased, suckling frequency increased (40x/d vs. 26x/d) (Quiniou and Noblet, 1999). This suggests that as milk production decreases, piglets will attempt to suckle more frequently to meet their nutritional needs. At weaning, piglets whose mothers were heat stressed during lactation experienced a lower average daily gain than those whose mothers were thermoneutrally housed (189 g/d vs. 241 g/d) (Quiniou and Noblet, 1999).

Williams et al. also showed a 20% decrease in feed intake for those animals heat stressed during lactation (2013). They also reported a decrease in feed intake for females of all treatments 4 to 7 days post-farrowing. This 20% decrease in feed intake did not

translate to a significant decrease in estimated milk production. Although there was no estimated difference in milk production, piglets whose mothers were kept in heat stress conditions during lactation were 0.5 kg lighter than piglets whose mothers were kept in thermoneutral environments (Williams et al., 2013).

#### Effect of *in utero* heat stress on carcass composition and growth

Gestational heat stress has also been shown to impact growth and carcass composition at slaughter. Boddicker et al. (2014) obtained offspring at weaning from primiparous crossbred gilts that had been housed in the Brody Environmental Chambers at the University of Missouri under either heat stress (28 to 34°C) or thermoneutral (18 to 22°C) conditions. Dams were kept in either heat stress or thermoneutral conditions for the entire gestation period or switched treatments at mid-gestation (TNHS or HSTN). At slaughter (19 weeks of age), animals that were exposed to *in utero* heat stress during the first half of gestation tended to have smaller longissimus dorsi areas than those animals exposed to thermoneutral conditions *in utero* (Boddicker et al., 2014). Along with smaller longissimus dorsi areas, pigs exposed to *in utero* heat stress during the first half of gestation had greater subcutaneous fat thickness than those exposed to thermoneutral conditions *in utero* (Boddicker et al., 2014). This increase in subcutaneous fat thickness and decrease in longissimus dorsi area were accompanied by higher levels of circulating insulin in those animals heat stressed *in utero* (Boddicker et al., 2014).

In opposition to the study by Boddicker et al. (2014), Cruzen et al. (2015) found barrows that had been exposed to heat stress during the first half of gestation tended to have greater loin eye area at 19 weeks of age. The data also suggested that there was no difference in subcutaneous back fat thickness between barrows heat stressed *in utero*

versus animals kept in thermoneutral conditions *in utero* (Cruzen et al., 2015). Although no significant differences were detected between treatments for loin eye area and subcutaneous fat depth, hot carcass weight and percent head, bone, and skin differed between treatments. Cruzen et al. (2015) determined that barrows exposed to *in utero* heat stress during the first half of gestation tended to have heavier hot carcass weights at slaughter compared to those that were thermoneutral during gestation. For example, head weight as a percentage of body weight was reduced in barrows that had been heat stressed *in utero* (Cruzen et al., 2015). Johnson et al. (2015a) also found a reduction in head weight of pigs heat stressed *in utero*. Similarly, barrows heat stressed *in utero* during the second half of gestation also displayed a reduction in percent carcass bone (Cruzen et al., 2015). Also, skin as a percentage of body weight tended to be lower for barrows subjected to heat stress *in utero* (Cruzen et al., 2015). Along with differences in carcass weight and composition, Johnson et al. (2015a) determined that pigs heat stressed *in utero* had a reduction in liver weight and tended to have a reduction in total viscera weight, as well.

Along with differences in carcass bone composition, pigs exposed to *in utero* heat stress also exhibited changes to protein accretion. For example, gestationally heat stressed pigs exhibit a reduction in protein accretion rate, as well as a reduction in whole body protein content (Johnson et al., 2015b). Similarly, the rate of lipid accretion tended to be increased in *in utero* heat stress pigs, and the ratio of lipid to protein accretion was shown to be increased in gestationally heat stressed pigs (Johnson et al., 2015b). These same gestationally heat stressed pigs had a reduction in overall feed efficiency (Johnson et al., 2015b).

## **Effects of nutritional and mixing stress on reproduction**

### Effect of nutritional status on reproduction

Various studies have been conducted attempting to understand the effect of differing nutritional status on such reproductive characteristics as ovulation rate, conception rate, litter size, and survival percentage. The data from these studies conflict, but do show that either overfeeding or underfeeding pregnant gilts or sows can cause a change in the developing litter of piglets. For instance, when pregnant gilts were fed high-energy diets of 8.1 Mcal of ME/day at a level of 1.95 kg/d from days 3 to 30 of gestation, females had more embryos and a higher percentage of embryo survival than those fed 1.60 kg/d of a diet containing 5.4 Mcal of ME/day ( $11.2 \pm 0.4$  vs.  $9.6 \pm 0.4$ ;  $83.7 \pm 2.7$  vs.  $71.3 \pm 2.7\%$ ) (Liao and Veum, 1994). Animals fed one of these treatment diets (8.1 Mcal vs. 5.4 Mcal) from days 3 to 30 of gestation had no difference in the number of CL per gilt ( $13.5 \pm 0.6$  vs.  $13.6 \pm 0.6$ ) (Liao and Veum, 1994).

Dyck and Strain also demonstrated that feeding pregnant gilts either 1.5 kg or 2.5 kg around the time of ovulation did not cause a change in ovulation rate (1983). In the same study, in contrast with the study conducted by Liao and Veum, the results indicated that pregnant females fed 1.5 kg/day from days 1-10 of gestation had more fetuses ( $11.0 \pm 0.3$ ) compared to females fed 2.5 kg/day during the same time period ( $9.5 \pm 0.4$ ) (Dyck and Strain, 1983). Those fed 1.5 kg/day also had a higher percent survival ( $86.2 \pm 2.1\%$ ) than those fed 2.5 kg/day ( $75.8 \pm 2.9\%$ ) (Dyck and Strain, 1983). In contrast to both of these studies, no differences in ovulation rate, percent survival, or the number of offspring were detected between females fed either a diet in which weight was simply

maintained and females that were fed a diet in which they gained 0.4 kg daily (Dyck, 1991).

#### Effect of mixing stress on reproduction

It is widely accepted that mixing gilts during gestation, especially in the first few weeks, may negatively affect embryonic development. Many studies have been conducted in order to determine if this widely held belief is true. Van Wettere et al. housed pregnant gilts (n=96) in either stalls or groups in which they were randomly mixed on days 3 or 4, 8 or 9, or not at all (2008). At 26 days of gestation, animals were slaughtered and reproductive characteristics such as ovulation rate, the number of embryos, embryo survival, embryo length, and empty uterine weight were analyzed. No significant differences were found among treatments, indicating that mixing pregnant gilts around days 3 or 8 of gestation does not impact the developing litter (van Wettere et al., 2008).

Similar results were reported from a study conducted by Cassar et al. in 2007. Pregnant sows were allotted to individual housing or were mixed into groups at days 2, 7, 14, 21, or 28 post-breeding. Mixed groups (n=15) contained two or three sows from each time-point post-breeding (2, 7, 14, 21, or 28 days). Sows were allowed to farrow and litter characteristics were analyzed. Mixing day did not affect farrowing rate or litter size (Cassar et al., 2007).

Repeated regrouping of pregnant gilts between the days of 0-34 of gestation also did not have an effect on the developing litter. Gilts were regrouped once per week into groups of four for six weeks. Those that were continuously regrouped were found to have higher skin lesion scores ( $5.80 \pm 0.12$ ) on the day of mixing than those that were not mixed

(3.06±0.09) (Soede et al., 2006). Although animals that were mixed weekly had higher lesion scores, there was again no difference in such characteristics as the number of embryos, ovulation rate, or uterine weight.

Knox et al. conducted a similar study in which gestating sows were mixed at different points during gestation in order to determine the mixing stress's effect on reproduction. Sows were assigned to one of the following treatments: gestation in an individual stall, mixed on day 3 to 7 of gestation, mixed on day 13 to 17 of gestation, or mixed 35 days after breeding. Conception rate was lowest in females mixed between 3 to 7 days of gestation (Knox et al., 2014). Farrowing rate was also lowest in sows mixed between 3 to 7 days of gestation, although no other differences were detected on reproduction (Knox et al., 2014).

### **Maintaining sows in a thermoneutral environment**

Swine producers work to raise their animals in a comfortable environment that will maximize performance. This environment can be described as an animal's thermoneutral zone (TNZ). This thermal range is the environment in which an animal's heat production neither increases nor decreases, but rather remains basal. It is in this thermal environment that an animal experiences optimum comfort and peak performance (Mount, 1974). Factors such as feed intake, physical activity, and insulation can largely affect an animal's TNZ (Ames, 1980). The TNZ is also defined as the zone between the lower critical temperature (LCT) and the upper critical temperature (UCT). If the ambient temperature drops below the LCT, the animal experiences cold stress and must increase heat production to maintain its body temperature (Ames, 1980). In contrast, if the ambient temperature rises above the UCT, the animal experiences heat stress and must

increase heat loss in order to maintain its body temperature (Ames, 1980). Performance can be negatively affected if animals are subjected to either cold or heat stress. Therefore, it is of great importance that swine producers raise their animals in the optimum thermal environment.

### **Methods to mitigate heat stress**

Modern swine producers have the option to cool females in breeding/gestation barns, farrowing barns, or in both. It is widely accepted that sows housed in farrowing rooms experience an increase in metabolic heat production, because they are lactating. This increase in metabolic heat production from lactation puts sows in farrowing at a greater risk for heat stress and a subsequent decrease in feed intake. This decrease in feed intake may translate into a decrease in milk production, further affecting the sow's litter by way of decreased piglet growth rates and lower weaning weights (Quiniou and Noblet, 1999; Williams et al., 2013). Metabolic heat production is lower during gestation than in farrowing, because females are limit fed during gestation in order to prevent excess weight gain before farrowing. Therefore, heat stress effects during gestation are less pronounced, leading many producers to put more resources into cooling farrowing rooms rather than gestation barns.

#### Natural vs. forced/mechanical ventilation

Swine producers ventilate their buildings in one of two ways: natural or mechanical ventilation. Naturally ventilated buildings feature curtains that can be lowered or raised to adjust airflow in the building (Hoff, 2012). Inlets and vents may also be used to allow for increased air flow (Hoff, 2012). Air flow over the pigs convectively cools the animals in warmer months. Many naturally ventilated buildings also feature

heaters for use during the winter months. These types of buildings are typically used for market hogs.

Mechanical ventilation systems can be identified by the use of fans to aid in ventilation. Two types of mechanical systems exist: negative pressure and positive pressure systems (Curtis, 1983). Negative pressure systems, the most common, use the difference in inside and outside air pressure to move outside air in through inlets. Positive pressure systems work by releasing air to the building's exterior via outlets, because the interior pressure exceeds that of the exterior (Curtis, 1983). Mechanically ventilated buildings are most often used for pigs that need careful control of their environment such as lactating sows and nursery pigs (Hoff, 2012).

Some buildings may be ventilated by a mixture of mechanical and natural ventilation. In such a case, a barn may utilize fans, curtains, and cooling pads. Cooling pads effectively cool incoming air by pulling the air through a wet, porous pad (Curtis, 1983). Buildings that feature a mixture of mechanical and natural ventilation are most often used for breeding/gestation barns (Hoff, 2012).

### **Conclusions**

The goal of this review was to describe reproduction in gilts, porcine fetal development throughout gestation, and the effects of heat stress, nutritional status, and mixing during gestation on reproduction in the gilt. Finally, the review described the ways in which producers work to ventilate their buildings and cool their animals.

Gestational heat stress has been shown to negatively affect the reproduction of female pigs. For example, gestational heat stress may cause a decrease in ovulation rates, decreased conception rates, lower birth rates, and fewer pigs per litter at farrowing.

The porcine fetus is rapidly developing during gestation, and heat stress during this time has the ability to negatively affect the fetus. Previous literature has shown negative effects of gestational heat stress on carcass composition of the developing fetus, such as a decrease in protein accretion and an increase in lipid deposition. This previous data proposed that heat stress *in utero* can negatively manifest itself in the offspring at slaughter, therefore suggesting that gestational heat stress may also affect the reproductive system of the developing piglet. Additional research to understand the effects of *in utero* heat stress on the developing fetus's reproductive capability at puberty will allow producers to better manage gilts during periods of high ambient temperatures.

## CHAPTER 3

### EFFECTS OF HEAT STRESS FROM WEEKS 4 TO 8 OF GESTATION ON THE DEVELOPMENT OF THE FETUS AND REPRODUCTIVE TRACT IN GILTS

#### SUMMARY

Gestational heat stress may lead to 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 heat stress (GHS; 28 to 38°C; 65 to 88% relative humidity; n=16) or thermoneutral (GTN; 17 to 22°C; 56 to 65% relative humidity; n=14) conditions during pregnancy. Gilts were housed in the Brody Environmental Chambers from weeks 4 to 8 of pregnancy before sacrifice during the 8<sup>th</sup> week of gestation for the collection of the reproductive tracts and fetal tissues. During pregnancy, GHS gilts had greater rectal temperature (38.5±0.04 vs. 38.0±0.04 °C; P<0.001), skin temperature (35.5±.2 vs. 28.7±0.2 °C; P<0.001), and respiration rate (44.3±2.6 vs. 19.5±2.7 breaths per min; P<0.001) compared with GTN. Sow was the experimental unit for analyses of fetal development. The weight of the pregnant tract (12.0±1.2 vs. 12.5±1.3 kg), number of viable conceptuses (13.8±0.8 vs. 15.3±0.9), number of non-viable conceptuses (0.3±0.2 vs. 0.1±0.2), the number of mummies (0.2±0.1 vs. 0.3±0.1), and the % survival (number of viable conceptuses/number corpora lutea; 89±4 vs. 90±5%) did not differ (P>0.10) for

GHS vs. GTN. Upon dissection, the weight of the fetus ( $82.3 \pm 3.6$  vs.  $84.9 \pm 3.8$  g), placenta ( $155.5 \pm 14.7$  vs.  $170.1 \pm 15.6$  g), fetal fluid ( $80.4 \pm 10.0$  vs.  $90.4 \pm 10.6$  g), and placental efficiency (fetal weight/placental weight;  $0.60 \pm 0.04$  vs.  $0.55 \pm 0.05$ ) did not differ ( $P > 0.10$ ) for GHS vs. GTN. The ratio of male to female fetuses was similar ( $P > 0.10$ ) for GHS ( $1.3 \pm 0.3$ ) and GTN ( $1.6 \pm 0.3$ ). The weight of male fetuses ( $86.2 \pm 3.8$  vs.  $86.4 \pm 4.0$  g), combined testis weight ( $34.2 \pm 1.4$  vs.  $32.8 \pm 1.5$  mg), and combined testis weight as a % of fetal weight ( $0.040 \pm 0.001$  vs.  $0.038 \pm 0.001$ ) did not differ ( $P > 0.10$ ) for GHS vs. GTN. The weight of female fetuses ( $81.2 \pm 3.6$  vs.  $83.5 \pm 3.8$  g), combined ovarian weight ( $25.2 \pm 1.0$  vs.  $26.1 \pm 1.1$  mg), and combined ovarian weight as a % of fetal weight ( $0.031 \pm 0.001$  vs.  $0.031 \pm 0.001$ ) did not differ ( $P > 0.10$ ) for GHS vs. GTN. The conclusion was that heat stress from weeks 4 to 8 of gestation in gilts did not change the growth of the fetus, placenta, ovary or testis at mid-gestation.

## INTRODUCTION

High ambient temperatures and humidity during the summer months 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 rates rather than those housed in cooler temperatures. Similarly, Warnick et al. (1965) determined that females housed at high ambient temperatures from conception through day 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.

## **MATERIALS AND METHODS**

### 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<sup>®</sup> (Merck Animal Health, De Soto, KS) at the University of Missouri Swine Research Complex (SRC) between September and February (Table 3.1). 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. While gilts were housed at the SRC, temperatures within the gestation barn

were recorded twice daily for replicates 2, 3, and 4 (Figures 3.1, 3.2, and 3.3, respectively).

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. Chamber temperature and humidity data was recorded automatically every 15 minutes using an Onset HOBO data logger (Bourne, MA). 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;  $152.9 \pm 2.8$  vs.  $152.0 \pm 3.0$  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 (Figures 3.4 and 3.5). The average temperature-humidity index (THI) was calculated for each hour gilts were in the environmental chambers, as well (Figure 3.6). 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 (Figure 3.7). 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 (Table 3.2). 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  $60 \pm 3$  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 Science 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 diameters 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 ovary 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

Gilts were exposed to one of two treatments (GTN or GHS). There were four replicates with approximately 12 gilts per treatment in each replicate. Data with one measurement per gilt (total uterine weight, empty uterine weight, uterine length, number of viable fetuses, etc.) were analyzed by using the general linear models procedure of SAS (PROC GLM). The model included the main effects of treatment, replicate, and treatment by replicate interaction. Data with multiple measurements per gilt (fetal weight, fetal length, placental weight, etc.) were analyzed by using the mixed model procedure in SAS (PROC MIXED). The model included the main effects of treatment, replicate, treatment by replicate, fetal sex, and treatment by sex interaction. The number of viable fetuses (litter size) was included as a covariate in the analyses. Sow nested within treatment and replicate was defined as random. Data are presented as least squares means  $\pm$  standard error of the least square mean. Means were considered significant at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

### Thermal Response, Feed Intake, and Body Weight Data Results

Gilts subjected to heat stress from days 30 to 60 of gestation had greater rectal temperature ( $38.5 \pm 0.04$  vs.  $38.0 \pm 0.04$  °C;  $P < 0.001$ ) compared with GTN (Figure 3.8). GHS gilts had greater skin temperature ( $35.5 \pm 0.2$  vs.  $28.7 \pm 0.2$  °C;  $P < .001$ ) than GTN (Figure 3.9). Heat stressed gilts also had greater respiration rate ( $44.3 \pm 2.6$  vs.  $19.5 \pm 2.7$  breaths per min;  $P < 0.001$ ) compared with GTN (Figure 3.10). Feed intake ( $1.99 \pm 0.003$

vs.  $1.99 \pm 0.003$  kg) was similar ( $P > 0.10$ ) between GHS and GTN gilts. Similarly, body weight did not differ ( $P > 0.10$ ) upon exit of the environmental chambers for females housed under GHS ( $160.1 \pm 1.96$  kg) or GTN ( $154.6 \pm 1.96$  kg) conditions.

### Slaughter Data Results

*Effects of heat stress on uterine and ovarian measures of the gilt.* Total uterine weight, empty uterine weight, and uterine length did not differ for GHS vs. GTN (Table 3.3). Empty uterine weight was found to differ between replicates, while total uterine weight and uterine length were similar between replicates 1, 2, 3, and 4 (Table 3.4). Ovarian weight, the number of corpora lutea (CL), and CL weight did not differ for GHS vs. GTN (Table 3.3). Similarly, ovarian weight, the number of CL, and CL weight did not differ between replicates (Table 3.4). Results are not available for follicular diameter.

*Effects of heat stress on litter and placental measures of the gilt.* No treatment differences were detected between GHS and GTN for total number of fetuses per litter, number of viable fetuses per litter, number of nonviable fetuses per litter, and the number of mummies per litter (Table 3.3). The number of mummies per litter differed between replicates, while the total number of fetuses, the number of viable fetuses, and the number of nonviable fetuses were similar between replicates (Table 3.4). Survival (number of fetuses/number of CL) also did not differ between GHS and GTN (Table 3.3). Survival (number of fetuses/number of CL) was also similar between replicates (Table 3.4). Implantation length was similar between GHS and GTN but was found to differ between replicates (Tables 3.3 and 3.4, respectively). Placental attachment area was similar between GHS and GTN, but placental attachment area tended to differ between replicates (Tables 3.3 and 3.4, respectively). The combined weight of the fetus, placenta,

and placental fluids did not differ for GHS vs. GTN but differed between replicates (Tables 3.3 and 3.4, respectively). The weight of the placenta and fluid also did not differ for GHS vs. GTN (Table 3.3). Placental weight and fluid weight were similar between replicates 1, 2, 3, and 4 (Table 3.4). Placental efficiency (fetal weight/placental weight) was similar between GHS and GTN but differed between replicates (Tables 3.3 and 3.4, respectively).

Fetal weight did not differ between GHS and GTN (Table 3.5). The weight of male fetuses and female fetuses also did not differ for GHS vs. GTN (Table 3.5). Similarly, fetal length was similar for GHS vs. GTN (Table 3.5). Replicate effects were observed for fetal weight, male fetal weight, female fetal weight, and fetal length (Table 3.6).

*Effects of heat stress on fetal ovarian and testes development.* Fetal ovarian weight and fetal testes weight did not differ for GHS vs. GTN, but replicate effects were observed (Tables 3.5 and 3.6, respectively). Ovarian weight as a percentage of body weight and testes weight as a percentage of body weight were similar for GHS vs. GTN (Table 3.5). Ovarian weight as a percentage of body weight and testes weight as a percentage of body weight also differed between replicates (Table 3.6). Female anogenital distance did not differ for GHS vs. GTN (Table 3.5). Similarly, male anogenital distance also did not differ for GHS vs. GTN (Table 3.5). Both male and female anogenital distances differed between replicates (Table 3.6).

## 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 indicates 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 stress conditions or thermoneutral conditions. Replicate effects were observed for such measures as fetal weight and gonadal weight in both males and females. Fetuses were heaviest from litters in replicate 4 and fetal gonads were heavier in replicate 4, as well. Gestation barn temperatures from days 0 to 30 were similar between replicates 3 and 4, and no such improved effects were observed in replicate 3. Therefore, it is difficult to define the cause of the differences observed between replicates. Similar replicate effects were observed for efficiency (fetal weight/placental weight), implantation length, and the combined weight of the fetus, placenta, and placental fluid. These replicate effects most likely are observed due to their relationship with replicate 4 fetuses. Overall, we conclude that gestational heat stress from weeks four to eight of gestation had no significant effect on the pregnancy of the dam.

Table 3.1. Gilt housing location from insemination to farrowing.<sup>1</sup>

Replicate	Location		
	Swine Research Center (SRC)	Environmental Chambers	Farrowing
1	August-September 2015	September-October 2015	December 2015
2	September-October 2015	October-November 2015	January 2016
3	December 2015-January 2016	January-February 2016	April 2016
4	January-February 2016	February-March 2016	May 2016

<sup>1</sup>Months and locations during which gilts were on trial.

Table 3.2. Composition of gestation/breeding diets (% as-fed basis).

Item	Gestation/Breeding diet
Corn	69.4
Soybean meal (48%)	15
Soy hulls	10
Choice white grease	1
Dicalcium phosphate	2.3
Limestone	1
Salt	0.5
Lysine	-
Vitamin premixes	0.5
Trace mineral premix	0.2
Zinc and biotin premixes	0.2

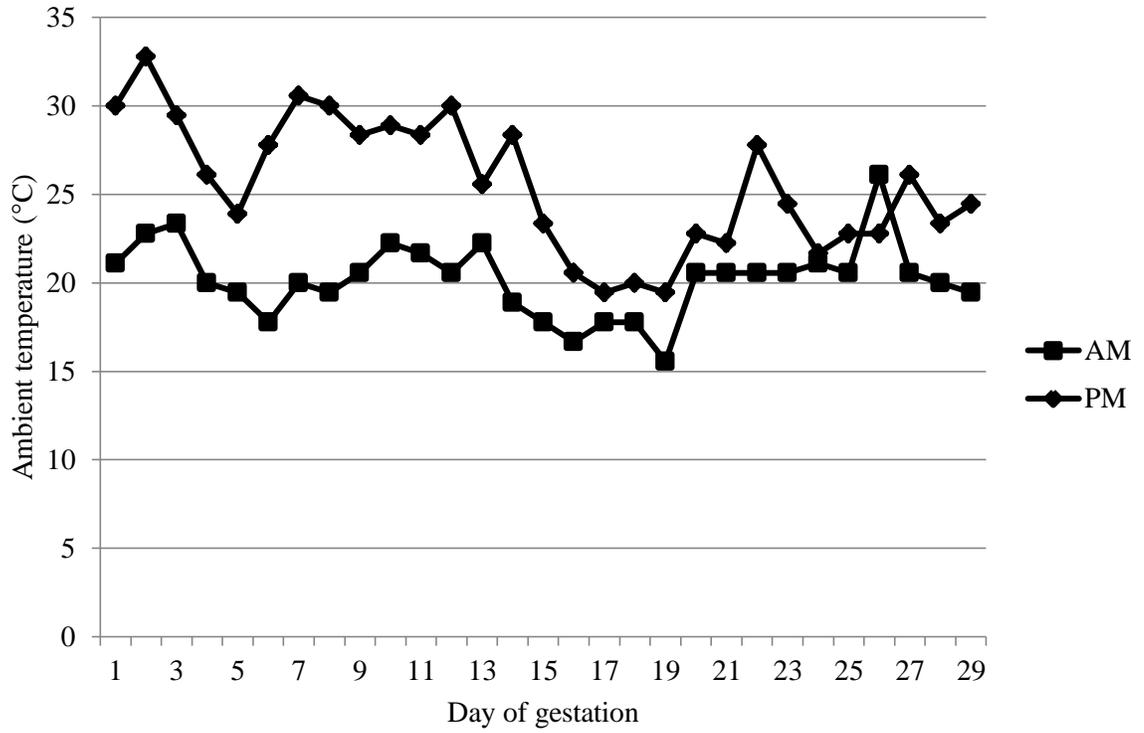


Figure 3.1. Ambient temperature of the Swine Research Center gestation barn during days 0 to 30 of gestation for replicate 2. Temperatures were recorded in both the morning (AM) and afternoon (PM).

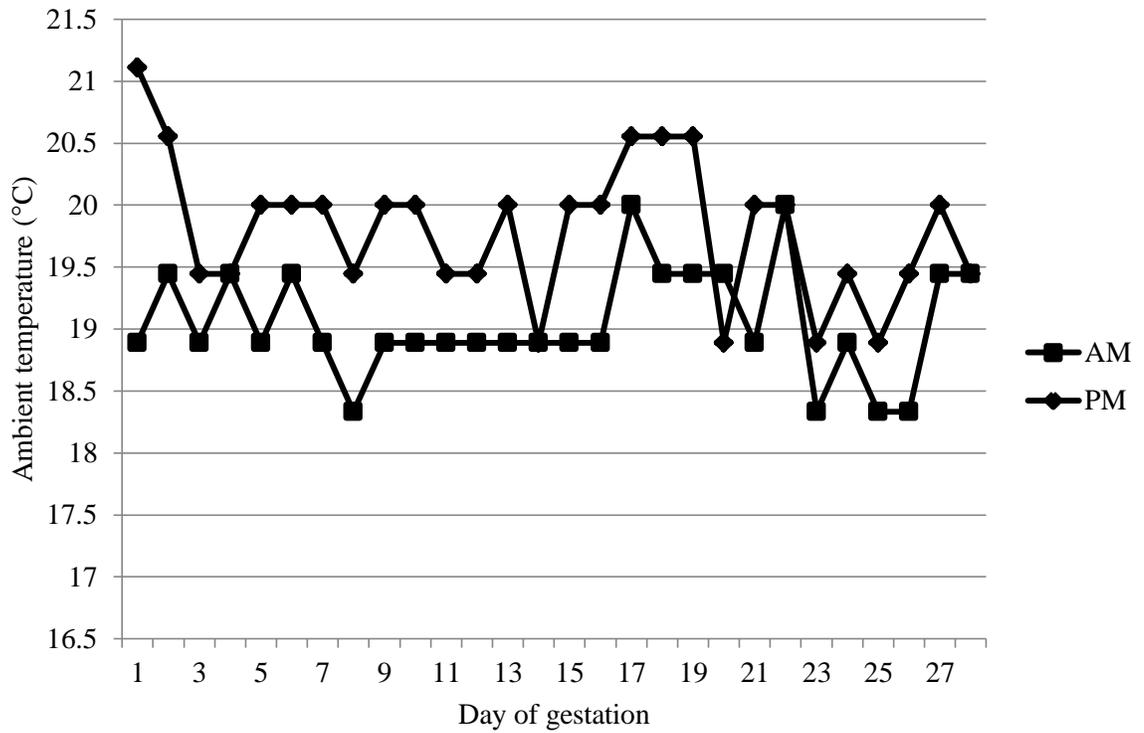


Figure 3.2. Ambient temperature of the Swine Research Center gestation barn during days 0 to 30 of gestation for replicate 3. Temperatures were recorded in both the morning (AM) and afternoon (PM).

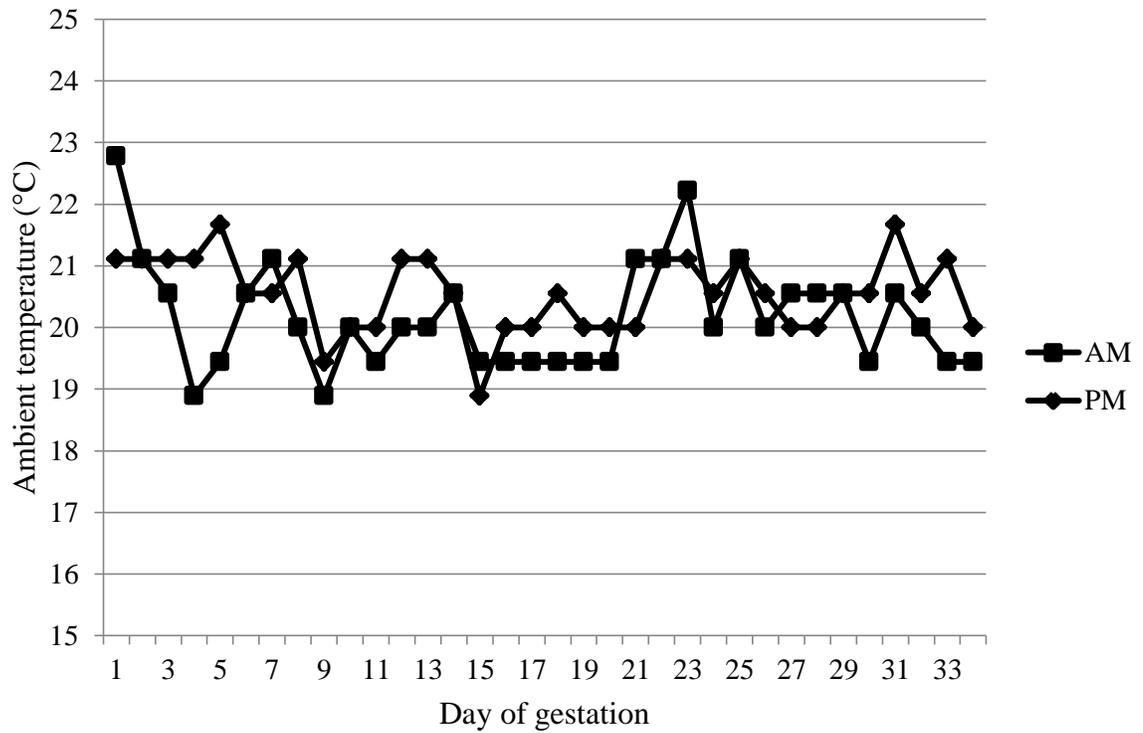


Figure 3.3. Ambient temperature of the Swine Research Center gestation barn during days 0 to 30 of gestation for replicate 4. Temperatures were recorded in both the morning (AM) and afternoon (PM).

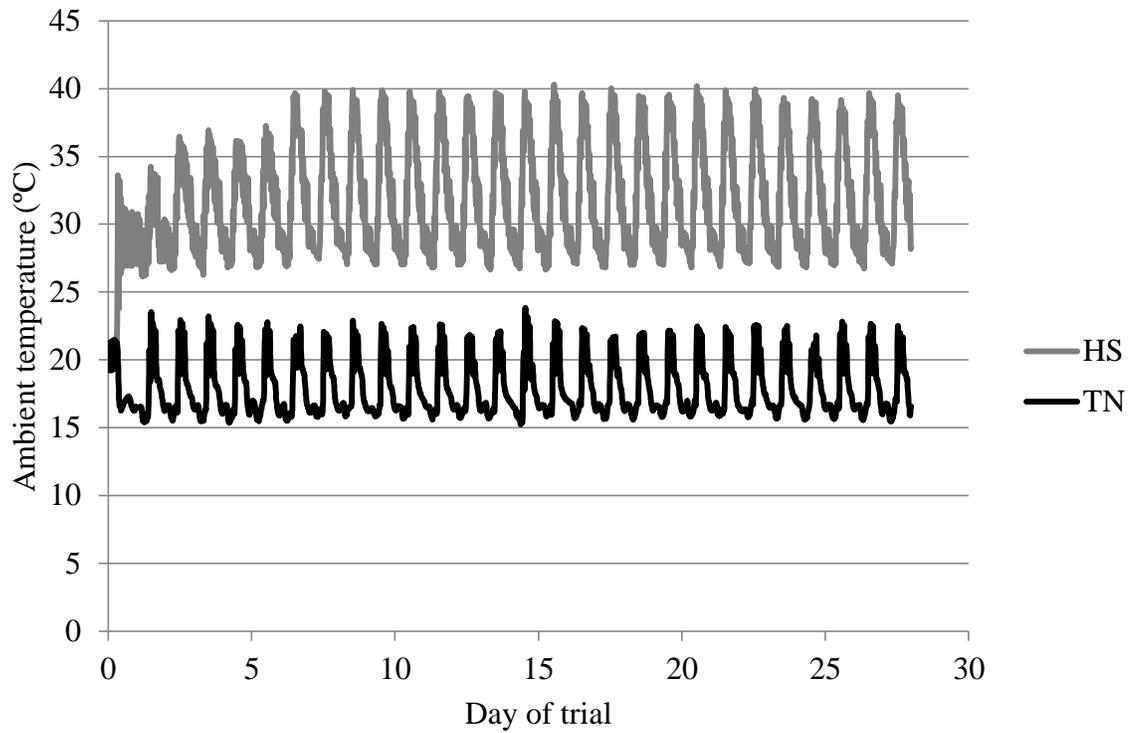


Figure 3.4. Diurnal temperature cycle that gilts were exposed to in either the heat stress (HS; 28 to 38°C) or thermoneutral (TN; 17 to 22°C) chamber. Temperature data was recorded every 15 minutes for the duration of the gilts' stay in the chambers.

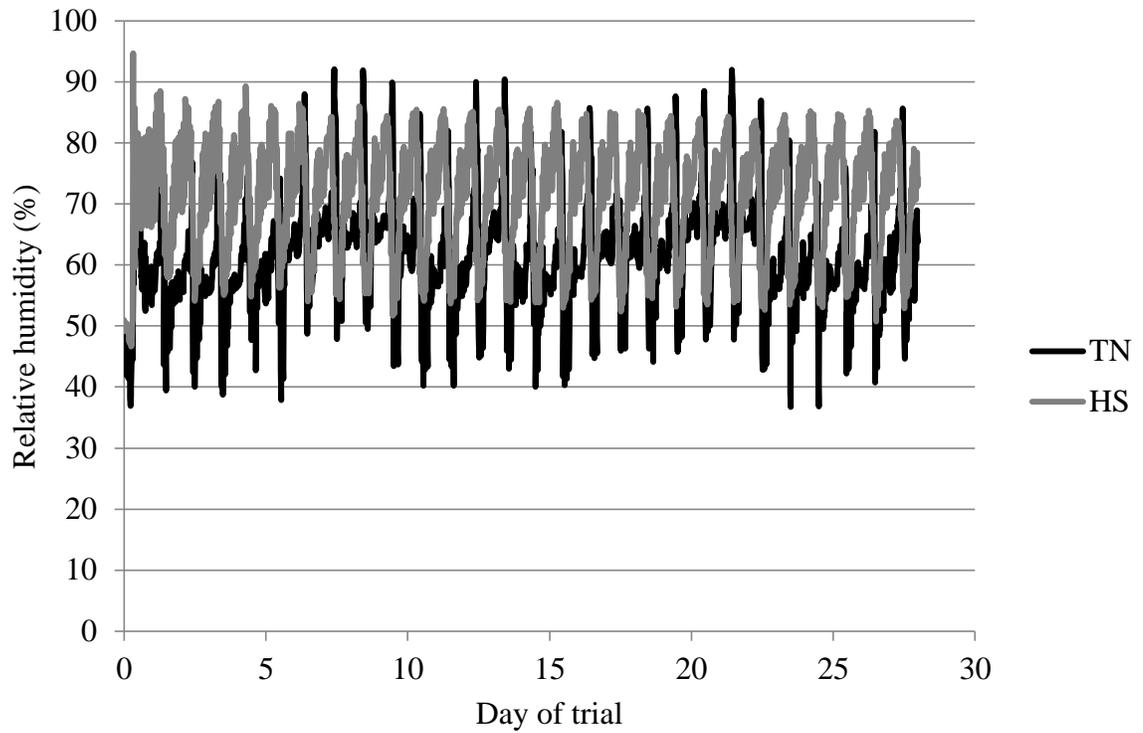


Figure 3.5. Diurnal humidity cycle that gilts were exposed to in either the heat stress (HS; RH 65 to 88%) or thermoneutral (TN; RH 56 to 65%) chamber. Humidity data was recorded every 15 minutes for the duration of the gilts' stay in the chambers.

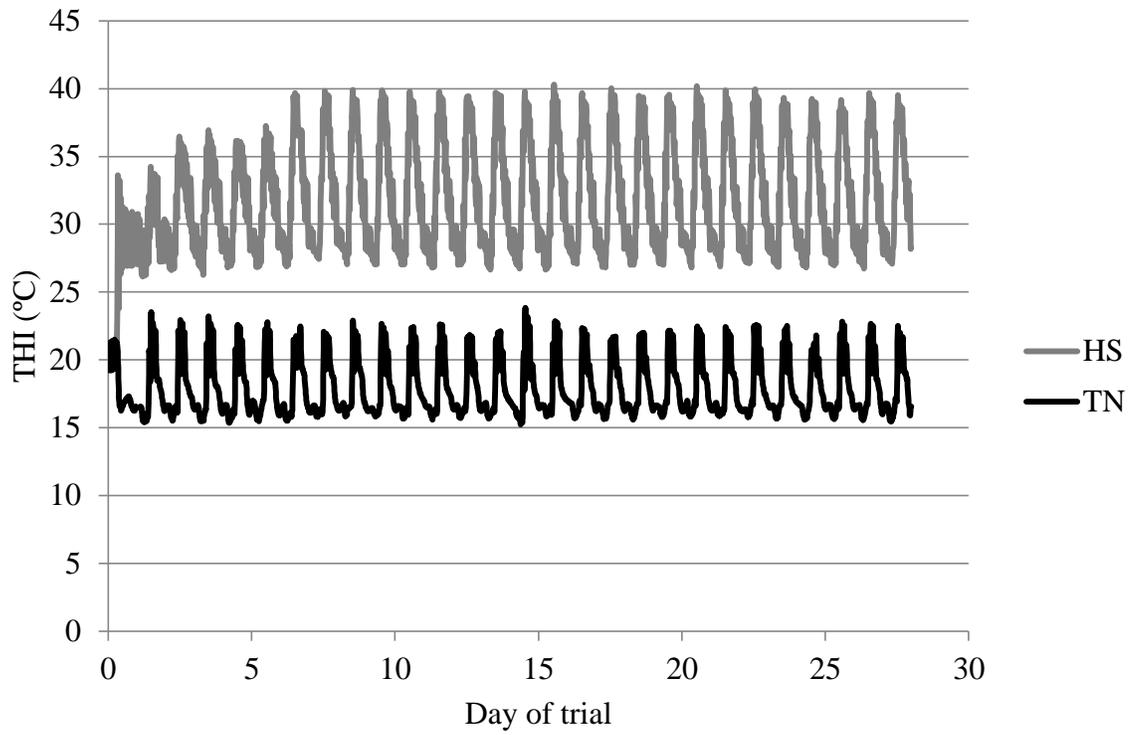


Figure 3.6. Hourly average temperature-humidity indices (THI) for gilts housed under cyclical diurnal GHS and GTN conditions from days 30 to 60 of gestation.

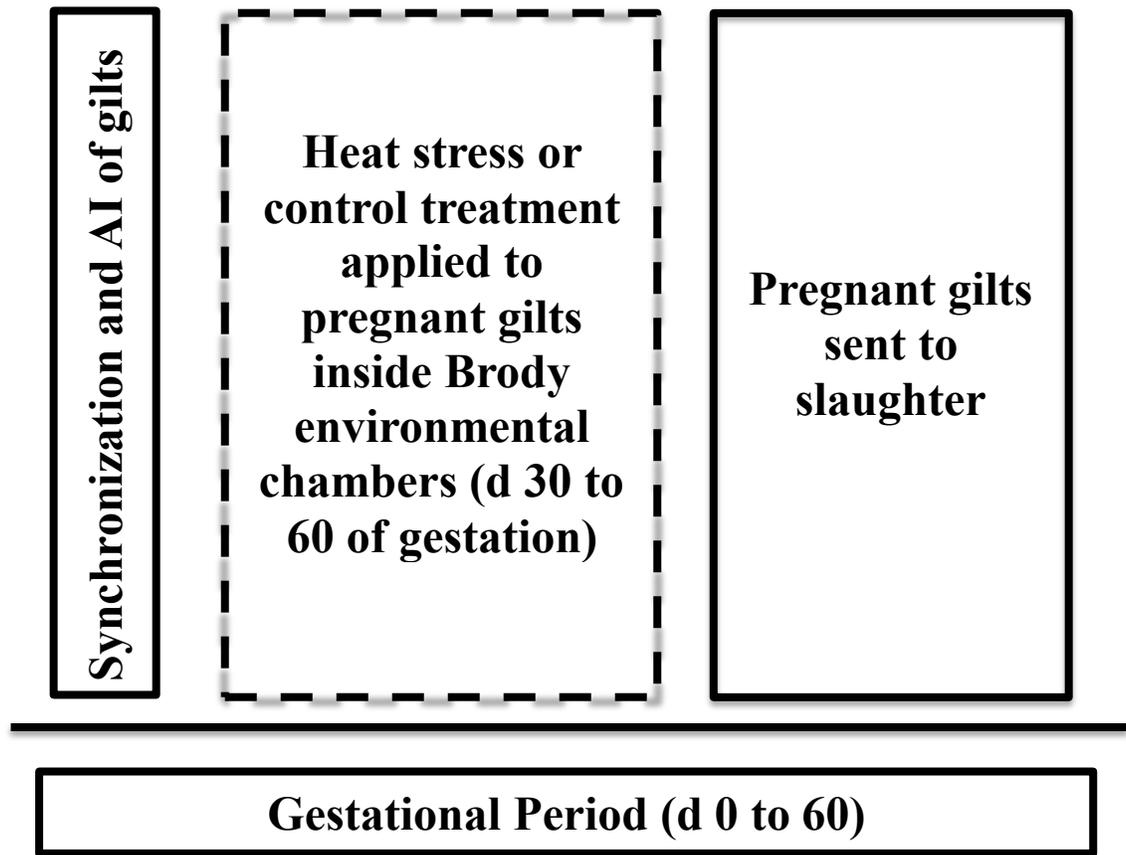


Figure 3.7. Experimental timeline for gilts exposed to HS or TN conditions during gestation.

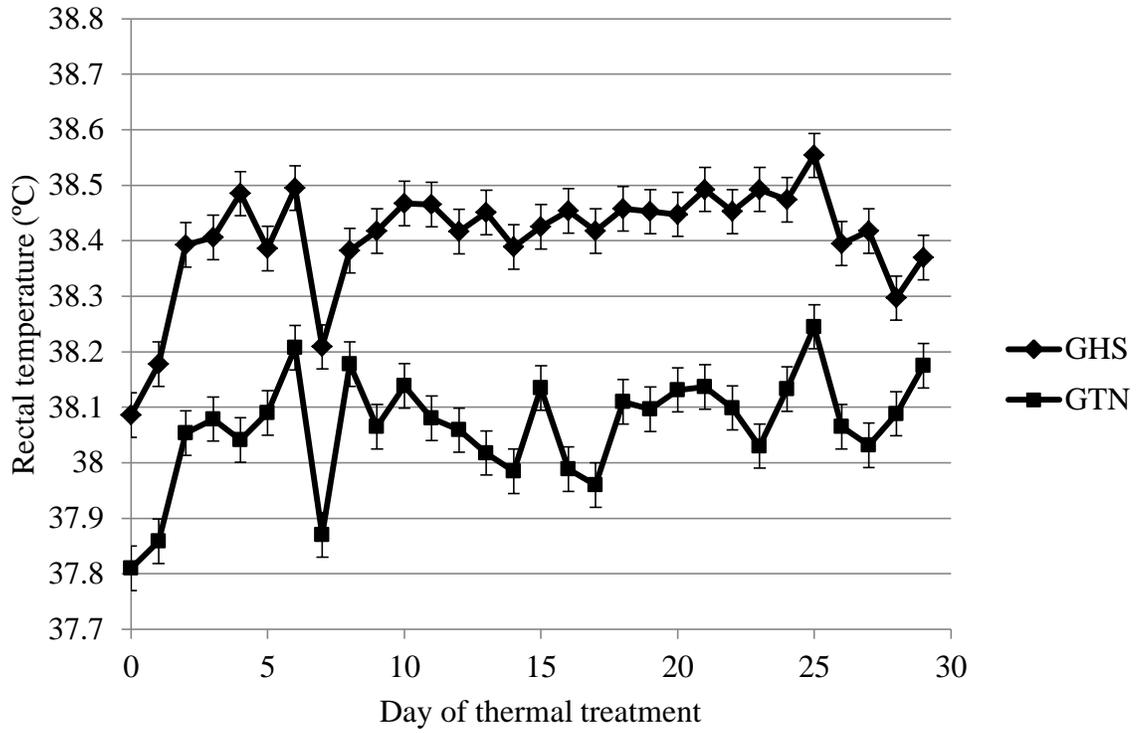


Figure 3.8. Average rectal temperatures of gilts exposed to HS or TN conditions from days 30 to 60 of gestation.

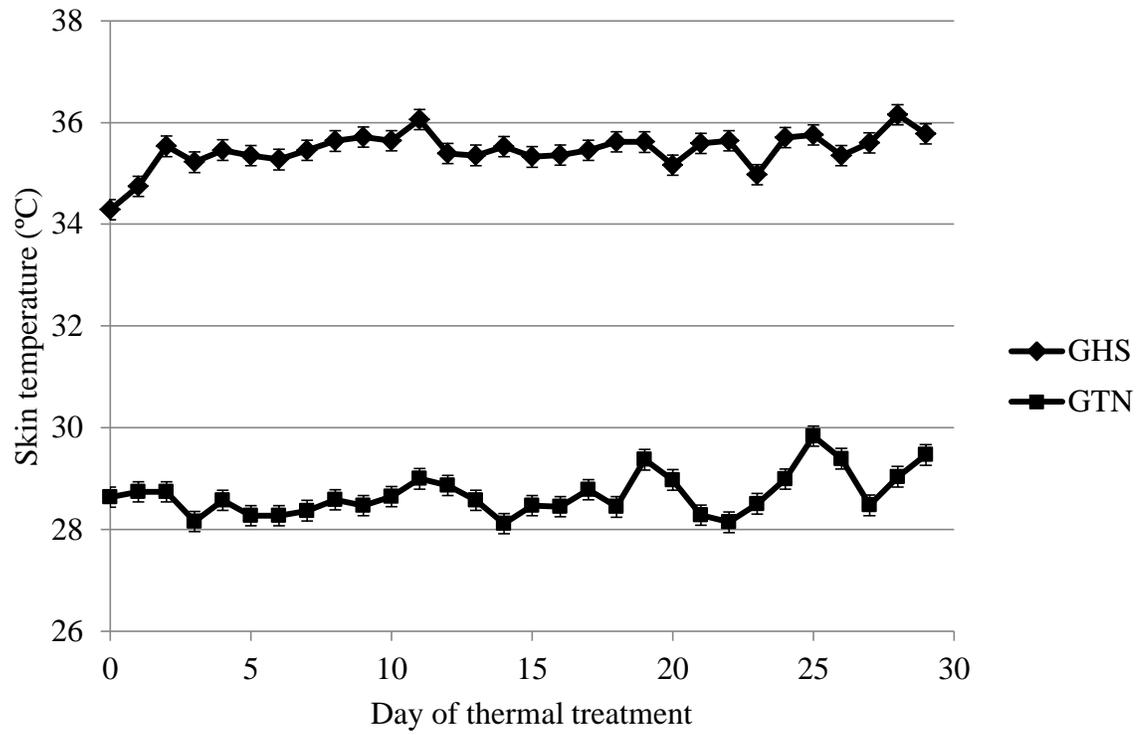


Figure 3.9. Average skin temperatures of gilts exposed to HS or TN conditions from days 30 to 60 of gestation.

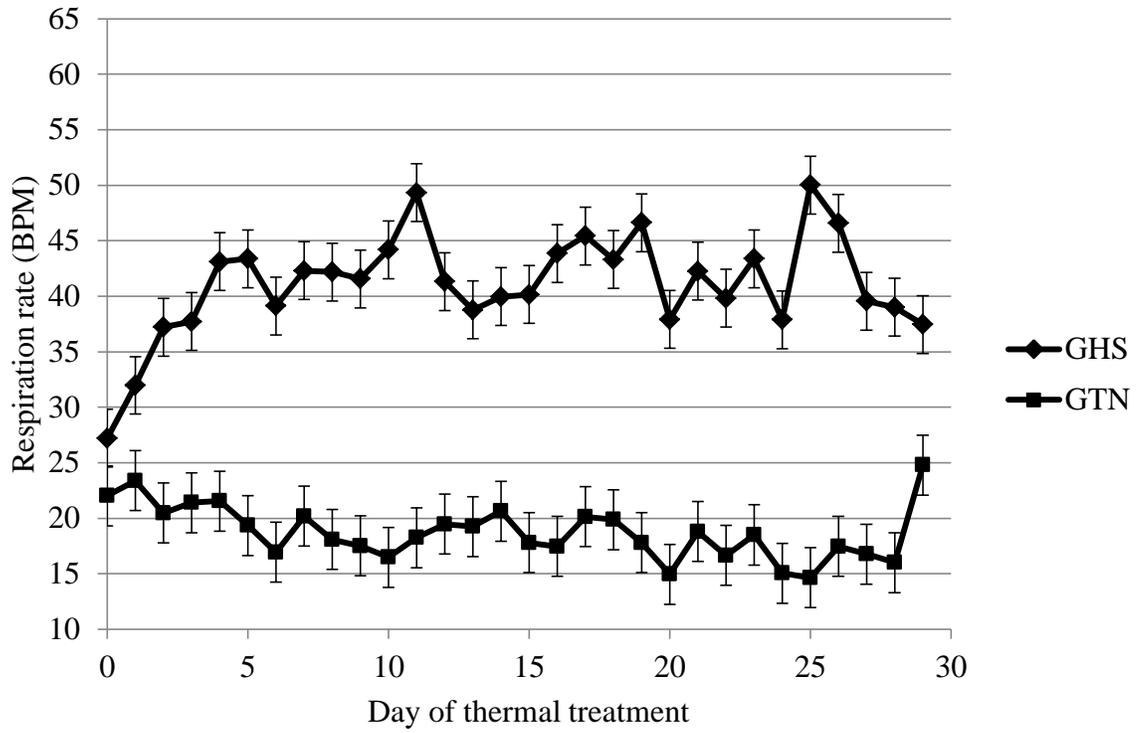


Figure 3.10. Average respiration rates (breaths per minute) of gilts exposed to HS or TN conditions during days 30 to 60 of gestation.

Table 3.3. Least squares means for the effects of heat stress from weeks 4 to 8 of gestation on uterine, ovarian, litter, and placental measures in gilts.

Item	Treatment (Trt)		P-value <sup>1</sup>
	GHS	GTN	
Number of pigs	16	14	-
Number of fetuses	228	223	-
<b>Uterine measures</b>			
Total uterine weight, kg	12.40±0.92	13.38±1.00	0.479
Empty uterine weight, kg	3.06±0.18	3.42±0.20	0.184
Uterine length, cm	345.35±20.06	377.75±21.66	0.283
Implantation length, cm	19.52±1.08	20.81±1.10	0.422
<b>Ovarian measures</b>			
Ovarian weight, g	17.21±1.22	17.35±1.31	0.938
Number of CL <sup>2</sup>	15.38±0.73	17.15±0.79	0.113
CL weight, g	1.15±0.06	1.00±0.07	0.120
<b>Litter measures</b>			
Total number of fetuses	14.4±0.8	16.3±0.8	0.105
Number of viable fetuses	13.8±0.7	15.3±0.8	0.168
Number of nonviable fetuses	0.3±0.7	0.4±0.2	0.777
Number of mummies	0.3±0.2	0.6±0.2	0.200
Survival (Number fetuses/number CL), %	91.6±3.7	89.1±4.0	0.652
<b>Placental measures</b>			
Placental attachment area, cm <sup>2</sup>	237.88±16.31	236.33±16.41	0.948
Combined weight (fluid, placental, fetal), g	334.24±20.26	378.64±20.56	0.148
Placental weight, g	154.2±13.4	180.1±13.5	0.198
Fluid weight, g	82.46±8.51	100.68±8.68	0.158
Efficiency (Fetal weight/placental weight), %	69.93±4.55	62.71±4.60	0.288

<sup>1</sup>Means differ at  $P<0.05$

<sup>2</sup>CL: Corpora lutea

Table 3.4. Least squares means for the effects of replicate on uterine, ovarian, litter, and placental measures in gilts exposed to heat stress or thermoneutral conditions from weeks 4 to 8 of gestation.

Item	Replicate (Rep)				<i>P</i> -value <sup>1</sup>
	1	2	3	4	
Number of pigs	8	8	7	7	-
Number of fetuses	129	113	101	108	-
<b>Uterine measures</b>					
Total uterine weight, kg	13.38±1.30	10.59±1.30	12.47±1.41	15.12±1.41	0.153
Empty uterine weight, kg	2.87±0.26	2.77±0.26	3.36±0.28	3.96±0.28	0.019
Uterine length, cm	355.25±28.36	323.25±28.36	382.96±30.64	384.54±30.64	0.425
Implantation length, cm	18.67±1.43	17.16±1.51	23.18±1.57	21.64±1.55	0.037
<b>Ovarian measures</b>					
Ovarian weight, g	20.74±1.72	15.85±1.72	15.00±1.86	17.53±1.86	0.132
Number of CL <sup>2</sup>	18.00±1.03	15.63±1.03	15.42±1.11	16.00±1.11	0.305
CL weight, g	1.15±0.09	1.02±0.09	1.00±0.10	1.12±0.10	0.622
<b>Litter measures</b>					
Total number of fetuses	16.13±1.07	14.13±1.07	14.71±1.15	16.54±1.15	0.380
Number of viable fetuses	15.88±1.00	13.75±1.00	14.04±1.08	14.54±1.08	0.468
Number of nonviable fetuses	0.00±0.28	0.25±0.28	0.38±0.30	0.79±0.30	0.304
Number of mummies	0.25±0.23	0.13±0.23	0.29±0.25	1.21±0.25	0.016
Survival (Number fetuses/number CL), %	88.13±5.18	89.34±5.18	91.00±5.60	92.89±5.60	0.931
<b>Placental measures</b>					
Placental attachment area, cm <sup>2</sup>	229.97±21.70	203.87±22.58	220.77±23.60	293.81±23.26	0.056
Combined weight (fluid, placental, fetal), g	358.95±26.68	293.59±28.20	316.21±29.41	457.02±29.02	0.004
Placental weight, g	172.39±17.61	141.11±18.63	157.96±19.42	197.02±19.14	0.252
Fluid weight, g	99.69±11.21	75.92±11.87	77.42±12.37	113.24±12.23	0.136
Efficiency (Fetal weight/placental weight), %	57.94±5.98	61.47±6.33	57.48±6.59	88.40±6.50	0.008

<sup>1</sup>Means differ at *P*<0.05

<sup>2</sup>CL: Corpora lutea

Table 3.5. Least squares means for the effects of heat stress from weeks 4 to 8 of gestation on fetal and gonadal development in gilts.

Item	Treatment (Trt)		<i>P</i> -value <sup>1</sup>
	GHS	GTN	
Number of pigs	16	14	-
Number of fetuses	228	223	-
<b>Fetal measures</b>			
Fetal weight, g	97.80±3.87	102.08±3.91	0.455
Fetal length, cm	15.43±0.21	15.17±0.21	0.404
Female fetal weight, g	96.24±4.37	96.36±4.49	0.985
Male fetal weight, g	100.49±4.08	106.91±4.02	0.283
<b>Female fetal gonad measures</b>			
Fetal ovarian weight, mg	27.819±0.867	26.721±0.949	0.418
Ovarian weight as a percentage of BW, %	2.99±0.09	2.94±0.10	0.734
Female anogenital distance, mm	2.461±0.190	2.187±0.198	0.342
<b>Male fetal gonad measures</b>			
Fetal testes weight, mg	37.419±1.770	36.994±1.717	0.868
Testes weight as a percentage of BW, %	3.82±0.13	3.60±0.12	0.236
Male anogenital distance, mm	31.803±0.508	32.241±0.490	0.551

<sup>1</sup>Means differ at *P*<0.05

Table 3.6. Least squares means for the effects of replicate on fetal and gonadal development in gilts exposed to heat stress or thermoneutral conditions from weeks 4 to 8 of gestation.

Item	Replicate (Rep)				<i>P</i> -value <sup>1</sup>
	1	2	3	4	Rep
Number of pigs	8	8	7	7	-
Number of fetuses	129	113	101	108	-
<b>Fetal measures</b>					
Fetal weight, g	88.55±5.08	77.67±5.38	81.58±5.61	151.96±5.53	<0.001
Fetal length, cm	14.13±0.27	14.22±0.29	14.35±0.30	18.51±0.30	<0.001
Female fetal weight, g	86.47±5.81	77.56±6.10	82.08±6.40	139.10±6.35	<0.001
Male fetal weight, g	92.30±5.26	80.04±5.63	83.32±5.85	159.14±5.69	<0.001
<b>Female fetal gonad measures</b>					
Fetal ovarian weight, mg	28.73±1.18	25.09±1.23	24.07±1.29	31.18±1.36	0.005
Ovarian weight as a percentage of BW, %	3.34±0.12	3.27±0.13	2.93±0.14	2.32±0.14	<0.001
Female anogenital distance, mm	3.37±0.26	2.31±0.27	1.42±0.28	2.20±0.28	<0.001
<b>Male fetal gonad measures</b>					
Fetal testes weight, mg	36.13±2.25	30.98±2.43	33.85±2.51	47.86±2.46	<0.001
Testes weight as a percentage of BW, %	3.96±0.16	3.87±0.17	4.03±0.18	2.99±0.18	0.001
Male anogenital distance, mm	30.19±0.64	29.85±0.69	28.89±0.72	39.16±0.70	<0.001

<sup>1</sup>Means differ at *P*<0.05

## CHAPTER 4

### REPRODUCTIVE PARAMETERS OF GILTS HEAT STRESSED FROM WEEKS 4 TO 8 *IN UTERO*

#### SUMMARY

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 °C; 65 to 88% relative humidity) or thermoneutral (GTN; n=25; 17 to 22 °C; 56 to 65% relative humidity) conditions as a developing fetus (*in utero*) from weeks 4 to 8 of pregnancy. All dams were moved to TN conditions before farrowing. After farrowing, 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 8<sup>th</sup> week of gestation, gilts that came from GTN (GTN-G1; n=55) and GHS (GHS-G1; n=50) dams were sacrificed for the collection of the reproductive tracts and fetal tissues. 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), respectively. 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.  $75\pm 3\%$ ) did not differ ( $P>0.10$ ) for GHS-G1 and GTN-G1. A sex-specific transgenerational effect on fetal weight was observed, because male fetuses from GHS-G1 had increased weight ( $129.0\pm 4.8$  vs.  $119.5\pm 4.5$  g) but female fetuses were similar ( $117.4\pm 4.7$  vs.  $115.8\pm 4.5$  g) (GHS-G1 vs. GTN-G1; Treatment by sex,  $P<0.012$ ). Placental weight was lesser in females vs. male ( $155.5\pm 5.7$  vs.  $170.1\pm 5.7$  g;  $P<0.001$ ), but placental efficiency (fetal weight/placental weight) did not differ between females and males ( $82.9\pm 2.3$  vs.  $80.5\pm 2.3$ ;  $P>0.10$ ) or GHS-G1 vs. GTN-G1 ( $P>0.10$ ). The conclusion was that *in utero* heat stress from weeks 4 to 8 of gestation had gender-specific transgenerational (first generation) effects.

## INTRODUCTION

It has been well documented that high ambient temperatures and humidity during the summer months may have detrimental effects on various aspects of reproduction, including breeding, gestation, farrowing, and lactation. Such effects include delayed puberty in gilts and longer estrous cycles. For example, Flowers et al. (1989) determined that gilts exposed to heat stress conditions from 180 days of age to 230 days of age showed a delay to puberty compared to gilts in control conditions. Similarly, Paterson et al. (1991) found that  $89.4\pm 4.13\%$  of gilts exposed to a mature boar during cold months reached puberty within approximately 60 days compared to  $74.0\pm 3.37\%$  of gilts during hot summer months. Edwards et al. (1968) demonstrated that gilts exposed to high ambient temperatures experienced longer estrous cycles than females in thermoneutral conditions. Heat stress may also cause a decrease in conception rate (Warnick et al.,

1965) and lower ovulation rates (Flowers et al., 1989). Teague et al. (1968) also demonstrated that females housed in low temperature and low humidity environments averaged 1.1 embryos per gilt more than females in high temperature and high humidity environments. Gilts housed under heat stress conditions during breeding may also return to estrus post-breeding at a higher rate than those in thermoneutral conditions (Omtvedt et al., 1971). Females heat stressed late in gestation may have a decrease in the number of live piglets farrowed per litter and a higher incidence of stillborn piglets (Omtvedt et al., 1971). Many negative effects of heat stress during lactation can be attributed to a decrease in feed intake. Quiniou and Noblet (1999) and Williams et al. (2013) found that sows housed in heat stress conditions ate less feed than sows in thermoneutral conditions. This decrease in feed intake may translate into decreased milk production and decreased piglet growth (Quiniou and Noblet, 1999).

Gestational heat stress has also 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 that 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. (2015b) 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. (2015b) 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 be better equipped to make decisions on the degree to which it is economically feasible to cool gilts in gestation.

## MATERIALS AND METHODS

### 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<sup>®</sup> (Merck Animal Health, De Soto, KS) at the University of Missouri Swine Research Complex (SRC) (Table 4.1). 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 Swine 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 approximately 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 Swine Teaching Farm where they stayed for the remainder of the study.

### Experimental Design

*Environmental chambers experimental design.* Once F<sub>0</sub> 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 (Figure 4.1).

*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 lactation diet (Table 4.2). 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 daily before boar exposure (Table 4.2). Gilts were fed 2.2 kg of feed per head in pens of eight G1-gilts per pen. Gilts were fed to maintenance. 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-G1 gilts 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  $60\pm 3$  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 Science 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 diameters 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 length was 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 was 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 replicates 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, replicate, and treatment by replicate interaction. Data with multiple measurements per gilt were analyzed with a model that included main effects of treatment, replicate, fetal sex, treatment by replicate interaction, and treatment by fetal sex interaction. Data means are expressed as least squares means  $\pm$  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**

### Farrowing Data Results

Litter size did not differ between GHS and GTN females that were housed in the Brody Environmental Chambers at the University of Missouri from days 30-60 of gestation (Table 4.3). Litter size was also similar between replicates (Table 4.4). Birth

weight was not affected by treatment for piglets born to GHS or GTN dams, although male piglets ( $1.28 \pm 0.02$  kg) tended to be heavier ( $P < 0.09$ ) than female piglets ( $1.25 \pm 0.02$  kg) (Table 4.3). Similarly, day three piglet body weights did not differ, while male piglets ( $1.60 \pm 0.03$  kg) tended ( $P < 0.10$ ) to weigh more than female piglets ( $1.57 \pm 0.03$  kg) (Table 4.3). Week one piglet body weights, week two piglet body weights, and week three piglet body weights (weaning weight) did not differ for GHS vs. GTN (Table 4.3). No differences in body weight from birth to weaning were observed between replicates (Table 4.4). Female piglet anogenital distance did not differ for offspring from GHS vs. GTN dams, and male piglet anogenital distance also did not differ for offspring from GHS vs. GTN dams (Table 4.3). Replicate effects were not detected for male or female anogenital distance (Table 4.4).

#### 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 (Tables 4.5 and 4.7, respectively). Ovarian weight did not differ between GHS-G1 and GTN-G1 females (Table 4.5). No replicate effect was observed for ovarian weight, as well (Table 4.7). The number of CL and the number of follicles did not differ for GHS-G1 vs. GTN-G1, but an effect of replicate was observed for both CL and follicle number (Tables 4.5 and 4.7, respectively).

*Effects of in utero heat stress on litter and placental measures of GHS-G1 and GTN-G1 gilts.* The number of viable fetuses and the number of nonviable fetuses was similar between GHS-G1 and GTN-G1 females (Table 4.5). An effect of replicate was observed

for the number of viable fetuses and the number of nonviable fetuses (Table 4.7).

Survival (number of fetuses/number of CL) did not differ for GHS-G1 vs. GTN-G1, but an effect of replicate was observed (Tables 4.5 and 4.7, respectively).

Implantation length was similar between GHS-G1 and GTN-G1 (Table 4.5). An effect of replicate was observed for implantation length (Table 4.7). Placental attachment area did not differ between GHS-G1 and GTN-G1, but a replicate effect was observed (Tables 4.5 and 4.7, respectively). Combined fluid, placental, and fetal weight did not differ between GHS-G1 and GTN-G1, but a replicate and sex effect was observed (Tables 4.5 and 4.7, respectively). Placental weight also did not differ for GHS-G1 vs. GTN-G1 females, although replicate and sex effects were observed (Tables 4.5 and 4.7, respectively). Male GHS-G1 fetuses tended ( $P < 0.09$ ) to have heavier placentas ( $178.3 \pm 8.4$  g) than male GTN-G1 fetuses ( $161.9 \pm 7.8$  g), female GHS-G1 fetuses ( $158.3 \pm 8.3$ ), and female GTN-G1 fetuses ( $152.8 \pm 7.9$ ). Placental fluid weight was similar between GHS-G1 and GTN-G1 (Table 4.5). Placental fluid weight differed between replicates (Table 4.6). Placental efficiency (fetal weight/placental weight) did not differ for GHS-G1 vs. GTN-G1 (Table 4.5). Placental efficiency was observed to differ between replicates (Table 4.7).

Fetal weight was similar between GHS-G1 and GTN-G1, although an effect of replicate was observed (Table 4.6). Overall, male fetuses ( $124.24 \pm 3.27$  g) were larger ( $P < 0.001$ ) than female fetuses ( $116.62 \pm 3.28$  g), and male GHS-G1 fetuses ( $129.00 \pm 4.77$  g) were larger ( $P < 0.012$ ) than male GTN-G1 fetuses ( $119.47 \pm 4.47$  g), female GHS-G1 fetuses ( $117.42 \pm 4.74$ ), and female GTN-G1 fetuses ( $115.82 \pm 4.52$  g). Similarly, male fetuses ( $16.49 \pm 0.14$  cm) were longer ( $P < 0.001$ ) than female fetuses ( $16.26 \pm 0.14$  cm),

while male GHS-G1 fetuses ( $16.72 \pm 0.21$  cm) were longer ( $P < 0.018$ ) than male GTN-G1 fetuses ( $16.25 \pm 0.19$  cm), female GHS-G1 fetuses ( $16.34 \pm 0.20$  cm), and female GTN-G1 fetuses ( $16.18 \pm 0.19$  cm). GHS-G1 and GTN-G1 fetuses did not differ in length and no effect of replicate was observed (Table 4.6). GHS-G1 females (49.8) tended ( $P < 0.09$ ) to have a lower percent of male fetuses than GTN-G1 females (54.5). Female anogenital distance did not differ between fetuses from GHS-G1 females and GTN-G1 females (Table 4.6). Female anogenital distance was not observed to differ between replicates (Table 4.6). Male anogenital distance was similar between fetuses from GHS-G1 and GTN-G1 females (Table 4.6). Male anogenital distance was different between fetuses from different replicates (Table 4.6).

#### Progesterone Analysis and Age at Puberty 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  $n=12/23$ ). Age at puberty was similar ( $P > 0.10$ ) between GHS-G1 ( $209.71 \pm 2.37$  d) and GTN-G1 ( $207.74 \pm 1.97$  d) females, although an effect of replicate was observed between replicates 1, 2, 3, and 4 ( $198.01 \pm 3.49$  vs.  $211.63 \pm 2.63$  vs.  $210.29 \pm 3.52$  vs.  $214.97 \pm 2.56$  d), respectively.

## DISCUSSION

In this study, the transgenerational effects of *in utero* heat stress on reproductive characteristics in gilts were studied. In order to develop *in utero* heat stressed animals, pregnant dams were housed under heat stress or thermoneutral conditions in the Brody Environmental Chambers from days 30 to 60 of gestation. Dams were moved to the MU Swine Teaching Farm for further farrowing. Litter size was similar between GHS and GTN treatments. Dams were heat stressed during mid gestation, by which point implantation has already occurred; as such, dams were not heat stressed during the critical period when litter size is first determined. This finding is in accordance with Williams et al. (2013) who also found no difference in litter size when pregnant gilts were heat stressed 20 days prior to farrowing. Although the gilts in the Williams et al. (2013) study were heat stressed later in gestation than dams in this study, it can be deduced that by exposing pregnant females to heat stress past the period of implantation, dams can be protected from a reduction in litter size. Similarly, piglet birth weights did not differ between treatments, nor did subsequent weights at 3 days, 1 week, 2 weeks, and 3 weeks (weaning) of age. Although milk production was not measured, it can be concluded that HS sows had no reduction in milk output due to the similar growth characteristics of piglets born to GHS or GTN sows. Male piglets also tended to weigh more than female piglets at birth. This finding was similar to that of Baxter et al. (2012) in which they determined that males weighed significantly more than females at birth. This tendency of weight difference was minimized throughout the duration of suckling, by which point males and females were of similar weight, indicating the ability of females to grow and develop at a similar rate as males.

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. No significant effects of *in utero* treatment were detected on litter, placental, ovarian, fetal, and uterine characteristics after slaughter data collection. 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 (Table 4.7). 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, placental attachment area, and fetal weight also showed effects of replicate (Table 4.7). These replicate effects may be attributed to one of two protocol deviations: differing sires and the inability to manage 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 successfully 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, in most instances 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-linked 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<sub>1</sub> female to compensate for any damage that may have occurred to the maternal X-chromosome during development of the F<sub>0</sub> ovary *in utero*. Conversely, the hemizygosity for the X-chromosome in the F<sub>1</sub> 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<sub>0</sub> 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 in litter characteristics such as uterine weight, uterine horn length, number of 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.

Table 4.1. G1 gilt housing location from birth to breeding/gestation.<sup>1</sup>

Replicate	Location			
	Farrowing	MU Swine Teaching Farm	MOF (Heat Checking)	MOF (Breeding/Gestation)
1	December 2015	January-April 2016	May-June 2016	September-October 2016
2	January 2016	February-May 2016	June-July 2016	October-November 2016
3	April 2016	May-September 2016	October-November 2016	January-February 2017
4	May 2016	June-October 2016	November-December 2016	February-March 2017

<sup>1</sup>Months and locations during which G1 females were on trial.

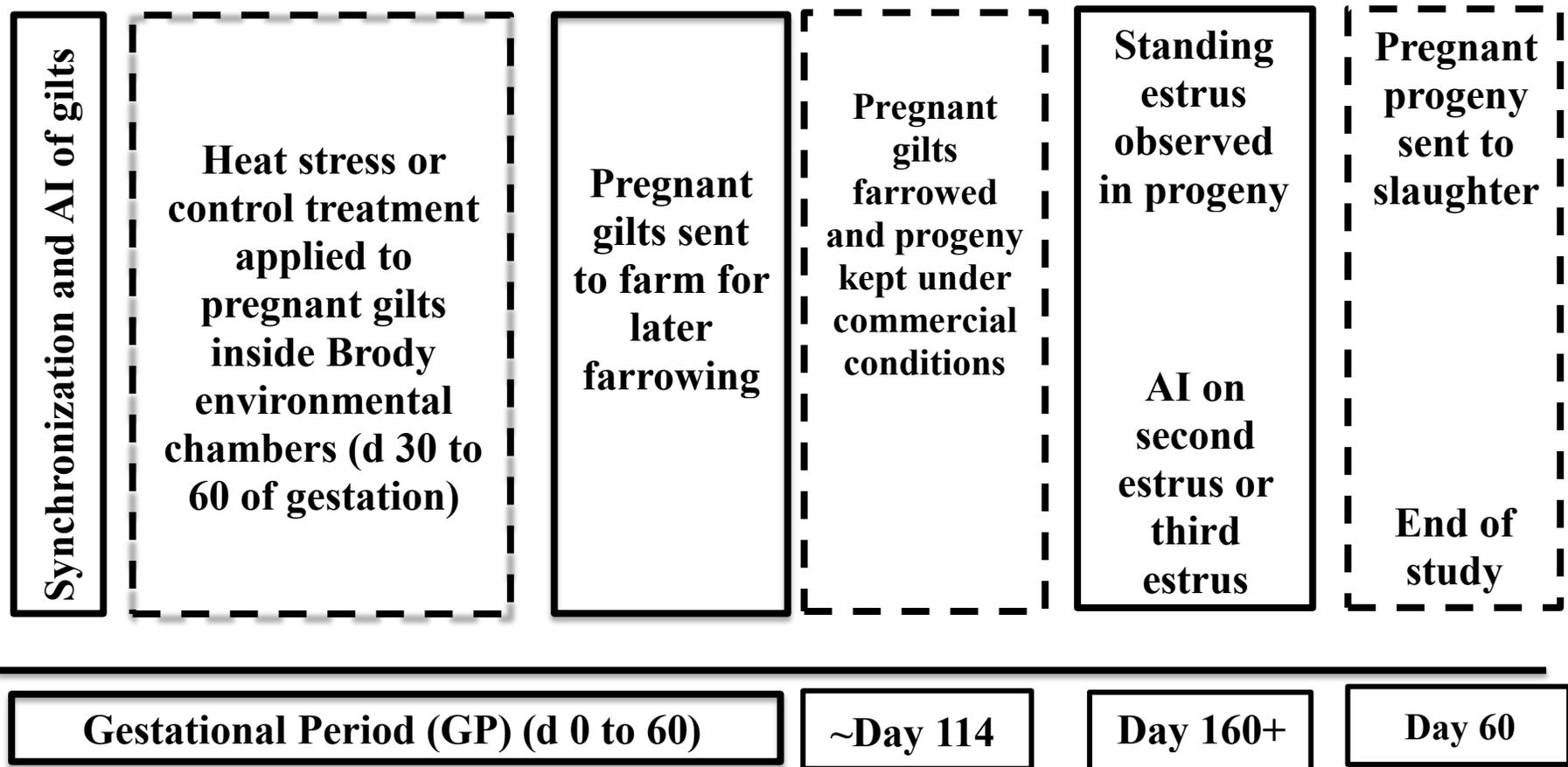


Figure 4.1. Experimental timeline for G1 gilts exposed to HS or TN conditions *in utero*.

Table 4.2. Composition of gestation/breeding and lactation diets (% as-fed basis)

Item	Gestation/Breeding diet	Lactation diet
Corn	69.4	64.6
Soybean meal (48%)	15	28.3
Soy hulls	10	-
Choice white grease	1	2.5
Dicalcium phosphate	2.3	2.4
Limestone	1	0.8
Salt	0.5	0.5
Lysine	-	0.1
Vitamin premixes	0.5	0.5
Trace mineral premix	0.2	0.2
Zinc and biotin premixes	0.2	0.2

Table 4.3. Least squares means for the effects of heat stress from weeks 4 to 8 of gestation at farrowing in gilts.

Item	Treatment (Trt)		<i>P</i> -value <sup>1</sup>
	GHS	GTN	Trt
Number of pigs	23	25	-
Number of piglets	314	346	-
<b>Litter measures</b>			
Litter size	13.6±0.7	13.6±0.6	0.636
<b>Weight measures</b>			
Birth weight, kg	1.26±0.03	1.28±0.03	0.587
Day 3 weight, kg	1.58±0.04	1.59±0.04	0.985
Day 7 weight, kg	2.29±0.06	2.30±0.06	0.977
Day 14 weight, kg	3.61±0.10	3.71±0.10	0.489
Wean weight, kg	5.37±0.16	5.63±0.15	0.249
<b>Neonatal gonad measurements</b>			
Female anogenital distance, mm	3.14±0.69	3.03±0.66	0.208
Male anogenital distance, mm	95.66±0.68	94.26±0.65	0.208

<sup>1</sup>Means differ at  $P < 0.05$

Table 4.4. Least squares means for the effects of replicate at farrowing in gilts exposed to heat stress or thermoneutral conditions from weeks 4 to 8 of gestation.

Item	Replicate (Rep)				<i>P</i> -value <sup>1</sup>
	1	2	3	4	Rep
Number of pigs	12	12	12	12	-
Number of piglets	163	157	167	173	-
<b>Litter measures</b>					
Litter size	13.00±0.90	13.08±0.90	13.92±0.90	14.43±0.91	0.958
<b>Weight measures</b>					
Birth weight, kg	1.25±0.04	1.26±0.04	1.30±0.04	1.26±0.04	0.771
Day 3 weight, kg	1.58±0.06	1.53±0.05	1.63±0.05	1.61±0.05	0.605
Day 7 weight, kg	2.19±0.09	2.26±0.09	2.43±0.09	2.30±0.09	0.298
Day 14 weight, kg	3.47±0.14	3.63±0.14	3.89±0.13	3.65±0.14	0.210
Wean weight, kg	5.26±0.22	5.37±0.22	5.79±0.22	5.58±0.22	0.339
<b>Neonatal gonad measurements</b>					
Female anogenital distance, mm	2.60±0.98	2.79±0.94	3.11±0.97	3.83±0.95	-
Male anogenital distance, mm	94.52±0.94	99.69±0.98	93.01±0.91	92.64±0.93	-

<sup>1</sup>Means differ at *P*<0.05

Table 4.5. Least squares means for the transgenerational effects of *in utero* heat stress from weeks 4 to 8 of gestation on uterine, ovarian, litter, and placental measures in gilts.

Item	Treatment (Trt)		P-value <sup>1</sup>
	GHS-G1	GTN-G1	
Number of pigs	50	55	-
Number of fetuses	629	716	-
<b>Uterine measures</b>			
Total uterine weight, kg	12.69±0.64	12.38±0.57	0.724
Weight of uterine contents, kg	8.75±0.57	8.73±0.51	0.976
Uterine length, cm	372.21±12.28	361.42±11.03	0.518
Implantation length, cm	21.46±0.74	21.12±0.70	0.738
<b>Ovarian measures</b>			
Ovarian weight, g	17.08±0.48	16.77±0.44	0.638
Number of CL	16.29±0.45	17.06±0.39	0.204
Number of follicles	34.58±1.60	35.32±1.31	0.722
<b>Litter measures</b>			
Number of viable fetuses	12.3±0.6	12.7±0.5	0.575
Number of nonviable fetuses	0.3±0.1	0.2±0.1	0.597
Survival (Number fetuses/number CL), %	76.8±3.7	74.5±3.3	0.651
<b>Placental measures</b>			
Placental attachment area, cm <sup>2</sup>	357.45±14.55	343.87±12.38	0.478
Combined weight (fluid, placental, fetal), g	386.05±15.46	370.44±14.67	0.468
Placental weight, g	168.3±8.0	157.3±7.6	0.326
Fluid weight, g	94.09±6.29	96.65±5.89	0.326
Efficiency (Fetal weight/placental weight), %	81.1±3.1	82.3±2.9	0.772

<sup>1</sup>Means differ at  $P < 0.05$

Table 4.6. Least squares means for the effects of replicate and transgenerational effects of *in utero* heat stress from weeks 4 to 8 of gestation on fetal and gonadal development in gilts.

Item	Treatment (Trt)		Replicate				P-value <sup>1</sup>	
	GHS-G1	GTN-G1	1	2	3	4	Trt	Rep
Number of pigs	50	55	15	36	17	38	-	-
Number of fetuses	629	716	163	416	247	530	-	-
<b>Fetal measures</b>								
Fetal weight, g	123.21±4.62	117.64±4.39	104.55±6.57	113.92±6.12	129.47±6.86	133.77±5.91	0.388	0.009
Fetal length, cm	16.53±0.20	16.22±0.19	16.00±0.28	16.18±0.26	16.52±0.30	16.79±0.25	0.267	0.18
<b>Fetal gonad measures</b>								
Female anogenital distance, mm	4.783±0.186	4.678±0.189	4.611±0.391	5.012±0.214	4.828±0.263	4.471±0.200	0.671	0.300
Male anogenital distance, mm	37.950±0.697	37.134±0.682	34.208±1.460	40.886±0.781	38.303±1.049	36.768±0.702	0.368	<0.001

<sup>1</sup>Means differ at  $P < 0.05$

Table 4.7. Least squares means for the effects of replicate and sex on uterine, ovarian, litter, and placental measures in gilts exposed to *in utero* heat stress or thermoneutral conditions from weeks 4 to 8 of gestation.

Item	Replicate				Sex		P-value <sup>1</sup>	
	1	2	3	4	Male	Female	Rep	Sex
Number of pigs	15	36	17	38	-	-	-	-
Number of fetuses	163	416	247	530	492	450	-	-
<b>Uterine measures</b>								
Total uterine weight, kg	9.87±0.97	11.66±0.71	15.00±1.00	13.59±0.69	-	-	0.003	-
Weight of uterine contents, kg	5.83±0.87	8.42±0.64	11.06±0.90	9.66±0.62	-	-	0.001	-
Uterine length, cm	362.89±18.41	331.61±14.09	430.33±19.12	342.43±13.64	-	-	0.002	-
Implantation length, cm	18.25±1.07	18.38±0.96	24.79±1.10	23.76±0.92	-	-	<0.001	-
<b>Ovarian measures</b>								
Ovarian weight, g	16.37±0.72	16.76±0.57	16.61±0.75	17.96±0.56	-	-	0.259	-
Number of CL <sup>2</sup>	16.72±0.68	15.71±0.47	16.59±0.71	17.66±0.45	-	-	0.044	-
Number of follicles	44.82±2.51	36.95±1.47	25.99±2.57	32.03±1.40	-	-	<0.001	-
<b>Litter measures</b>								
Number of viable fetuses	10.8±0.9	11.5±0.6	14.2±0.9	13.4±0.6	-	-	0.01	-
Number of nonviable fetuses	0.1±0.2	0.1±0.1	0.1±0.2	0.5±0.1	-	-	0.04	-
Survival (Number fetuses/number CL), %	64.6±5.6	72.9±4.3	88.9±5.8	76.3±4.1	-	-	0.04	-
<b>Placental measures</b>								
Placental attachment area, cm <sup>2</sup>	304.51±22.58	297.58±15.42	407.91±22.88	392.64±14.03	-	-	<0.001	-
Combined weight (fluid, placental, fetal), g	316.85±22.23	325.45±20.34	444.58±22.99	426.08±19.61	388.65±11.15	367.83±11.17	0.0001	0.003
Placental weight, g	148.3±11.5	133.6±10.6	196.6±11.9	172.8±10.2	170.1±5.7	155.5±5.7	0.002	<0.001
Fluid weight, g	64.71±9.38	78.36±8.03	117.04±9.37	121.35±7.68	-	-	<0.001	-
Efficiency (Fetal weight/placental weight), %	77.8±4.5	92.7±4.0	69.2±4.6	87.1±3.8	-	-	0.002	-

<sup>1</sup>Means differ at P<0.05

<sup>2</sup>CL: Corpora lutea

## **CHAPTER 5**

### **CONCLUSIONS, IMPLICATIONS, AND DIRECTIONS FOR FUTURE RESEARCH**

#### **CONCLUSIONS**

Females housed under heat stress conditions exhibited a physiological response in the attempt to cope with the thermal stress conditions imposed upon them. Heat stressed gilts expressed an increase in both rectal temperature and skin temperature, as well as an increase in respiration rate. By engaging a physiological response to their environment, gilts attempted to maintain a normal core body temperature and protect the developing pregnancy. Although heat stressed, no difference in feed intake values were detected. Gilts were limit fed throughout gestation, and it is possible that because gilts were fed a predetermined amount of feed daily, no difference in feed intake occurred.

No difference in reproductive performance was detected between gilts heat stressed from 30 to 60 days of gestation versus those kept in thermoneutral conditions during this same period. For instance, uterine, ovarian, litter, and placental measures were all similar between treatments. Fetal testes and ovarian development were also similar between GHS and GTN treatments. A study conducted in mice (Desaulniers et al., 2016) suggested that anogenital distance may be sensitive to heat stress, although no such results were observed during this study. Similarly, anogenital distances of piglets from

dams who had been housed in the Environmental Chambers did not differ between treatments. This lack of difference may have been caused by the difficult nature of measuring fetal and neonatal anogenital distance, potentially resulting in inaccurate data. At farrowing, it was also determined that GHS and GTN litters were of similar size, and no significant difference of birth weight was detected between piglets born to GTN or GHS females, although males tended to be heavier at birth. In general, male neonates tend to be of heavier weight commercially, as well.

The lack of significant results directly following heat stress may be attributed to an acclimation to the gestational heat stress treatment or the short treatment period. Under commercial conditions, temperatures are consistently varied and high ambient temperatures may last throughout the long summer months. The heat stress treatment applied to GHS gilts during this trial followed a diurnal pattern. This diurnal pattern consisted of a period of cooler temperatures during the overnight hours. Under commercial conditions, temperatures do not follow a set pattern in which the temperature drops considerably overnight.

After dissection of GHS-G1 and GTN-G1 pregnant reproductive tracts, many gross reproductive measures that may be indicative of reproductive performance did not vary. These parameters included uterine length, the number of CL and follicles, the number of viable fetuses, and implantation length. Replicate effects were detected for the above measures. These effects of replicate may have been influenced by the inability to effectively maintain the temperature of the gestation barn during the winter and summer months, or by the need to change the sire with which GHS-G1 and GTN-G1 females

were bred halfway through the study. Although replicate effects were detected, there was no detectable interaction between the GHS and GTN treatments and replicate.

Overall, male fetuses of both the GHS-G1 and GTN-G1 treatments were heavier and longer than female fetuses. Similarly, male GHS-G1 fetuses were heavier than male GTN-G1 fetuses. Male GHS-G1 fetuses also tended to have heavier placentas. This significant difference of body weight suggests an X-linked trait, in which damage to the X-chromosome at the molecular level may manifest as a difference in the male fetus. The QTL for body weight has been determined to lie on the X-chromosome, lending more credence to the hypothesis that molecular damage occurred within the developing fetal ovary.

## IMPLICATIONS

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 does not justify exposing females to heat stress during gestation. 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.

## **FURTHER RESEARCH**

Although it was determined that *in utero* heat stress affected ovarian development, the mechanism behind this effect is still unknown. It could be suggested that ovaries collected from females exposed to *in utero* heat stress and ovaries from those housed in thermoneutral conditions undergo genetic sequencing in order to further understand the ways in which the ovary was damaged. Understanding changes to the genetic fiber of the porcine ovary has the ability to allow producers to make informed decisions to better improve swine production throughout the world.

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## VITA

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