

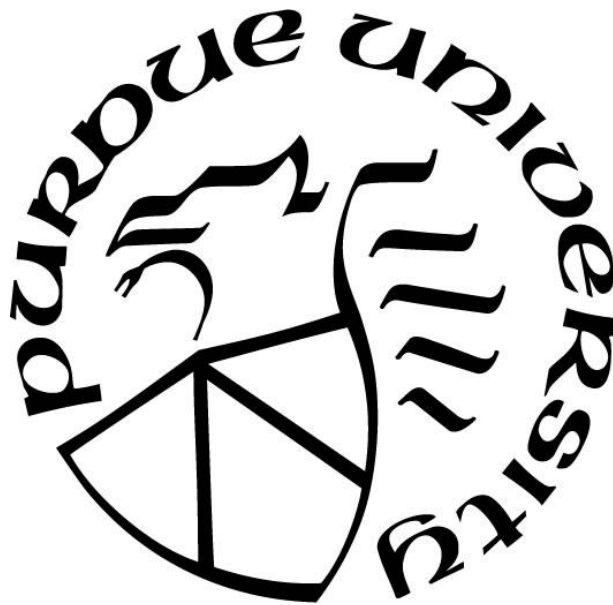
**USE OF ELECTRONICALLY-CONTROLLED FLOOR COOLING PADS
DURING HEAT STRESS IN THERMOREGULATORY AND
REPRODUCTIVE PERFORMANCES IN SWINE**

by
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ABSTRACT

Substantial economic losses occur in the swine industry during periods of high ambient temperatures. Heat stress produces physiological changes such as increased body temperature and respiration rate resulting in production losses from decreased reproductive performance, growth rate and feed intake. Heat stress in growing gilts delays puberty and decreases ovarian follicle numbers. In boars heat stress decreases semen quality. Electronically-controlled floor cooling pads were designed and constructed to assist pigs with thermoregulation by removing excess heat from pigs in a production facility. Based on this study, experiments were conducted to further investigate the effects of electronically-controlled cooling pads on physiological and reproductive performances in gilts and boars. A study was conducted on limit-fed gilts at 32°C and 35°C during short-term heat stress. Gilts exposed to short term heat stress at 32°C and 35°C had increased respiration rate, vaginal temperature and skin temperature. Gilts on electronically-controlled cooling pads exposed to short term heat stress at 35°C were able to minimize negative impacts of HS such as reduced respiration rate and vaginal temperature. A study was conducted with 24 boars which were exposed to cyclical heat stress for a duration of 3 days at 32°C and 35°C. Boars exposed to cyclical heat stress for 3 consecutive days at 32°C or 35°C which increased respiration rate and body temperature followed by a decrease in semen quality over several weeks. Boars cooled with electronically-controlled floor cooling pads had reduced physiological effects of heat stress as well as consistent semen quality post HS. The use of electronically controlled floor cooling pads have implications towards minimizing or removing the negative impacts of heat stress in gilts and boars.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Heat stress has negative implications on an animal's growth and reproductive performance, this in turn, has major impacts on profitability in the swine industry. Gilt development, or how a young female is managed from birth to puberty, is a critical driver in their lifetime reproductive performance and longevity (Holst and Leuwerke, 2017; Patterson and Foxcroft, 2019). Subsequently, this affects the profitability of sow farms. Currently in the U.S., over 90% of the sows and gilts in commercial production are bred using artificial insemination (AI) (Riesenbeck et al., 2011). To accomplish this, boars are housed off site at AI studs to provide the semen for use in artificial insemination. The boars used for artificial insemination impact farrowing rate and litter size, ultimately impacting profitability at the sow farm. Over the past few decades, advancements in boar and semen management have led to improvements in semen quality and storage, ultimately increasing production in sow farms. Despite these advancements, the swine industry faces many challenges regarding reproductive performance of the males and females used in breeding. Heat stress is one of the current main challenges within the swine industry affecting gilt development and their future performance as well as a reduction in semen quality and sperm production in boars (Evans and Doherty, 2001; Parrish et al., 2017).

Heat stress causes swine to alter their normal behavior and physiology to maintain homeostasis and has negative consequences on the animal's growth and reproductive performance. Major concerns for gilts during seasons of high temperatures is estrus expression and conception failures (Knox et al., 2013). Culling rates of females within the swine industry are the highest in gilts ranging from 38.5-51.1% (Li et al., 2018; Roongsitthichai et al., 2013). Li et al. (2018) and Roongsitthichai et al. (2013) found a high incidence of sows only producing one litter as well as 30% of the breeding herd being culled by parity 3 (Engblom et al., 2007). For a positive net economic return on investment on replacement gilts, females need to produce 3-5 litters (Stalder et al., 2003; Engblom et al., 2016; Gruhot et al., 2017; Rohrer et al., 2017; Patterson and Foxcroft, 2019). The primary reason of culling sows prior to parity 3 is due to reproductive failure (Balogh et al., 2015; Engblom et al., 2007; Segura-Correa et al., 2011; Tani et al., 201; Engblom et al., 2007). Balogh et al. (2015) documented 40-51% of the breeding herd being culled was due to

insufficient reproductive performance with high proportion of breeding herd culls due to delayed puberty (Roongsitthichai et al., 2013). Regarding boars, heat stress reduces semen output and quality of developing sperm cells at various stages of sperm cell development (Knox et al., 2008).

1.2 Gilt Reproductive Development

Gilts management from birth to puberty is a critical factor that plays into sow longevity and productivity (Patterson and Foxcroft, 2019). Gilt development starts at birth (Patterson and Foxcroft, 2019) where the gilt's reproductive tract is in an immature state and continues to develop during the post-natal period. For example, at birth, the gilt's ovary is immature and incapable of ovulation, but contains all of the oocytes that the female will ever have stored in primordial, primary and secondary follicles (Christenson et al., 1985). Additionally, the uterus is immature at birth and will grow in size and develop uterine tissue layers and glands from birth until 6-8 weeks after birth (Bartol et al., 2017).

Puberty can be defined in many ways including the first ovulation, the onset of regular cyclicity, or the ability to conceive and maintain pregnancy. Gilts typically begin cyclicity at 5-7 months of age and a body weight of about 80-120 kg (Roongsitthichai et al., 2013, Geisert, 2020). The onset of cyclicity occurs when the female experiences a decrease in sensitivity to negative feedback on GnRH, resulting in follicular growth (tertiary follicles) producing large quantities of the hormone estradiol (Soede et al., 2011). When estradiol concentrations peak, a positive feedback loop is initiated which results in an LH surge that initiates ovulation. This is frequently defined as puberty.

After the pubertal ovulation, the gilt will begin a regular estrous cycle that is approximately 21 days in length (18-22 days) consisting of a follicular phase of 5-7 days and luteal phase of 13-15 days (Soede et al., 2011). The follicular phase is characterized by growing follicles that acquire dominance, the LH surge and ending with ovulation. The follicular phase can also be divided into the proestrus and estrus periods based on the animal's behaviors where they exhibit standing heat during estrus. Formation of ovulatory follicles occurs in the proestrus period where estradiol (E2) secretion is increasing. This is followed by the estrus period where the gilt exhibits sexual receptivity and ovulation occurs. The estrus period lasts 2-3 days. Following ovulation, the luteal phase begins which is longer in duration than the follicular phase and is characterized by the

development of the corpus luteum (CL) on the ovary. The luteal phase can be divided into the metestrus and diestrus periods based on the timing of development of the CL. Metestrus immediately follows ovulation where the corpus hemorrhagicum (CH) is present on the ovary and the CL is beginning to develop. Once the CL is completely formed and secreting the hormone progesterone the animal is in diestrus.

The first pubertal estrus is generally less fertile; this estrus typically has fewer follicles developing on the ovary resulting in fewer ovulations (Geisert, 2020). This impacts management practices as farms usually will not inseminate gilts at their first estrus, but instead breed them at their second or third estrus (Geisert, 2020; Kraeling et al., 2015). This management practice has resulted in an increased ovulation rate which has led to larger litter sizes (Knox et al., 2019; Young et al., 1990; MacPherson et al., 1977; Grigoriadis et al., 2001; Gaughan et al., 1997; Beltranena et al., 1991).

In order to time artificial insemination with ovulation, sow farms perform estrus detection to identify when females are in estrus to know when to inseminate. Estrus detection is done daily with use of a heat check boar (Knox, 2016) starting in gilts around 140-160 days of age (Miller et al. 2011). Gilts who are in estrus show interest in the boar and stand with stiffened muscles and erect ears (Pedersen, 2007).

However, predicting the time that ovulation occurs during the estrus period is very difficult. On average, females ovulate about 2/3 of the way through the estrus period. However, there is great variation in this with times ranging from less than 24 hours to 64 hours after the onset of estrus (Kemp and Soede, 1996). Fertility is maximal if semen is placed into the female's reproductive tract 0-24 hours ahead of ovulation (Kemp and Soede, 1996). Semen used for artificial insemination has a lifespan of approximately 24 hours in the gilt's reproductive tract, therefore, industry practice is to inseminate two-three times during estrus at an interval of 12-24 hours (Geisert, 2020). This ensures viable sperm in the oviduct at time of ovulation to allow for fertilization of oocytes by sperm that will occur in the oviduct (Geisert, 2020).

1.2.1 Factors That Affect Puberty in Females

Breed, nutrition, exposure to boars and season can have an influence on age at puberty. Durocs reach puberty at older ages in comparison to Hampshire, Large Whites and Landrace

(Evans and Doherty, 2001). Crossbred gilts reach puberty at a younger age and have fewer non-productive days (Bidanel et al., 1996). Restricting energy intake as well as reducing feed intake in gilts can delay puberty (den Hartog and van Kempen, 1980, den Hartog and Noordewier, 1984). Gilts exposed to boars by either visual or physical contact will reach puberty at a younger age. Roongsitthichai et al. (2014) found growth rate and boar contact had an effect on first observed estrus. Gilts that had their first contact with a boar <150 days of age had an earlier first standing estrus compared to gilts exposed to boars >150 days (Roongsitthichai et al., 2014). Patterson and Foxcroft (2019) reported that 12-43% of gilts are noncyclic after 30 days of boar exposure. Reasons for these percentages could be due to an ineffective estrus detection, silent estrus, disorders in sexual organs or gilt being prepubertal (Stancic et al., 2011).

1.2.2 Reproductive Longevity in Females

Reproductive longevity in gilts is dependent on age at puberty and first service and can be an indicator of future sow reproductive performance and longevity (Tart et al., 2013; Rohrer et al., 2017). Gilts that reach puberty at younger ages have increased lifetime productivity as indicated by increased productive days and increasing the likelihood of gilts farrowing multiple parities, resulting in being culled at a higher parity (Koketu et al., 2017; Li et al., 2018; Saito et al., 2011; Serenius and Stalder, 2007; Tart et al., 2013; Sterning et al., 1998; Patterson et al., 2010; Roongsitthichai et al., 2013; Wijesena et al., 2017). Gilts which reach puberty at an earlier age tend to have their first conception at a younger age. Earlier age of first conception results in gilts who will produce more litters before removal resulting an increase productive life (Knauer et al. 2010; Koketsu et al., 1999; Yazdi et al., 2000). Upon farrowing, gilts who reach puberty later will have a greater wean-to-estrus interval after their first litter (Sterning et al., 1998), increasing their non-productive days. Saito et al. (2011) found that gilts with an earlier first mating had a higher parity removal, reproductive herd days, and lifetime total piglets born alive resulting in a greater longevity and efficiency.

1.3 Boar Reproduction

Reproductive efficiency within breeding herds begins with fertile sperm meeting fertile eggs within the female reproductive tract. The process of producing fertile sperm is highly

dependent on boar development and management in addition to the boar's inherent fertility. Boar fertility is measured in farrowing rate and total born or number born alive. Since there is no benchmark test for fertility, the current best predictor of a boar's fertility is evaluation of semen quality of individual ejaculates. Flowers (1997) found that ejaculates with greater than 60% total motility had similar fertility as determined by farrowing rate and total born. Therefore, total motility has been used as a predictor of the fertility of the ejaculate. Althouse (1998) also looked at sperm morphology as an indicator of fertility and found that ejaculates with greater than 80% normal spermatozoa did not differ in fertility. Taken together, semen quality cut offs have been created for individual ejaculates to be used in artificial insemination programs, where decreases in semen quality below these thresholds are known to have reduced fertility. However, semen quality does not explain all of the differences in fertility among boars as boar fertility can be affected by many factors including gestational and neonatal environments and management through puberty and into adulthood.

1.3.1 Boar Reproductive Development

Boar reproductive development begins when they are a fetus during gestation. Genes are expressed on the Y chromosome that allow germ cells to begin to divide and differentiate 20-40 days into gestation (Knox, 2001). Around day 60 of gestation testicles start to descend from the abdominal cavity into the scrotum (Knox, 2001). The number of sperm cells that a boar can produce is related to the number of Sertoli cells in the testicle. The Sertoli cells are commonly referred to as the "nurse cell" and function to develop sperm cells from round germ cells into elongated spermatozoa. Mitosis of Sertoli cells begins during gestation and continues into the post-natal period (McCoard et al., 2003; Flowers, 2015; Parrish et al., 2017). Griffin et al. (2006) showed that sperm production was greater in boars that were raised in litters of <10 piglets due to strategic cross fostering, suggesting that management during the neonatal period can impact adult sperm production.

The second, and less pronounced, phase of proliferation of Sertoli cells occurs just prior to puberty, around 3-4 months of age (Parrish et al., 2017; França et al., 2000). This proliferative phase is associated with the animal's pre-pubertal increase in FSH and testosterone resulting in increases in testicular size, Leydig cell number, and the onset of spermatogenesis. Follicle

stimulating hormone (FSH) produced around 3-4 months of age results in regulation of the second phase of proliferation in Sertoli cells prior to puberty that determines the total number of sperm that can be produced by the testes (Franca et al., 2005). As boars age, their sperm production continues to increase, reaching a plateau about 15-18 months of age.

Onset of puberty can be defined in various ways such as age when sexual behavior traits are expressed, age at first ejaculation, age when spermatozoa first appear in ejaculate, or age when ejaculate contains a threshold number of spermatozoa. Testosterone levels are relatively low in boars until about 4-5 months of age (Knox, 2001). Androgens, such as testosterone, play a role in male associated characteristics or behavior such as aggressiveness and libido (Knox, 2001). The first wave of spermatozoa begin production in the testicles at 5- 6 months of age but are not ejaculated for another 5-7 weeks to allow for maturation of the spermatozoa in the testicle and epididymis (Flowers, 2015). Training of boars for semen collection occurs between 7-9 months of age (Knox et al., 2008). As the boar continues to mature, from 6 months of age to about 18 months, sperm production increases due to an increase in volume of ejaculate and sperm cell concentration (Knox, 2001). Once the boar is considered fully mature (18 months of age) sperm production plateaus.

1.3.2 Spermatogenesis

Spermatogenesis is a continuous process of spermatogonia developing into spermatozoa that will appear in ejaculate approximately 45 days after the start of development. Spermatogenesis starts within the seminiferous tubules of the testicle. The seminiferous tubule consists of different segments that contain germ cells which are in different stages of development as well as Sertoli cells that support the germ cells through mitosis and meiosis (Parrish et al., 2017). In males, germ cells undergo a series of mitotic divisions that are followed by meiosis (Parrish et al., 2017). Spermatogonia, the pre-mature sperm cell, begin to develop and mature along the wall of the seminiferous tubule (Senger, 2012). As sperm cells mature, they migrate from the wall of the seminiferous tubule towards the lumen through the Sertoli cell cytoplasm. This process of the spermatogonia developing into a spermatozoa with an elongated head with a tail to be released into the lumen takes approximately 34-36 days. Spermatozoa leave the testicle via the rete testis into the caput (head) of the epididymis in a non-motile state. At this time, proteins are incorporated

into the membrane of the sperm cell that are essential for fertilizing the egg (Knox, 2001). Sperm cells then travel to the corpus of the epididymis where they gain their motility, finally traveling to the cauda epididymis to be stored for ejaculation. Additional proteins are added to the spermatozoa when in the cauda epididymis that play an important role in sperm fertility (Knox, 2001). Spermatozoa spend approximately 12-14 days in the epididymis. Full motility and fertilization capability of spermatozoa are obtained when diluted with seminal plasma prior to ejaculation (Knox, 2001).

Spermatogenesis is controlled by endocrine stimulations of growth factors, cytokines, and transcription factors by the Leydig and Sertoli cells in the testicle (Geisert, 2020). Spermatogenesis is controlled by the secretion of gonadotropic releasing hormone (GnRH) causing secretion of follicular stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior lobe of the pituitary. These gonadotropins stimulate the secretion of testosterone and estradiol from the testicular cells (Senger, 2012). LH acts on Leydig cells located within the testes, but outside the seminiferous tubules causing the secretion of testosterone that regulates Sertoli cell's function (Parrish et al., 2017). This testosterone is converted into estradiol and dihydrotestosterone by the Sertoli cell in response to the hormone FSH.

1.3.3 Factors That Affect Spermatogenesis

As reviewed by Flowers (2015), temperature, photoperiod, nutrition, collection frequency and housing all have an influence on immature sperm cells leaving the Sertoli cells. Spermatogenesis is highly dependent on temperature and is negatively impacted under elevated temperatures. Boar's have the capability to regulate testicular temperature. Cooling is achieved by the boar's testes residing outside the body cavity, low amounts of subcutaneous fat that reduces heat retainment and how the arterial and venous blood vessels enter and leave the testes (Parrish et al., 2017; Knox, 2001). This mechanism of blood flow to and from the testes is called the pampiniform plexus and consists of arterial blood vessels leaving the body anastomosing with venous blood vessels heading into the body, cooling the arterial blood from 102°F (body temperature of the boar) to 98°F (Knox, 2001). Increasing collection frequency of boars, beyond once a week, will produce fewer total sperm. Additionally, boars collected less than once a week, have an increased percentage of non-fertile and degenerate cells present in the ejaculate (Knox,

2001). Collection frequency of boars has a direct impact on spermatogenesis and semen quality. Knox et al., (2008) studied that in the US and Canada boars' resting period was typically 5-7 days across studs. Therefore, boars under 9 months of age should only be collected once a week and older boars should not be collected more than twice a week (Knox, 2001). Knox (2001) found that boars collected twice a week produced about 50 billion sperm per ejaculate and if collected once every 2 weeks produced up to 100 billion sperm cells in the ejaculate. Ideally boars should be collected once a week to assist in the maintenance of spermatogenesis as well as provide high quality semen.

1.3.4 Reproductive Longevity in Males

Due to constant improvements of genetics within the swine industry boars have a high turnover rate within studs. Majority of boars are culled within 1-2 years (Knox et al., 2008, Wang, 2017) due to lameness, semen quality and genetic improvement. Culling rate of boars is as high as 59% a year, with one likely cause is the overuse of young boars resulting in decreased semen quality.

1.4 Heat Stress

Heat stress disrupts a pig's core body temperature above their critical limit which has negative implications on performance. Heat stress is a critical concern in the swine industry effecting both females and males in breeding herds. Heat stress can alter an animal's normal behavior and physiology as they try to maintain homeostasis. Heat stress occurs when ambient temperatures exceed an animal's thermoneutral neutral zone (TNZ). In summer months, as temperature and humidity are elevated, reproductive efficiency within breeding herds is decreased significantly, resulting in both immediate and long-term production issues (St. Pierre et al., 2003). Boar studs report increased rates of unusable ("trashed") ejaculates during the summer months (Knox et al., 2008) resulting in either more boars being required to breed the same number of females or boars to be collected more frequently during the summer.

1.4.1 Thermoregulation and the Thermoneutral Zone

With a pig's inability to sweat they control heat loss by regulating their blood flow throughout their body. Providing an environment that allows a pig to maintain their normal body temperature and control heat loss during times of high ambient temperatures will result in optimal production. The thermal neutral zone is defined as a range in temperatures at which an animal can maintain internal temperatures with minimal metabolic regulation. The thermal neutral zone for gilts and boars (gilts; 70 to 100 kgs, boars; >70 kgs) ranges from 10-25°C (FASS, 2010). Gilts weighing 60-130 kgs can maintain their thermoneutral zone in a solid, wet concrete floor with no draft at 25°C or with a moderate draft up to 31°C (Lammers et al., 2007). Gilts (70 to 100 kgs) have an upper extreme of 35°C (FASS, 2010). Boars (> 280 lbs.) can maintain TNZ at 83°F in a solid, wet, concrete floor with no draft and 33°C with a moderate draft (Lammers et al., 2007). Boars, 70 to 100 kgs have an upper extreme limit of 35°C while boars >100 kgs. have an upper extreme limit of 32°C (FASS, 2010).

1.4.2 Animal's Response to Heat Stress

When pigs are not in their TNZ, they tend to regulate their body temperature via alterations to their physiology and behavior. These responses to heat stress can consist of pigs laying apart, maximizing contact with concrete, panting (increasing respiration rate), decreasing feed intake, and increasing water intake (Lammers et al., 2007). These responses to heat stress are a way the pig is able to maintain homeostasis during times of high ambient temperatures.

During high ambient temperatures animals tend to have a decrease in feed intake as a way to reduce metabolic heat production (Black et al., 1993; Baumgard and Rhoads, 2013; Renaudeau et al., 2008 Renaudeau et al., 2010). Increased water intake results an increase loss of electrolytes and hematocrit and hemoglobin levels (Ait-Boulahsen et al., 1995; Puvadolpirod and Thaxton, 2000; Zulkifli et al., 2009; Wang et al., 2018). As ambient temperatures increase, respiration rate, skin temperature and internal temperature increase (Cabezón et al., 2017; Lucy and Safranski, 2017). Heat dissipation through skin is an ineffective way at reducing their body temperature resulting in the pig increasing their respiration rate to dissipate the additional heat (Bouchama and De Vol., 2001). If this is ineffective, the animal will divert blood flow from the internal organs, such as the reproductive organs and digestive tract, to the skin. This shunting of blood and nutrient

flow compromises the GIT physical barrier allowing unwanted larger molecules into the submucosa (Baumgard et al. 2021)

1.4.3 Heat Stress Impacts on Female Reproduction

High ambient temperatures impair a female's pubertal development, development of ovarian follicles, ability to conceive, and embryonic survival (Krishnan et al., 2017; De Rensis et al., 2017). Pre-pubertal gilts exposed to heat stress have reduced circulating FSH and LH, decreased ovarian follicle numbers, and delayed puberty (Knox, 2019).

Heat stress in lactating sows leads to reduced feed intake, milk yield, reproductive performance and growth rate of piglets (Black et al., 1993). Reduction in milk yield results in a decrease in growth rate of piglets (Black, 1993). Schoenherr et al. (1989) observed a decrease in sow's body weight during lactation, milk yield and piglets average daily gain when exposed to a 30C ambient environment. Sows exposed to higher ambient temperatures during the summer months have shown to have a longer interval between wean-to-estrus or wean-to-mating (Clark et al., 1986). Auvigne et al. (2010) observed a higher fertility rate during winter months than summer months (86.4% vs 83.5%). Sows face seasonal infertility that may be influenced by heat stress and photoperiod (Auvigne et al., 2010; Lucy and Safranski, 2017). Sows exposed to heat stress conditions during gestation not only impact the sow, but can have impacts to the developing fetuses such as decreased litter size and in gilts and a decrease in sperm number and quality in boars (Lucy and Safranski, 2017; Lugar et al., 2018; Johnson et al., 2020).

1.4.4 Heat Stress Impacts on Male Reproduction

Knox et al., (2008) found that 1-10% of ejaculates were discarded due to poor motility and morphology related to season and individual boar. One of the main stressors that affects boar fertility is elevated temperatures. Boars exposed to temperatures of 26-29°C for 10-14 weeks results in reduced semen quality (Flowes 2015). Boars exposed to temperatures above 33C results in abnormal sperm 2 weeks post stressor for up to a duration of 3 weeks (McNitt and First, 1970). Elevated temperatures above 95°F for 3 consecutive days causes a reduction in sperm production and an increase in sperm abnormalities beginning 2 weeks post stressor (Parrish et al., 2017; Rockett et al., 2001; Cai et al., 2010; Rao et al., 2015).

A high percentage of cytoplasmic droplets in semen is negatively correlated with boar fertility (Waberski et al., 1994). Cytoplasmic droplets are located on either the terminal midpiece region (distal) or the around the neck (proximal) (Althouse, 1998) and are the most common abnormality seen in heat stressed boar ejaculates. Sperm cell abnormalities are not seen in ejaculates until 14 days after the heat stress begins due to the time required for spermatogenesis to occur relative to the sperm cells that are eligible for ejaculation. Early developing spermatogonia in the testicle are the most sensitive cells to impacts from heat stress (Parrish, 2019). Duration of decreased semen quality is due to the severity and length of the stress that the boar undergoes and which stages of sperm cell development of spermatogenesis were susceptible to the stressor (Knox, 2001).

Heat stress is caused by a combination of both temperature and humidity. McNitt and First (1970) evaluated how heat stress effects semen quality in boars that were exposed to 33°C (F) and 50% relative humidity for 72 hours. An environment of 33°C for 72 hours resulted in a decrease in total sperm count and an increase in percent abnormal spermatozoa around two weeks after exposure. Parrish et al. (2017) evaluated semen quality impact from heat stress via scrotal insulation in boars. Insulated sacks were constructed to cover the scrotum and under inguinal region allowing for isolation of the pampiniform plexus. Insulated sacks were able to increase scrotal temperature from 32°C to 34°C. The study consisted of a total of ten boars in either a CONTROL group (n=5) or INSULATED group (n=5) that were stressed for a total of 48 hours. Semen was collected 2 weeks prior to insulation and 6 weeks post insulation and collected three times a week. Overall, INSULATED boars had a decreased motility between days 30-35 and an increase in morphological abnormalities between days 19-37 post scrotal insulation. Additionally, 48 hours of scrotal insulation affects the meiosis stages of spermatogenesis resulting in an increase in primary abnormalities. This is likely due to how heat stress disrupts Sertoli cell-Sertoli cell junctional complexes of the blood-testes-barrier (Cheng and Mruk, 2012; Xu et al., 2016; Cai et al., 2010). Heat stress-induced sperm abnormalities typically recover following the normal length of spermatogenesis (Parrish et al., 2017; Parrish et al., 2019)

1.5 Management/Strategies for Minimizing and Alleviating Heat Stress

There is a need to identify environmental conditions where animals become heat stressed as it contributes to welfare and economic issues. Knowing what temperatures are ideal for each stage of the animal's life is necessary to provide environmental conditions that will maximize production

as well as provide a thermoneutral environment that is comfortable. Environmental modifications can be implemented that prevents the degree of HS an animal is exposed to or improve the heat exchange mechanisms between the animals and its surrounding environment (Mayorga et al., 2019; Renaudeau et al., 2012).

1.5.1 Current Industry Cooling Systems

There are multiple cooling systems available within the swine industry focused on indirect and direct cooling of animals. Air conditioning is increasing within boar studs because it is currently the most effective way to maintain temperatures within the boars' TNZ. The use of air conditioning in boar studs is only around 7%, meaning the majority of farms cool boars using evaporative and mechanical cooling (Knox et al., 2008). The swine industry highly relies on indirect cooling through the use of evaporative cooling cells to cool the ambient air as it enters the barn. This cool air is then circulated through the barn using fans. Additional direct cooling mechanisms can be used which consist of drip and snout cooling systems to help the boars cool their body temperatures (Bull et al., 1997).

Bull et al. (1997) evaluated mature gilts' (133 to 159 kg) preferences between snout coolers, drip coolers and conductive pads when undergoing continuous 10-hour heat stress conditions. Forty-two gilts were acclimated to warm temperatures ($27.6 \pm 2.5^{\circ}\text{C}$) a day prior to heat stress. Gilts were heat stressed continuously for 10 hours throughout the day ($34.2 \pm 2.8^{\circ}\text{C}$) and maintained in a warm environment for 14 hours throughout the night ($26.6 \pm 2.3^{\circ}\text{C}$) over 6 days. Relative humidity averaged 56% throughout study. Gilts were recorded on video tape throughout the entire study to document their behavior. The gilts were housed in free stalls equipped with either a drip cooler, snout cooler, or conductive cooling pad, and allowed to enter any stall they wanted. Drip coolers ran continuously at a rate of $59 \pm 0.9 \text{ mL/min}$ with mean drip water temperature of $26.2 \pm 1.2^{\circ}\text{C}$. Snout coolers operated continuously where a minimum of 12.3 L/s of cool air ($27.7 \pm 0.9^{\circ}\text{C}$) was delivered 30 cm above the pen floor. An 89 x 46 cm conductive cooling pad was evaluated that had iron pipe looped within it that continuously flushed tap water ($19.5 \pm 0.45^{\circ}\text{C}$) at a water flow rate of $3,953 \pm 579 \text{ mL/min}$. Surface temperature of a pad when animal was not laying on it ranged from 19.5 to 23.8°C . Respiration rate and rectal temperature were measured 3 and 7 hours into heat stress. Gilts spent 39.4% of their time in stalls

with cooling pads, 25.3% in drip cooling stalls, 15% in snout cooling stalls, and 20.3% with no cooling during heat stress. During warm nights, gilts spent 28.9% on cooling pads, 15.3% in drip cooling stalls, 17.6% in snout cooling stalls, and 38.2% with no cooling. Respiration rate was dramatically lower for gilts who spent majority of their time on cooling pads (72.7 breaths/min) when compared to drip cooling, snout cooling, and no cooling (102.7, 114.7 and 103.7 breaths/min). Rectal temperatures were similar between gilts under different cooling systems with lower rectal temperatures in gilts using cooling pads.

1.5.2 Floor Cooling Pads

Pigs lie down more than 19 hours within a 24-hour day or about 80% of the time (Huynh, 2004). Based on the fact that animals appear to prefer cooling pads to other methods of cooling, as well as the large amount of time that pigs are laying down, floor cooling pads have great potential for use in commercial swine production to help animals regulate their body temperature during heat stress.

Implementation of cooling pads have demonstrated a significant increase in feed intake and average daily gain during periods of high heat in growing pigs (Huhyn et al., 2004) in addition to an improvement of lying behavior.

Cabazon et al. (2017) evaluated the effect of floor cooling on late lactating sows undergoing acute heat stress. Ten multiparous sows were housed in farrowing crates with cooling pads and randomly assigned to receive a constant water flow (control) or 0.25, 0.55 or 0.85 L/min. The room was heated to a target temperature of 35°C and maintained for 160 minutes. All sows were exposed to temperatures without cooling for 60 minutes and then cooling pads began to flush a constant water flow based off treatment for 100 minutes. Respiration rate, vaginal temperature and skin temperature was measured 20 minutes before cooling pads were turned on and continued every 20 minutes until the end of heat stress. Rectal temperature was measured 20 minutes before cooling pad began flushing and at the end of heat stress. Cooling pads were able to alleviate increases in respiration rate and vaginal temperature within 20-40 minutes of use with 0.85L/min having the greatest reduction. Heat removal throughout the study was 193, 321 and 365 W for the 0.25, 0.55 and 0.85 L/min treatments, respectively, with heat removal being the greatest for 0.55 and 0.85 L/min. This study illustrates that the use of cooling pads is able to remove excess heat

from sows while reducing physiological indicators that are associated with increases in ambient temperatures.

1.6 Final Considerations

Seasonal infertility due to heat stress is a great economic loss for the swine industry. Current management practices used today are not effective at reducing such negative consequences of heat stress on gilts and boars therefore there is a need to find an alternative way to alleviate heat stress during warmer months. The use of electronically-controlled cooling pads is the most effective at removing heat from lactating sows in a way that reduces negative physiological traits and behaviors resulting from heat stress (Cabezón et al., 2017). Currently, there is no literature showing the use of cooling pads on boars as a way to alleviate the negative impacts from heat stress on the boar's physiology, behavior and reproductive performance. Therefore, the objective of this study is to evaluate the use of electronically-controlled cooling pads on gilt's behavior and physiology as well as a boar's physiology, behavior and reproductive performance.

1.7 References

- Ait-Boulahsen, A., J. D. Garlich, and F. W. Edens. 1995. Potassium Chloride Improves the Thermotolerance of Chickens Exposed to Acute Heat Stress. *Poultry Science* 74(1):75–87. doi: 10.3382/ps.0740075
- Althouse, G. C. and A. C. T. Dipl. n.d. Cytoplasmic Droplets on Boar Sperm Cells.
- Auvigne, V., P. Leneveu, C. Jehannin, O. Peltoniemi, and E. Sallé. 2010. Seasonal Infertility in Sows: A Five Year Field Study to Analyze the Relative Roles of Heat Stress and Photoperiod. *Theriogenology* 74 (1): 60–66. doi: 10.1016/j.theriogenology.2009.12.019.
- Balogh, P., W. Kapelański, H. Jankowiak, L. Nagy, S. Kovacs, L. Huzsvai, J. Popp, J. Posta, and A. Soltesz. 2015. The Productive Lifetime of Sows on Two Farms from the Aspect of Reasons for Culling. *Annals of Animal Science* 15(3): 747–58. doi:10.1515/sosd-2015-0020.
- Baumgard, L. H., and R. P. Rhoads Jr. 2013. Effects of Heat Stress on Postabsorptive Metabolism and Energetics. *Annual Review of Animal Biosciences* 1:311–337. doi: 10.1146/annurev-animal-031412-103644.
- Baumgard, L. H., E. A. Horst, E. J. Mayorga Lozano, A. Al-Qaisi, C.S. Shouse, S. K. Kvidera, S. Lei, J.T. Seibert, H.A. Ramirez, A. F. Keating, J.W. Ross, J.T. Selsby, R. Appuhamy, and R. P. Rhoads. 2021. Heat Stress Consequences of Gut Barrier Dysfunction. *Journal of Animal Science* 96(Suppl.2):217-218 (Abstr)
- Bartol, F.F., Wiley, A.A., George, A.F., Miller, D.J., and C.A. Bagnell. 2017. Postnatal reproductive development and the lactocrine hypothesis. *Journal of Animal Science* 95:2200-2210. doi: 10.2527/jas.2016.1144

- Beltranena, E., G. R. Foxcroft, F. X. Aherne, and R. N. Kirkwood. 1991. Endocrinology of Nutritional Flushing in Gilts. *Can. J. Anim. Sci.* 71:1063-1071.
- Bidanel, J., J. Gruand, and C. Legault. 1996. Genetic variability of age and weight at puberty, ovulation rate and embryo survival in gilts and relations with production traits. *Genetic Selection Evolution.* 28:103doi: 10.1186/1297-9686-28-1-103
- Black, J. L., B. P. Mullan, M. L. Lorsch, and L. R. Giles. 1993. Lactation in the Sow during Heat Stress. *Livestock Production Science.* 35:153-170. doi: 10.1016/0301-6226(93)90188-n.
- Bouchama A. and E.B. De Vol. Acid-base alterations in heatstroke. 2001. *Intensive Care Med.* 27(4):680-5. doi:10.1007/s001340100906
- Bull, R. P., P. C. Harrison, G. L. Riskowski, and H. W. Gonyou. 1997. Preference among Cooling Systems by Gilts under Heat Stress. *Journal of Animal Science* 75: 2078–83. doi: 10.2527/1997.7582078x
- Cabezón, F. A., A. P. Schinckel, J. N. Marchant-Forde, J. S. Johnson, and R. M. Stwalley. 2017. Effect of Floor Cooling on Late Lactation Sows under Acute Heat Stress. *Livestock Science.* 206:113–120. Doi: 10.1016/j.livsci.2017.10.017
- Cai, H., Y. Ren, X.-X. Li, J.-L. Yang, C.-P. Zhang, M. Chen, C.-H. Fan, X.-Q. Hu, Z.-Y. Hu, F. Gao and Y.-X. Liu. 2010. Scrotal Heat Stress Causes a Transient Alteration in Tight Junctions and Induction of TGF- β Expression. *International Journal of Andrology* 34:352–362. doi: 10.1111/j.1365-2605.2010.01089.x
- Cheng, C. Y. and D. D. Mruk. 2012. The Blood-Testis Barrier and Its Implications for Male Contraception. In: *Pharmacological Reviews.* 64:16–64.
- Christenson R.K., J.J. Ford, and D.A. Redmer. 1985. Maturation of ovarian follicles in the prepubertal gilt. *Journals of Reproduction and Fertility Ltd.* 33:21-36.
- Clark, J. R., A. Komkov, and L. F. Tribble. 1986. Effects of Parity, Season, Gonadotropin Releasing Hormone and Altered Suckling Intensity on the Interval to Rebreding in Sows. *Theriogenology* 26 (3): 299–308. doi: 10.1016/0093-691x(86)90149-4
- De Rensis, F., A. J. Ziecik and Roy N. Kirkwood. 2017. Seasonal Infertility in Gilts and Sows: Aetiology, Clinical Implications and Treatments. *Theriogenology.* 96:111–117.
- Den Hartog, L. A. and G. J. M. van Kempen. 1980. Relation between Nutrition and Fertility in Pigs.” *Netherlands Journal of Agricultural Science.* 28:211-227. doi:10.18174/njas.v28i4.17022.
- Den Hartog, L. A. and G. J. Noordewier. 1984. The Effect of Energy Intake on Age at Puberty in Gilts. *Netherlands Journal of Agricultural Science.* 32:263-280. Doi: 10.18174/njas.v32i4.16884
- Engblom, L., J. A. Calderón Díaz, M. Nikkilä, K. Gray, P. Harms, J. Fix, S. Tsuruta, J. Mabry, and K. Stalder. 2016. Genetic Analysis of Sow Longevity and Sow Lifetime Reproductive Traits Using Censored Data. *Journal of Animal Breeding and Genetics.* 133:138–144. doi: 10.1111/jbg.12177
- Engblom, L., N. Lundeheim, A.M. Dalin, and K. Andersson. 2007. Sow Removal in Swedish Commercial Herds. *Livestock Science* 106:76–86. doi: 10.1016/j.livsci.2006.07.002
- Evans, A. C. O., and J. V. O’Doherty. 2001. Endocrine Changes and Management Factors Affecting Puberty in Gilts. *Livestock Production Science.* 68:1-12. doi: 10.1016/s0301-6226(00)00202-5
- FASS. 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Federation of Animal Science Societies, Champaign IL

- Flowers, W. L. 2015. Factors Affecting the Efficient Production of Boar Sperm. *Reproduction in Domestic Animals*. 50(2):25–30. doi: 10.1111/rda.12529
- Flowers WL. Management of boars for efficient semen production. *J Reprod Fertil Suppl*. 1997; 52:67-78.
- França, L.R., G.F. Avelar, and F.F.L. Almeida. 2005. Spermatogenesis and Sperm Transit through the Epididymis in Mammals with Emphasis on Pigs. *Theriogenology* 63:300–318. doi: 10.1016/j.theriogenology.2004.09.014
- Gaughan, J.B., R.D.A. Cameron, G.McL. Dryden, and B.A. Young. 1997. Effect of Body Composition at Selection on Reproductive Development in Large White Gilts. *Journal of Animal Science*. 75:1764-1772. doi: 10.2527/1997.7571764x
- Geisert, R. D., P. Sutvosky, M. C. Lucy, F. F. Bartol and A. E. Meyer. 2020. Chapter 15 - Reproductive Physiology of Swine. In: *Animal Agriculture*, F. W. Bazer, C. Lamb and G. Wu, editor, Academic Press. page 263–81. doi: 10.1016/B978-0-12-817052-6.00015-X
- Griffin J.K., M.C. Seal, W. L. Flowers, 2006. Effect of neonatal environment on adult reproductive function in boars. *Journal of Animal Science*. 84:205.
- Grigoriadis, D.F., S.A. Edwards, P.R. English, and F. Davidson. 2001. The Effect of Oestrous Cycle Number, at Constant Age, on Gilt Reproduction in a Dynamic Service System. *Animal Science* 72:11–17. Doi:10.1017/S1357729800055508
- Gruhot, T.R., J.A. Calderón Díaz, T.J. Baas, and K.J. Stalder. 2017. Using First and Second Parity Number Born Alive Information to Estimate Later Reproductive Performance in Sows. *Livestock Science*. 196:22–27. doi: 10.1016/j.livsci.2016.12.009
- Huynh, T.T.T., A.J.A. Aarnink, H.A.M. Spoolder, M.W.A. Verstegen, and B. Kemp. 2004. Effects of Floor Cooling During High Ambient Temperatures on the Lying Behavior and Productivity of Growing Finishing Pigs. *Transactions of the ASAE*. 47(5):1773-1782. doi:10.13031/2013.17620
- Holst, S. and B. Leuwerke. 2017. “Success of sow herd depends on replacement gilt performance.” *Swine Health Quarterly*.
- Huynh, T.T.T., A.J.A. Aarnink, C.T. Truong, B. Kemp, and M.W.A. Verstegen. 2006. Effects of Tropical Climate and Water Cooling Methods on Growing Pigs’ Responses. *Livestock Science* 104(3): 278–291. doi: 10.1016/j.livsci.2006.04.029
- Johnson, J.S., Stewart, K.R., Safranski, T.J., Ross, J.W. and L.H. Baumgard. 2020. In utero heat stress alters postnatal phenotypes in swine. *Theriogenology*, 154:110-119. doi:10.1016/j.theriogenology.2020.05.013
- Kemp, B., and N.M. Soede. 1996. Relationship of Weaning-to-Estrus Interval to Timing of Ovulation and Fertilization in Sows. *Journal of Animal Science*. 74(5): 944–949. doi: 10.2527/1996.745944x
- Knauer, M., K.J. Stalder, T. Serenius, T.J. Baas, P.J. Berger, L. Karriker, R.N. Goodwin, R.K. Johnson, J.W. Mabry, R.K. Miller, O.W. Robison and M.D. Tokach. 2010. Factors Associated with Sow Stayability in 6 Genotypes. *Journal of Animal Science*. 88:3486–3492. doi:10.2527/jas.2009-2319
- Knox, R., D. Levis, T. Safranski and W. Singleton. 2008. An Update on North American Boar Stud Practices. *Theriogenology*. 70:1202–1208. doi: 10.1016/j.theriogenology.2008.06.036
- Knox, R.V. 2001. *The Anatomy & Physiology of Sperm Production in Boars*.

- Knox, R. V. 2016. Artificial Insemination in Pigs Today. *Theriogenology* 85: 83–93. doi: 10.1016/j.theriogenology.2015.07.009
- Knox, R. V., S. L. Rodriguez Zas, N. L. Slotter, K. A. Mc Namara, T. J. Gall, D. G. Levis, T. J. Safranski, and W. L. Singleton. 2013. An Analysis of Survey Data by Size of the Breeding Herd for the Reproductive Management Practices of North American Sow Farms. *Journal of Animal Science* 91: 433–445. doi: 10.2527/jas2012-5189
- Knox, Robert V. 2019. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Factors Influencing Follicle Development in Gilts and Sows and Management Strategies Used to Regulate Growth for Control of Estrus and ovulation. *Journal of Animal Science*. 97:1433–1445. doi: 10.1093/jas/skz036
- Koketsu, Y., H. Takahashi and K. Akachi. 1999. Longevity, Lifetime Pig Production and Productivity, and Age at First Conception in a Cohort of Gilts Observed over Six Years on Commercial Farms. *J. Vet Med Sci*. 61:1001–1005. doi:10.1292/jvms.61.1001
- Koketsu, Y., S. Tani, and R. Iida. 2017. Factors for Improving Reproductive Performance of Sows and Herd Productivity in Commercial Breeding Herds. *Porcine Health Management* 3:1. doi: 10.1186/s40813-016-0049-7
- Kraeling, R. R., and S. K. Webel. 2015. Current Strategies for Reproductive Management of Gilts and Sows in North America. *Journal of Animal Science and Biotechnology*. 6:3. doi: 10.1186/2049-1891-6-3
- Krishnan, G., M. Bagath , P. Pragna, M.K. Vidya, J. Aleena, P.R. Archana, S. Veerasamy and R. Bhatta, 2017. Mitigation of the Heat Stress Impact in Livestock Reproduction. *Intech*. doi:10.5772/intechopen.69091
- Lammers, P. J., D. R. Stender, and M.S. Honeyman. 2007. *Environmental Needs of the Pig. Niche Pork Production*.
- Li, Q., X. Yuan, Z. Chen, A. Zhang, Z. Zhang, H. Zhang, and J. Li. 2018. Heritability Estimates and Effect on Lifetime Reproductive Performance of Age at Puberty in Sows. *Animal Reproduction Science*. 195:207–215. Doi: 10.1016/j.anireprosci.2018.05.025
- Lucy, M.C., and T.J. Safranski. 2017. Heat Stress in Pregnant Sows: Thermal Responses and Subsequent Performance of Sows and Their Offspring. *Molecular Reproduction and Development* 84:946–956. Doi: 10.1002/mrd.22844
- Lugar, D.W., J.A. Proctor, T.J. Safranski, M.C. Lucy, and K.R. Stewart. 2018. In utero heat stress causes reduced testicular area at puberty, reduced total sperm production, and increased sperm abnormalities in boars. *Animal Reproduction Science*. 192:126-135. doi: 10.1016/j.anireprosci.2018.02.022
- MacPherson, R. M., F. D. Deb Hovell, and A. S. Jones. 1977. Performance of Sows First Mated at Puberty or Second or Third Oestrus, and Carcass Assessment of Once-Bred Gilts. *Animal Science*. 24:333–342.
- Mayorga, Edith J., David Renaudeau, Brett C. Ramirez, Jason W. Ross, and Lance H. Baumgard. 2019. “Heat Stress Adaptations in Pigs.” *Animal Frontiers: The Review Magazine of Animal Agriculture* 9:54–61. doi:10.1093/af/vfy035
- McCoard, S. A., T. H. Wise, D. D. Lunstra, and J. J. Ford. 2003. Stereological Evaluation of Sertoli Cell Ontogeny during Fetal and Neonatal Life in Two Diverse Breeds of Swine. *The Journal of Endocrinology*. 178:395–403. doi: 10.16677/joe.0.1780395
- McNitt, J. I., and N. L. First. 1970. Effects of 72-Hour Heat Stress on Semen Quality in Boars. *International Journal of Biometeorology*. 14: 373–380. doi: 10.1007/BF01462914

- Miller, P. S., R. Moreno, and R. K. Johnson. 2011. Effects of Restricting Energy during the Gilt Developmental Period on Growth and Reproduction of Lines Differing in Lean Growth Rate: Responses in Feed Intake, Growth, and Age at Puberty. *Journal of Animal Science* 89: 342–354. doi:10.2527/jas.2010-3111
- Parrish, J. J. 2019. Spermatogenesis, Heat Stress and Male Infertility. In: *Molecular Signaling in Spermatogenesis and Male Infertility*. Doi:10.1201/9780429244216-17
- Parrish, J. J., K. L. Willenburg, K. M. Gibbs, K. B. Yagoda, M. M. Krautkramer, T. M. Loether, and F. C. S. A. Melo. 2017. Scrotal Insulation and Sperm Production in the Boar. *Molecular Reproduction and Development*. 84: 969–978. doi: 10.1002/mrd.22841
- Patterson, J.L., E. Beltranena, and G.R. Foxcroft. 2010. The effect of gilt age at first estrus and breeding on third estrus on sow body weight changes and long-term reproductive performance. *Journal of Animal Science*. 88:2500-2513. doi: 10.2527/jas.2008-1756
- Patterson, J. and G. Foxcroft. 2019. Gilt Management for Fertility and Longevity. *Animals*. 9:434. Doi: 10.3390/ani9070434
- Pedersen, L. J. 2007. Sexual Behaviour in Female Pigs. *Hormones and Behavior*. 52:64–69. doi: 10.1016/j.yhbeh.2007.03.019
- Puvadolpirod, S. and J. P. Thaxton. 2000. Model of Physiological Stress in Chickens 1. Response parameters. *Poultry Science*. 79 (3): 363–369. doi: 10.1093/ps/79.3.363
- Rao, M., X.-L. Zhao, J. Yang, S.-F. Hu, H. Lei, W. Xia, and C.-H. Zhu. 2015. “Effect of Transient Scrotal Hyperthermia on Sperm Parameters, Seminal Plasma Biochemical Markers, and Oxidative Stress in Men.” *Asian Journal of Andrology*. 17: 668–675. Doi: 10.4103/1008-682X.146967
- Renaudeau, D., C. Anais, L. Tel, and J. L. Gourdine. 2010. Effect of Temperature on Thermal Acclimation in Growing Pigs Estimated Using a Nonlinear Function. *Journal of Animal Science*. 88:3715–3724. doi: 10.2527/jas.2009-2169
- Renaudeau, D., A. Collin, S. Yahav, V. de Basilio, J. L. Gourdine, and R. J. Collier. 2012. Adaptation to Hot Climate and Strategies to Alleviate Heat Stress in Livestock Production. *Animal*. 6:707–728. doi: 10.1017/S1751731111002448
- Renaudeau, D., M. Kerdoncuff, C. Anaïs, and J. L. Gourdine. 2008. Effect of Temperature Level on Thermal Acclimation in Large White Growing Pigs. *Animal*. 2:1619–1626. doi: 10.1017/S1751731108002814
- Riesenbeck, A. 2011. Review on International Trade with Boar Semen. *Reproduction in Domestic Animals*. 46:1–3. doi: 10.1111/j.1439-0531.2011.01869.x
- Rockett, J. C., F. L. Mapp, J. B. Garges, J. C. Luft, C. Mori, and D. J. Dix. 2001. Effects of Hyperthermia on Spermatogenesis, Apoptosis, Gene Expression, and Fertility in Adult Male Mice. *Biology of Reproduction* 65: 229–239. doi: 10.1095/biolreprod65.1.229
- Rohrer, G.A., A. J. Cross, C.A. Lents, J.R. Miles, D. J. Nonneman and L. A. Rempel. 2017. Genetic improvement of sow lifetime productivity. *Journal of Animal Science*, 95:11–12. (Abstr) doi: 10.2527/asasmw.2017.026
- Roongsitthichai, A., P. Cheuchuchart, S. Chatwijitkul, O. Chantarothai, and P. Tummaruk. 2013. Influence of Age at First Estrus, Body Weight, and Average Daily Gain of Replacement Gilts on Their Subsequent Reproductive Performance as Sows. *Livestock Science*. 151:238–245. doi: 10.1016/j.livsci.2012.11.004
- Saito, H., Y. Sasaki, and Y. Koketsu. 2011. Associations between Age of Gilts at First Mating and Lifetime Performance or Culling Risk in Commercial Herds. *The Journal of Veterinary Medical Science*. 73:555–559. doi: 10.1292/jvms.10-0040

- Schoenherr, W.D., T.S. Stahly and G.L. Cromwell. 1989. The effects of dietary fat or fibre addition on yield and composition of milk from sows housed in a warm or hot environment. *J. Anim. Sci.* 67: 482-495. doi: 10.2527/jas1989.672482x
- Segura-Correa, J.C., E. Ek-Mex, A. Alzina-López, and V. M. Segura-Correa. 2011. Frequency of Removal Reasons of Sows in Southeastern Mexico. *Tropical Animal Health and Production.* 43:1583–1588. doi:10.1007/s11250-011-9847-8
- Senger, P. L. 2012. Pathways to pregnancy and parturition (3rd ed.). Current Conceptions.
- Serenius, T. and K. J. Stalder. 2007. Length of Productive Life of Crossbred Sows Is Affected by Farm Management, Leg Conformation, Sow's Own Prolificacy, Sow's Origin Parity and Genetics. *Animal.* 1:745–750. doi: 10.1017/S175173110769185X
- Soede, N. M., P. Langendijk and B. Kemp. 2011. Reproductive Cycles in Pigs. *Animal Reproduction Science* 124:251–258. doi: 10.1016/j.anireprosci.2011.02.025
- St-Pierre, N. R., B. Cobanov and G. Schnitkey. 2003. Economic Losses from Heat Stress by US Livestock Industries. *Journal of Dairy Science* 86:52–77. doi:
- Stancic, I., B. Stancic, A. Bozic, R. Anderson, R. Harvey and Dragan Gvozdic. 2011. Ovarian Activity and Uterus Organometry in Delayed Puberty Gilts. *Theriogenology* 76:1022–1026. doi:10.1016/j.theriogenology.2011.05.004
- Sterning, M., L. Rydhmer and L. Eliasson-Selling. 1998. Relationships Between Age at Puberty and Interval from Weaning to Estrus and Between Estrus Signs at Puberty and After the First Weaning in Pigs. *American Society of Animal Science.* 76:353-359. doi: 10.2527/1998.762353x
- Tani, S., C. Piñeiro and Y. Koketsu. 2017. Characteristics and Risk Factors for Severe Repeat-Breeder Female Pigs and Their Lif/setime Performance in Commercial Breeding Herds. *Porcine Health Management.* 3:12. doi: 10.1186/s40813-017-0059-0
- Tart, J. K., R. K. Johnson, J. W. Bundy, N. N. Ferdinand, A. M. McKnite, J. R. Wood, P. S. Miller, M.F. Rothschild, M.L. Spangler, D.J. Garrick, S.D. Kachman and D.C. Ciobanu. 2013. Genome-Wide Prediction of Age at Puberty and Reproductive Longevity in Sows. *Animal Genetics.* 44:387–397. doi: 10.1111/age.12028
- Waberski, D., S. Meding, G. Dirksen, K. F. Weitze, C. Leiding and R. Hahn. 1994. Fertility of Long-Term-Stored Boar Semen: Influence of Extender (Androhep and Kiev), Storage Time and Plasma Droplets in the Semen. *Animal Reproduction Science.* 36:145-151. doi: 10.1016/0378-4320(94)90061-2
- Wang, C., J.-L. Li, H.-K. Wei, Y.-F. Zhou, S.-W. Jiang, and J. Peng. 2017. Linear Model Analysis of the Influencing Factors of Boar Longevity in Southern China. *Theriogenology.* 93:105–110. doi:10.1016/j.theriogenology.2017.01.038
- Wang, Y., P. Saelao, K. Chanthavixay, R. Gallardo, D. Bunn, S. J. Lamont, J. M. Dekkers, T. Kelly and H. Zhou. 2018. Physiological Responses to Heat Stress in Two Genetically Distinct Chicken Inbred Lines. *Poultry Science.* 97:770–780. doi: 10.3382/ps/pex363
- Wijesena, H. R., C. A. Lents, J-J Riethoven, M. D. Trenhaile-Grannemann, J. F. Thorson, B. N. Keel, P. S. Miller, M. L. Spangler, S. D. Kachman and D. C. Ciobanu. 2017. GENOMICS SYMPOSIUM: Using Genomic Approaches to Uncover Sources of Variation in Age at Puberty and Reproductive Longevity in Sows. *Journal of Animal Science.* 95:4196–4205. doi: 10.2527/jas2016.1334
- Xu, Y.-R., H.S. Dong and W.X. Yang. 2016. Regulators in the Apoptotic Pathway during Spermatogenesis: Killers or Guards?. *Gene.* 582: 97–111. doi: 10.1016/j.gene.2016.02.007

- Yazdi, M. H., L. Rydhmer, E. Ringmar-Cederberg, N. Lundeheim and K. Johansson. 2000. Genetic Study of Longevity in Swedish Landrace Sows. *Livestock Production Science*. 63: 255-264. Doi: 10.1016/s0301-6226(99)00133-5
- Young, L.G., G.J. King, J.S. Walton, I. Mcmillan and M. Klevorick. 1990. Reproductive Performance over Four Parities of Gilts Stimulated to Early Estrus and Mated at First, Second or Third Observed Estrus. *Canadian Journal of Animal Science*. 70,:483-492. doi: 10.4141/cjas90-060
- Zulkifli, I., A. Al-Aqil, A. R. Omar, A. Q. Sazili and M. A. Rajion. 2009. Crating and Heat Stress Influence Blood Parameters and Heat Shock Protein 70 Expression in Broiler Chickens Showing Short or Long Tonic Immobility Reactions. *Poultry Science*. 88:471–476. Doi: 10.3382/ps.2008-00287

CHAPTER 2. USE OF AN ELECTRONICALLY-CONTROLLED FLOOR COOLING PAD DURING HEAT STRESS ON THERMOREGULATION IN GILTS

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2.1 Abstract

The objective of this study was to evaluate the use of cooling pads on measures of physiological responses in heat-stressed gilts. Two experiments were conducted evaluating the frequency of flushing water (Experiment 1) and water flushing at set temperatures (Experiment 2) in the cooling pads. For experiment 1, gilts were randomly assigned into one of three treatment groups: Control (CN1) with no water flushing; or flushing 2.0 L of cool water over 30 seconds every 4 minutes (F4) or every 8 minutes (F8). For experiment 2, gilts were randomly assigned to three treatments: Control (CN2) with no water flushing; or flushing 2.0 L of cool water over 30 sec when sensors in the pads read 28.0°C (F28) or 29.5°C (F29.5). Both studies were conducted in a Latin square design and replicated 6 times each at one of two temperatures (32°C and 35°C) resulting in 12 replicates for each experiment. Gilts were limit fed twice a day at 2.4 kg/gilt/day before heating the barn. At the start of the heat cycle, the room temperature was gradually increased from 18°C to either 32 or 35 °C and maintained for 150 minutes. Respiration rates (RR), skin temperature through thermal imaging (ST) and vaginal temperature (VT) were collected twice before heat stress followed by every 20 minutes thereafter. The cooling pads were turned on following pre-heat stress physiological measurements. Pre-heat measurements (PreH), Post-heat measurements (PostH), and the change in value between PreH and PostH were evaluated using PROC MIXED of SAS 9.4. In experiment 1, the change in PreH and PostH for RR was lower in F4 and F8 compared to CN1 at both 32°C (8, -0.5, -0.7; $P=0.097$) and 35°C (14.2, 0.75, 1.6; $P=0.005$). The change between PreH and PostH in VT and ST were not different among treatments. At 35°C, PostH and the change in VT between PreH and PostH were lower for F4 and F8 compared to CN14 ($P\leq 0.01$). PostH RR were lower in F4 and F8 compared to CN1 at 35°C and 32°C ($P=0.0074$ and 0.0472). In experiment 2, for the change in PreH and PostH for RR were

lower for F28 and F29.5 compared to CN2 ($P=0.05$). The change in VT was slightly lower for F28 and F29.5 compared to CN2 ($P=0.05$). The change for ST was not different among treatments ($P>0.05$). Use of cooling pads flushing at a set time prevented physiological indicators of heat stress at 35°C in gilts and not at 32°C.

Key words: gilt, heat stress, physiological response

2.2 Introduction

Periods of high ambient temperatures results in high economical losses in the swine industry due to production efficiencies as well as a concern for animal welfare. High ambient temperatures result in physiological changes in animals in response to heat stress such as decrease feed intake, increased body temperature and respiration rate as a way to maintain homeostasis (Johnson et al., 2015). Decreases in feed intake results in negative consequences on growth and reproduction performances (Collin et al., 2001, Lammers et al., 2007).

In growing gilts, a reduction in feed intake will delay puberty and decrease ovarian follicle numbers. This causes a decrease in ovulation rate, farrowing rate, and total born (Bloemhof et al., 2013; Sasaki et al. 2018), ultimately impacting longevity within the breeding herd (Knox, 2019). Therefore, there is a need to improve environmental conditions for gilts during times of high ambient temperatures to reduce negative impacts associated with heat stress.

The majority of swine farms today cool the barns to try and keep the animals comfortable by using evaporative cooling cells. In a cool cell, outside air crosses a medium that is wetted by flowing or dripping water. As the warm air crosses the cool water, the water evaporates, reducing the temperature of the incoming air. Because the water evaporates, the cool cell adds some humidity to the barn. Therefore, the cool cell is most effective at cooling the incoming air when humidity in the air is low, allowing for the additional moisture created by the cool cell to be added to the air. In a large portion of the United States, humidity is high during the summer, to cool cell technology is not capable of maintaining barns in the comfort zone for pigs. Therefore, farms may use drippers or water sprayers to cool the pigs. Drippers are placed above the animals and drip cool water onto the skin of the animal. The heat on the animal's skin will then evaporate the water off of their skin, removing the heat from the animal. Again, this technology adds moisture to the air of the barn, so is less effective during periods of high humidity.

A recent discovery allows for cooling of the individual animal without adding humidity to the air. Electronically-controlled, floor cooling pads were designed and constructed to assist pigs with thermoregulation by removing excess heat from pigs in a production facility (Cabezón et al., 2017). These pads consist of an aluminum treaded plate covering copper pipes with a polyethylene base (Cabezón et al., 2017). The copper pipes are able to circulate water throughout the pad at either a certain time and/or when the sensors in the pad reach a set temperature (Cabezón et al., 2017a).

These pads have been tested in lactating sows and prevented physiological responses to heat stress where sows maintained on the pads had decreased respiration rate, skin temperature, vaginal temperature, and rectal temperature (Cabezón et al., 2017a).

Therefore, the objective of this study was to evaluate the use of electronically-controlled floor cooling pads in limit-fed gilts at reducing negative physiological effects during heat stress. Gilts were used as a preliminary study prior to evaluation of the pads for use in boars, so gilts were limit-fed as a means of mimicking management practices for pubertal aged boars. This was important as digestion of food increases an animal's metabolic heat production, so limit-fed gilts could be used as a model for boars. It was hypothesized that floor cooling pads would eliminate physiological responses to heating the barn to 32°C and 35°C for 140 minutes.

2.3 Materials And Methods

All animal procedures were approved by Purdue University Animal Care and Use Committee prior to initiation of the study (1804001739). Two experiments were conducted with twelve gilts with body weight of 151 ± 9 kgs in January of 2020. Gilts were limit-fed a common diet to meet or exceed NCR 2012 requirements offered twice a day at 2.4 kg/gilt/day before heat stress was applied. Gilts were individually housed in 2.2 x 0.6m farrowing crates equipped with nipple waterers and an electronically-controlled floor cooling pad.

2.3.1 Experiment 1

Twelve gilts were randomly assigned to one of three treatment groups for each replicate in a Latin square experimental design. All gilts received each of the three treatments throughout the study. Treatments consisted of 0.0L of cool water being flushed through cooling pad (CN1) or

flushing 2.0L of cool water through the pad over 30 seconds every 4 minutes (F4) or every 8 minutes (F8). Two replicates were performed each day for six consecutive days for a total of 12 replicates allowing for each gilt to receive each of the three treatments. Six of these replicates were performed where the barn was heated to a maximum of 32°C and 6 replicates to 35°C. Table 3.1 illustrates the overall ambient temperature and relative humidity throughout heat stress for all replicates for both 32°C and 35°C heat stress. The barns quickly warmed from ~18°C to 30°C, but the desired temperatures were not obtained until ≥ 80 mins of barn heating. Following feeding, two pre-heat measurements were taken to serve as baseline measurements for respiration rate (RR), skin temperature (ST), and vaginal temperature (VT) (methods described below). The room was then gradually heated to the desired temperature and RR, ST, VT were collected (0 mins) when the barn started heating. After first heat stress measurement (0 mins) cooling pads were turned on. RR, ST and VT were then collected every 20 minutes for 140 minutes as the barn was heated. The room was then cooled to 18°C for 2 hours in between the two daily replicates.

2.3.2 Experiment 2

Twelve gilts were randomly assigned to one of three treatment groups for each replicate in a Latin square experimental design. Treatments consisted of 0.0L of cool water being flushed through the cooling pad (CN2) or flushing 2.0L of cool water through the pad over 30 seconds when pad sensors reached 28°C (F28) or 29.5°C (F29.5). Two half-day replicates were performed each day for 6 days in the exact same manner as Experiment 1.

2.4 Gilt Heat Stress Measurements

Physiological measurements recorded were respiration rate (RR), skin temperature (ST) and vaginal temperature (VT). Skin temperature was captured via infrared thermal camera (accuracy $\pm 2\%$, emissivity=0.98, FLIR Model T440; FLIR Systems Inc.; Wilsonville, OR) behind the ear, and on the shoulder, rump and tail. FLIR Tools Software (version 5.13) was used to analyze all thermal images and average skin temperature was recorded. Respiration rate was recorded by trained technicians by counting the number of breaths for 15 seconds and then multiplying that number by 4 to calculate breaths/minute. Vaginal temperature was measured with a calibrated temperature recorder (iButton model 1921H, calibrated accuracy $\pm 0.10^\circ\text{C}$; resolution = 0.50°C ;

Dallas Semi-conductor, Maxim, Irving, TX) attached to a blank Eazi-Breed®Sheep CIDR (Zoetis, Parsippany, NJ). For all physiological measures, the values prior to the HS being applied (PreH) are compared to the values at the end of the HS period (PostH) in addition to the change in the values between the start and end of the heat stress period.

2.5 Gilt Behavior

Gilts were video-recorded using mounted cameras (Panasonic WV-CP254H, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) throughout the duration of the study. Behaviors were scored in Observer XT 11.5 (Noldus, The Netherlands) by trained individuals according to a predetermined ethogram (Figure 2.1) to quantify the amount of time gilts exhibited various postures such as standing, sitting and laying (lateral or sternal), as well as the gilt's position on the cooling pad.

2.6 Statistical Analysis

All analyses were performed using the MIXED procedure of SAS v9.4 (SAS Inst. Inc., Cary, NC) for Latin square treatment designs. Treatment and replicate were considered fixed effects when analyzing physiological parameters for PreH and PostH measurements. If PostH had significant differences then physiological parameters were analyzed with treatment, replicate and time as fixed effects with time as a repeated measurement and a compound symmetry covariance structure. Treatment was considered as a fixed effect for behavioral analyses. For all analyses $P < 0.05$ was considered significant and $0.05 \leq P \leq 0.10$ was considered a tendency. Data is presented as means \pm standard error (SE).

2.7 Experiment 1 Results

Physiological measures are shown in Figure 2.2 for Experiment 1. Respiration rate ($P=0.007$) in the PostH period was lower in F4 and F8 gilts compared to CN1 at 35°C (Figure 2.2A). The change in RR from the start to end of heat stress was lower in F4 (2 ± 2 BPM) and F8 (1 ± 2 BPM) gilts compared to CN1 (14 ± 5 BPM) at 35°C ($P=0.005$) and tended to be lower at 32°C (-1 ± 3 , -1 ± 3 8 ± 5 BPM, $P=0.097$). In CN1, RR was higher 120 and 140 minutes into heat stress compared to pre-heat measurements (31 ± 3 , 37 ± 4 BPM vs 23 ± 1 , $P < 0.05$; Figure 2.3). RR in CN1

gilts differed from PreH measurements starting at 120 minutes and continuing to 140 minutes ($P<0.01$, Figure 2.3A). F4 and F8 gilt's postHS RR did not differ in comparison to PreH measurements ($P>0.05$).

Vaginal temperature was lower in F4 and F8 gilts during the PostH period compared to CN1 at 35°C (39, 39, 39.2 °C; $P=0.009$; Figure 2.2B). Again, no differences were seen between F4 and F8 gilts. The change in VT from the start (PreH) to end (PostH) of heat stress at 35°C was less in F4 (0.2 ± 0.1 °C) and F8 (0.3 ± 0.1 °C) compared to CN1 (0.5 ± 0.1 °C) at 35°C ($P=0.001$) and tended to be lower at 32°C (0.1 ± 0.1 °C (F4), 0.1 ± 0.1 °C (F8) vs 0.5 ± 0.1 °C (CN1), $P=0.064$). Looking at changes in VT over time, VT was higher than preheat measurements beginning at 60 minutes into HS (Figure 2.3B) and continuing throughout the heat event ($P<0.01$) in CN1. Compared to CN1, F4 and F8 shown lower internal temperatures 60 minutes into HS and remained lower until cooling of the room (Figure 2.3B).

Gilts, regardless of treatment, had increased skin temperature when compared to pre-heat measurements ($P<0.001$). No differences in skin temperatures were seen among the treatments at either 32°C and 35°C ($P=0.303$, Figure 2.2C).

Gilt behavior did not differ between CN1, F8 and F4 throughout the study at either 32°C and 35°C (Table 3.2A). Over 80% of the time, gilts were laying regardless of treatment.

2.8 Experiment 2 Results

The average RR and ST increased in the postHS period compared to the preHS period for all treatments ($P<0.01$ (RR), $P<0.01$ (ST)) at 32C. However, no differences in postHS measures were observed among treatments for RR ($P=0.78$), VT ($P=0.99$), or ST ($P=0.99$) in gilts. The change in RR, VT, and ST between preHS and postHS measurements at 32°C or 35°C also did not differ ($P=0.75$ (RR), $P=0.90$ (VT), $P=0.49$ (ST), Figure 2.4A-C).

The average RR, VT, and ST increased in the postHS period compared to the preHS period for all treatments ($P<0.01$ (RR), $P<0.01$ (VT), $P<0.01$ (ST)) at 35C. However, no differences in postHS measures were observed among treatments for RR ($P=0.74$), VT ($P=0.75$), or ST ($P=0.68$) in gilts. The change in RR, VT, and ST between preHS and postHS measurements at 32°C or 35°C also did not differ ($P=0.51$ (RR), $P=0.35$ (VT), $P=0.49$ (ST), Figure 2.4A-C).

Behavior did not differ between treatments throughout the study for either 32°C and 35°C (Table 3.2B).

2.9 Discussion

As ambient temperature increases, an animal will alter their behavior and physiology to remain cool and prevent increases in body temperature in the most energy efficient manner they can (Angilletta, 2009). In response to elevated temperatures the animal's ST will increase first. This was seen in the current studies as the gilts' ST were elevated early in the heating event and remained elevated throughout, regardless of treatment. The next thing the animal will do is alter their behaviors. This may include things such as postural changes or increased water consumption. In the current study, water consumption was not measured, but behavior observations were made. Gilts spent >80% of their time laying on the cooling pad regardless of whether the cooling pad was circulating water (cooling) or not. Perhaps the aluminum top of the cooling pad was slightly cooler because it was made of metal, so all animals exposed a large surface area of their body to the cold metal top of the pad similar to study findings by Aarnink et al., 2006. A more plausible explanation is that gilts use the least amount of energy when laying down (Brown-Brandl et al., 2001, Aarnink et al., 2006, Bonneau et al., 2021), so all animals remained relatively inactive during the study as a means of not creating additional body heat from activity.

When the animal's body temperature continues to increase despite these behavioral changes, the animal will begin to pant or increase their RR to increase evaporative heat loss to try and reduce the skin temperature. In the current study, the RR increased only in the control animals after ~80minutes of barn heating and remained elevated until the barn cooled for the 35°C study, suggesting that at 35°C gilts were less able to regulate their body temperature with behavioral changes alone. At 32°C, the control gilts tended to have increased RR and VT after the barn was heated suggesting that at this temperature, behavioral changes were effective at removing heat from the animal. However, at 35°C, VT did increase in control animals by 0.5°C despite the increased RR, suggesting that panting was not as effective at removing heat from the animal at 35°C.

While the term "heat stress" is used in this study to define the different stages of elevated temperatures in the barn, it is unlikely that the gilts were actually experiencing stress if the assumed

definition of heat stress is when an animal is outside of their thermal zone of comfort. When this happens, the animal's body temperature will increase at a rapid and uncontrolled rate. This was not observed in the current study with a short term, 140-minute heating event. While the animals were not likely heat stressed, they did have to alter their physiology to maintain their body temperatures when the barn was heated. One difference between this study and normal management of gilts would be the fact that the gilts were fed only a small meal prior to heating the barn, where normally gilts would be fed *ad libitum* during this developmental period. Increased feed intake could increase their metabolic heat production, making them more sensitive to the increased ambient temperatures (Collin et al., 2007; Kumar et al., 2016). Based on the limited increases in VT in the current study, it is unlikely that the gilts would venture outside of their thermal comfort zone with the added heat from increased feed intake at 32°C or 35°C. The increased feed intake may have resulted in earlier increases in RR and VT, however.

The floor cooling pads used in this study have been evaluated in lactating sows where water was continually flushed through the pads at three different water flow rates as the barn was heated to 35°C (Cabezón et al., 2017a). Cooling pads were able to maintain or decrease RR and VT in lactating sows during the barn heating event (100 minutes), despite elevations in skin temperatures. Similar findings are reported here in gilts where despite elevated skin temperatures, gilts housed on the floor cooling pads had decreased increases in their RR or VT at 32°C or 35°C compared to control. The cooling pads effectively removed excess heat from the animal allowing them to maintain constant RR and VT during the study. It should be noted that in the lactating sow study, the barn was heated rapidly over ~22 min and then maintained for 100 min while in the gilt study, the barn was slowly heated to desired temperature which took ~80 minutes. Therefore, the gilts were only experiencing the desired maximal temperature for ~40 minutes. This could be one reason that the changes in RR and VT were not as extreme as in the lactating sows (10bpm increase in RR in gilts vs 35bpm increase in lactating sows). Another explanation for this is that lactating sows are larger animals, with larger body mass, are fed *ad libitum* during lactation, and are producing large quantities of milk, all of which increase the animal's metabolic heat production. This would make the lactating sow alter their behavior and physiology quicker in response to elevated ambient temperatures compared to a limit-fed gilt.

This trial also evaluated different cooling pad water flushing operations where water either flushed through the pad at a set time frequency or when the sensors on the pad reached a set temperature. Flushing water at a set time, either every 4 or 8 minutes, was effective at reducing physiological alterations in gilts in response to the elevated temperatures. However, operating the pad with water flushing at a pre-determined temperature was not effective at eliminating these physiological responses as in Experiment 2, all gilts had elevated RR and VT, regardless of treatment. So, future work with the pads should be performed with water flushing at a set time.

2.10 Conclusion

When studying a short-term heat stress in limit-fed gilts, environmental temperatures need to reach at least 35°C to induce physiological changes in the gilt in response to the elevated temperature. When using a cooling pad to prevent physiological heat stress in swine, the operation of the cooling pads should be planned based on water flushing through the pad at a set time frequency. More specifically, water flush rates at a frequency of every 8 minutes is as effective as every 4, so a less frequent flush rate can be used to conserve water. Cooling pads are effective at removing physiological changes in limit-fed gilts to increased ambient and skin temperatures.

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2.12 References

- Aarnink, A. J. A., J. W. Schrama, M. J. W. Heetkamp, J. Stefanowska and T. T. T. Huynh. 2006. Temperature and Body Weight Affecting Fouling of Pigs Pens. *Journal of Animal Science*. 84:2224-2231. doi: 10.2527/jas.2005-521
- Angilletta, M. J. Jr. 2009. Thermoregulation. In *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Incorporated.
- Bonneau, M., N. Poulet, D. Beramice, L. Dantec, L. Canario and J.-L. Gourdine. 2021. Behavior Comparison During Chronic Heat Stress in Large White and Creole Pigs Using Image-Analysis. *Frontiers in Animal Science*. doi: 10.3389/fanim.2021.784376
- Bloemhof, S., P. K. Mathur, E. F. Knol and E. H. van der Waaij. 2013. Effect of Daily Environmental Temperature on Farrowing Rate and Total Born in Dam Line sows. *Journal of Animal Science*. doi: 10.2527/jas2012-5902

- Brown-Brandl, T. M., R. A. Eigenberg, J. A. Nienaber and S. D. Kachman. 2001. Thermoregulatory profile of a newer genetic line of pigs. *Livestock Production Science*. 71:253-260. doi: 10.1016/S0301-6226(01)00184-1
- Bull, R. P., P. C. Harrison, G. L. Riskowski, and H. W. Gonyou. 1997. Preference among Cooling Systems by Gilts under Heat Stress. *Journal of Animal Science* 75: 2078–83. doi: 10.2527/1997.7582078x
- Bouchama A. and E.B. De Vol. Acid-base alterations in heatstroke. 2001. *Intensive Care Med*. 27(4):680-5. doi:10.1007/s001340100906
- Cabezón, F.A., A.P. Schinckel, and R.M. Stwalley. 2017. Thermal capacity of hog-cooling pad. *Applied Engineering In Agriculture* 33.6:891-899. doi: 10.13031/aea.12333
- Cabezón, F. A., A. P. Schinckel, J. N. Marchant-Forde, J. S. Johnson, and R. M. Stwalley. 2017a. Effect of Floor Cooling on Late Lactation Sows under Acute Heat Stress. *Livestock Science*. 206:113–120. Doi: 10.1016/j.livsci.2017.10.017
- Collin, A., J. van Milgen, S. Dubois and J. Noblet. 2001. Effect of High Temperature on Feeding Behaviour and Heat Production in Group-Housed Young Pigs. *The British Journal of Nutrition* 86:63–70. doi: 10.1079/BJN2001356
- FASS. 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Federation of Animal Science Societies, Champaign IL
- Johnson, J. S., M. Abuajamieh, M. V. Sanz Fernandez, J. T. Seibert, S. K. Stoakes, J. Nteeba, A. F. Keating, J. W. Ross, R. P. Rhoads and L. Baumgard. 2015. Thermal Stress Alters Postabsorptive Metabolism During Pre- and Postnatal Development. In *Climate Change Impact on Livestock: Adaptation and Mitigation*, edited by Veerasamy Sejian, John Gaughan, Lance Baumgard, and Cadaba Prasad, 61–79. New Delhi: Springer India.
- Knox, Robert V. 2019. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Factors Influencing Follicle Development in Gilts and Sows and Management Strategies Used to Regulate Growth for Control of Estrus and ovulation. *Journal of Animal Science*. 97:1433–1445. doi: 10.1093/jas/skz036
- Kumar, S., S. V. Singh, P. Pandey, N. Kumar and O. K. Hooda. 2016. Estimation of metabolic heat production and methane emission in Sahiwal and Karan Fries heifers under different feeding regimes. *Vet World*. 9:496-500. doi: 10.14202/vetworld.2016.496-500
- Lammers, P. J., D. R. Stender, and M.S. Honeyman. 2007. Environmental Needs of the Pig. *Niche Pork Production*.
- Sasaki, Y., M. Fujie, S. Nakatake and T. Kawabata. 2018. Quantitative Assessment of the Effects of Outside Temperature on Farrowing Rate in Gilts and Sows by Using a Multivariate Logistic Regression Model. *Animal Science Journal*. 89:1187–1193. doi: 10.1111/asj.13048
- St-Pierre, N. R., B. Cobanov and G. Schnitkey. 2003. Economic Losses from Heat Stress by US Livestock Industries. *Journal of Dairy Science* 86:52–77. doi: 10.3168/jds.S0022-0302(03)74040-5

Table 2.1: Average room temperature and relative humidity throughout heat stress replicates

^aExperiment 1: Heat stress gilts (CN1), gilts on electronically-controlled floor cooling pad flushing water every 4 minutes (F4) or every 8 minutes (F8) under 32°C and 35°C ambient conditions

^bExperiment 2: Heat stress gilts (CN2), gilts on electronically controlled cooling pad flushing at 28°C (F28) or flushing at 29.5°C (F29.5) under 32°C and 35°C ambient conditions

	Experiment 1 ^a				Experiment 2 ^b			
	Temperature (°C)		Relative Humidity (%)		Temperature (°C)		Relative Humidity (%)	
	32°C	35°C	32°C	35°C	32°C	35°C	32°C	35°C
Before trial	17.10 ± 0.57	19.85 ± 0.87	65 ± 5	72 ± 4	18.15 ± 0.25	17.49 ± 0.80	65 ± 8	73 ± 7
0 min	30.50 ± 0.40	32.75 ± 1.00	58 ± 3	52 ± 3	31.6 ± 1.00	32.67 ± 0.37	58 ± 9	51 ± 4
20 min	31.58 ± 0.66	33.64 ± 0.70	58 ± 4	53 ± 3	31.48 ± 0.52	31.98 ± 0.41	65 ± 6	60 ± 1
40 min	31.70 ± 0.69	33.79 ± 0.57	58 ± 4	56 ± 2	31.56 ± 0.09	32.59 ± 0.49	69 ± 4	62 ± 1
60 min	31.77 ± 0.34	34.51 ± 0.55	56 ± 3	56 ± 1	31.73 ± 0.12	33.00 ± 0.57	71 ± 3	62 ± 2
80 min	31.74 ± 0.54	35.02 ± 0.51	58 ± 4	56 ± 2	32.16 ± 0.11	33.45 ± 0.56	72 ± 2	63 ± 3
100 min	31.42 ± 0.48	34.98 ± 0.28	59 ± 3	56 ± 2	32.52 ± 0.19	33.63 ± 0.55	72 ± 2	63 ± 3
120 min	32.47 ± 0.49	35.32 ± 0.13	56 ± 3	56 ± 2	32.71 ± 0.16	33.90 ± 0.55	72 ± 2	65 ± 2
140 min	32.17 ± 0.53	35.60 ± 0.11	58 ± 2	55 ± 3	33.01 ± 0.15	34.21 ± 0.52	72 ± 2	65 ± 2

Behavior	Position
Standing	Head in feeder, head near front of the pad, butt at the rear of the crate
Sitting	Head laying on feeder, head laying on front of pad, butt at the rear of the crate
Laying (sternal or lateral)	Head laying on feeder, head laying on front of pad, butt at the rear of the crate

Figure 2.1: Ethogram used for behavior analysis

Experiment 1: Heat stress gilts (CN1), gilts on electronically-controlled floor cooling pad flushing water every 4 minutes (F4) or every 8 minutes (F8) under 32°C and 35°C ambient conditions

Experiment 2: Heat stress gilts (CN2), gilts on electronically controlled cooling pad flushing at 28°C (F28) or flushing at 29.5°C (F29.5) under 32°C and 35°C ambient conditions

Table 2.2: Comparison of behaviors in heat stress gilts

^aExperiment 1: Heat stress gilts (CN1), gilts on electronically-controlled floor cooling pad flushing water every 4 minutes (F4) or every 8 minutes (F8) under 32°C and 35°C ambient conditions

^bExperiment 2: Heat stress gilts (CN2), gilts on electronically controlled cooling pad flushing at 28°C (F28) or flushing at 29.5°C (F29.5) under 32°C and 35°C ambient conditions

A	Experiment 1 ^a							
	32°C				35°C			
	CN1	F4	F8	P-value	CN1	F4	F8	P-value
Sitting, %	5.65±4.3	7.30±4.08	2.15±0.77	0.5664	2.54±1.72	5.88±3.19	3.37±1.53	0.8865
Standing, %	6.00±1.34	9.87±1.55	4.93±2.02	0.2256	10.69±5.63	10.60±2.83	7.34±2.61	0.2549
Laying, %	89.44±3.92	83.43±4.00	88.53±2.72	0.4468	86.78±5.97	83.52±4.12	89.30±3.34	0.3314
Sternal, %	8.05±4.39	7.57±4.20	4.93±2.02	0.8167	0.91±0.60	9.42±6.19	6.04±5.08	0.4037
Lateral, %	85.43±4.21	79.99±4.08	83.61±3.47	0.6083	85.87±6.02	74.10±9.25	83.26±6.16	0.643

B	Experiment 2 ^b							
	32°C				35°C			
	CN1	F4	F8	P-value	CN1	F28	F29.5	P-value
Sitting, %	3.23±1.16	6.02±2.75	5.58±2.66	0.6903	2.54±1.72	5.88±3.19	3.37±1.53	0.5642
Standing, %	12.58±3.45	11.98±3.01	13.74±2.72	0.9419	10.69±5.63	10.60±2.83	7.34±2.61	0.7922
Laying, %	84.18±3.33	82.00±3.65	80.69±3.97	0.8123	86.78±5.97	83.52±4.12	89.30±3.34	0.6781
Sternal, %	7.44±5.39	0.83±0.68	1.49±1.45	0.3347	0.91±0.60	9.42±6.19	6.04±5.08	0.4395
Lateral, %	76.74±5.78	81.18±3.70	79.20±4.18	0.7922	85.87±6.02	74.10±9.25	83.26±6.16	0.4992

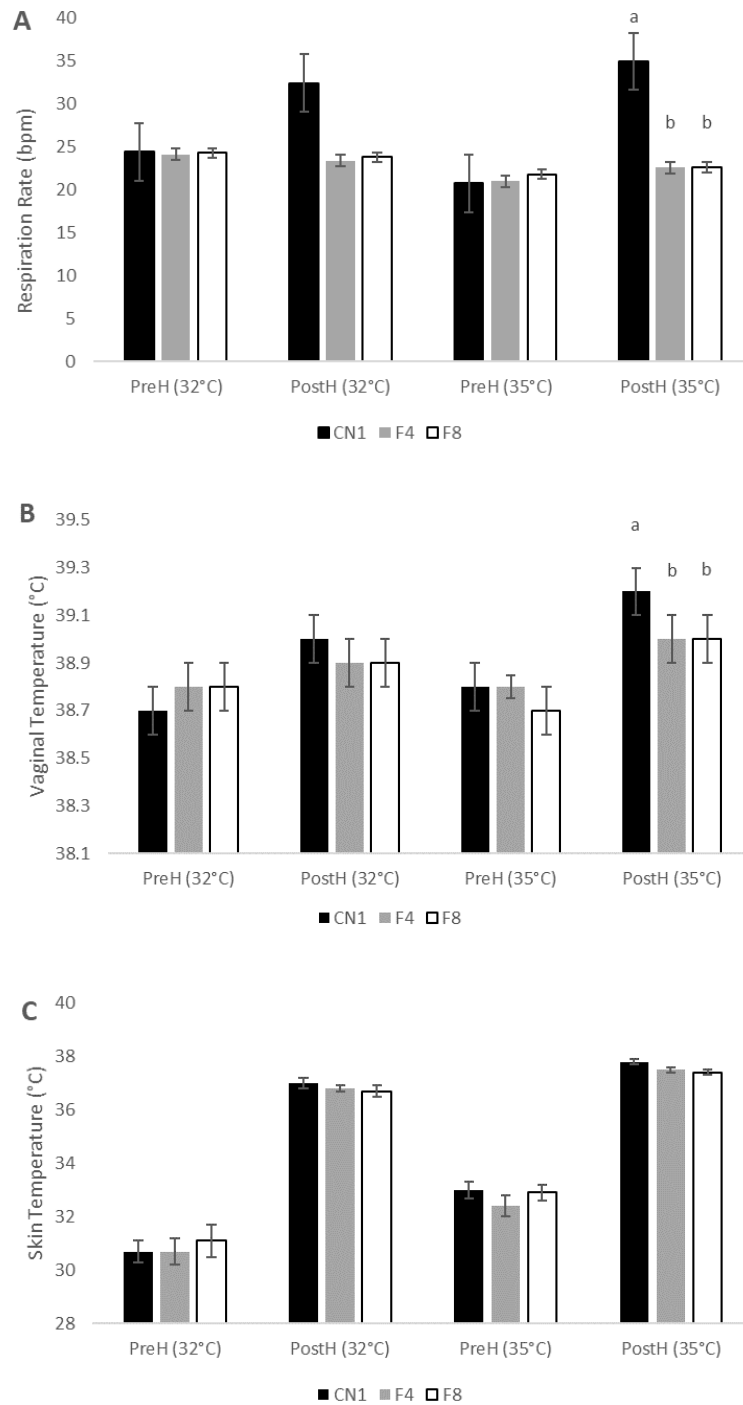


Figure 2.2: Comparison of respiration rate

(A), vaginal temperature (B) and skin temperature (C) in heat stress gilts (CN1), gilts on electronically-controlled floor cooling pad flushing water every 4 minutes (F4) or every 8 minutes (F8) under 32°C and 35°C ambient conditions (Experiment 1).

^{a,b} Different superscripts represent different means at $P < 0.05$

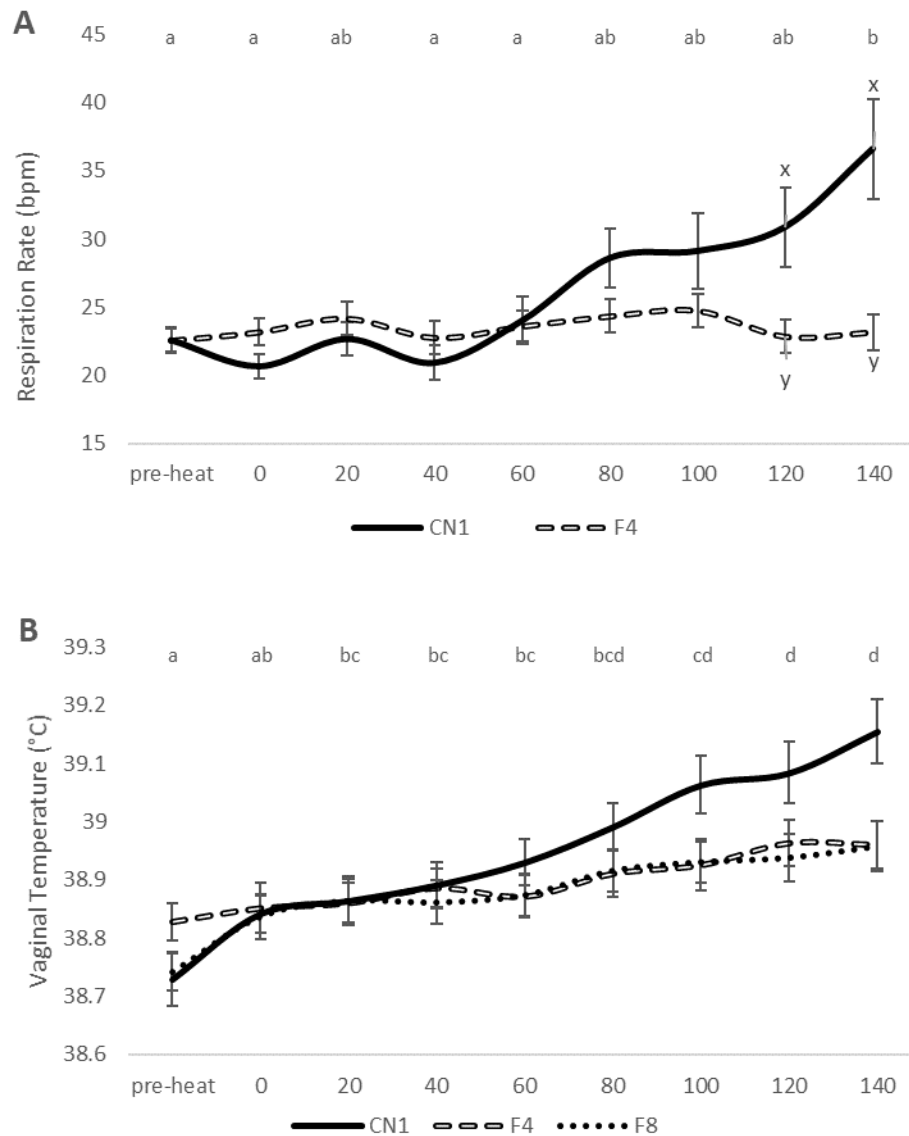


Figure 2.3: Comparison of respiration rate

(A) and vaginal temperature (B) over time in heat stress gilts (CN1), gilts on electronically-controlled floor cooling pad flushing water every 4 minutes (F4) or every 8 minutes (F8) under 35°C ambient conditions (Experiment 1).

^{a,b,c,d} Different superscripts represent different means in time at $P < 0.05$

^{x,y} Different superscripts represent different means in trt*time at $P < 0.05$

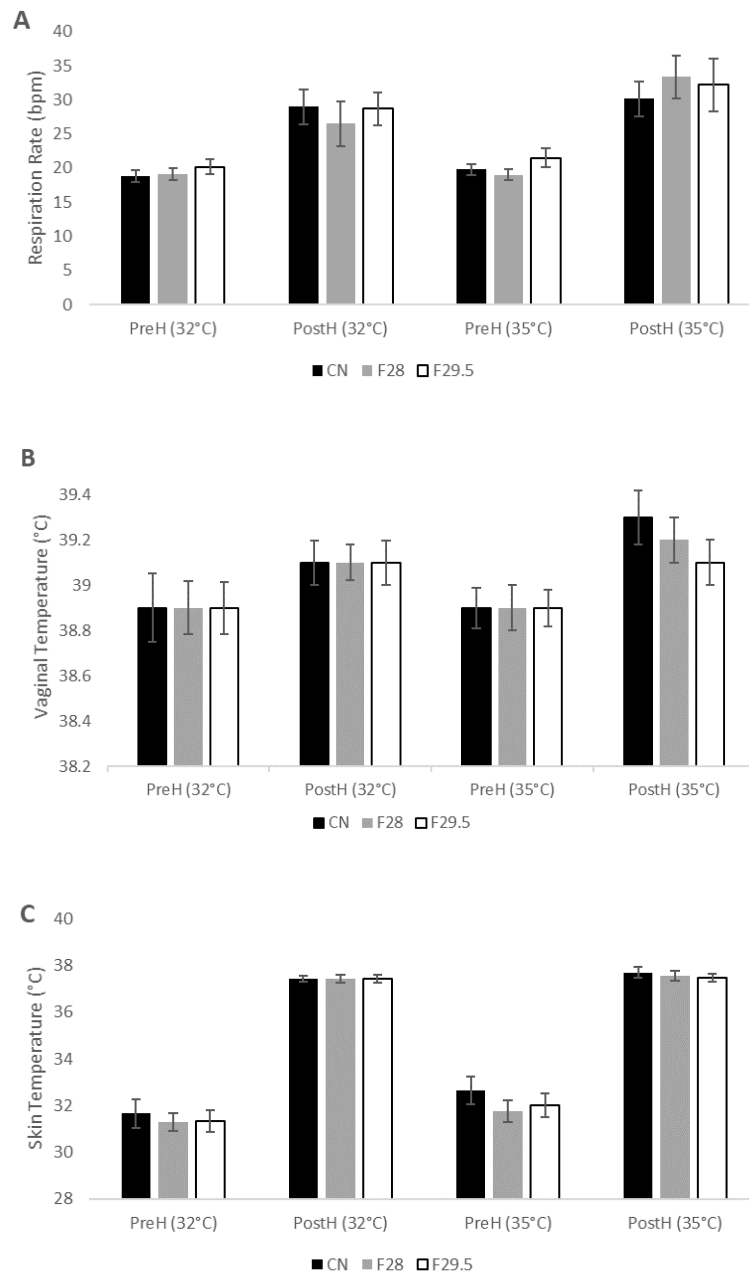


Figure 2.4: Comparison of respiration rate

(A), skin temperature (B) and vaginal temperature (C) in heat stress gilts (CN2), gilts on electronically controlled cooling pad flushing at 28°C (F28) or flushing at 29.5°C (F29.5) under 32°C and 35°C ambient conditions (Experiment 2).

CHAPTER 3. USE OF AN ELECTRONICALLY-CONTROLLED FLOOR COOLING PAD DURING HEAT STRESS ON THERMOREGULATORY AND REPRODUCTIVE PERFORMANCE IN BOARS

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3.1 Abstract

The study objective was to evaluate the use of an electronically-controlled floor cooling pads' ability to reduce physiological indicators of heat stress and reduction in semen quality due to heat stress in two experiments conducted at 32°C (Experiment 1) and 35°C (Experiment 2). Twenty-four boars were housed in 2.2 x 0.6 meters farrowing crates and randomly assigned to either CONTROL (n=12; 0.0L water flushed through the pad) or FLUSH (n=12; 2.0 L of water flushed every 8 minutes or when sensors reached 27°C, which ever occurred first) treatments. Boars were subjected to a cyclical heat stress or a duration of 3 days. Each day the room was gradually heated from 28°C to 32°C for the first 2 hours and then from 32°C to 35°C and remained at 35°C for 8 hours before being cooled to 28°C overnight. Humidity remained greater than 60% for the 3 days. Cooling pads remained on and flushing for the FLUSH treatment 24 hours per day for the full three days. Physiological indicators of heat stress consisted of respiration rate (RR), rectal temp (RT) and skin temps (ST) taken on the ear, shoulder, rump and tail recorded 20 minutes into heat stress and then every 2 hours for the duration of the heat stress, as well as 2 hours after the room was cooled. Testicle temperature (TT) was taken using an infrared camera one time a day after 10 hours of heat right before the room was cooled. Semen was collected twice before heat stress to determine a base line semen quality for each boar to be used as covariates in the statistical evaluation. Semen collection began on day 1 following heat stress and continued weekly for 29 days in Experiment 1 and 43 days in Experiment 2. Evaluation of semen consisted of volume, total sperm, viability, CASA characteristics such as motility and progressive motility and sperm and acrosome morphology. FLUSH boars had lower RR, ST, TT and RT compared to CONTROL boars (P<0.05). FLUSH boars maintained normal semen quality during weekly collection following heat stress whereas CONTROL boars had reduced semen quality, more

profound at 35° compared to 32°C. iCa⁺ and PCO₂ increased in CONTROL boars at 35C compared to FLUSH boars. Cooling pads successfully eliminated physiological indicators of heat stress in boars.

Key words: boar, heat stress, physiological response, semen quality

3.2 Introduction

Previous studies have shown increases in respiration rate, which disrupts the balance of O₂ and CO₂ within the body, during periods of elevated ambient temperatures (Lammers et al., 2007; Cabezon et al., 2017; Lucy and Safranski, 2017; Bouchama and De Vol., 2001). As temperature increases over time pigs typically decrease their feed intake and over-hydrate resulting in a loss of electrolytes and a decrease in blood gas parameters (Black et al., 1993; Baumgard and Rhoads, 2013; Reneaudeau et al., 2008; Reneaudeau et al., 2010; Ait-Boulahsen et al., 1995; Puvadolpirod and Thaxton, 2000; Zulkifli et al., 2009; Wang et al., 2018). Pigs exposed to high ambient temperatures will alter their behaviors and physiology, which can have negative consequences on growth and reproduction.

Specifically, in boars, exposure to elevated ambient temperatures causes a decrease in semen quality and a subsequent reduction in boar fertility (Flowers, 2015; McNitt and First, 1970). As ambient temperatures increase, the percentage of ejaculates with poor semen quality increases, resulting in fewer usable ejaculates for creating insemination doses for breeding females (Knox et al., 2008). The most typical changes to semen quality are a decrease in motility as well as an increase in morphological abnormalities (Parrish et al., 2017; Rockett et al., 2001; Cai et al., 2010; Rao et al., 2015; McNitt and First, 1970). The timing of reduced semen quality and the severity of the decrease in quality depends on the severity of temperature increase and duration of time the temperatures remain elevated. Research conducted previously can be divided into short-term elevations, defined as up to 1 week, and long-term elevations lasting >1 week. This distinction comes from a study by Cameron and Blackshaw (1980) that showed after 7 days of elevated temperatures, boars were more negatively impacted compared to 3, 4 or 5 days. Short-term elevations seem to negatively affect sperm motility and morphology (McNitt and First, 1970, Cameron and Blackshaw, 1980), whereas long-term elevations will also reduce sperm production (Flowers, 1997, 2015). It can take up to about 2 weeks for these alterations in the semen to appear.

This is because spermatogenesis takes approximately 39 days in boars, and increased temperatures can differentially affect the developing cells in the testicle where cells undergoing meiosis are more susceptible to damage from elevated temperature (Parrish et al., 2017). Additionally, the negative alterations in the semen may remain for 5 weeks after the increased temperatures subside (Parrish et al., 2017). A natural example of long-term heat stress is seen with the changes in season where during the summer months, the ambient temperatures remain elevated for multiple weeks. With global warming we are seeing additional increases in fluctuations and peak daily temperatures, providing swine with short-term elevations in temperature.

While the swine industry has added management strategies such as drippers and fans to minimize the impact of elevated temperatures on animals, there is still room for improvement. Electronically-controlled floor cooling pads have been designed to assist pigs with heat loss (Cabezón et al., 2017). Studies have shown that the use of electronically-control floor cooling pads to be effective in assisting lactating sows with heat loss resulting in decreasing respiration rate as well as internal and skin temperatures in sows due to conductive heat exchange but has not been studied fully with boars (Cabezón et al., 2017a). Therefore, the study objective was to evaluate the use of electronically-controlled floor cooling pads during periods of short-term elevated temperature on boar's thermoregulation, semen production, and semen quality. The hypothesis is that cooling pads will effectively remove negative impacts of elevated ambient temperature on boar's physiology and semen production and quality.

3.3 Materials And Methods

Two studies were conducted to evaluate the effects of heat stress on boars. Both studies were approved by Purdue University Animal Care and Use Committee (1804001739). Experiment 1 was conducted from February-April 2020 and consisted of 24 boars divided into two replicates of 12 boars each that were exposed to barn temperatures of 32°C with $\geq 60\%$ humidity for three days (Table 3.1) followed by weekly semen collection for 29 days. Experiment 2 was conducted from May-July 2020 on the same 24 boars in two replicates except the barn temperatures were maintained at 35°C for three days (Table 3.1) followed by weekly semen collection for 43 days. Boars were terminally crossbred (York/Landrace x Duroc) boars born and raised at Purdue University in May 2019 and were 9 months of age at the start of Experiment 1.

3.3.1 Cooling Pad Structure and Setup

Electronically-controlled floor cooling pads consisted of an aluminum treaded plate covering copper pipes with a polyethylene base bottom as described in the paper Cabezon et al., 2017. The copper pipes are able to circulate water throughout the pad at either a certain flush time or when the sensors in the pad reach a certain set temperature (Cabezon et al., 2017a). The pads were installed in the floor of sow farrowing crates that were 2.2 x 0.6m in size.

3.3.2 Treatments

Boars were housed in individual farrowing crates (2.2 x 0.6m) equipped with a floor cooling pad. For Experiment 1, boars were randomly assigned to one of two treatments: water flow of 0.0 L of water (CONTROL, n=12); or 2.0 L of water flushed every 8 minutes or when sensors reached 27°C, whichever occurred first (FLUSH, n=12) and the barn was heated to 32°C. For Experiment 2, boars were randomly re-allotted to the same two treatments and the study repeated at a barn set temperature of 35°C. For both studies, boars were moved into farrowing crates before 1200 h on day 0 (before heat stress) and moved out the morning of day 4 back to the boar stud.

3.3.3 Heat Stress and Physiological Measurements

Boar body weight was recorded the day before (day -1) heat stress and the day following heat stress (day 4) in Experiment 2. At 630 every morning, boars were fed 3.4kg of a boar-specific diet designed to meet or exceed NRC (2012) requirements. Pre-heat physiological measurements were taken twice before the barn temperature increased on day 1, and these values were averaged together for a baseline value for each boar. All cooling pads were turned on after last pre-heat measurement on day 1 and pads assigned FLUSH treatment began to flush at this time. The barn was then gradually heated from 28°C to 30°C immediately following the second pre-heat measurement. The barn was held at 30°C for 2 hours and then increased from 30°C to either 32°C or 35°C, for Experiments 1 and 2, respectively (Table 3.1). The barns were then maintained at these maximum temperatures for an additional 8 hours. The barns were then cooled from the maximal temperature to 28°C overnight to mimic a typical cyclical environmental heat stress. This barn heating schedule was repeated for three consecutive days during which relative humidity was

maintained at $\geq 60\%$ using humidifiers. Temperature and humidity were recorded every 5 minutes using data loggers (HOBO, accuracy $\pm 0.2^\circ\text{C}$; Onset, Bourne, MA) placed in farrowing crates at pig level. All cooling pads remained on throughout the day and night for 3 consecutive days. The timeline of the study design is shown in Figure 3.1.

Physiological measurements collected were skin temperature (ST), rectal temperature (RT) and respiration rate (RR) collected twice prior to heating the barn, 20 minutes after the barn started heating, and every 2 hours for 12 hours (Pre-heat1, Pre-heat2, 20 mins, 2 hrs, 4 hrs, 6 hrs 8 hrs, 10 hrs, and 12 hrs; Figure 3.1). Skin temperature was recorded via an infrared thermal imaging camera (accuracy $\pm 2\%$, emissivity=0.98, FLIR Model T440; FLIR Systems Inc.; Wilsonville, OR). Images were captured to record ST behind ear (STear), shoulder (STshoulder), rump (STrump) and tail (STtail). Testicle temperature (TT) was also captured at both pre-heat measurements and again at 10 hrs after initiation of barn heating with an infrared thermal camera. FLIR Tools Software (version 5.13) was used to analyze all thermal images. Respiration rate was recorded by number of flank movements (number of breathes) for 15 seconds and then multiplied by 4 to record breathes/minute. Rectal temperatures were measured using thermistor thermometer with a 9.5 cm probe (Cooper Atkins TM99A, Middlefield, CT, U.S.A.) with a temperature reading range of -40°C - 160°C and accuracy of $\pm 0.5\%$.

3.3.4 Blood Collection

Boars were snared and blood was collected via jugular venipuncture into a serum separator tube at 1200 h on day 0 (pre-heat stress; PHS), day 1 (acute heat stress; AHS), and day 3 (chronic heat stress; CHS). One milliliter of blood was placed into a microcontainer containing lithium heparin (BD Microtainer™ Capillary Blood Collector) to prevent clotting. This sample was evaluated using a CHEM8+ cartridge on an i-Stat machine (i-Stat 1, Abbott, Orlando, FL, U.S.A.). The blood components measured were sodium (Na, mmol/L), potassium (K, mmol/L), ionized calcium (iCa, mmol/L), glucose (mg/dL), hematocrit (hct, % PCV), hemoglobin (hgb, g/dL), pH, carbon dioxide partial pressure (PCO₂, mmHg), oxygen partial pressure (PO₂, mmHg), base excess (BE, mmol/L), bicarbonate (HCO₃, mmol/L), total carbon dioxide (TCO₂, mmol/L), and oxygen situation (sO₂, %).

3.3.5 Semen Collection And Evaluation

Prior to and following heat stress, boars were housed individually in a boar barn equipped with 2.2 x 0.6m crates and two 1.1 x 1.3m semen collection pens. Boars were trained for semen collection using the gloved-hand method and were on a routine weekly semen collection schedule. When semen was collected, raw ejaculate was evaluated on farm for volume (g), concentration (SpermaCue, Minitube, USA) and motility (brightfield microscopy, subjective scoring). Semen was then diluted 1:5 with BTS extender with antibiotics (Beltsville Thawing Solution, Minitube, USA) and transported approximately 19 km to the Purdue University laboratory. At the lab, samples were further diluted (when necessary) and warmed for 15 minutes at 35°C before loading 3 microliters of sample into a Leja® slide (IMV, USA) chamber to be evaluated using a computer-assisted semen analysis system (CASA, CerosII, Hamilton Thorne) for motility, progressive motility and kinematic parameters. Kinematic parameters consisted of amplitude of lateral head displacement (alh), beat cross frequency (bcf), distance curvilinear (dcl), distance straight line (dsl), linearity (lin), straight line (str), (vap), velocity curvilinear (vsl), velocity straight line (vsl) and wobble (wob). A separate 1 mL of diluted semen was preserved with 50 µl of 10% formalin for evaluation of spermatozoa morphology. Two-hundred sperm cells were hand counted on phase-contrast microscope using a 40x objective and classified as normal or containing proximal droplets, distal droplets, distal midpiece reflex (DMR), or head/tail abnormalities. Using oil immersion and the 100x objective, an additional 100 morphologically normal sperm cells were classified as having a normal or abnormal acrosome morphology. Sperm concentration and viability was assessed using a Nucleocounter® (Reproductive Provisions, USA). Nucleocounter® samples were diluted to appropriate concentrations according to manufacturer's specifications in Sp100 or BTS, for evaluation of total concentration and viability, respectively. Total sperm in ejaculate was calculated by $(\text{volume (mL)} \times \text{total cells (million/mL)}) \times \text{dilution ratio} / 1000$. Total non-viable cells in the ejaculate were calculated by $((\text{non-viable (million/mL)} / 1000) \times \text{volume (mL)}) / \text{total sperm in ejaculate}$. Total percent non-viable was calculated by $\text{total non-viable in ejaculate} / \text{total sperm cells in ejaculate}$.

Semen quality was analyzed for all 24 boars 2 weeks before heat stressing and averaged as covariates in the statistical model. Semen collection continued on the same day every week beginning the day following the 3d heat stress (day 4) and continuing weekly. Experiment 1 ended at 29 days post heat stress and Experiment 2 was continued until 43 days post heat stress.

3.3.6 Statistical Evaluation

All analyses were performed using Proc MIXED in SAS v9.4 (SAS Inst. Inc., Cary, NC) for repeated measures. Compound-symmetry structure was used to nest boar within treatment, which was included as a random effect for all analyses. A Tukey-Kramer means comparison method was used to evaluate differences between time and day. The slice option was used to evaluate treatment by time or treatment by day interactions. For all analyses, a $P < 0.05$ was considered significant and $0.05 \leq P \leq 0.10$ was considered a tendency.

Treatment, day and time were treated as fixed effects with time as a repeated measurement when analyzing physiological indicators. Environmental variables such as room temperature and relative humidity were included as covariates. Other variables included as covariates consisted of boar set (first 12 or second 12 of boars) and if a boar had to be cooled during the study. Pre-heat stress measurements were included as a covariate for all physiological measures. Treatment and day were treated as fixed effects with day as a repeated measurement when analyzing I-STAT blood parameters with pre-heat stress measurements included as covariates and a compound symmetry covariance structure. Treatment and day were treated as fixed effects with day as a repeated measurement and pre-heat stress measurements as covariates for semen quality parameters. Covariates were removed from model if $P > 0.10$.

3.4 Results

3.4.1 Physiological Indicators of Heat Stress

Table 3.1 illustrates the overall ambient temperature and relative humidity throughout heat stress for all 3 days. In both Experiment 1 and 2, desired barn temperatures of 32°C and 35°C were achieved by 4 hours of barn heating, or within 2 hours of increasing the set temperature up from 30°C.

All physiological indicators of stress are shown in Table 3.2. No differences were seen in body weight change during heat stress in boars at 35°C. All boars consumed all of their feed every day during heat stress in both treatments for both experiments. Boars, regardless of treatment, had significantly greater RR on day 2 then on day 1 and 3 at 32°C (62 vs 48 and 53 bpm, $P < 0.0001$). Likewise, ST was significantly lower on day 3 on the ear (37.1 vs 37.0, $P = 0.036$), rump (36.4 vs

36.3, $P=0.007$), tail (36.4 vs 36.3 $P<0.001$) and shoulder (36.6 vs 36.6, $P=0.003$ shoulder) regardless of treatment at 32°C. At 35°C, boars had significantly greater RR and RT on day 1 compared to day 3 (91 vs 77 bpm; $P<0.01$, 38.5 vs 38.3, $P<0.01$).

Overall RR, RT, ST (ear, shoulder, rump), and TT were lower in FLUSH boars compared to CONTROL ($P<0.008$) in both Experiment 1 and 2. FLUSH boars had significantly lower RR all 3 days at 32°C when compared to CONTROL boars (Table 3.3). Within CONTROL boars at 32°C, day 2 and 3 had significantly greater RR compared to day 1 (Table 3.3). No other physiological indicators of heat stress had treatment by day interactions at 32°C. At 35°C, FLUSH boars had significantly lower RT during heat stress (day 1, 2 & 3) and CONTROL boars had higher RT on day 1 compared to day 3 (Table 3.4). Figure 3.3 shows the effect of day of heat stress on the average RT, ST and TT at 35°C. RT was highest on d1 of heat stress compared to day 2 and 3. STear and STshoulder were higher on days 1 and 2 of heat stress compared to day 3. STrump and STtail were higher on day 2 compared to d 1 and 3. TT did not differ by day of the heat stress. Figures 3.4 and 3.5 shows RR, RT and STshoulder over the duration of the cyclical heat stress events each day for 32°C and 35°C, respectively. FLUSH boars had significantly lower RR, RT, STshoulder beginning 4 hours into heat stress and continuing through the duration of the sampling period when compared to CONTROL boars at 32°C (Figure 3.3A-C). At 35°C, FLUSH boars RR was lower than CONTROL at 20 minutes into heat stress and continued throughout the heat stress event (Figure 3.4A). However, RT tended to be lower in FLUSH boars than CONTROLS at 2 hours, significantly lower by 4 hours into heat stress and STshoulder wasn't lower until 6 hours into heat stress (Figure 3.4B,C).

Table 3.5 shows all i-STAT blood parameters by treatment and experiment for each of the three timepoints blood was collected (PHS, AHS, CHS). At 32°C, no i-STAT measurements were different between CONTROL and FLUSH boars, except a tendency for glucose to be lower in FLUSH boars when compared to CONTROL ($P=0.0804$). At 35°C, FLUSH boars had increased iCa²⁺, pH, and PCO₂ and decreased sO₂ during the AHS phase and an increased K⁺ in the CHS phase.

When the data was evaluated to compare PHS to heat stress (AHS+CHS), FLUSH boars had higher hct during heat stress at 35°C in comparison with CONTROL boars (37.27 vs 33.86%PCV; $P=0.0488$). There was a tendency for higher hgb in FLUSH boars (12.67 vs

11.53g/dL; $P=0.0525$) during heat stress. K^+ was higher in FLUSH boars during heat stress (4.80 vs 4.30mmol/L; $P=0.0069$). When all three phases were included in the statistical model (PHS, AHS, and CHS), PCO_2 was higher in FLUSH boars (42.0 vs 37.25mmHg; $P=0.0213$) due to a treatment by phase interaction ($P=0.0353$) where FLUSH boars had greater PCO_2 during AHS when compared to CONTROL (44.35 vs 35.44mmHg). iCa^{2+} levels in CONTROL boars were lower during AHS compared to CONTROL boars during CHS ($P=0.0387$) and FLUSH boars during AHS ($P=0.0151$). There was a tendency for pH to differ between treatments by phase with FLUSH boars in AHS being lower than CONTROL boars during CHS (7.45 vs 7.49).

Figure 3.6 depicts changes in i-STAT blood parameters by phase of heat stress (PHS, AHS and CHS) when boars were exposed to ambient temperatures of 35°C. For glucose, PHS was higher than AHS, with CHS being intermediate to both (81.75, 75.65 and 77.95; $P=0.0229$). For hct, PHS tended to be higher than AHS and was significantly higher than CHS (41.75, 36.13, 34.95% PCV). Likewise, PHS hgb tended to be higher than AHS ($P=0.0626$) and was significantly higher than CHS ($P=0.047$, 13.34, 12.29 and 11.89)

3.4.2 Semen Quality Post Heat Stress

Table 3.6 shows all of the semen parameters from boars following the 3-day heat stress for both Experiments 1 and 2. At 32°C, FLUSH boars had significantly lower volume compared to CONTROL ($P=0.0078$), without a corresponding decrease in total sperm ejaculated ($P=0.9221$). FLUSH boars had increased motility compared to CONTROL boars, driven by differences on day 22 and 29 post heat stress (Figure 3.7). Normal morphology was numerically higher in FLUSH boars compared to CONTROL ($P=0.1098$) with a tendency for lower proximal droplets ($P=0.0587$). Regardless of treatment, non-viable sperm, proximal droplets, DMRs, and tail abnormalities varied by days post heat stress increasing on days 15-29 (Figure 3.8).

At 35°C, FLUSH boars had significantly higher volume, motility, progressive motility, percent normal morphology and normal acrosome with a tendency for higher total sperm in ejaculate (Table 3.6). The improvement in normal morphology stemmed from decreased proximal droplets, distal droplets, and tail abnormalities in FLUSH boars. While not significant, FLUSH boars had numerically lower percent non-viable sperm cells compared to control boars (16.1 vs 18.4 %, $P=0.2301$). There was a treatment by day interaction where FLUSH boars had higher

motility on d15-29 and progressive motility d15-36 post heat stress when compared to CONTROL boars exposed to 35°C ambient temperature (Figure 3.9A-B). FLUSH boars had higher normal morphology ($P=0.0055$) on days 15 and 22 due to a decrease in proximal droplets in comparison to CONTROL boars at 15, 22 and 29 d post heat stress ($P=0.0344$, Figure 3.10A-B). Additionally, 22 d post heat stress, FLUSH boars had increased percent normal acrosomes compared to CONTROL boars ($P=0.0014$, Figure 3.11).

3.5 Discussion

The present study indicates that the use of electronically controlled cooling pads is an effective method to alleviate heat stress in boars and the associated decreases in semen quality. Boars tend to regulate their body temperature via alterations to their physiology and behavior to maintain homeostasis during times of high ambient temperatures. Boars from 70 to 100 kgs body weight have an upper extreme limit of 35°C while boars >100 kg have an upper extreme of 32°C (FASS 2021), providing the justification for testing both temperatures in the current study as boars weighed 176 +/- 15 kgs at the start of Experiment 1 and 217 +/- 4 kgs. at the start of Experiment 2. Boars exposed to ambient temperatures above their upper extreme limit except for brief periods should be provided with cooling methods.

As ambient temperature increases, animals' skin temperature will increase and they will divert blood flow from the internal organs, such as the reproductive organs and digestive tract, to the skin in an attempt to remove additional heat. The animal will also alter their behaviors such as making postural changes or increasing water intake to remove excess heat. When these methods are not effective, the animal will increase their respiration rate to allow for evaporative heat loss. When this is not effective, an increase in the animal's body temperature will occur. Exposure to elevated temperatures as in Experiment 1 (32°C) and Experiment 2 (35°C) resulted in an increase in RR, ST, and RT compared to baseline measurements, which was also observed by Cabezon et al. 2017 in lactating sows and reviewed by Lucy and Safranski (2017) in pregnant sows. This indicates that the conditions used in this study were effective at inducing physiological changes in boars in response to the elevated temperatures. The alterations in physiological parameters were more extreme in the 35°C compared to 32°C, where for example, RR averaged 114 bpm at 35°C and only 71 bpm at 32°C.

As ambient temperatures increase, animals tend to decrease feed intake as a way to reduce their metabolic heat production (Baumgard and Rhoads 2013; Reneaudeau et al., 2008; Reneaudeau et al 2010; Black et al. 1993). No differences were seen in this trial in feed intake which could be due to boars being limit fed 3.4 kg of feed in one meal/day compared to studies in lactating sow studies where sows were fed ad libitum (Cabezón et al., 2017). Additionally, previous work in growing pigs has indicated that under elevated temperatures, pigs will remove electrolytes in their urine resulting in decreased circulating levels (Holmes and Grace, 1975). In the current study, there were no differences seen between treatments for i-STAT parameters at 32°C but at 35°C we found that K⁺ was decreased in CONTROL boars compared to FLUSH. Also, hct and hgb were decreased in CONTROL boars compared to FLUSH boars throughout the duration of the study. One potential explanation for this difference could be due to CONTROL boars having increased water intake. Water intake was not measured in this study, however, animals undergoing heat stress tend to increase water consumption resulting in a loss of electrolytes, and decreased hematocrit and hemoglobin levels (Ait-Boulahsen et al. 1995; Puvadolpirod and Thaxton 2000; Zulkifli et al 2009; Wang et al 2018).

The current study evaluated blood parameters on day 1 (AHS) and 3 (CHS) of elevated temperatures. There were no differences in blood parameters among treatments during AHS or CHS in Experiment 1 at 32°C. However, in Experiment 2 at 35°C, CONTROL boars had lower iCa and PCO₂ during the AHS phase, and lower K⁺ during CHS. Subjecting pigs to heat stress can result in respiratory alkalosis due to increasing respiration rate (Patience et al., 2005). When respiration rate is increased, the balance of breathing in oxygen and breathing out CO₂ is disrupted, therefore, decreasing the PCO₂ in the blood (Cottrell et al., 2020; Patience et al., 2005), which is an explanation for the decreased PCO₂ seen in this study. Boars appeared to acclimate to the elevated temperatures as the PCO₂ was not different on day 3 (CHS). Wang et al. (2018) observed similar results in chickens where heat stressed chickens had lower PCO₂ levels and K⁺ during acute heat stress (4 hours post heat treatment) and decreased PCO₂, hct, and hgb during chronic heat stress (6 days post heat treatment). In the current study, decreased K⁺ was not seen until day 3 (CHS) of elevated temperatures. When chickens undergo heat stress, their increased respiratory rates cause respiratory alkalosis and a subsequent reduction in blood ionized calcium levels (Odom et al., 1984). In the present study, there are indicators that respiratory alkalosis was occurring as

RR was greatly increased at 35°C and blood pH tended to be higher in CONTROL boars, likely contributing to the decreased iCa⁺.

Knox et al., (2008) found that 1-10% of ejaculates were discarded due to poor motility and morphology during the warm seasons of the year, which has been confirmed by Flowers (1997, 2015) who found boars exposed to temperatures above 26-29°C for 10-14 weeks resulted in reduced semen quality. Heat stress associated with season could be considered a long-term, or chronic stress, which has different effects on semen output and quality compared to a short-term stress, which is more likely to only impact semen quality (Cameron and Blackshaw, 1980). A review from Parrish et al. (2017) concluded that boars exposed to temperatures above 33°C results in abnormal sperm 2 weeks post stressor for up to a duration of 3 weeks. McNitt and First (1970) evaluated how heat stress effects semen quality in boars exposed to 33°C and 50% humidity for 72 hours and found a decreased total sperm count and increased percent abnormal spermatozoa around two weeks after exposure to the stressor. In the present studies, a 3-day heat stress at 32°C resulted in decreased motility beginning 22 days after the end of the period of elevated temperatures, with no changes seen in sperm cell morphology. In this experiment, semen was only collected for 29 days following the period of elevated temperatures, which was likely not long enough to see the semen quality return to normal levels. However, when the barns were heated to 35°C and semen was collected for an additional 43 days, motility and sperm cell morphology were decreased beginning on day 15 and continued through day 29 following the period of elevated temperatures. The reduction in semen quality starting earlier at 35°C suggests that more developing sperm cells in the testicle were negatively impacted by the elevated temperatures compared to 32°C. Regardless, this study concludes that boars exposed to cyclical increases in ambient temperatures above 32°C for 3 days will cause decreases in semen motility and morphology without a corresponding decrease in sperm cell production. This is consistent with studies evaluating short-term (<7d) elevated temperatures in boars (Cameron and Blackshaw, 1980).

Utilization of cooling pads in boars was able to alleviate all of the physiological adaptations to increased elevated temperatures in boars at both 32°C and 35°C. Skin temperatures increased on both CONTROL and FLSUH boars as ambient temperature increased, however this was greater in CONTROL boars compared to FLUSH. FLUSH boars were then able to prevent a subsequent

increase in RR or RT, meaning the cooling pad was capable of removing the excess heat from the boar for them to maintain homeostasis during elevated temperatures. In fact, at 32°C, FLUSH boars had a slight decrease in RR and RT from 8-10 hours of elevated temperatures, suggesting the cooling pads were removing more heat than the boars were producing. The production losses typically occur when the boar's RT increases. Boars housed on cooling pads maintained a rectal temperature of 38°C while CONTROL boars increased by 0.5-0.9°C. Therefore, cooling pads are an effective method to alleviate the negative impacts of elevated ambient temperature on boars.

3.6 Conclusion

Cyclical heat stress for 3 consecutive days at 32°C or 35°C with >65% humidity resulted in boars having increase RR and RT followed by a decrease in semen quality over several weeks. Boars that were getting cooled during the period of elevated ambient temperatures with the use of electronically controlled cooling pads did not exhibit physiological indicators of heat stress resulting in better semen quality post heat stress when compared to boars who were not getting cooled. Overall, electronically-controlled cooling pads minimized or removed the negative impacts of heat stress on semen quality in boars.

3.7 Acknowledgments

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3.8 References

- Ait-Boulahsen, A., J. D. Garlich, and F. W. Edens. 1995. Potassium Chloride Improves the Thermotolerance of Chickens Exposed to Acute Heat Stress. *Poultry Science* 74(1):75–87. doi: 10.3382/ps.0740075
- Baumgard, L. H., and R. P. Rhoads Jr. 2013. Effects of Heat Stress on Postabsorptive Metabolism and Energetics. *Annual Review of Animal Biosciences* 1:311–337. doi: 10.1146/annurev-animal-031412-103644.
- Black, J. L., B. P. Mullan, M. L. Lorsch, and L. R. Giles. 1993. Lactation in the Sow during Heat Stress. *Livestock Production Science*. 35:153-170. doi: 10.1016/0301-6226(93)90188-n.
- Bouchama A. and E.B. De Vol. Acid-base alterations in heatstroke. 2001. *Intensive Care Med*. 27(4):680-5. doi:10.1007/s001340100906

- Bull, R. P., P. C. Harrison, G. L. Riskowski, and H. W. Gonyou. 1997. Preference among Cooling Systems by Gilts under Heat Stress. *Journal of Animal Science* 75: 2078–83. doi: 10.2527/1997.7582078x
- Cabezón, F. A., A. P. Schinckel, J. N. Marchant-Forde, J. S. Johnson, and R. M. Stwalley. 2017. Effect of Floor Cooling on Late Lactation Sows under Acute Heat Stress. *Livestock Science*. 206:113–120. Doi: 10.1016/j.livsci.2017.10.017
- Cameron RD, Blackshaw AW. 1980. The effect of elevated ambient temperature on spermatogenesis in the boar. *J Reprod Fertil*. May;59(1):173-9. doi: 10.1530/jrf.0.0590173.
- Cai, H., Y. Ren, X.-X. Li, J.-L. Yang, C.-P. Zhang, M. Chen, C.-H. Fan, X.-Q. Hu, Z.-Y. Hu, F. Gao and Y.-X. Liu. 2010. Scrotal Heat Stress Causes a Transient Alteration in Tight Junctions and Induction of TGF- β Expression. *International Journal of Andrology* 34:352–362. doi: Cottrell,
- Cottrell, J. J., Furness, J. B., Wijesiriwardana, U. A., Ringuet, M., Liu, F., DiGiacomo, K., Leury, B. J., J Clarke, I., & Dunshea, F. R. 2020. The Effect of Heat Stress on Respiratory Alkalosis and Insulin Sensitivity in Cinnamon Supplemented Pigs. *Animals* 10(4), 690. <https://doi.org/10.3390/ani10040690>
- FASS. 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Federation of Animal Science Societies, Champaign IL
- Flowers, W. L. 2015. Factors Affecting the Efficient Production of Boar Sperm. *Reproduction in Domestic Animals*. 50(2):25–30. doi: 10.1111/rda.12529
- Flowers WL. Management of boars for efficient semen production. *J Reprod Fertil Suppl*. 1997; 52:67-78.
- C.W. Holmes, N.D. Grace. 1975. A note on the metabolism of Ca, P, Mg, Na, and K by pigs growing at a high ambient temperature. *Anim. Prod.*, 21, p. 34
- Huynh, T.T.T., A.J.A. Aarnink, H.A.M. Spoolder, M.W.A. Verstegen, and B. Kemp. 2004. Effects pf Floor Cooling During High Ambient Temperatures on the Lying Behavior and Productivity of Growing Finishing Pigs. *Transactions of the ASAE*. 47(5):1773-1782. doi:10.13031/2013.17620
- Knox, R., D. Levis, T. Safranski and W. Singleton. 2008. An Update on North American Boar Stud Practices. *Theriogenology*. 70:1202–1208. doi: 10.1016/j.theriogenology.2008.06.036
- Lammers, P. J., D. R. Stender, and M.S. Honeyman. 2007. Environmental Needs of the Pig. Niche Pork Production.
- Lucy, M.C., and T.J. Safranski. 2017. Heat Stress in Pregnant Sows: Thermal Responses and Subsequent Performance of Sows and Their Offspring. *Molecular Reproduction and Development* 84:946–956. Doi: 10.1002/mrd.22844
- McNitt, J. I., and N. L. First. 1970. Effects of 72-Hour Heat Stress on Semen Quality in Boars. *International Journal of Biometeorology*. 14: 373–380. doi: 10.1007/BF01462914
- NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.
- Odom, T. W., P. C. Harrison and W.G. Bottje. 1984. Effects of Thermal-Induced Respiratory Alkalosis on Blood Ionized Calcium Levels in the Domestic Hen. *Poultry Science*. 65: 57-573. doi: 10.3382/ps.0650570.
- Patience, J. F., J. F. Umboh, R. K. Chaplin, and C. M. Nyachoti. 2005. Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. 96:205-214. doi: 10.1016/j.livprodsci.2005.01.012

- Parrish, J. J., K. L. Willenburg, K. M. Gibbs, K. B. Yagoda, M. M. Krautkramer, T. M. Loether, and F. C. S. A. Melo. 2017. Scrotal Insulation and Sperm Production in the Boar. *Molecular Reproduction and Development*. 84: 969–978. doi: 10.1002/mrd.22841
- Puvadolpirod, S. and J. P. Thaxton. 2000. Model of Physiological Stress in Chickens 1. Response parameters. *Poultry Science*. 79 (3): 363–369. doi: 10.1093/ps/79.3.363
- Rao, M., X.-L. Zhao, J. Yang, S.-F. Hu, H. Lei, W. Xia, and C.-H. Zhu. 2015. “Effect of Transient Scrotal Hyperthermia on Sperm Parameters, Seminal Plasma Biochemical Markers, and Oxidative Stress in Men.” *Asian Journal of Andrology*. 17: 668–675. Doi: 10.4103/1008-682X.146967
- Renaudeau, D., C. Anais, L. Tel, and J. L. Gourdine. 2010. Effect of Temperature on Thermal Acclimation in Growing Pigs Estimated Using a Nonlinear Function. *Journal of Animal Science*. 88:3715–3724. doi: 10.2527/jas.2009-2169
- Renaudeau, D., M. Kerdoncuff, C. Anaïs, and J. L. Gourdine. 2008. Effect of Temperature Level on Thermal Acclimation in Large White Growing Pigs. *Animal*. 2:1619–1626. doi: 10.1017/S1751731108002814
- Rockett, J. C., F. L. Mapp, J. B. Garges, J. C. Luft, C. Mori, and D. J. Dix. 2001. Effects of Hyperthermia on Spermatogenesis, Apoptosis, Gene Expression, and Fertility in Adult Male Mice. *Biology of Reproduction* 65: 229–239. doi: 10.1095/biolreprod65.1.229
- St-Pierre, N. R., B. Cobanov and G. Schnitkey. 2003. Economic Losses from Heat Stress by US Livestock Industries. *Journal of Dairy Science* 86:52–77. doi: 10.3168/jds.S0022-0302(03)74040-5
- Wang, C., J.-L. Li, H.-K. Wei, Y.-F. Zhou, S.-W. Jiang, and J. Peng. 2017. Linear Model Analysis of the Influencing Factors of Boar Longevity in Southern China. *Theriogenology*. 93:105–110. doi:10.1016/j.theriogenology.2017.01.038
- Wang, Y., P. Saelao, K. Chanthavixay, R. Gallardo, D. Bunn, S. J. Lamont, J. M. Dekkers, T. Kelly and H. Zhou. 2018. Physiological Responses to Heat Stress in Two Genetically Distinct Chicken Inbred Lines. *Poultry Science*. 97:770–780. doi: 10.3382/ps/pex363
- Zulkifli, I., A. Al-Aqil, A. R. Omar, A. Q. Sazili and M. A. Rajion. 2009. Crating and Heat Stress Influence Blood Parameters and Heat Shock Protein 70 Expression in Broiler Chickens Showing Short or Long Tonic Immobility Reactions. *Poultry Science*. 88:471–476. Doi: 10.3382/ps.2008-00287

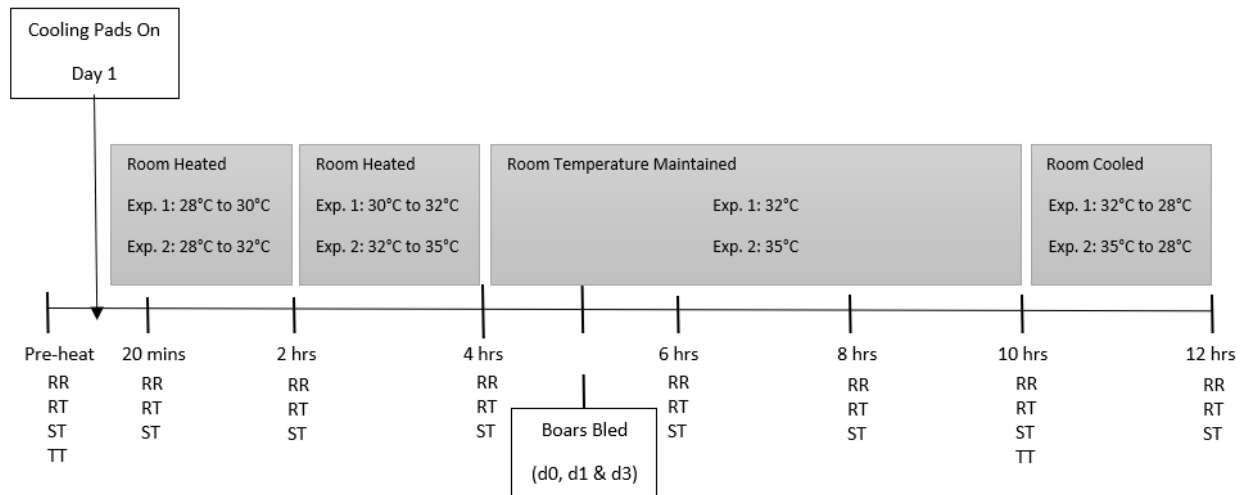


Figure 3.1: Graphical depiction of the study for heating of the barns and collecting physiological measurements during the heat stress event. This figure describes a single day. Heat stress was repeated for 3 consecutive days.

Experiment 1: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 32°C.

Experiment 2: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C.

Behavior	Position
Standing	Head in feeder, head near front of the pad, butt at the rear of the crate
Sitting	Head laying on feeder, head laying on front of pad, butt at the rear of the crate
Laying (sternal or lateral)	Head laying on feeder, head laying on front of pad, butt at the rear of the crate

Figure 3.2: Ethogram used for behavior analysis

Experiment 1: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 32°C.

Experiment 2: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C.

Table 3.1: Average room temperature and relative humidity throughout heat stress replicates.

^aExperiment 1: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 32°C.

^bExperiment 2: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C.

	Experiment 1 ^a		Experiment 2 ^b	
	Temperature (°C)	Relative Humidity (%)	Temperature (°C)	Relative Humidity (%)
20 mins	24.5 ± 0.3	76.5 ± 0.3	28.6 ± 0.1	73.3 ± 0.6
2 hours	31.1 ± 0.1	63.6 ± 0.3	32.9 ± 0.1	66.0 ± 1.1
4 hours	32.7 ± 0.1	63.7 ± 0.8	34.9 ± 0.1	65.0 ± 0.6
6 hours	32.8 ± 0.1	61.3 ± 1.0	35.3 ± 0.1	62.2 ± 0.5
8 hours	32.7 ± 0.1	58.4 ± 1.4	35.2 ± 0.1	66.8 ± 0.6
10 hours	32.8 ± 0.1	58.5 ± 1.3	35.4 ± 0.1	62.5 ± 1.1
12 hours	25.5 ± 0.1	63.5 ± 0.8	28.8 ± 0.1	61.1 ± 0.5

Table 3.2: Comparison of physiological indicators between CONTROL and FLUSH boars

^aExperiment 1: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 32°C.

^bExperiment 2: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C.

	Experiment 1: 32°C ^a						Experiment 2: 35°C ^b					
	Means ± SE		p-value				Means ± SE		p-values			
	CONTROL	FLUSH	trt	time	day	trt*time	CONTROL	FLUSH	trt	time	day	trt*time
Respiration Rate, bpm	71 ± 2	38 ± 1	<0.0001	<0.0001	<0.0001	<0.0001	114 ± 3	55 ± 2	<0.0001	0.0001	0.0003	<0.0001
Rectal Temperature, °C	38.5 ± 0.1	38.0 ± 0.1	<0.0001	<0.0001	0.2300	<0.0001	38.9 ± 0.1	38.0 ± 0.1	<0.0001	<0.0001	0.0021	0.0021
Ear Temperature, °C	37.4 ± 0.1	36.8 ± 0.1	<0.0001	<0.0001	0.0026	<0.0001	37.9 ± 0.1	36.9 ± 0.1	0.0002	<0.0001	<0.0001	<0.0001
Shoulder Temperature, °C	37.0 ± 0.1	36.7 ± 0.1	<0.0001	<0.0001	0.0033	<0.0001	37.9 ± 0.1	37.1 ± 0.1	0.0076	<0.0001	<0.0001	<0.0001
Rump Temperature, °C	36.9 ± 0.1	35.9 ± 0.1	<0.0001	<0.0001	0.0007	<0.0001	37.9 ± 0.1	36.9 ± 0.1	0.0029	<0.0001	<0.0001	<0.0001
Tail Temperature, °C	36.9 ± 0.1	35.8 ± 0.1	<0.0001	<0.0001	<0.0001	<0.0001	37.6 ± 0.1	36.3 ± 0.1	0.0008	<0.0001	<0.0001	<0.0001
Left Testicle Temperature, °C	36.2 ± 0.2	34.4 ± 0.4	0.0014	---	0.3200	---	37.4 ± 0.1	35.9 ± 0.2	<0.0001	---	0.7951	---
Right Testicle Temperature, °C	36.3 ± 0.2	34.4 ± 0.4	0.0004	---	0.2880	---	37.3 ± 0.1	35.8 ± 0.2	0.0002	---	0.9926	---
Change in Body Weight, lbs	---	---	---	---	---	---	-5.25 ± 2.99	-0.79 ± 2.44	0.2600	---	---	---

Table 3.3: Comparison of physiological indicators between heat stressed boars (CONTROL) and boars on electronically-controlled cooling pad flushing water every 8 minutes and 27°C (FLUSH) by day in Exp. 1 (32°C).

^{a,b,c} different subscripts represents different means in trt*day at P<0.05 within row.

	CONTROL Means ± SE			FLUSH Means ± SE			p-value trt*day
	day 1	day 2	day 3	day 1	day 2	day 3	
Respiration Rate (RR) bpm	60 ± 4 ^a	81 ± 4 ^b	73 ± 4 ^b	37 ± 3 ^c	44 ± 2 ^c	33 ± 2 ^c	<0.0001
Rectal Temperature (RT), °C	38.5 ± 0.05	38.4 ± 0.05	38.5 ± 0.05	38.0 ± 0.1	38.0 ± 0.1	38.0 ± 0.1	NS
Ear Temperature (STear), °C	37.4 ± 0.2	37.4 ± 0.1	37.3 ± 0.1	36.9 ± 0.1	37.0 ± 0.1	36.6 ± 0.1	NS
Shoulder Temperature (STshoulder), °C	37.0 ± 0.2	37.1 ± 0.1	36.9 ± 0.2	36.2 ± 0.2	36.5 ± 0.2	36.2 ± 0.2	NS
Rump Temperature (STrump), °C	37.0 ± 0.2	36.9 ± 0.1	36.8 ± 0.1	35.9 ± 0.2	36.2 ± 0.1	35.8 ± 0.2	NS
Tail Temperature (STail), °C	37.0 ± 0.2	36.9 ± 0.1	36.8 ± 0.1	35.7 ± 0.1	35.9 ± 0.2	35.7 ± 0.1	NS
Left Testicle Temperature (TT), °C	35.7 ± 0.4	36.5 ± 0.2	36.2 ± 0.4	32.9 ± 0.7	35.7 ± 0.4	34.7 ± 0.9	NS
Right Testicle Temperature (TT), °C	35.9 ± 0.4	36.6 ± 0.2	36.3 ± 0.4	32.8 ± 0.8	35.7 ± 0.4	34.6 ± 0.8	NS

Table 3.4: Comparison of physiological indicators between heat stressed boars (CONTROL) and boars on electronically-controlled cooling pad flushing water every 8 minutes and 27°C (FLUSH) by day in Exp. 2 (35°C).

^{a,b,c} different subscripts represents different means in trt*day at P<0.05

	CONTROL Means ± SE			FLUSH Means ± SE			p-value trt*day
	day 1	day 2	day 3	day 1	day 2	day 3	
Respiration Rate, bpm	121 ± 6	116 ± 5	106 ± 6	62 ± 3	54 ± 3	49 ± 3	NS
Rectal Temperature, °C	39.1 ± 0.1 ^a	38.9 ± 0.1 ^{ab}	38.7 ± 0.1 ^b	38.0 ± 0.1 ^c	37.9 ± 0.1 ^c	38.0 ± 0.1 ^c	0.0027
Ear Temperature, °C	38.0 ± 0.2	38.1 ± 0.2	37.6 ± 0.1	37.0 ± 0.2	37.0 ± 0.2	36.7 ± 0.2	NS
Shoulder Temperature, °C	38.0 ± 0.2	38.1 ± 0.2	37.6 ± 0.2	37.1 ± 0.2	37.2 ± 0.2	37.0 ± 0.2	NS
Rump Temperature, °C	37.9 ± 0.2	38.0 ± 0.2	37.7 ± 0.2	36.8 ± 0.2	37.1 ± 0.2	36.8 ± 0.2	NS
Tail Temperature, °C	37.6 ± 0.2	37.8 ± 0.2	37.3 ± 0.1	36.3 ± 0.2	36.5 ± 0.2	36.2 ± 0.2	NS
Left Testicle Temperature, °C	37.4 ± 0.2	37.5 ± 0.3	37.4 ± 0.2	35.9 ± 0.2	36.0 ± 0.4	35.8 ± 0.3	NS
Right Testicle Temperature, °C	37.3 ± 0.2	37.4 ± 0.3	37.4 ± 0.1	35.9 ± 0.2	35.7 ± 0.4	35.8 ± 0.4	NS

Table 3.5: Comparison of i-STAT parameters

^aExperiment 1: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 32°C.

^bExperiment 2: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C

		Experiment 1: 32°C ^a			Experiment 2: 35°C ^b		
		Means ± SE		p-values	Means ± SE		p-values
		CONTROL	FLUSH		CONTROL	FLUSH	
Pre-Heat Stress	Na ⁺ (mmol/L)	144.5 ± 1.19	143.25 ± 0.41	0.2450	143.16 ± 0.64	142.83 ± 0.59	0.6663
	K ⁺ (mmol/L)	4.28 ± 0.2	4.2 ± 0.13	0.7463	4.58 ± 0.19	4.62 ± 0.11	0.8554
	iCa ²⁺ (mmol/L)	1.37 ± 0.01	1.35 ± 0.01	0.3719	1.31 ± 0.01	1.30 ± 0.02	0.7675
	Glucose (mg/dL)	95.25 ± 1.11	84.75 ± 1.94	0.0804	80.58 ± 2.13	82.83 ± 2.54	0.4723
	Hct (% PCV)	35.25 ± 1.93	35.88 ± 1.75	0.8354	44.5 ± 4.53	39.67 ± 2.29	0.1795
	Hgb (g/dL)	12 ± 0.66	12.18 ± 0.6	0.8644	13.43 ± 0.70	13.48 ± 0.78	0.9480
	pH	7.43 ± 0.03	7.45 ± 0.01	0.4884	7.44 ± 0.02	7.47 ± 0.02	0.2927
	PCO ₂ (mmHg)	50.23 ± 2.37	48.61 ± 0.98	0.7070	45.92 ± 2.78	43.33 ± 2.71	0.4141
	PO ₂ (mmHg)	38 ± 3.49	35.38 ± 2.29	0.7028	42.08 ± 5.54	44.45 ± 6.16	0.6985
	BE (mmol/L)	8.75 ± 1.11	9.88 ± 0.74	0.4824	6.5 ± 0.53	8.17 ± 0.83	0.3093
	HCO ₃ (mmol/L)	33.1 ± 0.64	33.71 ± 0.69	0.7076	30.58 ± 0.62	31.5 ± 0.99	0.4705
	TCO ₂ (mmol/L)	34.75 ± 0.63	35.13 ± 0.72	0.8272	32 ± 0.72	32.92 ± 1.05	0.7590
	sO ₂ (%)	70.25 ± 6.81	67.63 ± 3.68	0.8190	71.42 ± 4.19	76.5 ± 5.21	0.4428
Acute Heat Stress	Na ⁺ (mmol/L)	143.75 ± 1.49	143.13 ± 0.64	0.5576	143.75 ± 0.51	142.91 ± 0.44	0.2891
	K ⁺ (mmol/L)	4.33 ± 0.13	4.61 ± 0.19	0.2205	4.24 ± 0.10	4.56 ± 0.17	0.1716
	iCa ²⁺ (mmol/L)	1.32 ± 0.01	1.35 ± 0.02	0.2222	1.19 ± 0.08	1.34 ± 0.01	0.0097
	Glucose (mg/dL)	82 ± 9.53	82.88 ± 2.43	0.8812	77.42 ± 2.25	73.73 ± 2.30	0.2507
	Hct (% PCV)	37.25 ± 2.32	37.38 ± 1.43	0.9668	34.33 ± 0.85	38.09 ± 2.09	0.3059
	Hgb (g/dL)	12.68 ± 0.8	12.7 ± 0.48	0.9805	11.68 ± 0.29	12.95 ± 0.71	0.1712
	pH	7.48 ± 0.02	7.45 ± 0.03	0.4710	7.51 ± 0.01	7.45 ± 0.02	0.0392
	PCO ₂ (mmHg)	45.23 ± 3.98	49.01 ± 3.77	0.3798	35.44 ± 1.02	44.35 ± 2.38	0.0073
	PO ₂ (mmHg)	40.75 ± 6.26	35.88 ± 6.71	0.4799	39.67 ± 1.83	37.45 ± 2.87	0.7233
	BE (mmol/L)	9.5 ± 1.32	9.88 ± 1.20	0.8142	7.53 ± 2.08	6.55 ± 1.25	0.5545
	HCO ₃ (mmol/L)	33.1 ± 1.67	33.68 ± 1.31	0.7247	29.38 ± 1.22	30.4 ± 1.07	0.4340
	TCO ₂ (mmol/L)	34.5 ± 1.71	35.13 ± 1.42	0.7162	34.25 ± 4.69	31.73 ± 1.06	0.4101
	sO ₂ (%)	74 ± 8.25	59.5 ± 8.53	0.2120	83.5 ± 5.93	69.82 ± 4.56	0.0463
Chronic Heat Stress	Na ⁺ (mmol/L)	143.25 ± 0.25	143 ± 0.46	0.8141	143.8 ± 0.58	143 ± 0.57	0.3349
	K ⁺ (mmol/L)	4.4 ± 0.13	4.26 ± 0.09	0.5540	4.36 ± 0.08	5.03 ± 0.27	0.0080
	iCa ²⁺ (mmol/L)	1.36 ± 0.01	1.35 ± 0.01	0.7789	1.33 ± 0.01	1.31 ± 0.02	0.7379
	Glucose (mg/dL)	80.5 ± 5.69	75.63 ± 3.43	0.4075	77.2 ± 2.05	78.64 ± 2.19	0.6677
	Hct (% PCV)	35 ± 2.68	35.88 ± 2.03	0.7712	33.3 ± 1.58	36.45 ± 2.16	0.4109
	Hgb (g/dL)	11.88 ± 0.91	12.19 ± 0.69	0.7606	11.34 ± 0.54	12.39 ± 0.73	0.2750
	pH	7.44 ± 0.03	7.43 ± 0.1	0.9516	7.49 ± 0.01	7.5 ± 0.03	0.7432

Table 3.4 continued

PCO ₂ (mmHg)	46.1 ± 1.93	49.48 ± 2.29	0.4333	39.42 ± 0.86	39.84 ± 2.64	0.8986
PO ₂ (mmHg)	29.25 ± 3.90	29.75 ± 1.36	0.9420	36.3 ± 1.11	39.82 ± 5.44	0.5908
BE (mmol/L)	6.75 ± 1.65	8.63 ± 0.60	0.2451	6.4 ± 0.62	6.75 ± 0.84	0.8381
HCO ₃ (mmol/L)	39.98 ± 1.34	32.9 ± 0.68	0.2433	29.75 ± 0.53	29.99 ± 0.74	0.8558
TCO ₂ (mmol/L)	32.5 ± 1.32	34.25 ± 0.73	0.3123	30.9 ± 0.53	31.17 ± 0.78	0.9322
sO ₂ (%)	54.5 ± 8.45	49.75 ± 7.65	0.6791	73.3 ± 2.06	69.18 ± 5.14	0.5609

Table 3.6: Comparison of semen quality parameters

^aExperiment 1: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 32°C.

^bExperiment 2: Heat stress boars (CONTROL) or boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C.

^cDays post 3-d heat stress event

	Experiment 1: 32°C ^a					Experiment 2: 35°C ^b				
	Means ± SE		p-values			Means ± SE		p-values		
	CONTROL	FLUSH	trt	Day ^c	trt*day	CONTROL	FLUSH	trt	Day ^c	trt*day
Volume, g	190 ± 8	158 ± 8	0.0079	0.6566	NS	210 ± 8	235 ± 7	0.0099	0.1493	NS
Total Sperm in Ejaculate, billion	30.5 ± 2.1	31.1 ± 2.0	0.9221	0.1074	NS	35.6 ± 1.4	38.2 ± 1.3	0.0753	0.0919	NS
Non-viable Sperm, %	16.5 ± 1.3	13.1 ± 1.1	0.1716	0.0017	NS	18.4 ± 1.1	16.1 ± 1	0.2301	0.0005	NS
Motility, %	84.8 ± 1.0	88.7 ± 0.7	<0.0001	0.0049	0.0521	72.2 ± 1.7	83.0 ± 1.2	<0.0001	<0.0001	0.0064
Progressive Motility, %	85.1 ± 13.4	76.4 ± 1.6	0.5594	0.3037	NS	52.3 ± 2.1	70.0 ± 1.7	<0.0001	0.0004	0.0011
Normal Morphology, %	77.6 ± 2.0	82.5 ± 1.2	0.1098	0.1800	NS	79.6 ± 2.1	87.5 ± 1.4	0.0055	0.0001	NS
Proximal Droplets, %	4.0 ± 0.9	1.7 ± 0.5	0.0587	<0.0001	NS	6.8 ± 1.3	2.2 ± 0.5	<0.0001	0.0001	0.0344
Distal Droplets, %	6.9 ± 1.0	4.3 ± 0.6	0.1287	0.0131	NS	8.8 ± 0.9	6.0 ± 0.8	0.0242	0.3563	NS
DMR, %	1.9 ± 0.4	2.3 ± 0.4	0.7456	0.1551	NS	3.5 ± 0.6	3.6 ± 0.5	0.7987	0.1644	NS
Tail Abnormailites, %	9.6 ± 0.8	9.2 ± 0.9	0.7284	0.0001	NS	1.3 ± 0.2	0.7 ± 0.1	0.0007	0.0007	0.0075
Normal Acrosome, %	---	---	---	---	---	91.5 ± 0.9	94.3 ± 0.4	0.0014	0.0003	0.0111

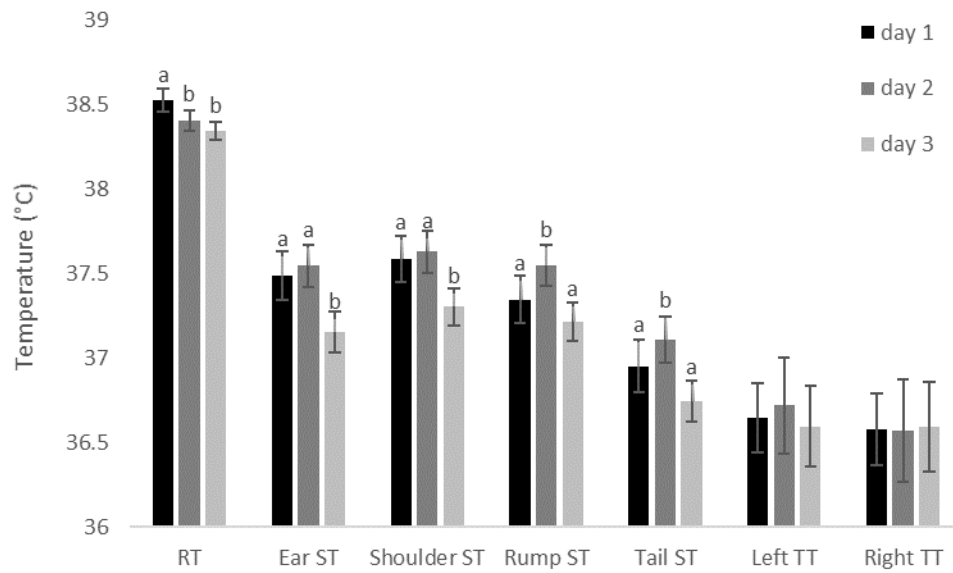


Figure 3.3: Comparison of rectal temperature and skin temperature (ear, shoulder, rump, tail, left testicle and right testicle) by day in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C ambient conditions (Experiment 2).

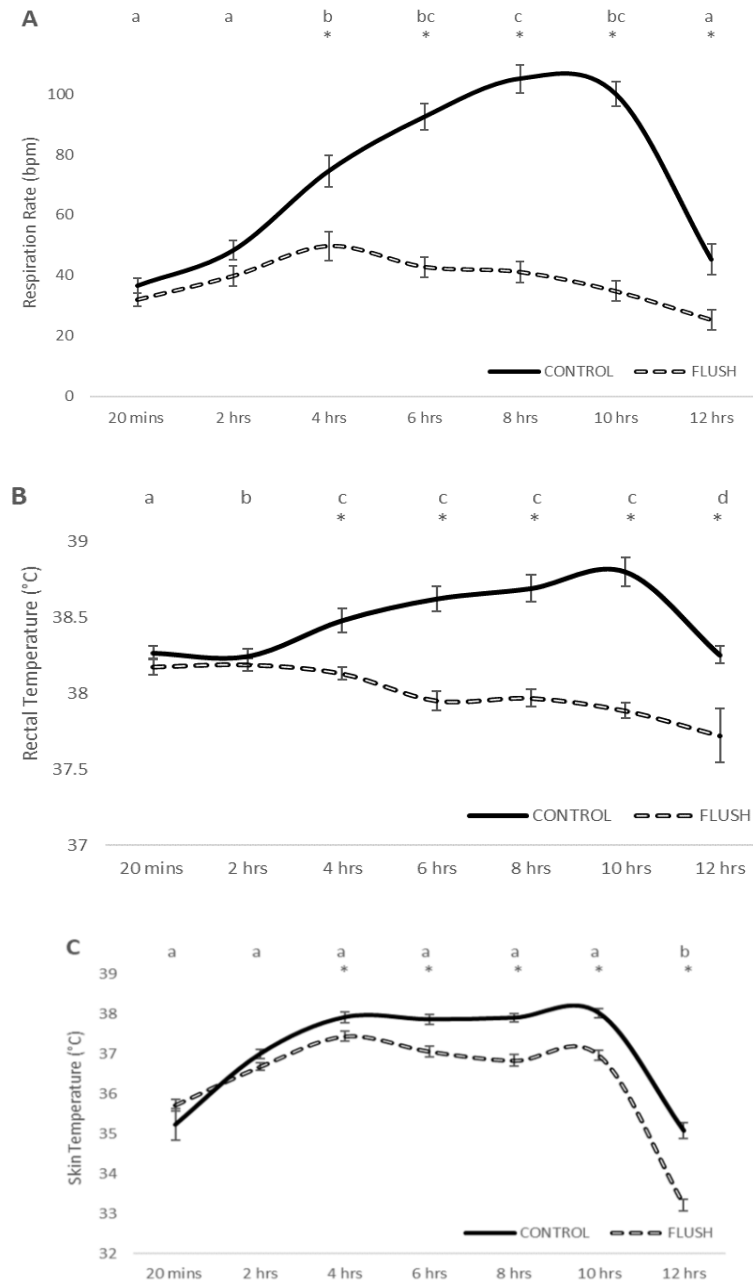


Figure 3.4: Comparison of respiration rate

(A), rectal temperature (B) and skin temperature (shoulder) (C) over time in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 32°C ambient conditions (Experiment 1).

a,b,c,d different subscripts represent different mean in time at $P < 0.05$

* different subscript represent different means in trt*time at $P < 0.05$

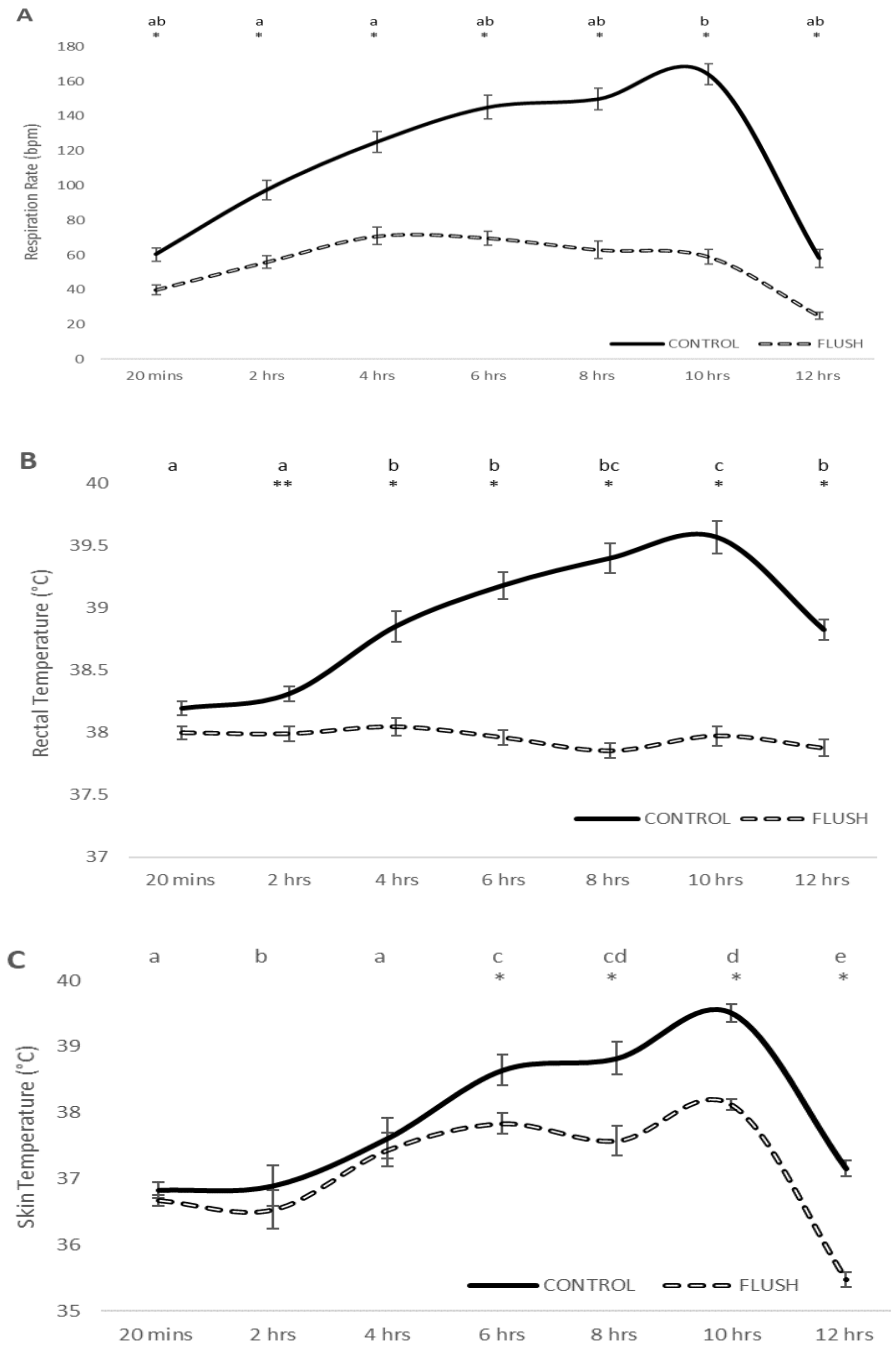


Figure 3.5: Comparison of respiration rate

(A), rectal temperature (B) and skin temperature (shoulder) (C) over time in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes and 27°C under 35°C ambient conditions (Experiment 2).

a,b,c,d different subscripts represent different mean in time at $P < 0.05$

* different subscripts represent different means in trt*time at $P < 0.05$

** different subscripts represent different means in trt*time at $0.5 < P < 0.05$

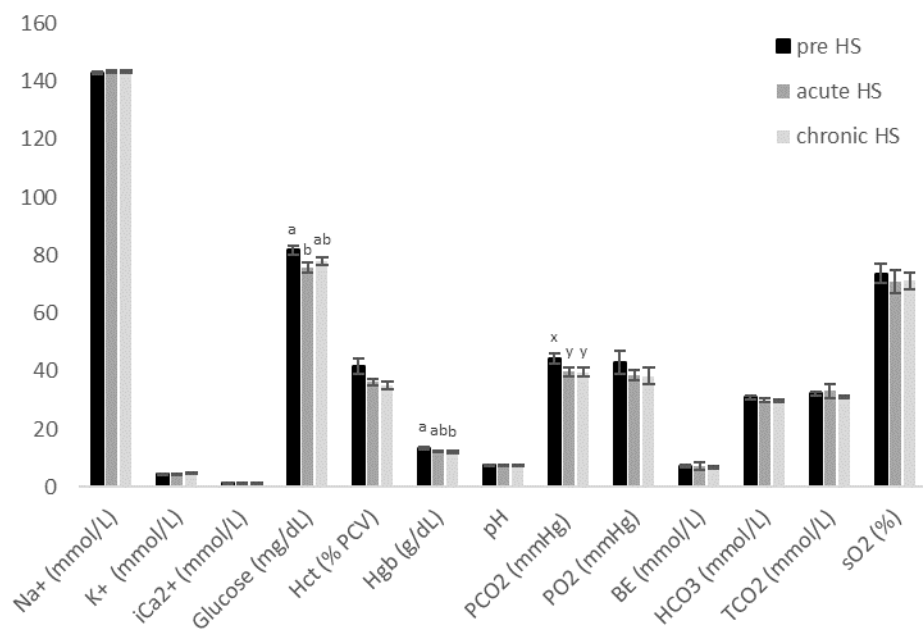


Figure 3.6: Comparison of i-STAT measurements by day in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes or 27°C under 35°C ambient conditions (Experiment 2).

^{a,b,c} different subscripts represent different mean in time at $P < 0.05$

^{x,y,z} different subscripts represent different mean in time at $0.05 < P < 0.10$

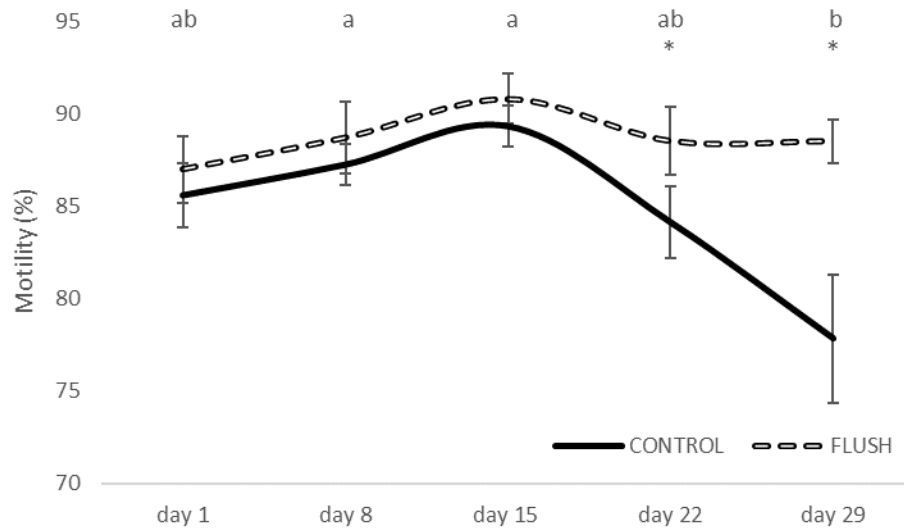


Figure 3.7: Comparison of percent motility over time in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes or 27°C under 32°C ambient conditions (Experiment 1).

^{a,b} different subscripts represent different mean in time at $P < 0.05$

* different subscripts represent different means in $\text{trt} \times \text{time}$ at $P < 0.05$

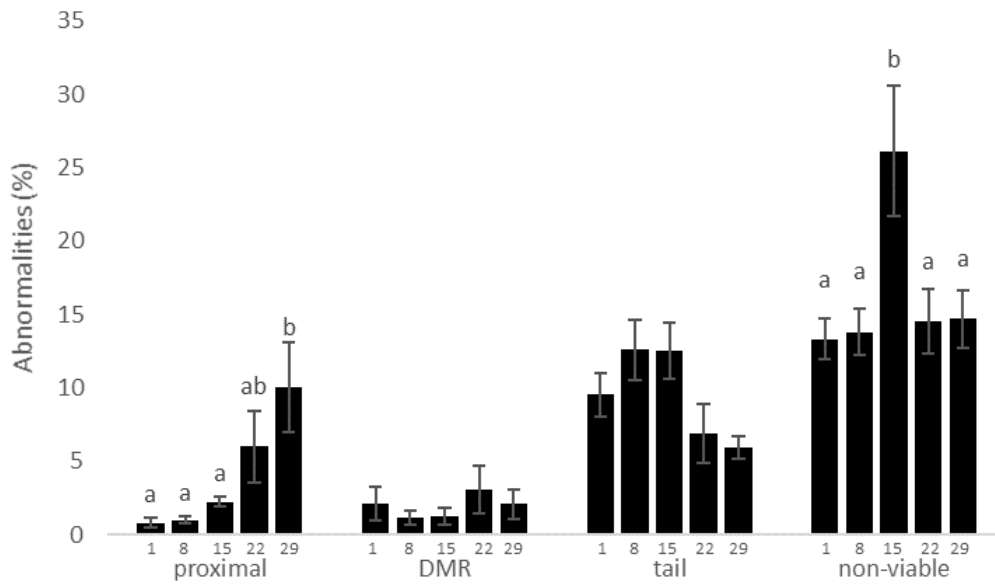


Figure 3.8: Comparison of non-viable sperm, proximal droplets and distal droplets over days in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes or 27°C under 32°C ambient conditions (Experiment 1).

^{a,b} different subscripts represent different mean in time at $P < 0.05$

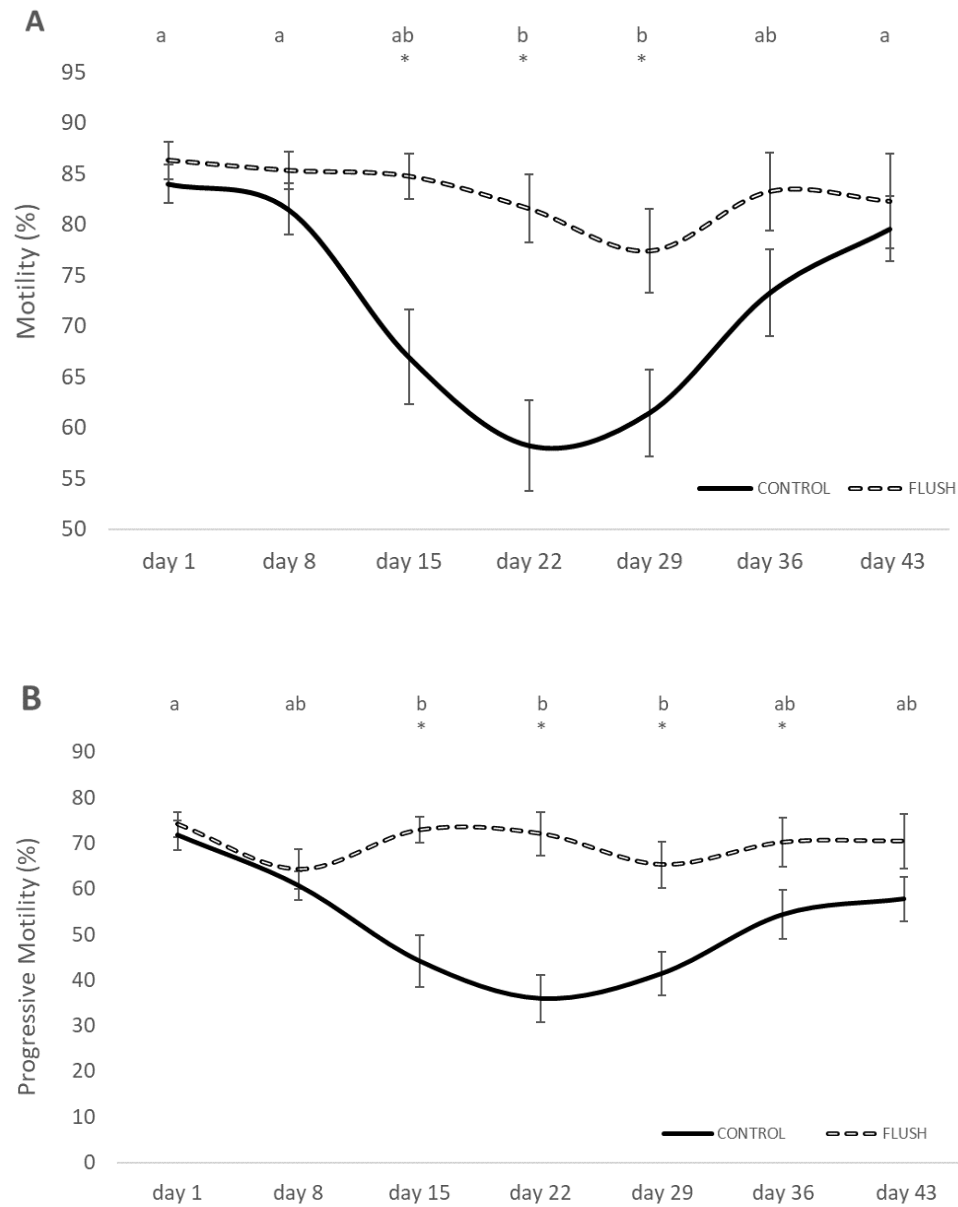


Figure 3.9: Comparison of percent motility

(A) and percent progressive motility over time in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes or 27°C under 35°C ambient conditions (Experiment 2).

^{a,b} different subscripts represent different mean in time at $P < 0.05$

* different subscripts represent different means in $\text{trt} \times \text{time}$ at $P < 0.05$

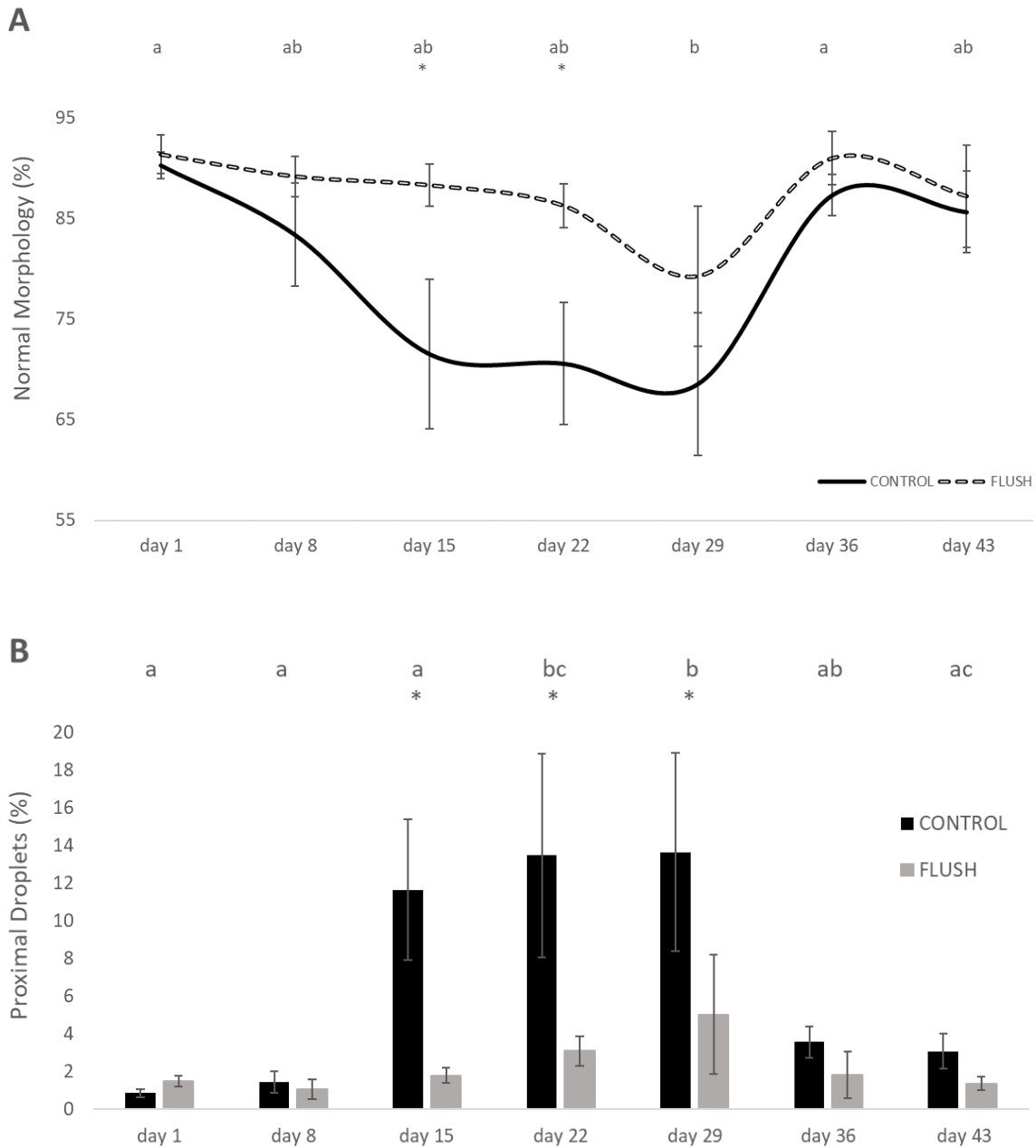


Figure 3.10: Comparison of normal morphology

(A) and proximal droplets (B) over time in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes or 27°C under 35°C ambient conditions (Experiment 2).

^{a,b,c} different subscripts represent different mean in time at $P < 0.05$

* different subscripts represent different means in $\text{trt} \times \text{time}$ at $P < 0.05$

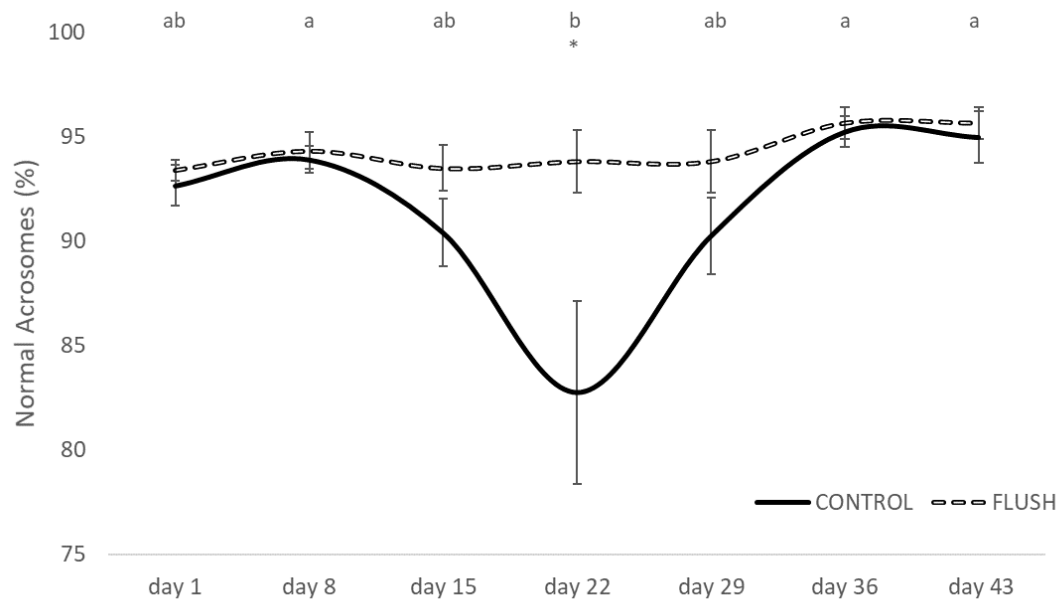


Figure 3.11: Comparison of normal acrosomes over time in heat stressed boars (CONTROL) and boars on electronically-controlled floor cooling pad flushing water every 8 minutes or 27°C under 35°C ambient conditions (Experiment 2).

^{a,b} different subscripts represent different mean in time at $P < 0.05$

* different subscripts represent different means in trt*time at $P < 0.05$

CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

During periods of elevated ambient temperatures, animal's will alter their behavior and physiology in an attempt to maintain the body temperature within their thermoneutral zone. When the animal is unsuccessful at maintaining body temperature, there are negative implications on the animal's growth and reproductive performance, resulting in financial losses to the swine industry.

Heat stress occurs when the body cannot get rid of excess heat resulting in increase heart rate and internal temperatures. As ambient temperatures increase, skin temperatures increase which is followed by behavior changes such as increase water consumption, decrease feed intake and increased time spent laying down as a way to reduce energy to maintain homeostasis. As heat duration increases, the animal will begin to increase their respiration rate to increase evaporative heat loss to try and prevent increases in their body temperature. When this becomes inefficient, the animal's body temperature will increase. In boars, this increase in body temperature will also induce an increase in testicular temperature which can have negative impacts on semen quality. The length of time over which the boar is unable to cool his body temperature into normal ranges and the severity of the increase in body temperature determines how significant the impacts in semen quality are and for how long the semen quality is decreased.

Farms have adopted many management practices to reduce the negative impacts of elevated temperatures on pigs. Barns are equipped with cool cell technology which cools incoming air by evaporative heat loss. However, these cool cells are only able to reduce the incoming air by ~5-8°C and is more effective during low humidity. The majority of the areas in the U.S. where pigs are raised is on the east coast, through the midwestern corn belt, and some in the south, all have relatively high humidity during the summer months with the exception of the western plains. Therefore, cool cell technology can help, but is relatively ineffective during the summer months in the U.S. Farms also use misters, drippers and fans to try and cool pigs. These technologies again can be useful, but are typically not adequate during the summer months. Therefore, the swine industry needs an alternative that can keep boars cool during the summer.

Some boar studs being constructed today are fully air conditioned as a means to regulate ambient temperatures for boars throughout the entire year. This is an added expense for the boar

stud industry, but, in theory, can eliminate the elevations in ambient temperatures throughout the summer months. One substantial benefit to air conditioning is the fact that the air conditioner will reduce humidity in the barn. While humidity may not be a large driving factor for heat stress in young boars, it likely plays more of a role in the older boars that have a larger body mass. So, this is a benefit for the air conditioned barns. Air conditioning adoption is challenging since many boar studs are older barns that have been converted to house boars. Therefore, adding air conditioning is not always a viable option. Therefore, many farms are looking for economical alternatives that can be added to existing barns.

The electronically controlled floor cooling pads developed at Purdue University hold great promise for use in the boar stud sector to cool boars in barns where air conditioning is not a viable option. Currently the pads have been designed for use in lactating sows, so the design may have to be altered slightly for use in boars. One consideration is that sows urinate towards the back of the crate and boars urinate in a forward direction. With the current pad design, urine pooled underneath the boars during the trials, which would need to be rectified for use in boars. Today, it is unclear what the cost of the cooling pads will be as the studies in this thesis used prototypes built at Purdue. Once a final pad design is established, an economic analysis should be conducted to determine the affordability of the pads.

This thesis has demonstrated that the use of electronically-controlled floor cooling pads has the implications towards minimizing or removing the negative impacts of elevated ambient temperatures. Seen in chapter 2, gilts exposed to short term heat stress at 32°C had no differences between pre-heat measurements when compared to control measurements for vaginal temperature and respiration rate. Showing that heat stress at 32°C for a short duration of time elicits a mild heat stress in limit fed gilts and behavioral changes alone can maintain homeostasis. 80 minutes of 35°C increased respiration rate (14 ± 5 BPM) and vaginal temperature ($0.5 \pm 0.1^\circ\text{C}$). Though there was an increase in respiration rate and vaginal temperature, limit fed gilts heat stressed for a short duration of time at 32°C and 35°C did not expose gilts to a heat stress that would place them outside their thermal neutral zone. Future studies should test a longer-term heat stress to evaluate gilt's response to heat stress.

Chapter 3 highlights the boar's response to heat stress at 32°C and 35°C for 3 days with the use of electronically-controlled cooling pads. This study allows for a more in-depth evaluation

of the effects that heat stress has on boars physiological and reproductive performance. 32°C and 35°C ambient temperatures did not impact feed intake likely due to boars being limit fed and fed in the morning before ambient temperatures increased. As boars were exposed to high ambient temperatures (32°C and 35°C) skin temperatures increased. Increases in respiration rate and vaginal temperature were also observed with an average increase in respiration rate of 79 bpm at 32°C and 126 bpm at 35°C and vaginal temperature of 0.7°C and 1.7°C. No differences were seen in blood parameters at 32°C but increases in potassium, hematocrit, hemoglobin and partial CO₂ was decreased in 35°C. Once potential explanation for this difference in potassium, hematocrit and hemoglobin could be due to a behavior change of increasing water consumption as temperatures increase. As respiration rate increases the balance of breathing in oxygen and breathing out CO₂ is disrupted decreasing partial CO₂ in the blood. Ambient temperatures below 35°C decreased semen quality 15 days post heat stress and continued for duration of 4 weeks. Motility and morphology decreased below industry standards 15-29 days post heat stress. Ambient temperatures of 32°C had a decrease in semen quality 29 days post heat stress but remained above industry standards with no decreases in morphology.

Boars exposed to 32°C and 35°C experienced heat stress while boars at 32°C experienced a mild heat stress. 32°C ambient temperatures for 3 days increased physiological indicators of heat stress, while only increasing internal temperature by 0.7 °C. No differences were seen in blood parameters suggesting that though the boar experienced heat stress, it was not significant enough to elicit biological changes in the boar. Semen quality did have a decrease 29 days post heat stress but remained above industry standards (70% motile and 70% normal). 35°C ambient temperatures for 3 consecutive days increased physiological indicators while increasing internal temperature by 1.7°C and respiration rate by 126 bpm. Decreases in partial CO₂ show that the increase in respiration rate at 35°C was significant enough to disrupt CO₂ and O₂. 35°C resulted in semen quality below industry standards for a duration of 3 weeks. Overall, 32°C exposed boars to heat stress but did not result in biological changes nor decreases in semen quality below industry standards.

Boars housed on electronically-controlled floor cooling pads did not exhibit any physiological changes associated with heat stress, nor any negative changes in semen production or quality following a short-term heat stress. Use of electronically controlled cooling pads were

able to remove the physiological indicators of heat stress by minimizing respiration rate, internal temperature and skin temperature in gilts and boars. Additionally, electronically-controlled cooling pads were effective at minimizing negative decreases in semen quality due to heat stress at both 32°C and 35°C. Overall, the studies presented in this thesis further confirm the negative impacts of heat stress has on the swine industry and provide an effective method for alleviating heat stress that should continue to be investigated for use in the boar stud sector.