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CORTISOL IN HAIR AS A MEASURE OF CHRONIC STRESS DURING SOW GESTATION AND THE PATTERN OF CORTISOL IN BLOOD DURING PARTURITION IN SOWS

BY

TALIA EVERDING

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Animal Science

South Dakota State University

2021

THESIS ACCEPTANCE PAGE

Talia Everding

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

°C	Degrees Celsius
ACTH	Adrenocorticotropic hormone
AUC	Area under the curve
AvgPC	Average piglet cord blood cortisol
AvgPW	Average piglet birth weight
BoLP	Time of birth of the last piglet
cm	Centimeter
CRH	Corticotropin-releasing hormone
d	Day(s)
dL	Deciliter(s)
FI	Feed intake three days prior to and the day of parturition
g	Gram(s)
h	Hour(s)
HCC	Hair cortisol concentration(s)
HPA	Hypothalamic-pituitary-adrenal axis
kg	Kilogram(s)
LPPoTF	Time to last piglet as a percentage of total farrowing time

m	Meter(s)

MaxCPoLP	Time to maximum cortisol as a percentage of time of last piglet

MaxCPoTF	Time to maximum cortisol as a percentage of total farrowing time

mg	Milligram

min	Minute(s)

MinCPoTF	Time to minimum cortisol as a percentage of total farrowing time

mL	Milliliter(s)

ng	Nanogram(s)

pg	Picogram(s)

s	Second(s)

SAS	Statistical analysis system

TF	Total farrowing time, from birth of first piglet until expulsion of last placental part

μg	Microgram(s)

μL	Microliter(s)

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ABSTRACT

CORTISOL IN HAIR AS A MEASURE OF CHRONIC STRESS DURING SOW GESTATION AND THE PATTERN OF CORTISOL IN BLOOD DURING PARTURITION IN SOWS

TALIA EVERDING

2021

Cortisol is known as the stress hormone, as it influences many metabolic processes to maintain glucose homeostasis during stressful experiences, including physical and psychological stress. It can be detected in biological matrices such as blood and hair and is released rapidly during sudden stressors and continuously during long-term stress. Blood cortisol fluctuates rapidly in response to acute stressors like pain, exertion, and fear; in hair cortisol accumulates steadily over the period of hair growth and may be useful for detecting chronically elevated cortisol resulting from long-term stress. The objective of this research was to, 1) determine the influence of a simulated chronic stress scenario on hair cortisol concentrations (HCC), 2) determine HCC of sows in two different gestation housing systems as a marker of chronic stress, and 3) examine the pattern of blood cortisol during parturition in the sow.

In the US and internationally, gestation stalls have received consumer criticism because of the way they limit sow movement and natural behaviors. However, the data are conflicting as to whether gestation stalls cause poorer welfare than group housing, as injuries and stress may result from mixing unfamiliar sows. In study 1, a total of 18 gilts in 2 groups were used. In group 1, 6 gilts from one pen were split into 3 pens of 2 gilts. In group 2, 12 gilts were mixed from separate group pens into 4 pens of 3 gilts. Mixing

occurred on d0. All gilts were assigned to 1 of 2 treatments, ACTH or Control. Treatment gilts were repeatedly administered ACTH (thrice in group 1 and twice in group 2), and Control gilts were administered saline at the same timepoints. Hair was shaved on d0 and on d21 after mixing, hair growth was collected. HCC was not affected by ACTH administration, but mixing unfamiliar gilts in new pens caused a significant increase in HCC. Administration of ACTH may not be adequate for simulating chronic stress in pigs, but HCC is an effective matrix for evaluating in pigs. In study 2, 34 sows were housed in gestation stalls and 32 sows were housed in group pens from breeding until approximately d111 of gestation. Hair samples were collected on d37 and d111, representing early and late gestation, and were analyzed for cortisol. Sows were categorized as parity 0-1, 2-3, or ≥4, and data were analyzed using the PROC MIXED procedure in SAS. Stall-housed sows had higher hair cortisol than group-housed sows, and stall-housed gilts and parity 1 sows had higher HCC than all other females regardless of housing system. Hair cortisol concentrations tended to be higher in late gestation than in early gestation for all females; HCC was not affected by time in gilts, and stall-housed gilts had higher HCC than group-housed gilts.

In study 3, the pattern of cortisol secretion during parturition was examined using a total of 9 farrowing periods from 7 primi- and multiparous females. Females had previously been surgically fitted with cephalic vein catheters, and blood samples were collected every 15 min from the birth of the first piglet until the expulsion of the last placental part. Piglet birth times and weights were recorded, and data were analyzed using the PROC CORR function of SAS. Smaller litters were associated with a higher minimum maternal cortisol, which occurs closer to or after the birth of the last piglet. In

large litters, maximum cortisol may occur earlier in relation to the birth of the last piglet, and minimum cortisol is more likely to occur at the beginning of parturition. Larger, more robust piglets in smaller litters are associated with higher maternal cortisol at the onset of parturition and promote shorter farrowing duration. Maternal cortisol appears to be strongly influenced by fetal cortisol. However, sow cortisol at the onset of parturition may be reflective of the total litter size and expected total farrowing time.

HCC is an effective matrix for identifying elevated cortisol over prolonged periods of stress in pigs and may be used to identify chronic stress in gestating sows. Maternal cortisol at parturition is strongly influenced by fetal cortisol and is not likely to be useful as an indicator of sow welfare during that period.

1.0 LITERATURE REVIEW

1.1 Sow housing and consumer preferences

The animal agricultural industry has seen a trend in consumers demanding more humanely or ethically-raised products (Zhao & Hamm, 2010). This trend occurs as consumers become more educated and affluent and can afford to have different standards for the products they consume (Summers, 2016), as evidenced by the demand for more organic labeling, free-trade and local products, and ethically-raised animal products (Johnston et al., 2011). One of the recent areas of concern for consumers has been sow housing, particularly in gestation. The U.S. population has grown progressively more educated since the 1940s (Schmidt, 2018), and Ryan et al. found that more educated survey-responders were less likely to support the use of gestation stalls (2015). This may be indicative of an inevitable continued trend towards improving perceived animal welfare in animal agriculture.

Gestation stalls have increased in prevalence since the 1950s (Ryan et al., 2015) and have become the standard in commercial swine production in the U.S. In 2012, approximately 75.8% of the United States sow herd was housed in gestation stalls (Schulz and Tonsor, 2015). Stalls became popular for sow confinement because of the ability to feed sows individually. Gestating sows can be competitive and sows that eat quickly in a group setting are able to chase other sows away from their ration, causing subordinate sows to receive inadequate nutrition (Li et al., 2017). Stalls also allow farm workers to examine each female individually and easily give injections if necessary, as sows are able to run away or hide in a group (Patterson-Kane, 2018). Stall-housed sows

are also less likely to become injured and lame, as group-housed sows often injure their legs and feet when interacting with other females (Anil et al., 2007).

Gestation stalls are generally 0.61 m wide and 2.1 m long and made of metal bars with a metal feeder or concrete trough in front to provide individual rations to each sow. Most sows are between 1.5 and 1.8 m long (McGlone, 2013), so the stall allows them to stand, lie down, and shift forward and back, but not turn around. This design has drawn criticism because of how it restricts sow movement and natural behaviors like moving freely and interacting with other pigs (Ryan et al., 2015).

In the European Union, a ban on gestation stall use after 28 days of gestation was instituted in 2003, with the goal of having phased out gestation stall use by 2013 (The Council of the European Union, Council Directive 200/120/EC, 2008). In Canada, swine producers are expected to comply with Canadian Quality Assurance standards, which include the animal welfare requirements in the Code of Practice for the Care and Handling of Pigs. The most recent iteration of the Code requirements state that any barn built or rebuilt after July 2014 must house pregnant sows in gestation stalls no longer than 28 days post-breeding unless provided exercise (Canadian Pork Council & National Farm Animal Care Council, 2014). South Korea requires that by 2030 all sows must be moved to group housing 6 weeks after breeding (Min et al., 2020).

In the United States, public opinion became strong enough that individual states began banning the use of gestation stalls in the early 2000s (Schultz & Tonsor, 2015). As a result, many companies in the United States recognized this demand from consumers and set a precedent for not accepting pork from sows that were housed in gestation stalls. For example, restaurants and retailers like McDonalds (2012) and Costco (Schutt, n.d.)

have announced they will source their pork products from pigs born on farms that use group housing and will phase out suppliers using gestation stalls. Pork producers also have acknowledged the demand for stall-free pork, and since 2007 Smithfield, the world's leading pork producer, has stopped using gestation stalls in approximately half of their farms worldwide (Smithfield Foods, 2017). In addition, sow farms contracted by Smithfield are being encouraged to convert their farms to group housing systems by the end of 2022 (Smithfield Foods, 2020). Other pork-producing companies like Hormel (2017) and Cargill (2014) have also pledged to phase out gestation stalls in their company-owned farms.

The proposed solution to potential welfare issues related to stall-housing of pregnant pigs is housing sows in group pens (Arey & Edwards, 1998), which consumers support more than stall housing (Ryan et al., 2015). However, group housing causes welfare challenges as well. When unfamiliar sows are mixed into a group, they fight in order to establish a social ranking (Arey & Edwards, 1998). This can cause stress (Salak-Johnson, 2017), as well as injuries leading to pain and lameness (Cador et al., 2014). The design and management of group housing affects sow welfare, and several factors can influence its effectiveness at improving sow welfare, such as dynamic or static groups, group size, space allowance per sow, pen shape (Arey & Edwards, 1998), type of flooring or presence of bedding (Cador et al., 2014), and feeding strategy (Hulbert & McGlone, 2006; Chapinal et al., 2010).

1.2 Measuring animal welfare

Good animal welfare is generally defined as animal handling, housing, and daily care that results in a state of fitness and a feeling of well-being. These criteria were formalized in 1979 by the Farm Animal Welfare Council as the Five Freedoms, which were later refined into the criteria used today: 1) freedom from hunger and thirst; 2) freedom from discomfort; 3) freedom from pain, injury, and disease; 4) freedom to express normal behavior; and 5) freedom from fear and distress (Farm Animal Welfare Council, 2009). The inadequacy in one of these freedoms can result in stress, which is a state of threatened homeostasis: for example, hunger, boredom, isolation, and thermal discomfort, among others (Etim et al., 2014). In the context of sow welfare, whether these freedoms are met, and thus if sows are experiencing stress, can be measured by a number of metrics, either individually or collectively. Sow productivity, which can include measures such as farrowing rate, litter size, longevity, and wean-to-estrus interval, can reflect her health and fitness (Salak-Johnson, 2017; Iida, Piñeiro, & Koketsu, 2020), and productivity is relatively easy to quantify objectively. Sow behavior can be an indicator of her mental well-being and emotional state, but behavior patterns can be difficult to measure and interpret (Barnett et al., 2001; Bakeman & Quera, 2011). Biological markers can reflect both her health and mental well-being (Etim et al., 2014); these, like productivity, are objective measures and relatively easy to quantify. However, productivity, behavior, and biological markers can be influenced by additional factors such as genetics, nutritional factors, and individual personality and ability to react to stress. As a result, comparing sow housing systems and their effect on sow welfare is difficult using the established metrics of sow productivity, sow behavior, and other biological markers.

1.3 Sow productivity

Sow productivity determines her profitability in the herd and her value to the farm (Rodriguez-Zas et al., 2003). Profitability is important, as a farm typically invests in a sow for three to four parities before she becomes profitable (Rodriguez-Zas et al., 2003). Many factors affect sow productivity, and decades of research have examined sow productivity in different housing systems. Several of these have been well-researched and reviewed: farrowing rate, number of piglets per litter, lameness and longevity, and wean-to-estrus interval.

1.3.1 Farrowing rate

Farrowing rate is a common metric for determining reproductive success. Farrowing rate is generally defined as the number of sows that farrow divided by the number of sows that are serviced (Young et al., 2010).

A meta-analysis by McGlone et al. (2004) examined papers published between 1970 and 2002, three of which compared both stall and group housing and identified no significant difference in farrowing rates (81% and 76% for stall and group housing, respectively). Bates et al. (2003) reported data from 388 sows followed for multiple parities, with 1315 total records. This study noted a higher farrowing rate for group-housed females (94%), who were mixed 3-4 days post-breeding, compared to stall-housed females (89%). Hulbert and McGlone (2006) used 80 gilts housed in stalls and 80 gilts in groups of 5. Group-housed gilts tended to have lower farrowing rates than stall-housed (68% and 78%, respectively). Karlen et al. (2007) compared 640 sows in groups

of 85 sows on deep litter with stall-housed sows and found lower farrowing rates for group-housed females (66%) compared to stall-housed (77%). Johnston and Li (2013) compared small pens with 6 or large pens with 26 sows in each to stall-housed sows, using a total of 815 sows. In their study, the group-housed sows were mixed at 35 days of gestation and had lower farrowing rates (92% and 95% for large and small pens, respectively) than stall-housed (98%). Knox et al. (2014) found that in a study using 1436 sows, group-housed sows mixed 14 and 35 days after breeding had a similar farrowing rate compared to stall-housed sows (88%, 91%, and 93%, respectively). However, sows mixed 3-7 days after breeding had lower farrowing rates than stall-housed sows (83%).

Farrowing rate may be affected by elevated HPA activity because it can disrupt estrus and embryo implantation (Arey & Edwards, 1998). Embryo implantation occurs 12 to 15 days after breeding (Li et al., 2015), which means that litters are particularly sensitive to stress until at least 15 days of gestation. Inconsistency in reported farrowing rates may be a result of the severity, duration, and timing of stress for group-housed females, in addition to a combination of other factors, including sow genetics, age and experience with different housing systems, types of flooring, feeding strategies, and herd health, among others. It is therefore difficult to conclude that housing system alone plays a definitive role in farrowing rates.

1.3.2 Litter size

Litter size, or the number of piglets born per litter, is one of the greatest contributors to sow productivity (Legault, 1985), so much research has compared housing systems to identify any effect of group housing on litter size. Similar to farrowing rate,

litter size can be influenced by stress at implantation, particularly for subordinate females who may experience greater stress during mixing (Arey & Edwards, 1998), so group housing may compromise litter size by increasing early embryo mortality. However, research has found little difference in litter size between sow housing systems. In the meta-analysis done by McGlone et al. (2004), the total litter size in 9 different studies was not different. In subsequent research, many authors identified no difference in litter size between group-housed and stall-housed sows (Bates et al., 2003; Hulbert & McGlone, 2006; Jansen et al., 2007; Karlen et al., 2007; Chapinal et al., 2010). Two studies observed that group-housed sows had greater litter sizes (Séguin, Barney, & Widowski, 2006; Lammers et al., 2007). However, in the study by Lammers et al. (2007), gilts were housed exclusively in stalls, which could have reduced the average litter size for stall-housed females. In contrast, Li et al. observed a tendency for smaller litter sizes from group-housed sows compared to stall-housed (2014). Average litter size is strongly influenced by a number of other factors not related to housing, including but not limited to genetics, nutrition, gilt management factors such as growth rate and age at first service, and semen quality and AI technician skill (Lawlor & Lynch, 2007). Further, in the studies discussed above, mixing time, group size, and feeding system are not consistent, which could also influence severity and duration of aggression and the consequent embryo survival. For these reasons, it is not surprising that litter size is not consistently affected by housing system.

1.3.3 Lameness and longevity

A major cost of sow farm operation is replacing sows, and it is recommended to cull sows between parity 5 and 9 to optimize their profitability (Bergman et al., 2018); however, sows are generally culled between parity 3 and 5, 8.6% of which are culled because of lameness (Poulson et al., 2020). Lameness is also a metric for welfare, as injury is painful and can affect sow comfort, and sow lameness during lactation can also increase the risk of piglet mortality (Iida, Piñeiro, & Koketsu, 2020). Shorter lifespan due to sows' inability to maintain high reproductive output is also considered a welfare concern (Serenius & Stalder, 2006), and reduced life expectancy is an indicator of poor welfare (Broom, 1991). Therefore, reducing lameness and lengthening sow longevity is necessary for improving sow welfare and mitigating costs related to sow replacement.

Sows housed in group pens are more likely to experience lameness (Koketsu and Iida, 2017), which is associated with greater likelihood of being culled younger (Iida, Piñeiro, & Koketsu, 2020). In one study, lameness was associated with a greater occurrence of mummified fetuses (Pluym et al., 2013), and sows with hoof abnormalities have been noted to have lower litter weights at weaning (Fitzgerald et al., 2012). However, the effect of housing system on sow longevity and consequent long-term productivity may be difficult to assess because of the many differences in group housing systems (Stalder et al., 2004).

1.3.4 Wean-to-estrus

Wean-to-estrus interval is the number of days between weaning and when the sow expresses standing estrus. A shorter wean-to-estrus interval reduces the number of non-productive days a sow spends in the barn (Poleze et al., 2006) and increases her average

number of parities per year. Wean-to-estrus, like all the previous measures discussed is strongly influenced by many factors, some of which are nutrition, lactation length, season, genetics, or disease (Poleze et al., 2006). However, there is some evidence that wean-to-estrus interval is influenced by housing system during the previous gestation. For example, Lammers et al (2007) reported a shorter wean to estrus interval for stall-housed sows, using observations from 957 litters. However, Bates et al., 2003 reported a greater return to estrus within 7 days in group-housed sows based on 1315 observations. Other studies with considerable samples sizes reported no difference in wean-to-estrus interval (Jansen et al., 2007 used 937 sows; and Johnston and Li, 2013 used 815 sows). Like the previous reproductive productivity measures, wean-to-estrus interval is highly variable and does not appear to be strongly influenced by housing system.

1.4 Behavioral measures of welfare

One of the ways to measure welfare is by observing behavior. Scientists have long studied behavior in an attempt to correlate patterns of behavior with emotions or mental state, and many behaviors have been associated with the attempt to escape either physical or psychological discomfort, such as pain or fear (Dawkins, 2008). Behaviors commonly measured that are associated with stress and poor welfare in sows include stereotypical behaviors, posture, and agonistic interactions (Barnett et al., 2001; Anil et al., 2002; Bench et al., 2013).

1.4.1 Stereotypical behavior

Stereotypical behaviors are considered indicators of poor welfare because they develop as symptoms of frustration, boredom, restraint, or persistent fear (Barnett et al., 2001; Chapinal et al., 2010). Stereotypical behaviors for sows include sham chewing (chewing while no food is present); head waving; bar-biting (biting bars of fences or crates); and licking, chewing, or nosing of available objects (Vieuille-Thomas, Pape, & Signoret, 1995). Pigs are naturally explorative animals and in natural conditions spend much of their time rooting and chewing (Brunberg et al., 2016). When these behaviors are unable to be performed, sows redirect these behaviors to their surroundings. Behaviors can also be directed at other animals, such as tail-biting, which causes pain, injury, and stress on the recipient of biting (Brunberg et al., 2016; Bench et al., 2013). Stall-housed sows have been noted to express more stereotypical behaviors than group-housed (Conte et al., 2014; Hulbert & McGlone, 2006; Chapinal et al., 2010); but tail-biting is not possible for stall-housed sows, as they are confined, and thus it is seen only in group-housing settings (Bench et al., 2013).

1.4.2 Posture

Posture behavior may also be used as a measure of welfare. Frequency of posture change; lying, standing, and sitting duration; and duration of posture change may reflect sow discomfort (Anil et al, 2002). It was demonstrated that more restrictive stalls corresponded to a longer duration of posture change, which may reflect greater discomfort associated with more restricted movement (Anil et al., 2002). Marchant and Broom (1996) noted that stall-housed sows took more time to lie down than group-housed sows, which suggests stall housing results in greater sow discomfort.

Posture may also be monitored to determine presence and severity of lameness. Sows spend less time standing, more time lying, and change positions less frequently when acutely lame (Roca et al., 2016); and stride length, number of steps, and hunching the back may reflect lameness (Grégoire et al., 2013). Measuring lameness has been used to compare injury risk in different sow housing systems (Cador et al., 2014), as fighting on slippery floors can cause injuries leading to lameness in group housing systems (Johnston & Li, 2013).

Harris et al. (2006) observed a tendency for more lameness in group-housed sows than stall-housed sows at the end of gestation. Anil et al. (2007) noted a higher occurrence of foot lesions that may lead to lameness in group-housed sows compared to stall-housed. Karlen et al. (2007), however, noted a higher rate of culling due to lameness in stall-housed sows compared to group-housed sows on deep bedding. Based on the inconsistencies among studies, occurrence of lameness due to fighting and injury may be more strongly influenced by the type or presence of bedding, type of flooring, number of sows per pen, floor space allowance, and feeding system rather than housing in stalls versus group pens.

1.4.3 Agonistic interactions

One argument against group pens is that sows fight for hierarchy when introduced to unfamiliar pen-mates, which may lead to injuries and stress. Injuries cause pain and can reduce longevity, and aggression can cause intense periods of stress (Greenwood et al., 2014). Combined, these experiences may lessen the welfare of group-housed females, who would be better protected from aggression and injury in an individual stall (Mack et

al., 2014). Aggression can also occur during feeding time in competitive feeding systems, where higher ranking sows can confront lower-rank sows and gain access to their feed (Salak-Johnson, 2017), which can result in frequent stress and inadequate nutrition for the subordinate sows. Aggression can occur in non-competitive feeding systems like electronic sow feeders as well, as sows establish a hierarchy and priority to enter the feeder. While waiting to enter the feeder, they may also interact aggressively and bite each other's vulvas (Bench et al., 2013; Remience et al., 2008).

Behavioral measures are more difficult to collect and define than objective values like reproductive performance. Behavior can be observed in real-time or on recordings. Observing in real-time is time-consuming, and it is possible to miss key observations. Recording animal movements with cameras and later analyzing the recording allows the observer to rewind and reanalyze a segment, but this can also be time-consuming, and using and maintaining cameras can be challenging (Bakeman & Quera, 2011). In addition, behaviors must be defined and categorized in order for viewers to consistently measure. For example, Anil et al. (2002) defined the process of a sow lying down in order to determine the time elapsed as a sow moved from standing to lying. Grégoire et al. (2013) defined 7 types and 3 levels of severity of foot lesion, and Elmore et al. (2011) categorized and defined multiple behaviors and postures in order to examine sow interaction with enrichment materials. These methods are effective for recording and analyzing behavior, but they are time-consuming and require strict definitions to maintain consistency.

1.5 Biological responses to stress

Researchers have long known that stress causes a physical response in animals, beginning with altered behavior and the activation of the autonomic nervous system, which also influences the immune system (Moberg & Mench, 2000). Monitoring the activity of the immune system and directly measuring the chemicals released via the autonomic nervous system can provide objective measures of the biological response to stress. While behavior measures often depend on the interpretation of a potentially biased observer, biological measures of stress are independent of personal opinion and are therefore more objective (Meagher, 2009).

1.5.1 Immune measures

It has long been thought that stress suppresses the immune system, but research over the last several decades has suggested that the role of stress in the immune system is more complicated and nuanced (Apanius, 1998). Some measurable components of the immune system are white blood cells like neutrophils and lymphocytes, immunoglobulins, cytokines, or acute-phase proteins. It is thought that corticosteroids increase the neutrophil:lymphocyte ratio, which is therefore a symptom of stress and inflammation (Karlen et al., 2007); however, McGlone noted that neutrophil:lymphocyte ratio has not yielded consistent results when comparing housing systems (2013).

According to Zhao et al., immunoglobulins G and M increase as an immune reaction to proteins altered by oxidative stress, but they did not detect a difference in immunoglobulins G and M in milk between group- or stall-housed sows (2013). Similarly, immunoglobulin A increases in response to stress (Goumon et al., 2018), but

Merlot et al. (2017) did not detect a difference in immunoglobulin A in milk of sows housed in stalls versus that of sows in group pens.

Acute-phase proteins are indicators of tissue damage and have been noted to respond to long-term stress scenarios (Chapinal et al., 2010). They are released in order to maintain homeostasis in response to tissue damage, inflammation, infection, and stress (Sorrells et al., 2007). Their role in responding to psychological stress is not well understood, and studies on gestation housing have not identified a difference in acute phase proteins in stall- or group-housed sows (Chapinal et al., 2010; Sorrells et al., 2007).

The synthesis of acute-phase proteins is mediated by interleukin-6, tumor necrosis factor-α and interleukin-1β, which are pro-inflammatory cytokines, some of which can also be used as markers of stress (Murata et al., 2004). However, no difference was noted in interleukin-6 or tumor necrosis factor-α between housing systems (Grün et al., 2013), and an earlier study did not detect a difference in tumor necrosis factor-α or any other analyzed cytokine between group- or stall-housed sows (Sorrells et al., 2007).

Although it is thought that stress alters the immune system, the evidence of immune reactivity to stress is conflicting and depends on many factors including genetics, age, social status, and the type of stress (Salak-Johnson & McGlone, 2007). As evidenced by the reported results discussed above, immune measures have not been shown to be widely effective in measuring chronic stress during gestation.

1.5.2 Sympathetic-adrenal-medullary axis

During periods of stress, the sympathetic-adrenal-medullary axis is activated, which causes a release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla (Martínez-Miró et al., 2016). Catecholamines prepare the body to react to a threat, causing vasodilation and increased heart rate (Martínez-Miró et al., 2016). Heart rate has therefore been used as a measure of welfare or stress status in gestation housing systems (Von Borell et al., 2007). Harris et al. (2006) and McGlone et al. (2004) did not note a difference in heart rate between sows in different housing systems; however, Marchant et al. (1997) noted that stall-housed sows had higher basal heart rate and greater heart rate increase during feeding than group-housed females. They attributed this difference to increased sympathetic activation due to the potentially stressful experience of eating next to a dominant sow, and possibly lower physical fitness of stall-housed sows.

Catecholamines are released rapidly into the bloodstream, and their half-life is generally between 10-100 seconds in circulation (Young, 2011). Norepinephrine is thought to be very variable in blood because of how quickly it can break down in circulation (Einarsson et al., 2008) and catecholamines are highly variable and unstable in saliva (Martínez-Miró et al., 2016). As such, they are impractical and not commonly used to measure stress in gestating sows.

1.5.3 Hypothalamic-pituitary-adrenal axis

The most commonly used objective measure of stress or wellbeing is the activity of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is comprised of

corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and glucocorticoids, generally cortisol or its metabolites.

The hypothalamus releases CRH as a response to physical or psychological stress and is the first initiator of the “fight, flight, or freeze” response to a perceived threat (Contoreggi, 2015). Corticotropin-releasing hormone triggers the release of ACTH from the pituitary and is negatively regulated by the subsequent release of glucocorticoids from the adrenal cortex (Contoreggi, 2015). It is also active in fetal development, and CRH is secreted by the placenta and the fetus, which, combined with maternal CRH secretion, play complicated roles in fetal development and the initiation of parturition (Fliers et al., 2014).

The target of ACTH is the adrenal cortex, where cortisol is synthesized and released within 3-5 minutes of stimulation (Spencer & Deak, 2017). The release of progesterone and prostaglandin F2α metabolite are also affected by ACTH. As pregnancy progresses, maternal response to external stressors results in attenuated ACTH and glucocorticoid response, possibly in order to protect the fetus from maternal glucocorticoids. The fetus and placenta also release ACTH during gestation (Fliers et al., 2014). Injection of ACTH has been used widely as a model for acute stress because it can cause a spike in cortisol that imitates stressful situations; this has been demonstrated in many species, including cattle, swine, goats, lynx, and humans (Negrao et al., 2004; Otten et al., 2004; Terwissen et al., 2013; Endo et al., 2018; Nye et al., 1999).

Cortisol is the major glucocorticoid in most mammals and is released from the adrenal cortex as a result of ACTH stimulus; it is commonly considered the “stress hormone” (Spencer & Deak, 2017). As stress in this discussion is defined as a state of

threatened homeostasis, this includes both physical stress and some psychological stressors (Gerber et al., 2012; Spencer & Deak, 2017). Psychological stress stems from the perception of a threat and the expected need for escape, while physical stress may not include a psychological element and may only be the physical action of addressing the threat. For example, cortisol may be elevated in a stall-housed sow experiencing psychological stress of isolation and boredom; a group-housed sow may have elevated cortisol because of both psychological and physical stress from pain and exertion during a fight for hierarchy; and elevated cortisol in a sow during parturition may be attributable to psychological and physical stress of pain as well as energetic demands of labor (Lawrence et al., 1997). Therefore, it may be difficult to attribute cortisol response to either physical or psychological stress when both are present (Viru et al., 2010). Cortisol is released during various types of stressors because it plays a major metabolic role, in combination with catecholamines, in preparing the body to react to a potential threat. Because fast-twitch muscles use glucose as a primary energy source and are involved in rapid, powerful bursts of activity (Baskin et al., 2015), glucose availability is critical for addressing a potential danger. Cortisol's primary metabolic role is to increase circulating blood glucose, which it does in concert with glucagon and epinephrine during periods of acute stress (Sapolsky et al., 2000). It stimulates lipolysis, proteolysis, and glycogenolysis and gluconeogenesis in the liver over several hours as preparation for the next stress event (Sapolsky et al, 2000). Cortisol is known to increase during aerobic exercise, and cortisol is critical for exercise performance, as induced cortisol deficiency increases perceived exertion and maximum heart rate (Kanaley & Hartman, 2002). Cortisol also rises postprandially, in order to correct for potential hypoglycemia induced by insulin

secretion (Sapolsky et al., 2000). Chronically elevated cortisol promotes the development of insulin resistance, which during pregnancy reduces the female's ability to utilize glucose from the bloodstream and thus increases glucose availability for the fetus (Herrera, 2000).

Cortisol is affected by circadian rhythm and typically peaks at the time of wakening (early morning for diurnal animals and evening for nocturnal animals). In many animals, basal cortisol is secreted in an ultradian pattern, about 60 minutes between pulses (Spencer & Deak, 2017), although this pattern has not been identified in pigs (Mormède et al., 2007).

During periods of stress where the "fight, flight, or freeze" reaction is perceived to be necessary to escape a threat, the HPA axis is consistently activated in response (Contoreggi, 2015). Cortisol is therefore a reliable indicator of stress and can be measured to identify HPA activation.

1.6 Cortisol analysis from short-, medium-, and long-term measures

Cortisol can be assayed from multiple types of biological samples, including blood, saliva, feces and urine, and hair. These substances represent cortisol secretion over different periods of time. Blood and saliva represent a relatively short timeframe with cortisol being secreted into blood and saliva within minutes of a stressful stimulus (Guzik et al., 2006; Bozovic et al., 2013); feces and urine represent longer periods of cortisol circulation, from 2 to 48 hours (Hay et al., 2000; Palme et al, 2005) and hair accumulates cortisol over long periods of time, from weeks to months (Bacci et al., 2014).

1.6.1 Blood and saliva

Cortisol in blood can be detected using either serum or plasma, including both free (biologically active) and bound (biologically inactive) cortisol (Bozovic et al., 2013). Blood cortisol in serum is relatively stable, as was observed in serum from cattle where cortisol decreased only 12% over 2 days when samples were stored at room temperature (22-26 ºC) (Reimers et al., 1983). The main challenge to using blood cortisol as a marker of stress in pigs is that a pig must be restrained to collect blood, either held by hand or secured with a snare depending on pig size/weight. Restraint causes stress to the animal, as could hearing or seeing other pigs being restrained, and any activation of the HPA axis can affect blood cortisol within several minutes, as cortisol can rise immediately post-stressor (Guzik et al., 2006; Spencer & Deak, 2017). A permanent or semi-permanent catheter may also be used. Surgical or non-surgical implantation of a catheter can be expensive and labor-intensive, and the process itself may cause stress (Spencer & Deak, 2017). An implanted catheter may be exteriorized and exit from the animal's skin, such as catheterization of the vena cava or the jugular vein via the auricular (ear) vein (Matte, 1999). Alternately, a vascular access port may be placed at the end of the catheter, allowing the catheter and port to be implanted under the animal's skin. The port can then be accessed using a Huber needle (Swindle et al., 2005). The vascular access port reduces risk of infection, catheter dislodging, and loss of patency, which are more likely to occur with exteriorized catheters, and individual housing and possibly a protective jacket over the catheter is required to prevent dislodgment of the catheter. However, vascular access ports are more expensive than externalized catheters (Swindle et al., 2005). Sampling blood from a catheter involves minimal animal restraint once the catheter is placed, so

repeatedly taking samples is effective for determining cortisol change over the course of several hours, accounting for circadian rhythm, feeding, or stressors. However, for the purposes of understanding cortisol release in relation to gestation housing, blood sampling using catheters is practical for only individually housed sows, because in group housing other sows would chew on and dislodge the exteriorized catheter or the Huber needle from a sow whose blood was being sampled.

Cortisol in saliva reflects free cortisol (Hellhammer et al., 2009), and can be collected non-invasively in swine, as they will voluntarily chew on a cotton ball or rope, which will soak up the saliva (Bushong et al., 2000) and can then be extracted from the ball or rope into a collection tube. This is advantageous in that it does not cause stress and an associated spike in cortisol (Mormede et al., 2007). Cortisol in blood is detectable in saliva within 2-3 minutes (Bozovic et al., 2013), so handling an animal to obtain a saliva sample may cause an increase in salivary cortisol. Studies have reported good correlation ($r = 0.80$) between salivary and serum cortisol after snaring stress (Cook et al., 1996). Salivary cortisol does have an advantage in that it is very stable and will not easily degrade during handling and processing; it is stable at 5 degrees C for up to 3 months, and the concentration decreases 9% per month stored at room temperature (Garde & Hanse, 2005). This means that samples do not need to be immediately frozen after collection, and there is little risk of samples degrading and values decreasing while being prepared for analysis.

Short-term measures of cortisol may be adequate to identify HPA activation within a short window of time (before the potential stress of handling alters cortisol levels), but they are impractical for determining chronic stress because they only identify

a short period of time. One time point cannot accurately account for circadian variation and the average cortisol secretion over one day; therefore a chronic state of stress cannot be accurately assessed based on one sample time. For example, a blood sample collected at waking will have elevated cortisol compared to a sample collected midday due to circadian pulsatility of cortisol (Spencer & Deak, 2017). The sample collected at waking is not necessarily reflective of the subsequent pattern of cortisol secretion throughout the day. Further, it has been shown that calves and pigs under chronic stress have basal blood cortisol similar to non-stressed animals, suggesting a blunting of the HPA response in chronic stress. Normal cortisol secretion patterns may be disrupted during chronic stress, with elevated cortisol at night, when it is usually low. As a result, blood and salivary cortisol are not useful for identifying chronic stress (Mormède et al., 2007). This is relevant for sow housing research because collecting blood or salivary cortisol to assess housing stress may not account for circadian fluctuation or reflect abnormal secretion of cortisol that may occur during periods of mixing stress or the stress of confinement and isolation.

1.6.2 Feces and urine

Cortisol in blood circulation is metabolized and excreted in feces and urine (Palme et al., 2005). Cortisol metabolites can therefore be measured in fecal and urine samples as a reflection of prior glucocorticoid activity in the blood.

Fecal samples can be easily and non-invasively collected. In pigs, about 7% of circulating cortisol is excreted in the feces, and peak cortisol excretion is reached 48 hours after HPA activation (Palme et al., 2005). Excretion time depends on digesta

passage time: therefore, species, type of feed, and feed intake affect the lag between the stressor and the appearance of cortisol metabolites in the feces (Palme, 2012). While blood cortisol can fluctuate widely throughout a 24-hour period, fecal metabolites represent cumulative secretion of cortisol and account for diurnal variation, making it less useful for identifying a short, acute stressor, but total collection may be practical for a stressful period of several hours up to several days (Palme, 2012). Carlsson et al. (2007) determined that cortisol from a single fecal sample varied dramatically (CV=8-114%) compared to the cortisol found in a total 24-hour fecal collection, so it is advised to collect total fecal excretion for the time period of interest and analyze a homogenized sample. There are some challenges to handling and processing fecal samples. Cortisol metabolites are sensitive to further metabolism by bacteria present in the feces, so samples must be frozen quickly after collection and are not stable like blood and saliva samples. Commercial assay kits for cortisol cannot be used, as they may not be sensitive enough to detect metabolites, so antibodies or kits must be chosen carefully in order to adequately measure the broad spectrum of cortisol metabolites present in the feces (Palme, 2012).

In swine, the majority of cortisol metabolites are excreted in urine (Palme et al., 2005), and peak excretion occurs 2-3 hours after an acute stressor (Hay et al., 1999). Urine can be collected non-invasively, but animals require constant observation in order to collect and freeze samples as soon as urination occurs (Pol et al., 2002), which may make collections of several hours or days prohibitively labor-intensive. Hay et al. (1999) inserted urinary catheters, but they did not discuss the potential stress and discomfort the presence of urinary catheters may cause. Urine concentrations of cortisol can vary

dramatically based on the volume of urine, so urinary cortisol metabolites must be standardized with another consistently excreted metabolite like creatinine (Pol et al., 2002). Urinary cortisol metabolites appear more quickly after a stressor than fecal metabolites do, but because it also represents an accumulation of cortisol secretion total urine can be used as a measure of cortisol secretion over several hours or days (Hay et al., 1999). However, it is not practical for use as a measure of chronic HPA activation.

Fecal or urinary metabolites of cortisol are simple to collect from individually-housed sows using metabolism crates and urinary catheters (Hay et al., 1999; Le Goff & Noblet, 2000); however, these methods are not possible to replicate in a group housing setting, as unconstrained sows will not urinate and defecate in one spot. Collecting individual samples in a group pen would also be challenging and would likely require constant surveillance in order to collect individual samples as they were excreted, minimizing risk of contamination from the manure of other sows.

1.6.3 Hair

Recently there has been increasing interest in cortisol deposited in hair as a measure of long-term HPA activation, particularly in farm animals (Heimbürge et al., 2020a). The challenges of the aforementioned biological matrices and their inadequacies for measuring long-term stress have led researchers to investigate hair as a stable and easily-collected matrix for cortisol deposition.

Collecting hair is minimally invasive and stressful, and a small, acute stressor is unlikely to be detectable in a hair sample (Creutzinger et al., 2017; Heimbürge et al., 2019). According to Heimbürge et al. (2020a), animals can be stalled or in a group pen,

and hair can be shaved using an electric trimmer and collected with a vacuum cleaner with a paper filter in the tube. Diurnal variation is accounted for as the hair grows, as repeated fluctuations are incorporated into the hair at equal intervals (Heimbürge et al., 2019). Hair cortisol is very stable, and hair samples protected from moisture and UV light are thought to be stable for months to years (Heimbürge et al., 2019). Like fecal and urinary cortisol, there is a delay between activation of the HPA axis and the deposition of cortisol in the hair. Bacci et al. (2014) estimated that the time it takes for sow hair to grow from the follicle and appear at the surface of the skin may be 15 days. Based on observations of the variation in hair growth in the sow herd at the South Dakota State University Swine Research and Teaching Facility, this likely depends greatly on the individual animal, ambient temperature, age, and genetics, among other factors. Hair is therefore not useful for examining HPA activity over short periods like the previously discussed matrices.

The exact mode of cortisol deposition into hair is not fully known. In human research, it has been suggested that substances in sweat are incorporated into hair more predominantly than via blood flow to the hair follicle (Kintz, 2006). However, as pigs do not sweat, it is more likely that blood supply to the hair follicle is the major route of deposition in hair. There is also potential for external contamination to affect hair cortisol; aqueous solutions cause the hair shaft to swell, which facilitates cortisol diffusion in or out of the hair (Otten et al., 2020). In one trial, pig hair cortisol concentrations were increased by urine contamination but not saliva, and feces reduced cortisol concentrations similarly to samples soaked in water, probably because of the low

concentrations of cortisol metabolites in pig feces, and cortisol leaching out when swelled in water (Otten et al., 2020).

There are some challenges to hair cortisol analysis. The period of investigation must be long enough for sufficient hair to grow, as short hairs and samples of small mass (<50 mg) are difficult to collect and handle. The body region of collection must be consistent, as hair growth and cortisol concentration differ by region (Heimbürge et al., 2019). Laboratory analysis is labor-intensive, as external contaminants must be removed without extracting the analyte (MacBeth et al., 2010), and hairs must be examined for damage, as damaged hair may incorporate more external cortisol than undamaged hairs (Otten et al., 2020). These strict requirements for hair quality necessitate great attention and care from the technician preparing the samples for analysis.

1.7 Conclusion

Consumers perceive group housing to be better for sow welfare, but traditional methods of measuring welfare such as sow reproductive productivity, behavior, and biological measures of stress have proven to be inconsistent and inconclusive. Cortisol may be useful for identifying HPA activation in response to housing stress, and hair may be a useful matrix for measuring long-term cortisol secretion as a measure of chronic stress.

1.8 Research objectives

The objective of this research was to 1) determine the influence of a simulated chronic stress scenario on HCC, 2) determine HCC of sows in two different gestation

housing systems as a marker of chronic stress, and 3) examine the pattern of blood cortisol during parturition in the sow.

2.0 ASSESSMENT OF HAIR CORTISOL ACCUMULATION FOLLOWING CHRONIC INDUCED STRESS

2.1 Introduction

Some studies have concluded that hair cortisol may not be a reliable metric for chronic stress in swine (Casal et al., 2017; Wiechers et al., 2021). Others identified a correlation between tail lesions and HCC, and a tendency for lameness to affect HCC (Carroll et al., 2018), which may indicate a correlation between overall stress caused by aggression and HCC. Additionally, fluctuations in HCC have been identified throughout the reproductive cycle in sows (Bacci et al., 2014), suggesting that hair does incorporate varying levels of circulating cortisol at different stages of reproduction. To our knowledge no studies had been conducted comparing HCC of control pigs with chronically stressed pigs.

It has been demonstrated that application of ACTH can induce elevated serum cortisol in swine (Mwanza et al., 2000). Repeated ACTH injections have also been shown to increase hair cortisol concentrations in dairy cattle, goats, lynx, and other mammals (del Rosario et al., 2011; Terwissen et al., 2013; Endo et al., 2018), but little research has demonstrated this result in swine. Repeated ACTH injections may model chronic stress by increasing serum cortisol over an extended period of time (Heimbürge et al., 2020a), so the purpose of this trial was to quantify the increase in serum cortisol after injection with ACTH and identify the corresponding increase in hair cortisol after repeated injections.

2.2 Materials and Methods

The experimental protocols used in this study were approved by the South Dakota State University Institutional Animal Care and Use Committee (IACUC #2003-016A).

2.2.1 Animals and experimental design

The study was conducted at the South Dakota State Swine Education and Research Facility in Brookings, South Dakota. A total of 18 gilts (PIC 1050 x Duroc, 104.5 ± 7.9 kg) were assigned to two treatments. Nine gilts were assigned to the ACTH treatment and 9 were assigned to the control treatment; each treatment was balanced for weight and for equal distribution of hair and skin coloration as assessed visually. This was done to account for any influence of darker hair color on HCC, which Heimbürge et al. (2020b) observed in pigs. The trial took place in two groups. In Group A, 6 gilts (3 control, 3 ACTH) were moved from a single group pen in the wean to finish facility to 3 pens (2 pigs/pen, 1.83 × 2.39 m) in the sow barn in March. In April in Group B, 12 gilts (6 control, 6 ACTH) were selected from 8 different pens in the wean to finish barn containing 5-6 pigs/pen, moved to the sow barn, and housed in 4 pens (1.83 x 2.39 m) with 3 pigs/pen.

2.2.2 Chronic stress model and sample collection

Repeated ACTH injections have been shown to increase hair cortisol concentrations in multiple animals (del Rosario et al., 2011; Terwissen et al., 2013; Endo et al., 2018), so it was thought that repeated ACTH injections may model chronic stress by increasing serum cortisol over an extended period of time (Heimbürge et al., 2020a). Blood samples were to be collected in order to verify that the selected dose of ACTH was

sufficient to increase blood cortisol to double the basal concentration, approximately 40-60 ng/mL (Clapper, 2008). Serial blood collections were to occur -10, 10, 20, 30, 60, 90, 120, and 150 min adjacent to administration of ACTH in order to characterize the change in serum cortisol.

2.2.3 Treatment and sample collection

Thirty-five days prior to movement to the sow barn (d-35), hair was shaved from the entire haunch of each gilt (Figure 2.1). At d0, 6 gilts in Group A and 4 gilts in Group B (2 control and 2 treatment) were administered TKX (50 mg each of telazol, ketamine, xylazine) 2.5 ml/kg body weight via intramuscular injection for anesthesia. An attempt was made to insert ear vein catheters but was unsuccessful; in Group B, minor procedural changes were made but insertion of ear vein catheters was unsuccessful. Hair samples were collected from the previously shaved area while animals were anesthetized. After recovery from anesthesia (approximately 6 h after administration of anesthesia) all treatment gilts were administered an intramuscular injection of ACTH (Cosyntropin acetate #23912, Cayman Chemical, Ann Arbor, Michigan) at a concentration of 10 µg/kg body weight. All control gilts received an intramuscular injection of 10 µL sterile saline/kg body weight. In Group A, the same dose of ACTH was administered at d7 and d14. In Group B, only 1 subsequent injection was given at d11 at a dosage of 8 µg/kg body weight because the addition of ACTH needed for the final injection was not obtained before termination of the trial. At d21, hair was collected from the previously shaved area. All hair samples were stored in paper envelopes in the dark at room temperature before analysis. Animals were fed the standard SDSU gestation diet ad

libitum from d0-d35. Pens were cleaned every two days to reduce potential contamination of hair by urine and feces.

2.2.4 Lab analysis

Samples were examined, damaged hairs and debris were removed, and samples were weighed. Samples ≤50 mg were placed into 2 mL cryogenic vials (Corning #430659) and >50 mg were placed in to 5 mL disposable glass culture tubes (Fisher Scientific #14-961-26). The samples were washed thrice in methanol (40 μL/mg sample), blotted dry between rinses, then left to dry overnight. Samples were then frozen with liquid nitrogen and powdered by hand using a mortar and pestle; 25 mg of powdered hair were weighed into 0.6 mL tubes. The analyte was extracted using 0.5 mL methanol per 25 mg of powdered hair, and the samples incubated for 16-24 h on a rotator. Tubes were centrifuged at 20 °C for 15 min at 2150 x *g*, and the supernatant was collected and transferred to a 12 millimeter glass culture tube. To ensure all extracted steroids were recovered, the powdered hair sample was rinsed 2 times by adding 0.5 mL of fresh methanol, then gently vortexing (40 s), centrifuging, and pooling supernatants. The pooled supernatant was dried at 38 °C under a gentle stream of nitrogen gas and reconstituted with 200 μL of extraction buffer from cortisol assay kit, and samples were frozen at -80 °C until analysis with enzyme immunoassay (EA65, Oxford Biomedical, Rochester Hills, MI, USA). The optical density (OD) value was read at 450 nanometers after 30 min on a SpectraMax 190 absorbance plate reader (Molecular Devices, Sunnyvale, CA, USA). A standard curve of OD value versus cortisol concentration was generated, and hair cortisol concentration was then determined according to the standard

curve and expressed in pg cortisol per mg powdered hair. According to the manufacturer, cross-reactivity of the antibody used for the cortisol kit is as follows: cortisol (100.00%), prednisolone (66.9%), 11-deoxycortisol (58.1%), cortisone (15.9%), prednisone (13.7%), 17-hydroxyprogesterone (5.4%), dexamethasone (4.6%), estriol 4.5%, estrone (4.1%), d-aldosterone (3.6%), progesterone (3.5%) 6-β-hydroxycortisol (3.4%), trans dehydroandrosterone (1.9%), testosterone (1.7%), corticosterone (1.4%), and pregnenolone (1.3%).

2.2.5 Statistical analysis

The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to confirm the homogeneity of variance and to analyze for outliers. Then data were analyzed using PROC MIXED procedure of SAS with pig as experimental unit. In the model, the main effects of ACTH treatment, time, and their interactions were tested with the group of pigs as the blocking factor and TKX administration as a random effect. Tukey's adjusted means test was used to detect differences where statistical significance and tendencies were set at $P \leq 0.05$ and $P < 0.10$, respectively, for all statistical tests.

2.3 Results and Discussion

Considering all gilts, there was a tendency ($P = 0.084$, SEM = 0.6) for average HCC of ACTH gilts (5.9 pg/mg) to be lower than average HCC of control gilts (7.3 pg/mg), which is partly due to control gilts having slightly, but not significantly, higher initial HCC before the application of the treatment (Figure 2.2). The effect of time was significant ($P = 0.002$, SEM = 0.6), where post-treatment HCC for all gilts was higher

(8.0 pg/mg) than pre-treatment levels (5.3 pg/mg). Hair cortisol concentrations of control gilts rose 41% from pre- to post-treatment, and HCC of ACTH-treated gilts rose 62%, but there was no interaction (P = 0.874) between treatment and time, which would have indicated that only ACTH administration caused an increase in HCC. Group B received 1 less injection than Group A, which may have attenuated the post-treatment increase in HCC.

Figure 2.3 shows the change in HCC from pre- to post-treatment for both control and ACTH gilts in Group A compared to Group B. There was no difference between ACTH and control gilts in Group A, which may be attributable to the small sample size. Overall, the HCC of gilts in Group A decreased from pre- to post-treatment (-0.7 pg/mg), as compared to Group B, which increased 4.5 pg/mg from pre- to post-treatment (P = 0.002, SEM = 0.8). As described in Section 2.2.1, the gilts in Group A were not mixed with unfamiliar females but were divided from a group of 6 into 3 groups of 2 on d0. In Group B, however, the 12 gilts were re-sorted into unfamiliar social groups of 3 at d0 when moved into the sow barn. Group B gilts had a greater increase in HCC than Group A control gilts (Figure 2.3), which suggests that Group B gilts experienced stress from the mixing process that confounded any potential increase in HCC due to ACTH injection.

We were unable to document an increase in blood cortisol because the ear vein catheters proved infeasible. However, TKX at 2.2 mg/kg body weight is known to elevate serum cortisol for 220 minutes after injection in gilts (Clapper, 2008). In addition, many other studies have identified an increase in blood cortisol for more than two hours following an injection of ACTH in pigs (Otten et al., 2004; Sautron et al., 2015; Larzul et

al., 2015). We also observed that all gilts that received ACTH on d0 vomited within an hour of injection. We are therefore confident that the animals experienced multiple periods of elevated cortisol throughout the trial. However, despite the known effects of ACTH on blood cortisol, this study failed to identify an increase in HCC following repeated ACTH injections. Heimbürge et al. (2020a) conducted a similar trial where gilts (90.3 ± 10.6 kg) were injected with 2 mg ACTH per animal (approximately twice the concentration used in this trial) every 2 days for 4 weeks; their results also failed to identify increased HCC in ACTH-treated gilts compared to control gilts. The authors speculated that an attenuated cortisol response, reduced hair growth, and external contamination could be reasons for the lack of difference in HCC between control and ACTH-treated gilts. Water and fecal contamination can decrease HCC, and contamination with urine can increase HCC (Otten et al., 2020); Heimbürge et al (2020a) noted that more distal segments of pig hair, which may have split ends, had higher HCC. If they analyzed damaged hairs instead of selecting intact hairs and trimming damaged ends, the values they reported, which were 6-10 times higher than the values found in this trial, may be largely attributable to external contamination leaching into the damaged hair shaft, confounding than any potential treatment effect.

In this trial, mixing unfamiliar animals in Group B caused a significant increase in HCC compared to Group A that was not mixed (P = 0.002, SEM = 0.8), while there was no significant effect overall of ACTH injection between control and ACTH-treated gilts on post-treatment HCC (P = 0.259, SEM = 1.1). This suggests that in order to cause chronic stress that is detectable in hair, mixing unfamiliar animals and creating social stress is more effective than repeated ACTH administration. Mixing unfamiliar pigs in

groups has been noted to increase salivary cortisol (Parrot & Misson, 1989; Jansen et al., 2007; Hemsworth et al., 2016), so it is likely that in this study mixing unfamiliar pigs created a sufficiently prolonged stress that hair growth over 21 days was able to capture a detectable increase in HCC, which repeated ACTH injections was not able to do. Future research, therefore, should utilize mixing stress rather than ACTH in order to create chronically elevated and prolonged blood cortisol to measure in the hair.

2.4 Conclusion

HCC was not affected by ACTH administration, but mixing unfamiliar gilts in new pens caused a significant increase in HCC. ACTH administration may not be adequate for simulating chronic stress in pigs, but HCC is an effective matrix for evaluating in pigs. Future research should consider using prolonged social stress as a less expensive and more effective model for chronic stress than repeated ACTH administration.

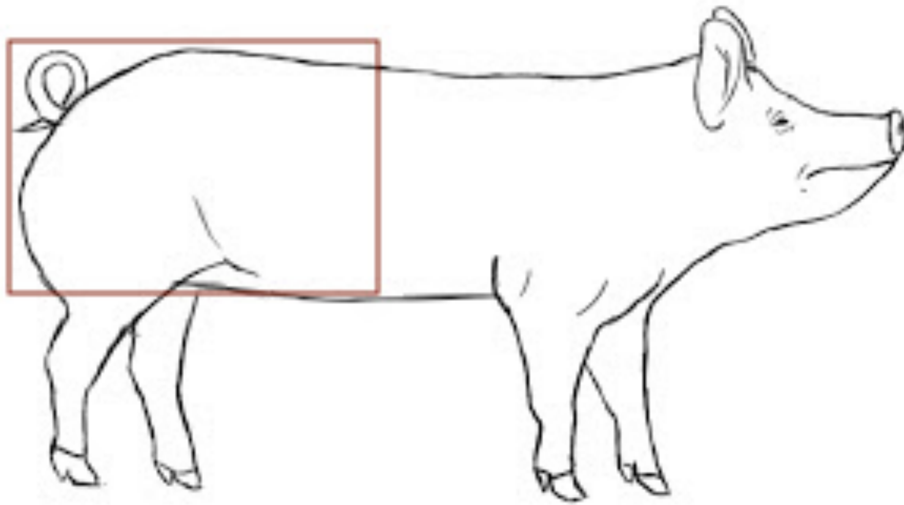

Figure 2.1 Hair collection site, shaved at d-35, d0[1], and d21

[1]D0 refers to the day treatment or control injections were begun.

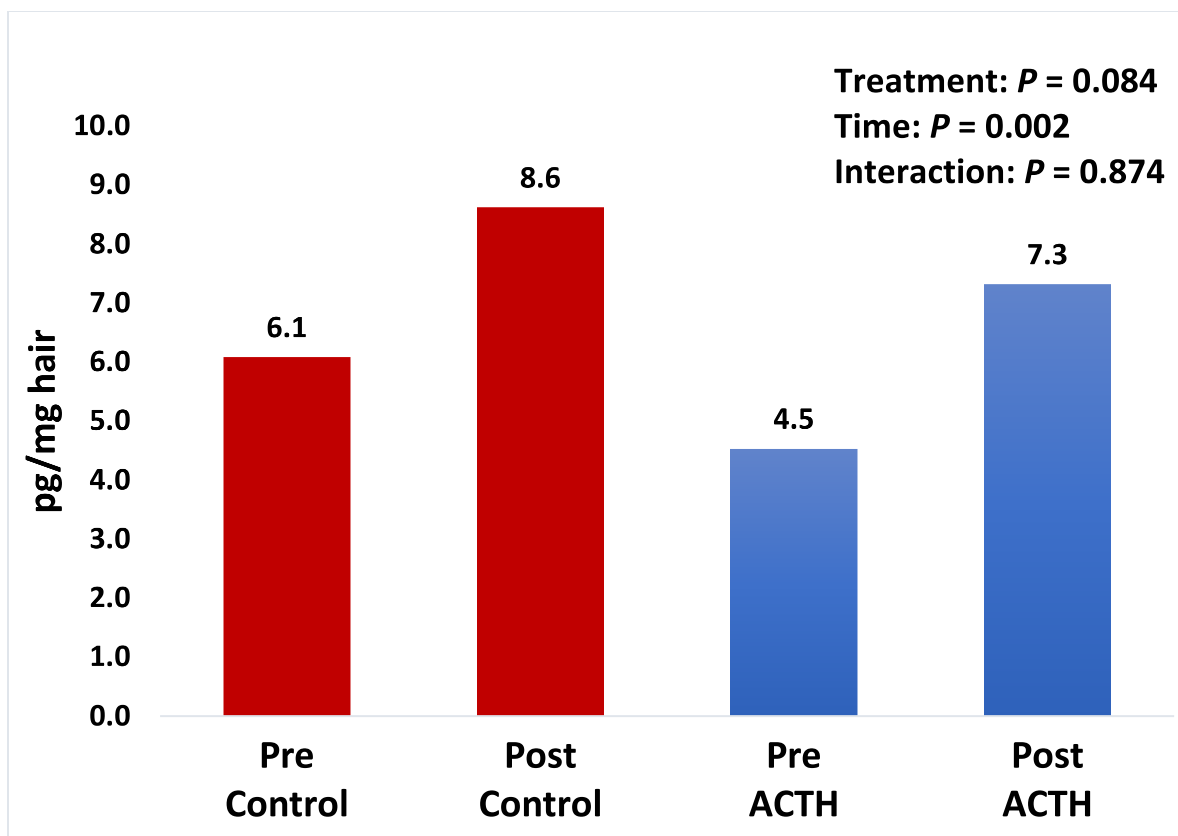

Figure 2.2 HCC from pre- and post-treatment[1] in control and ACTH-treated gilts[2]

[1]Pre-treatment refers to hair growth between d-35 and d0 (day of first treatment or control injection), and post-treatment refers to hair growth between d0 and d21.

[2]Significantly different means denoted by superscript a and b where $P \leq 0.05$.

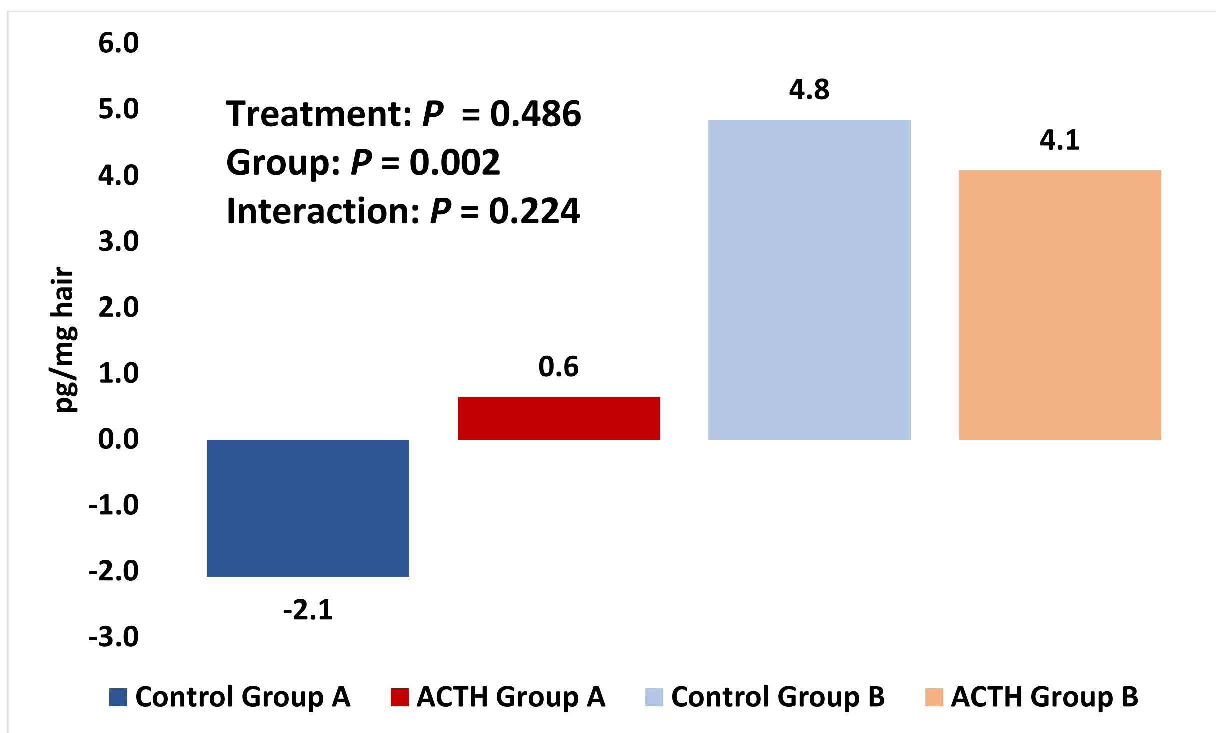

Figure 2.3 Difference in HCC from pre- and post-treatment[1] in control and ACTH-treated gilts in groups A and B[2]

[1]Pre-treatment refers to hair growth between d-35 and d0 (day of first treatment or control injection), and post-treatment refers to hair growth between d0 and d21.

[2]Significantly different means denoted by superscript a and b where $P \leq 0.05$.

3.0 QUANTIFYING CORTISOL IN HAIR AS A CHRONIC STRESS BIOMARKER IN GROUP-HOUSED AND STALL-HOUSED SOWS DURING GESTATION

3.1 Introduction

In the US sows are typically housed in stalls during gestation for a variety of reasons. Individual housing provides a safe environment for both the sows and the employees caring for them, protecting both from injury and aggression from other sows. Stall housing also allows each female to receive an individual ration without competition from other females (Salak-Johnson, 2017). Additionally, stall housing is economically efficient and allows for more sows to be housed in fewer square feet of space, resulting in lower building costs than most modern group-housing facilities (Buhr et al., 2010).

However, stall housing has been criticized for limiting sows' movement and natural behaviors, thereby reducing perceived sow welfare in comparison to group-housed females. Pork consumers have demanded higher welfare standards for gestating sows, and there has been an international trend to ban gestation stalls in an attempt to improve sow welfare. However, the research conclusions are conflicting as to whether sows housed in stalls experience poorer welfare than sows in group housing, and the potential negative effect of group housing on sow performance has been debated, along with the economic consequences of instituting a ban on gestation stalls, both from building and renovation costs and the potential for decreased sow productivity in group housing.

Many studies have examined the effect of housing on welfare parameters such as behavior, reproductive productivity and longevity, and concentrations of biological

measures like cortisol in an attempt to determine the long-term consequences of stall housing on sow welfare. Hair cortisol may be useful for identifying chronic stress, as it is not confounded by circadian variation or acute stress associated with handling. However, little research to date has utilized HCC to examine the long-term circulation of cortisol as a measure of chronic stress in gestating pigs. The purpose of this study was to determine the HCC of sows in two different housing systems.

3.2 Materials and Methods

The experimental protocols used in this study were approved by the South Dakota State University Institutional Animal Care and Use Committee (IACUC #17-072A and 18-064A). Daily animal care followed standard SDSU swine unit protocol and the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Third Ed., 2010).

3.2.1 Animals and experimental design

The study was conducted in the sow barn at the South Dakota State University Swine Education and Research Facility in Brookings, SD from November 2018 until June 2019. A total of 66 multiparous and primiparous females (PIC 1050) were assigned to 1 of 2 experimental treatments, stall-housed or group-housed. The SDSU sow herd is managed in a batch farrowing system, and each experimental treatment was assigned to two breed groups. The stall-housed sows (n=50) were housed in gestation stalls (0.61 m x 1.98 m) from breeding until d111 ± 1.4 of gestation. The first group (n=22) was bred in November 2018, and the second group (n=28) was bred in December 2018. The group-housed sows (n=39) were moved to 3 group pens (8.53 m x 8.20 m) approximately 48 h

after breeding. Each group pen held 10-15 sows, and the groups were dynamic because of the introduction or removal of non-experimental herd sows. Gilts and small parity 1 sows were housed in 1 group pen, and multiparous sows were housed in the other 2 group pens. The first group (n=21) was bred in February 2019, and the second group (n=18) was bred in March 2019. All females were housed in stalls at least 5 d prior to breeding. Sows were moved from their assigned housing system to farrowing crates at d111 ± 1.1 of gestation.

All females were included in 1 of 2 nutrition trials being conducted during this experiment. The stall-housed sows were on a trial examining dietary antioxidants fed in gestation and lactation (Hernandez et al., 2021). There were 4 treatments, receiving a standard gestation diet plus a carrier top dress including either a phytochemical oil, whole yeast cell, gamma-tocopherol, or nothing (control). The group-housed sows were on a trial examining varying dietary lysine:energy ratios in gestation (Bruhn, 2020). There were 3 treatments: constant lysine:energy ratio throughout gestation (control, n=12), higher lysine:energy ratio from d90-110 of gestation (PF1), or lower lysine:energy ratio from d2-89 and higher:energy ratio from d90-110 of gestation (PF2). In both nutrition trials, gestation diets were formulated to meet or exceed gestation nutrient requirements according to NRC (2012) and daily feed allotment was managed similarly across both groups; thus differences in diets between the trials were not expected to impact female response to housing. Further details on the nutritional studies can be found in Hernandez et al., (2021) and "Impact of Altering Lys:Energy Ratio During Gestation on Sow Productivity, Piglet Robustness, and Piglet Post-Wean Growth Performance" (Bruhn, 2020).

3.2.2 Hair sample collection

Approximately 400 cm^2 on the right hip and 200 cm^2 from the right shoulder (Figure 3.1) was shaved at 6.0 ± 3.8 d post-breeding and hair was discarded. The hip section was then shaved and samples collected at d37, d74, and d111 of gestation. The shoulder was shaved and samples collected at d111 of gestation. All samples were stored in individual paper envelopes and kept in a dark drawer until analysis. Samples from the hip from d37 and d111 of gestation were analyzed for cortisol concentration. Samples from the hip at d74 and samples from the shoulder were not analyzed in this experiment.

3.2.3 Lab analysis

Samples were analyzed according to the procedures described in Chapter 2, with slight modification. Instead of powdering the samples with mortar and pestle, they were ground with a Retsch MM310 mixer mill at 30 hertz.

3.2.4 Sample selection for analysis

A total of 64 stall-housed sows and 59 group-housed sows were shaved and samples collected 6.0 ± 3.8 d post-breeding. At d111, 50 stall-housed and 39 group-housed sows were shaved and samples collected. Overall, 14 stall-housed sows and 20 group-housed females were removed from the trial due to failure to become or remain pregnant, removal from group pen due to injury or illness, or insufficient hair growth. During the lab selection and analysis, a further 16 samples from stall-housed sows and 7 from group-housed females were removed due to inadequate sample size or poor sample

quality (badly damaged or soiled). In total, samples from 34 stall-housed sows and 32 group-housed sows were analyzed for HCC and included in the statistical analysis. The final analysis contains the following number of samples from each dietary treatment: phytochemical oil (n=1), whole yeast cell (n=11), gamma-tocopherol (n=9), and control (n=13). The final analysis contains the following number of samples from each treatment: control (n=12), PF1 (n=10), PF2 (n=10).

3.2.5 Statistical analysis

The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to confirm homogeneity of variance and to analyze for outliers. Data were analyzed using the PROC MIXED procedure of SAS with sow as the experimental unit. In the model, main effects of housing system, time, and their interactions were tested using parity as a random effect. In order to analyze differences by parity, sows were assigned to 1 of 3 parity groups, 0-1 (n=23), 2-3 (n=17), and ≥4 (n=26). Gilts were also analyzed separately (Stall n=7, Group n=8). To assess the impact of litter size, sows were assigned to 1 of 3 total born categories, ≤14 (n=22), 15-17 (n=20), and ≥18 (n=24). Tukey's adjusted means test was used to detect differences where statistical significance and tendencies were set at $P \leq 0.05$ and $P < 0.10$, respectively, for all statistical tests.

3.3 Results and Discussion

Sows housed in stalls exhibited higher HCC (49.4 pg/mg) than sows housed in group pens (19.8 pg/mg, $P < 0.001$, SEM = 8.0), which may indicate stalled sows experience greater stress during gestation. Figure 3.2 shows that stalled females in Parity

group 0-1 ($P < 0.001$, SEM = 9.0) and Parity 2-3 ($P = 0.049$, SEM = 11.0) had higher HCC than their group-housed counterparts, and there was a tendency for stall-housed Parity ≥4 sows to have higher HCC than the group-housed Parity ≥4 sows ($P = 0.078$, SEM = 8.6). This is consistent with behavioral observations of stall-housed females, who exhibit more frequent stress behaviors such as sham chewing and oronasofacial stereotypies compared to females in group pens (Chapinal et al., 2010). However, sows in group pens may face stress, pain, and reduced longevity due to factors not present in individual stall housing, such as opportunities to fight for hierarchy, experience aggression, and be injured by other sows (Salak-Johnson, 2017). In this trial, we did not record any behavioral measures to determine if group-housed sows demonstrated stress-induced behaviors, particularly during mixing, and therefore we cannot claim that group-housed sows were not stressed during gestation; however, the higher HCC overall in stalled females indicates that the degree or duration of stress experienced by sows in group pens was less than that experienced by stall-housed sows.

There was a tendency for time to influence HCC (Figure 3.3), with an increase in HCC during the last third of gestation (Early = 29.4 pg/mg, Late = 39.8 pg/mg, $P = 0.06$, SEM = 8.0). The HCC of stalled sows increased 39% from early to late gestation and 26% in group housed sows, but the increase due to time was not significantly different between treatment groups (Figure 3.3). Figure 3.4 shows the change in HCC from early to late gestation timepoints in each parity group; Parity 0-1 increased by 5% of the early value, Parity 2-3 increased by 61%, and Parity ≥4 increased by 78%. However, there was no parity by time interaction ($P = 0.601$), and without a larger sample size it is difficult to speculate on whether these results are biologically important. An increase in HCC with

progressing pregnancy is not unexpected, as both fetal and maternal cortisol rise as parturition approaches. Fetal cortisol increases as gestation progresses (Silver & Fowden, 1989) and in many mammals plays a role in inducing parturition (Decaluwe et al., 2012; Wood, 2013); it is thought that fetal space restriction *in utero* is one of the signals that begins the process of parturition (Senger, 2012). Cortisol is critical for late fetal development, assisting in lung maturation (Guo et al., 2014), skeletal growth in sheep (Fowden et al., 1995), gastrointestinal development, and the transition from trans-placental glucose supply to liver glycogen and gluconeogenesis (Fishman et al., 2018; Fowden et al., 1995). Maternal cortisol also contributes to fetal growth by promoting maternal insulin resistance (Herrera, 2000), which reduces the female's ability to utilize glucose from the bloodstream and thus increases glucose availability for the litter. As glucose is the primary nutrient crossing the placenta, this promotes fetal growth in late gestation (Herrera, 2000). In addition to providing greater glucose concentrations in late gestation, there is some thought that the maternal body catabolizes lean tissue in late gestation, generally d100, as maternal protein retention becomes insufficient to meet fetal growth requirements. This means the maternal body must mobilize lean tissue in order to accommodate fetal protein deposition (Ramirez-Camba & Levesque, n.d.). The catabolism of lean tissue is facilitated by cortisol (Viru & Viru, 2004), which may also contribute to heightened HCC in late gestation.

It is thought that larger litters may cause higher maternal cortisol in late gestation, as has been observed in sheep (Alon et al., 2021). It has also been noted in mice that larger litters are associated with greater maternal anxiety in late gestation (D'Amato et al., 2006), possibly as a survival adaptation because of the higher reproductive value of a

larger litter. In this trial, litter size was affected by parity grouping (*P* = 0.02, SEM = 0.5), and Parity ≥4 females had more total piglets born (16.8) than Parity 0-1 (15.1). Litter size of Parity 2-3 was similar to Parity ≥4 (16.9). However, litter size did not affect overall HCC (*P* = 0.212), although there was a tendency (P = 0.067) for sows that farrowed litters between 15-17 total born to have higher HCC in late gestation than in early gestation.

Maternal cortisol also rises in late gestation, possibly in part because corticosteroid-binding globulin rises in conjunction with estrogen (Hay et al., 2000), and estradiol peaks dramatically in late gestation in swine (Senger, 2012). As glucocorticoids have anti-inflammatory actions, the increase of cortisol bound to corticosteroid-binding globulin in late gestation may be in preparation for potential puerperal infection and rapid availability of cortisol to counter an inflammatory reaction (Nenke et al., 2017). It may also be that cortisol increases with the sows' discomfort in late gestation, and larger sows may experience greater discomfort, particularly when housed in stalls. It has been noted that sow discomfort in gestation stalls appears to increase from mid to late gestation, possibly because greater body weight and size cause changing positions to become more difficult (Anil et al., 2006). However, no published data indicates that this is also true for group-housed females or correlates sow cortisol in late gestation with discomfort.

There was a significant effect of parity grouping on HCC. Parity 0-1 females, those experiencing their first or second pregnancies, exhibited higher HCC than older sows (Parity ≥2) (Figure 3.4). Parity 0-1 females had 49.9 pg/mg, Parity 2-3 had 26.9 pg/mg, and Parity ≥4 had 28.4 pg/mg HCC (*P* < 0.001, SEM = 3.8). Stalled Parity 0-1 females had higher HCC than all other females (Figure 3.2), suggesting that young

females in stalls experience the most stress out of all other parity and housing groups. These data also demonstrate that Parity 0-1 females in group housing do not experience significantly higher stress than their experienced counterparts, which indicates the experience of being bred for the first time and carrying a litter does not cause significantly greater stress to a naïve female compared to multiparous females when housed in pens. This result is consistent with Roelofs et al. (2019), who, similarly, did not detect a difference in hair cortisol between multiparous and primiparous females in group housing (2019).

Figure 3.5 shows that gilts in stalls had higher HCC than the group-housed gilts (Stall = 82.7 pg/mg, Group = 19.9, $P < 0.001$, SEM = 10.9). At the SDSU sow facility prior to entering the breeding herd all gilts are housed in group pens of approximately 8 females per pen in the gilt development wing. It is generally recommended to acclimate gilts to stall housing for two to three weeks prior to boar exposure (Epp, 2020; Rutllant et al., 2018), as individual housing is stressful for social animals like pigs (Barnett et al., 1985), and stress can disrupt estrus, prolong the estradiol peak, and decrease early embryo survival (Roongsitthichai et al., 2011). At SDSU, gilts are moved to individual stalls approximately four weeks prior to breeding. Despite this acclimation period, our results indicate that gilts housed in stalls throughout gestation experience higher stress than group-housed gilts. Gilts in group housing were also mixed with small Parity 1 sows and were trained to use the electronic sow feeder upon entry to the group housing system within 1 day after breeding, which could also cause stress. However, these results show that a new social group and adjustment to using the ESF were insufficient to increase HCC in group-housed gilts significantly above the experienced sows in group housing.

This is supported by other research that has found that housing gilts and parity 1 sows together reduces aggression-related injuries compared to housing young females with older, large sows (Li, 2014). Figure 3.5 also shows that there was no increase in HCC from early to late gestation in gilts regardless of housing, and no difference in the degree of increase from early to late gestation between treatment groups. This could be because smaller females experience less discomfort and are less influenced by the space restriction of the stall relative to the larger, wider females whose maternal weight is greater.

There may be consequences to chronically heightened cortisol in all stages of gestation for litter productivity and long-term sow productivity. Treatment of pregnant women with glucocorticoids has been associated with reduced birth weights (Newham et al., 2001); and treating sows during early, mid-, or late gestation with hydrocortisone acetate to glucocorticoid levels similar to psychological stress resulted in lower offspring birth weights (Kranendonk et al., 2006). Lower birth weights are associated with higher pre-weaning mortality (Feldpausch et al, 2019), which affects both economic gain and animal welfare considerations. Otten et al. (2015) observed that the pre-weaning mortality rate of male piglets was increased in sows treated with hydrocortisone acetate in mid-gestation. Low body weight at weaning increases the rate of mortalities in the nursery phase (Larriestra et al, 2006), and Kranendonk et al. (2006) found not only lower birth weight but also lower weaning weights in piglets from sows who were treated with hydrocortisone acetate in gestation. Pre-natal stress could influence piglet body weight past weaning, as repeatedly mixing sows during mid- or late gestation was associated with lower piglet body weights 35 days after weaning (Jarvis et al., 2006). Piglets from

sows experiencing elevated glucocorticoids during gestation may be more likely to have poorer welfare, demonstrating more anxiety-related behavior or aggression in novel situations and higher pain score during tail docking (Otten et al., 2015). In addition, gilts raised from mothers who had experienced stress during gestation appeared more restless and uncomfortable in the periparturient period (Jarvis et al., 2006; Rutherford et al., 2014), and in one trial tended to be more aggressive towards piglets (Jarvis et al., 2006); both frequent posture changes and sow aggression can result in newborn piglets being injured or killed, contributing to pre-weaning mortality. Therefore, chronic stress in sows can detrimentally affect the productivity and welfare of their offspring. Our results suggest that sows housed exclusively in stalls are more likely to experience chronic stress than group-housed females and may therefore produce less economically productive offspring with lower welfare status.

As previously discussed, elevated glucocorticoids throughout gestation can also promote the development of insulin resistance, which persists through lactation. While insulin resistance allows the maternal body to redirect more glucose to fetal growth, it may force the sow to mobilize adipose tissue before parturition in order to maintain pregnancy-related tissues. Insulin resistance developed over the course of gestation may also affect sow performance into lactation, as it has been noted to reduce feed intake in early lactation (Mosnier et al., 2010). Young sows whose bodies are still growing may be more detrimentally affected by the need to mobilize body tissue to meet fetal energy demands, as young growing sows need additional nutrients to maintain their own growth and long-term health as compared to a sow at mature size. This is particularly relevant because our data indicate that young sows housed in stalls are more likely to have

elevated cortisol (Figure 3.3), so the detrimental effects of developing insulin resistance and the subsequent tissue catabolism may have a greater impact on her long-term productivity than they would on a multiparous sow who has already reached mature size. Insulin resistance has been noted to be greater in gilts than in multiparous sows, and it may contribute to lengthening wean-to-estrus interval (Père & Etienne, 2007). Our results show that gilts and parity 1 sows in stalls have higher HCC during gestation than all other females, which may reduce their long-term health and productivity in comparison to the group-housed gilts and parity 1 sows.

3.4 Conclusion

Stall-housed sows had higher hair cortisol than group-housed sows, and stall-housed gilts and parity 1 sows had higher HCC than all other females regardless of housing system. HCC tended to be higher in late gestation than in early gestation for all females. Litter size did not affect HCC. HCC was not affected by time in gilts, and stall-housed gilts had higher HCC than group-housed gilts.

Table 3.1 Sow demographics

Sow treatment		
Items	Stall	Group
Sows per treatment	34	32
Parity		
No. P0	7	8
No. P1	4	4
No. P2	4	6
No. P3	6	1
No. P4	8	2
No. P5	5	6
No. P6	0	5

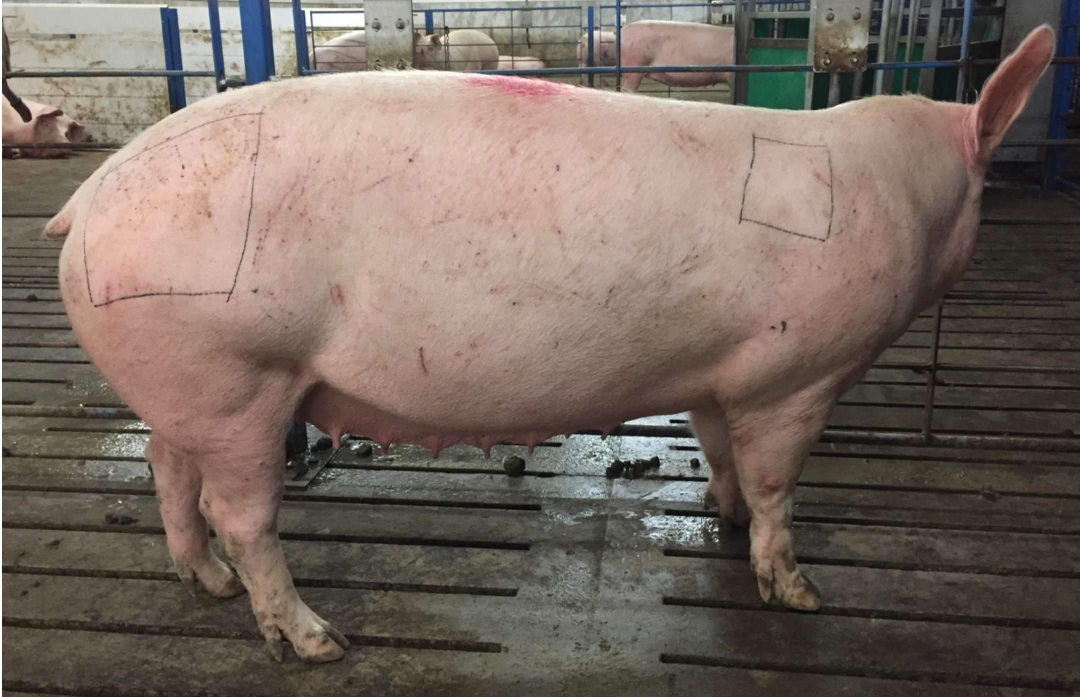

Figure 3.1 Sampled regions

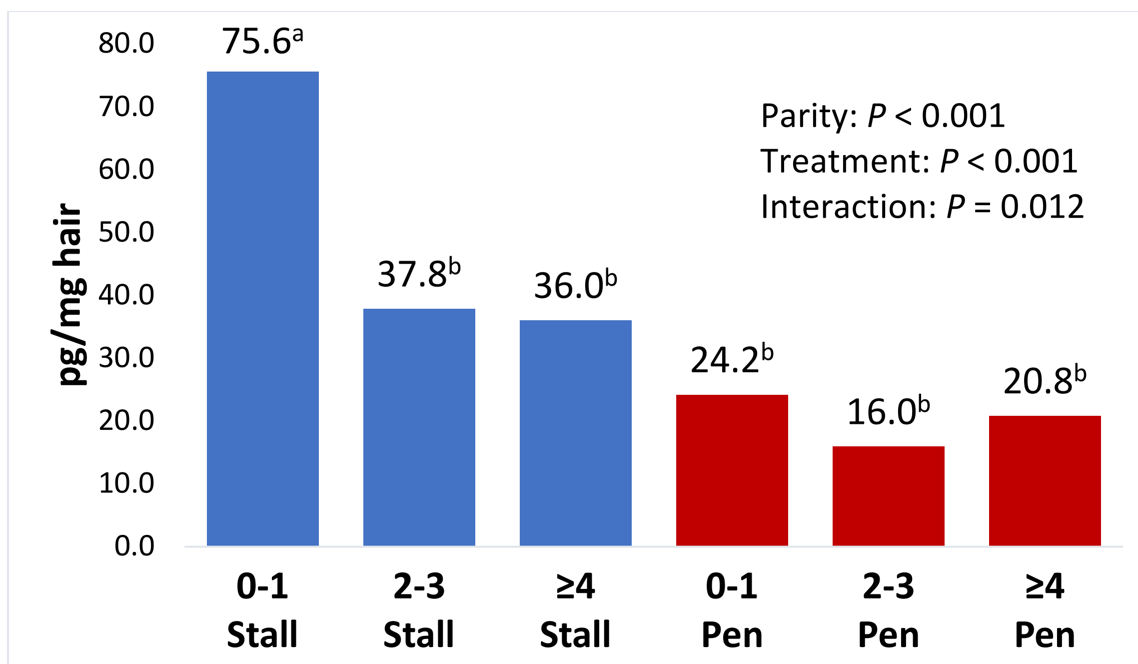

Figure 3.2 HCC between parity categories[1] in stall- and group-housed females[2,3]

[1]Parity 0-1 refers to gilts and sows that have successfully completed one parity, 2-3 refers to sows successfully completing 2 or 3 parities, and ≥4 refers to sows successfully completing 4 or more parities.

[2]Significant difference between means denoted by superscript a, b, or c where $P \leq 0.05$.

[3]0-1 Stall n=11, 2-3 Stall n=10, Stall ≥4 n=13; 0-1 Group n=12, Group 2-3 n=7, Group ≥4 n=13.

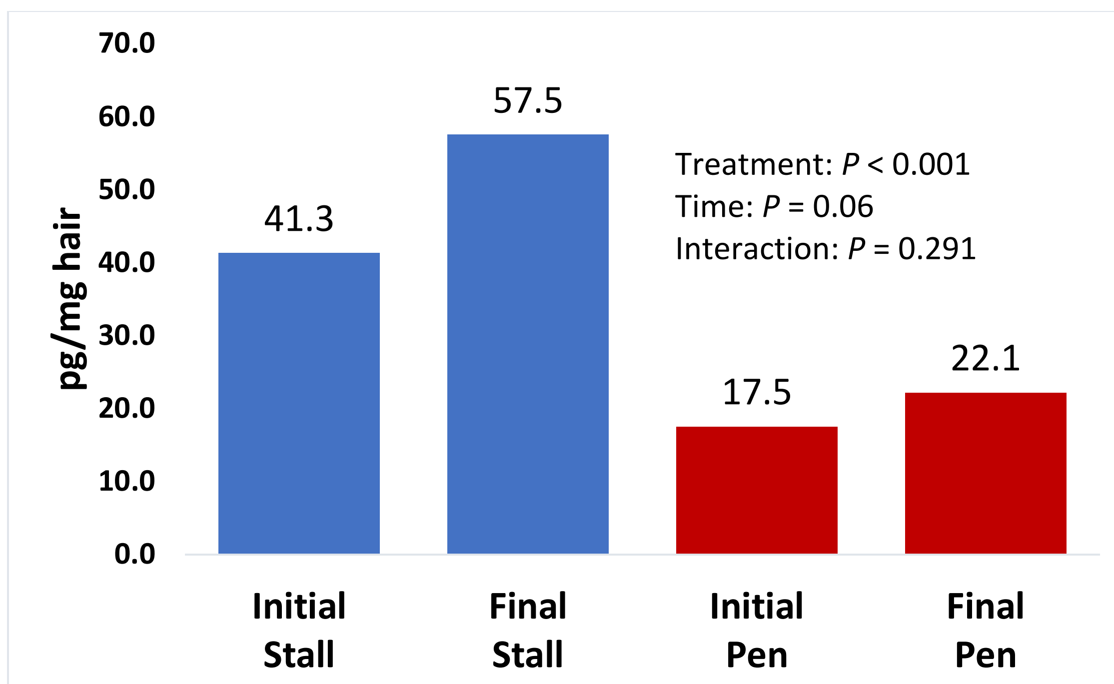

Figure 3.3 HCC from early to late gestation[1] for stall- and group-housed females[2,3]

[1]Early gestation refers to the hair grown from d0 (breeding) and shaved at d37 of gestation, representing the first third of gestation. Late gestation refers to hair grown from d74 and shaved at d111 of gestation, representing the last third of gestation.

[2]Significant difference between means denoted by superscript a, b, or c where $P \leq 0.05$.

[3]Stall n=34, Group n=32.

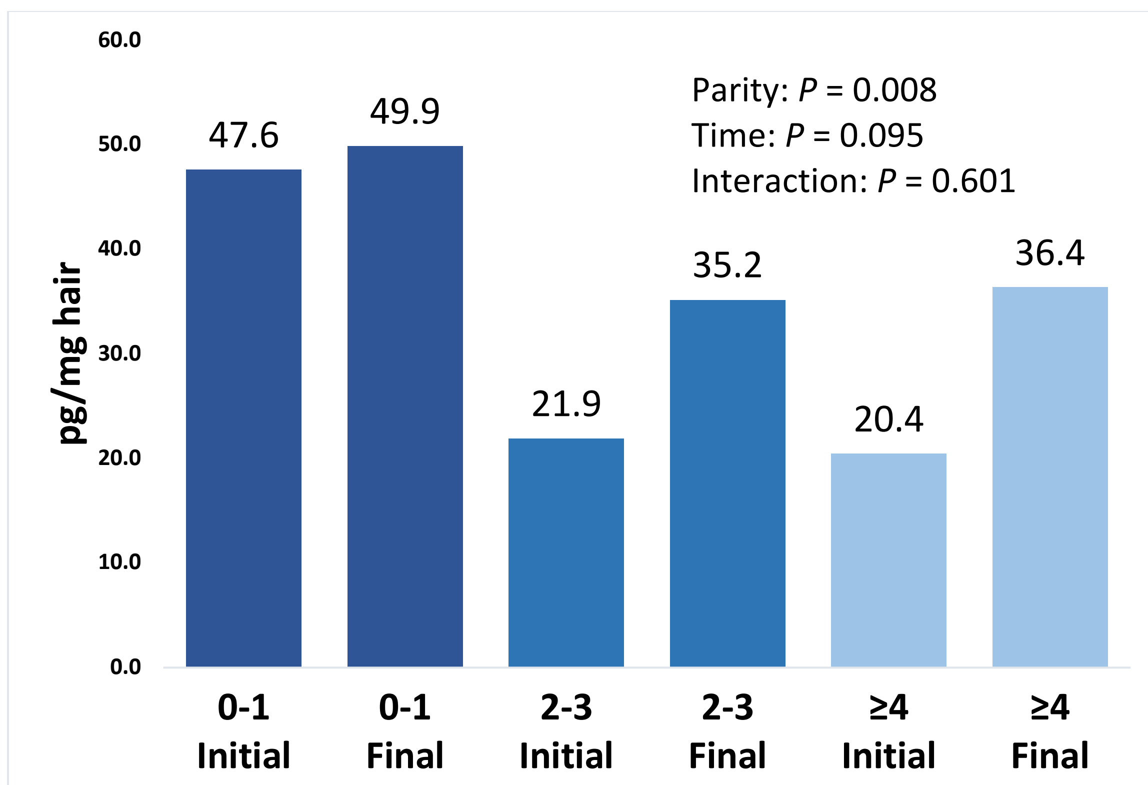

Figure 3.4 HCC between parity groups[1] from early to late gestation [2,3,4]

[1]Parity 0-1 refers to gilts and sows that have successfully completed 1 parity, 2-3 refers to sows successfully completing 2 or 3 parities, and ≥4 refers to sows successfully completing 4 or more parities.

[2]Early gestation refers to the hair grown from d0 (breeding) and shaved at d37 of gestation, representing the first third of gestation. Late gestation refers to hair grown from d74 and shaved at d111 of gestation, representing the last third of gestation.

[3]Significant difference between means denoted by superscript a or b where $P \leq 0.05$.

[4]Parity 0-1 n=23, Parity 2-3 n=17, Parity ≥4 n=26.

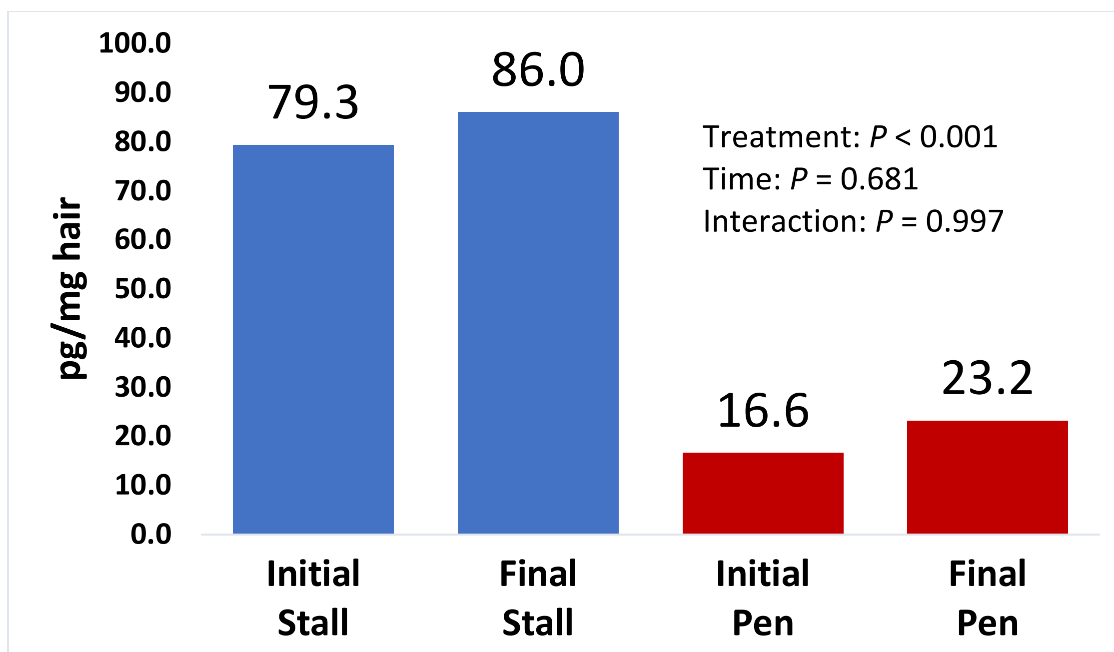

Figure 3.5 HCC between early and late gestation[1] of stall- or group-housed gilts[2,3]

[1]Early gestation refers to the hair grown from d0 (breeding) and shaved at d37 of gestation, representing the first third of gestation. Late gestation refers to hair grown from d74 and shaved at d111 of gestation, representing the last third of gestation.

[2]Significant difference between means denoted by superscript a, b, or c where $P \leq 0.05$.

[3]Stall n=7, Group n=8.

4.0 CORTISOL SECRETION DURING PARTURITION

4.1 Introduction

The role of cortisol during parturition is not well elucidated. Cortisol is known as the stress hormone, in part because it controls many metabolic processes in order to maintain glucose homeostasis during stressful experiences (Spencer & Deak, 2017). Little is known about how cortisol fluctuates in response to the pain and energetic demand of labor, as well as how cortisol fluctuates in gilts who experience parturition for the first time. The objective of this study was to investigate the pattern of cortisol secretion during parturition in primi- and multiparous sows, in context to litter characteristics and farrowing performance.

4.2 Materials and methods

The experimental protocols used in this study were approved by the South Dakota State University Institutional Animal Care and Use Committee (19-006A).

4.2.1 *Animals and housing*

The study was conducted in the sow barn at the South Dakota State University Swine Education and Research Facility in Brookings, SD from May 2020 until March 2021. A total of 7 primiparous (n = 6) and multiparous (n = 1) PIC 1045 females previously surgically fitted with cephalic vein catheters with a vascular access port (Swindle et al., 2005) were housed, handled, and fed according to farm standards during gestation, and were moved from gestation housing to farrowing crates at approximately d111 of gestation.

4.2.2 *Sample collection*

At 0600 h on d113 of gestation, a bandage with 4% lidocaine cream was placed on the port for 1 h to numb the skin. Once numb, the port area was cleaned with betadine scrub and 70 percent isopropanol, and a catheter with a 1.9 cm Huber needle and 46 cm of tubing (Access Technologies, Norfolk, VA) was used to access the port using sterile technique. To ensure the needle remained embedded in the port from d113 until completion of farrowing, a bandage was glued over the access site, the catheter was secured to the neck with glue and bandages, and a sterile syringe (10 mL) was placed on the end of the catheter. Vet wrap was crossed over the female's shoulders and under her girth, and the syringe tucked under the vet wrap at her withers (Figure 4.1) in between blood collections. A 4 mL blood sample was collected into a 6 mL syringe at 0700 and 1900 h from d113 of gestation until onset of farrowing, defined as the birth of the first piglet, to characterize cortisol changes due to circadian rhythm, which is known to affect cortisol levels (Martínez-Miró et al., 2016). Beginning on d114 of gestation at 0700 h, females received 24-hour supervision until completion of farrowing to ensure onset of farrowing was detected; at the onset of farrowing a 4 mL blood sample was collected every 15 minutes until one hour after farrowing was complete, which was deemed as the last expulsion of placenta. After each blood draw, a minimum of 4 mL physiological saline and 3 mL heparinized saline (10 IU/mL) was given to maintain sow blood volume and catheter patency. Two mL of each blood sample was placed in a serum vacutainer (BD Vacutainer 366668, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) and 2 mL in a vacutainer with sodium heparin (#455051, Greiner Bio-One, Kremsmünster, Austria); tubes were placed on ice until centrifugation. At the birth of

piglets, blood was gently stripped from the umbilical cord and placed into 3 mL serum vacutainer (BD Vacutainer 366668, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) and placed on ice until centrifugation. Cord blood sample was collected from as many piglets as possible within a litter. All blood samples were centrifuged at 2400 × *g* for 15 min within 6 hours of blood draw; serum or plasma was transferred into microcentrifuge tubes and stored at -20 ^{o}C until analysis. Time of birth, piglet birth order, and piglet birth weight were recorded. Administration of oxytocin or farrowing assistance were recorded.

The series of blood collections were completed for each female in a single gestation and for 3 females, collections were completed during their first and second farrowings, for a total of 10 sets of samples.

4.2.3 *Piglet vigor assessment*

A piglet vigor assessment was conducted at the birth of each piglet according to the guidelines presented in Table 4.1. Piglet vigor assessment was designed to reduce inconsistencies due to individual subjectivity such that scores would be consistent across multiple people and therefore not based on a previously published method.

4.2.4 *Laboratory analysis*

Plasma and serum concentrations of cortisol were analyzed in triplicate by radioimmunoassay (RIA) using the commercially available ImmuChem Coated Tube Cortisol kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. Briefly, 25 µL of sample were added to an anti-cortisol tube, 1.0 mL of

Cortisol^{125}I was added, and then vortexed. Tubes were incubated for 45 minutes in a water bath at 37 ± 1°C. Finally, tubes were decanted and counted using a gamma counter calibrated for ^{125}I.

4.2.5 *Statistical analysis*

Data were analyzed using the PROC CORR function in SAS to generate Pearson correlation coefficients for all variables considered. Variables considered were initial sow cortisol (sow cortisol at the onset of farrowing); average, maximum, and minimum sow cortisol throughout parturition; AUC (area under the curve generated by the pattern of cortisol secretion throughout parturition); litter size (total born) and total litter birth weight; AvgPW (average piglet birth weight); AvgPC (average piglet cord blood cortisol); TF (total farrowing time, from first piglet until last placenta); BoLP (time to the birth of the last piglet); LPPoTF (time to last piglet as a percentage of total farrowing); birth interval (average time elapsed between piglet births in each litter); MaxCPoTF (time to maximum cortisol as a percentage of total farrowing time); MaxCPoLP (time to maximum cortisol as a percentage of time to birth of last piglet); MinCPoTF (time to minimum cortisol as a percentage of total farrowing time); and the sum of total feed intake 3 days prior to and on the day of farrowing (FI). Statistical significance and tendency were set at $P \leq 0.05$ and $P \geq 0.1$, respectively. Area under the curve was calculated using the AUC function from the DescTools package in RStudio Version 1.3.1073.

4.3 Results

Attempts were made to analyze the sow serum samples for cortisol using a radioimmunoassay but variability in assay results among and within samples was very high. There was a persistent presence of fibrin clots in the serum samples. During the assay, attempts were made to avoid the clot or partially remove the clot before sampling; however, inconsistent results remained. When all fibrin clots were removed completely and samples reanalyzed, cortisol values for all samples were very low (2-3 µg/dL), suggesting that the cortisol had been bound in the fibrin clots and was removed upon removal of the clots. Because cortisol can be assayed in either serum or plasma according to the assay protocol plasma was used to assess sow cortisol. The occurrence of fibrin clots was not observed with cord blood samples, so serum was used for cortisol analysis of piglets.

In one parity sample collection set, one sow had very high sow cortisol (2+ SD above the average of other females) and was removed from analysis, so 9 farrowings from 7 females were analyzed. Figure 4.2 shows the pattern of cortisol secretion for all 10 farrowings collected. Table 4.2 shows the variation in the data analyzed from the 9 farrowings, excluding the outlying sow, and Table 4.3 shows all Pearson's correlation coefficients and *P*-values for each correlation. There were numerous significant correlations. To facilitate discussion, descriptions of specific correlations are listed below by subject.

There was very little variation in average piglet vigor scores for each litter, so these data were not analyzed. The optimal vitality score possible was 4, and the poorest was 9; the vitality score across litters was 4.55 ± 0.32.

4.3.1 *Litter characteristics*

Litter size and TF were positively correlated (r = 0.75, *P* = 0.019) (Figure 4.3); litter size was also positively correlated with birth interval (r = 0.69, *P* = 0.040), BoLP (r = 0.87, *P* = 0.003), and LPPoTF (r = 0.8, *P* = 0.010). There was a tendency for a positive correlation between LPPoTF and TF (r = 0.63, *P* = 0.070). Litter size and AvgPW were negatively correlated (r = -0.85, *P* = 0.004).

4.3.2 *Sow cortisol*

Several pre-farrow samples were not able to be analyzed because of the fibrin clots, so samples from only 4 females were analyzed. Pre-farrow samples were not different from morning to evening samples (1.87 ± 1.13 µg/dL and 1.88 ± 1.78 µg/dL, respectively). There was no notable difference between average cortisol from primiparous and multiparous sows (7.94 ± 1.79 µg/dL and 5.1 ± 2.24 µg/dL, respectively).

Maximum sow cortisol was positively correlated with average sow cortisol (r = 0.66, *P* = 0.052). There was no other significant correlation between maximum sow cortisol and any other variables tested. Average cortisol correlated positively with initial cortisol (r = 0.8, *P* = 0.010) and minimum cortisol (r = 0.71, *P* = 0.031). Initial cortisol tended to correlate positively with minimum cortisol (r = 0.6, *P* = 0.090) (Figure 4.4).

4.3.3 *Sow cortisol and litter characteristics*

Initial and average sow cortisol did not correlate strongly or significantly with most variables tested. Initial cortisol tended to negatively correlate (r = -0.61, P = 0.080) with LPPoTF, and average sow cortisol negatively correlated (r = -0.68, P = 0.046) with LPPoTF, as illustrated in Figures 4.5 and 4.6, respectively. This indicates that when initial or average cortisol was higher, total farrowing time was closer to the time of the last piglet.

Minimum cortisol was negatively correlated with litter size (r = -0.77, P = 0.015; Figure 4.7), TF (r = -0.72, P = 0.030) (Figure 4.8) BoLP (r = -0.83, P = 0.006) (Figure 4.9), birth interval (r = -0.80, P = 0.040), and LPPoTF (r = -0.84, P = 0.004). Minimum cortisol tended to positively correlate with average piglet weight (AvgPW) (r = 0.64, P = 0.063).

Minimum cortisol correlated negatively with average piglet cortisol (AvgPC) (r = -0.68, P = 0.044). There was a tendency for AvgPC to correlate positively with litter size (r = 0.6, P = 0.090) and litter weight (r = 0.6, P = 0.090). AvgPC also correlated positively with LPPoTF (r = 0.81, P = 0.008), as shown in Figure 4.10.

There was a negative correlation between MaxCPoLP and litter size (r = -0.68, P = 0.045), TF (r = -0.87, P = 0.002), BoLP (r = -0.88, P = 0.002), LPPoTF (r = -0.81, P = 0.009), and birth interval (r = -0.94, $P < 0.001$). There was a positive correlation between MaxCPoLP with AvgPW (r = 0.71, P = 0.033).

Initial cortisol correlated positively with MinCPoTF (r = 0.92, $P < 0.001$). When initial cortisol was below 5 ug/dL, minimum cortisol occurred in the first 20% of farrowing, and when initial cortisol was above 5 ug/dL minimum cortisol occurred in the last 10% of farrowing. Similarly, average cortisol positively correlated with MinCPoTF

(r= 0.75, *P* = 0.019); generally, when average sow cortisol was below 8 ug/dL, minimum cortisol occurred in the first 20% of farrowing, and when average cortisol was above 8 ug/dL minimum cortisol occurs in the last 10% of farrowing Both of these patterns are shown in Figure 4.11. Minimum cortisol also correlated positively with MaxCPoLP (r = 0.73, *P* = 0.025).

Figure 4.12 illustrates the correlation between MinCPoTF and LPPoTF (r = -0.67, *P* = 0.047). MinCPoTF tended to correlate with AvgPW (r = 0.64, *P* = 0.062), where heavier piglet weight corresponded with minimum sow cortisol occurring in the last 10% of farrowing.

There was a tendency for AUC to be positively correlated with average cortisol (r = 0.6, *P* = 0.085) and TF (r = 0.64, *P* = 0.063). AUC was negatively correlated with MaxCPoTF (r = -0.76, *P* =0.017).

The sum of feed intake 3 days prior and the day of parturition was negatively correlated with AUC (r = -0.7, *P* = 0.035). There was also a tendency for a negative correlation between FI and TF (r = -0.66, *P* = 0.053) and BoLP (r = -0.66, *P* = 0.053). FI correlated negatively with litter size (r = -0.73, *P* = 0.025) (Figure 4.13), indicating that sows carrying larger litters ate less feed in preparation for parturition. There was no correlation between feed intake and litter weight.

4.4 Discussion

The objective of this study was to investigate the pattern of cortisol secretion during parturition in relation to litter characteristics and farrowing performance.

The positive correlation between litter size, TF, piglet birth interval, BoLP, and LPPoTF makes sense, as larger litters have long been known to result in longer duration of farrowing (Baxter et al., 2013), and the phase of piglet expulsion (the period between the birth of the first and last piglet, after which only placenta and other uterine fluids are expelled) typically increases with larger litters (Van Djik et al., 2005). Additionally, some research has shown that longer farrowing durations are associated with time of last placenta being closer to the time of last pig (Björkman et al., 2017), so the positive relationship observed herein between LPPoTF and TF is also consistent with previous work. It is generally acknowledged that larger litters typically have smaller piglets (Rutherford et al., 2013), and in this trial, litter size negatively correlated with AvgPW.

Fetal cortisol is known to play a role in inducing parturition (Decaluwe et al., 2012; Wood, 2013), and it is thought that fetal space restriction in utero may be one of the signals that begins the process of parturition (Senger, 2012). Fetal cortisol promotes enzymes responsible for converting progesterone to estradiol. It also promotes the production of prostaglandin from the placenta, which contributes to the onset of uterine contractions and the regression of the corpora lutea, which also reduces progesterone circulation (Senger, 2012). In humans it is also generally accepted that maternal cortisol during parturition is largely influenced by fetal cortisol (Gitau et al., 2001). The increase of maternal cortisol during parturition has been suggested to be associated with pain (Nagel et al., 2019), but because the HPA axis responds to pain and exertion it is difficult to clearly demonstrate what stimulates its release during labor (Lawrence et al., 1997; Viru et al., 2010). In this study, we recorded farrowing assistance but were unable to identify a pattern of cortisol release after farrowing assistance such as manually pulling

piglets from the vagina, which would illustrate hormonal reaction to pain. Our sample size was small and variation in cortisol secretion was high, so a larger study would be necessary to determine if additional painful stimuli during labor affects cortisol secretion in the sow. Analgesics and other painkillers during labor have not influenced CRH, ACTH, and cortisol during human labor (Bergant et al., 1998; Gitau et al., 2001). Gitau et al. (2001) also observed that maternal cortisol rose with fetal cortisol in response to assisted delivery and concluded that maternal cortisol likely rose in response to fetal discomfort. Further, Jarvis et al. (1998) did not observe a change in cortisol in sows administered opioids during parturition, and Ison et al. (2018) reported no difference in salivary cortisol of sows administered ketoprofen or placebo after parturition. This suggests that maternal cortisol rises primarily in response to fetal cortisol and not in response to pain of labor. We can therefore be confident that the maternal cortisol changes observed over the course of parturition are strongly influenced by fetal cortisol secretion and may not be influenced by the pain of labor or additional painful stimuli during parturition.

In humans, older gestational age and more advanced fetal development is associated with higher maternal cortisol at delivery (Goldkrand et al., 1976). In swine larger litters lead to higher incidence of intrauterine growth-restricted pigs (Matheson et al., 2018). Our results also showed that total litter weight had no relationship with sow cortisol variables, which suggests that the total mass of the litter is less influential on maternal cortisol than the number of fetuses secreting cortisol during parturition. It may therefore be speculated that a large litter of less developmentally mature fetuses may be unable to influence maternal cortisol to the extent of more developed piglets.

The negative correlations between minimum cortisol and litter size, TF, birth interval, BoLP, and LPPoTF, and positive correlation with AvgPW are not surprising because of the previously established association between litter size and the variables above (Van Djik et al., 2005; Baxter et al., 2013). These results suggest that when litter size is smaller, minimum cortisol is higher, which may be because larger, more developmentally mature piglets secrete more cortisol during parturition.

We also observed that minimum cortisol correlated negatively with AvgPC, which is unexpected, as greater piglet cortisol should be reflected in higher minimum sow cortisol, assuming maternal cortisol is strongly influenced by fetal cortisol, which was established earlier. AvgPC tended to correlate positively with both litter size and litter weight, so these results do not elucidate whether total litter mass or number of piglets primarily influences piglet cortisol. AvgPC and LPPoTF were positively correlated which is not consistent with previous results. Because AvgPC and litter size tended to be positively correlated, and we have established the assumption that smaller litters with heavier piglets may contribute more strongly to maternal cortisol, we would expect that average piglet cortisol and LPPoTF would be negatively correlated, as our results indicate that LPPoTF is generally higher in larger litters. However, these results must be interpreted with caution, as AvgPC was not consistently collected within litters. In some litters only two samples were collected, and the values were highly variable, ranging from 4-250 ug/dL. Additionally, LPPoTF may be less biologically relevant to piglet parameters and may only be useful for considering farrowing performance of the sow. These results must therefore be interpreted with caution and in context to the other measures collected.

We saw evidence that in smaller litters minimum cortisol was likely to occur near the end of farrowing, and maximum cortisol was likely to occur near the birth of the last piglet. In contrast, in larger litters minimum cortisol was likely to occur near the beginning of farrowing, and cortisol was likely to peak earlier relative to the birth of the last piglet. This was demonstrated by the negative correlation between MaxCPoLP and litter size, TF, BoLP, LPPoTF, and BI. Higher average piglet weights and shorter farrowing durations are more likely to occur in smaller litters (Rutherford et al., 2013; Baxter et al., 2013), so these results all suggest that in larger litters peak cortisol is secreted earlier relative to the last piglet than in smaller litters. Initial and average cortisol correlated positively with MinCPoTF, and both of these results indicate that when initial or average cortisol were low, minimum cortisol occurred early in parturition, and greater initial or average cortisol was associated with minimum cortisol occurring near the end of farrowing. This is somewhat unexpected, as litter size was not associated with initial or average cortisol but litter size appears to be associated with the timing of minimum and maximum cortisol secretion. This pattern was also seen where minimum cortisol also positively correlated with MaxCPoLP, indicating that when minimum sow cortisol was greater (as it may be in a smaller litter), the maximum sow cortisol occurred closer to or after the birth of the last piglet. Additionally, MinCPoTF negatively correlated with LPPoTF; in cases where minimum cortisol occurred earlier (which was observed in larger litters), the birth of the last pig occurred closer to the end of farrowing (which was also observed in larger litters); however, this pattern was strongly influenced by one data point and is not likely to be strongly biologically relevant, although it is consistent with previously discussed results. We observed that MinCPoTF tended to positively correlate

with AvgPW, where heavier piglet weight corresponded with minimum sow cortisol occurring in the last 10% of farrowing; this is also consistent with our previous results, which indicated that heavier piglets are likely to occur in smaller litters, and smaller litters appear to secrete the least cortisol at the end of farrowing. However, litter size had no relationship with MinCPoTF, so this apparent trend may not be important and these results must be interpreted with caution.

The timing of minimum and maximum cortisol in relation to litter size may be related to the influence of fetal cortisol on maternal cortisol over time. In larger litters where farrowing takes longer, more piglets are in utero for a longer period of active labor and may secrete more cortisol over time, leading to maximum maternal cortisol being reached before the majority of piglets have been born. In smaller litters where the last piglet is born earlier in the process of farrowing, it may be that even though the larger piglets are secreting more cortisol at the onset of parturition (such that minimum cortisol does not occur until nearer the end of parturition), they may all be born by the time the sow's cortisol fully reflects the piglets' cortisol secretion. We also saw that when minimum cortisol was lower, maximum cortisol was likely to occur earlier relative to the birth of the last piglet; this pattern was strongly influenced by one data point and is unreliable on its own, but also helps illustrate that in litters where minimum cortisol is lower (which occurs in larger litters), maximum cortisol is also reached earlier in the process of parturition. The timing of minimum cortisol may also be a reflection of how long after the birth of the last piglet samples were collected in this trial. In a smaller litter with a shorter piglet expulsion period, there was more collection time without the influence of piglet cortisol on the sow, where only placenta was expelled. This may have

allowed more time for the sow's cortisol to drop to basal levels, whereas a sow with a longer piglet expulsion period had less time during farrowing uninfluenced by piglet cortisol.

The positive relationships between average cortisol and minimum, maximum, and initial cortisol were as expected because all of these variables were used in the calculation of average cortisol. This is relevant because initial cortisol may be useful for predicting average cortisol across farrowing. If these results were replicated with a larger sample size, it may be reasonable to conclude that a single blood sample at the initiation of parturition may be adequate for estimating average and minimum cortisol throughout parturition. This would be more practical for estimating sow cortisol during parturition than collecting blood continuously with the use of a temporary or indwelling catheter or subjecting the sow to repeated venipuncture. This may be useful for predicting prolonged farrowing duration, as minimum cortisol negatively correlated with TF. Therefore a sow with relatively low cortisol at the onset of parturition may be at risk for prolonged farrowing and longer birth intervals and may warrant more attention from farrowing technicians in order to prevent stillbirths.

The tendencies for AUC to correlate positively with average cortisol and TF were not unexpected, as cortisol secretion and farrowing time were used to calculate AUC, so it is logical that a longer farrowing duration or higher average cortisol would result in a higher AUC. Additionally, it also makes sense that AUC was negatively correlated with MaxCPoTF, as a sow whose cortisol peaked earlier in parturition was also more likely to have a prolonged farrowing and therefore a longer period of cortisol collection. Although FI was negatively correlated with AUC, it is unlikely that FI has a strong biological

association with cortisol secretion, as FI was unrelated to average, initial, maximum, or minimum cortisol. There was a tendency for a negative correlation between FI and TF, so it is more likely that the relationship between FI and AUC can be explained by the prolonged cortisol secretion that occurred during a longer parturition.

Interestingly, feed intake correlated negatively with litter size, indicating that sows carrying larger litters ate less feed in preparation for parturition. This is counterintuitive, as larger litters would be expected to be heavier and require more nutrients than smaller litters. A larger litter may also increase the sow's heat increment, and in a warm farrowing room the influence of a large litter may suppress the sow's appetite and her voluntary feed intake prior to parturition. There was, however, no correlation between FI and litter weight, so the relationship between FI and litter size may be coincidental. Feed intake is commonly restricted prior to parturition, so no studies to our knowledge have specifically addressed the influence of litter size or weight on voluntary feed intake in sows before parturition. There was also a tendency for a negative correlation between FI and TF and BoLP. It is logical to assume that a sow who had eaten recently prior to parturition would have more robust energy stores and would be better prepared for the exertion of labor. However, as was discussed earlier, TF and BoLP were shorter in smaller litters, and because FI was negatively correlated with litter size it is not clear whether the relationship between FI and TF is simply a reflection of litter size and its influence on FI. Some research has shown that providing sows ad libitum access to feed two days prior to parturition did not affect farrowing duration (Gourley et al., 2020). It is therefore possible that the relationships between FI and TF and BoLP are coincidental or attributable to litter size.

The results of this trial illustrate the pattern of cortisol secretion in the sow during parturition. Cortisol clearly plays a significant, if complicated, role during parturition. As previously discussed, fetal cortisol is thought to trigger a cascade of events leading to the reduction in circulating progesterone and the increase in circulating prostaglandins (Senger, 2012). Cortisol is also known to play a role in collagen remodeling in the placenta, and it is thought that the increase in fetal cortisol at parturition may assist the fetuses in rupturing the placenta and fetal membranes to facilitate birth (Wang et al., 2020).

4.5 Conclusion

Smaller litters are associated with a higher minimum maternal cortisol, which occurs closer to or after the birth of the last piglet. Further, maximum cortisol may occur earlier in relation to the birth of the last piglet in large litters, and minimum cortisol is more likely to occur at the beginning of parturition in larger litters. Thus, larger, more robust piglets in smaller litters are associated with higher maternal cortisol at the onset of parturition and promote shorter farrowing duration. Finally, maternal cortisol appears to be strongly influenced by fetal cortisol, such that maternal cortisol is not likely to be useful as a measure of welfare during parturition. However, sow cortisol at the onset of parturition may be reflective of the total litter size and expected total farrowing time.

Table 4.1 Piglet vigor assessment

Score	1	2	3
Breathing	Easy breaths, no struggling	Breathing slower, but managing on their own	Raspy, hard breathing
Birth	Born out of a sac	Born in a sac	
Movement	Movement towards sow/teats within 5 minutes	Stationary	
Color	Pink, healthy	Pale, anemic	

Table 4.2 Summary of the variation in data analyzed for Pearson correlations, including 9 farrowings from 7 females[1]

Variable	Mean	Max	Min	SD
Initial cortisol (µg/dL)	4.53	7.69	1.50	2.31
Maximum cortisol (µg/dL)	12.52	18.02	7.51	3.43
Minimum cortisol (µg/dL)	3.17	6.08	0.91	1.72
Average cortisol (µg/dL)	7.00	10.35	2.69	2.29
AUC	3081.47	4805.23	1265.61	1243.69
Litter size	15.89	20.00	12.00	3.02
Litter weight (kg)	21.35	24.80	18.20	2.24
AvgPW (kg)	1.38	1.69	1.01	0.22
AvgPC (µg/dL)	84.74	125.24	39.68	37.97
TF (min)	396.67	620.00	320.00	142.58
Time to max cortisol (min)	263.22	361.00	198.00	71.00
Time to min cortisol (min)	175.22	605.00	0.00	205.20
BoLP (min)	346.44	595.00	240.00	163.58
Birth interval (min)	20.94	30.44	17.14	7.44
FI (kg)	21.84	27.10	17.10	3.42

[1]AUC = area under the curve; AvgPW = average piglet birth weight; AvgPC = average piglet cord blood cortisol; TF = total farrowing time from birth of first piglet to expulsion of last placental part; BoLP = time of birth of the last piglet; FI = feed intake.

Table 4.3 Pearson's correlation coefficients and *P*-values for 9 farrowings from 7 females[1]

	Maximum cortisol	Minimum cortisol	Average cortisol	AUC	Litter size	Litter weight	AvgPW	AvgPC	TF	BoLP	LPPoTF	Birth interval	MaxCPoTF	MaxCPoLP	MinCPoTF	FI
Initial cortisol	0.36	0.60	0.80	0.54	-0.43	-0.08	0.53	-0.27	-0.13	-0.29	-0.61	-0.27	-0.46	0.31	0.92	-0.16
	0.346	0.090	0.010	0.131	0.251	0.834	0.141	0.489	0.742	0.443	0.080	0.474	0.210	0.414	0.000	0.677
Maximum cortisol		0.28	0.66	0.43	-0.35	-0.01	0.45	-0.35	-0.02	-0.20	-0.37	-0.06	-0.33	-0.01	0.50	-0.07
		0.468	0.052	0.252	0.359	0.981	0.222	0.357	0.968	0.612	0.324	0.879	0.389	0.987	0.172	0.850
Minimum cortisol			0.71	-0.02	-0.77	-0.51	0.64	-0.68	-0.72	-0.83	-0.84	-0.80	-0.02	0.73	0.55	0.29
			0.031	0.954	0.015	0.157	0.063	0.044	0.030	0.006	0.004	0.010	0.952	0.025	0.128	0.453
Average cortisol				0.60	-0.43	-0.27	0.43	-0.52	-0.19	-0.40	-0.68	-0.38	-0.43	0.32	0.75	-0.20
				0.085	0.243	0.487	0.244	0.153	0.621	0.287	0.046	0.318	0.244	0.408	0.019	0.608
AUC					0.28	0.00	-0.27	0.02	0.64	0.47	0.06	0.48	-0.76	-0.50	0.44	-0.70
					0.461	0.999	0.474	0.961	0.063	0.200	0.876	0.189	0.017	0.169	0.240	0.035
Litter size						0.56	-0.85	0.60	0.75	0.87	0.80	0.69	-0.08	-0.68	-0.43	-0.73
						0.120	0.004	0.090	0.019	0.003	0.010	0.040	0.837	0.045	0.248	0.025
Litter weight							-0.04	0.60	0.25	0.37	0.43	0.22	0.20	-0.22	0.10	-0.44
							0.916	0.090	0.523	0.327	0.252	0.561	0.598	0.561	0.795	0.239
AvgPW								-0.37	-0.73	-0.80	-0.75	-0.70	0.18	0.71	0.64	0.53
								0.326	0.025	0.009	0.021	0.036	0.640	0.033	0.062	0.140
AvgPC									0.38	0.56	0.81	0.54	0.39	-0.50	-0.39	-0.42
									0.313	0.120	0.008	0.137	0.299	0.173	0.304	0.265
TF										0.96	0.63	0.94	-0.60	-0.87	-0.15	-0.66
										<.0001	0.070	0.000	0.087	0.002	0.694	0.053
BoLP											0.79	0.95	-0.39	-0.88	-0.32	-0.66
											0.011	<.0001	0.296	0.002	0.397	0.053
LPPoTF												0.77	0.17	-0.81	-0.67	-0.43
												0.015	0.661	0.009	0.047	0.242
Birth interval													-0.42	-0.94	-0.32	-0.52
													0.255	0.000	0.398	0.154
MaxCPoTF														0.39	-0.49	0.32
														0.295	0.180	0.401
MaxCPoLP															0.34	0.49
															0.368	0.177
MinCPoTF																-0.09
																0.825

[1]AUC = area under the curve; AvgPW = average piglet birth weight; AvgPC = average piglet cord blood cortisol; TF = total farrowing time from birth of first piglet to expulsion of last placental part; BoLP = time of birth of the last piglet; LPPoTF = time to last piglet as a percentage of total farrowing time; MaxCPoTF = time to maximum cortisol as a percentage of total farrowing time; MaxCPoLP = time to maximum cortisol as a percentage of time to last piglet; MinCPoTF = time to minimum cortisol as a percentage of total farrowing time; FI = feed intake.

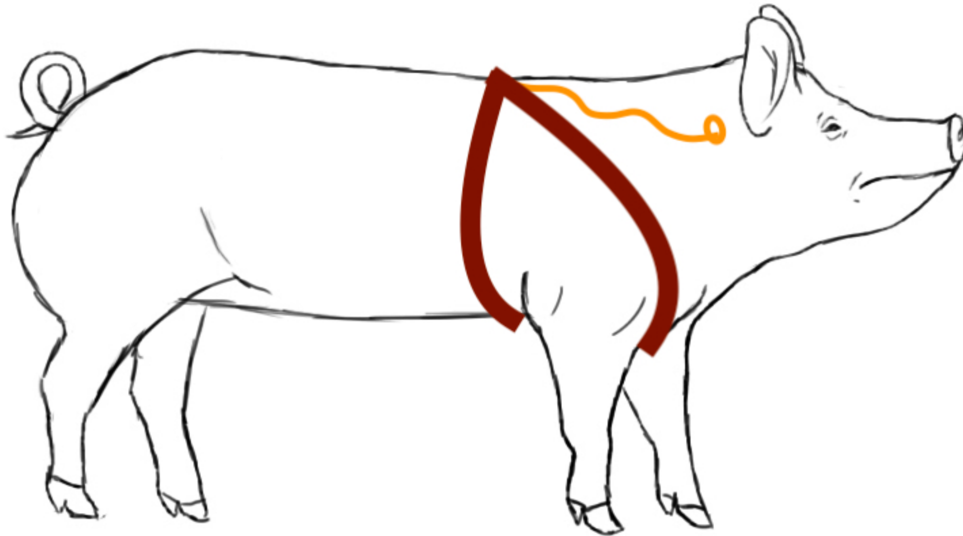

Figure 4.1 Catheter placement with syringe-holding belt[1]

[1]Catheter was placed d113 of gestation and was removed one hour after the last expulsion of placenta.

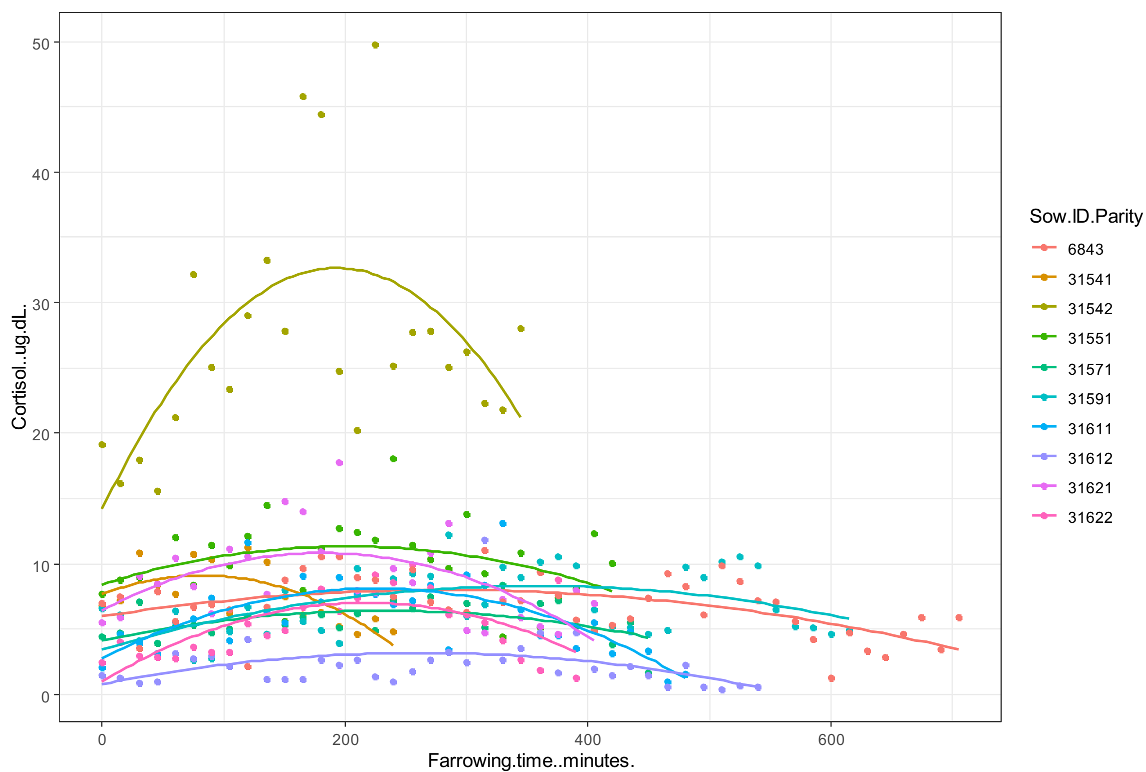

Figure 4.2 Sow[1] cortisol (μg/dL) during each farrowing event[2]

[1]Sow ID is listed along the right side, and the last digit of each sow ID corresponds to her parity number.

[2]Collection occurred every 15 min from the birth of the first piglet until 1 hr after the expulsion of the last placental part.

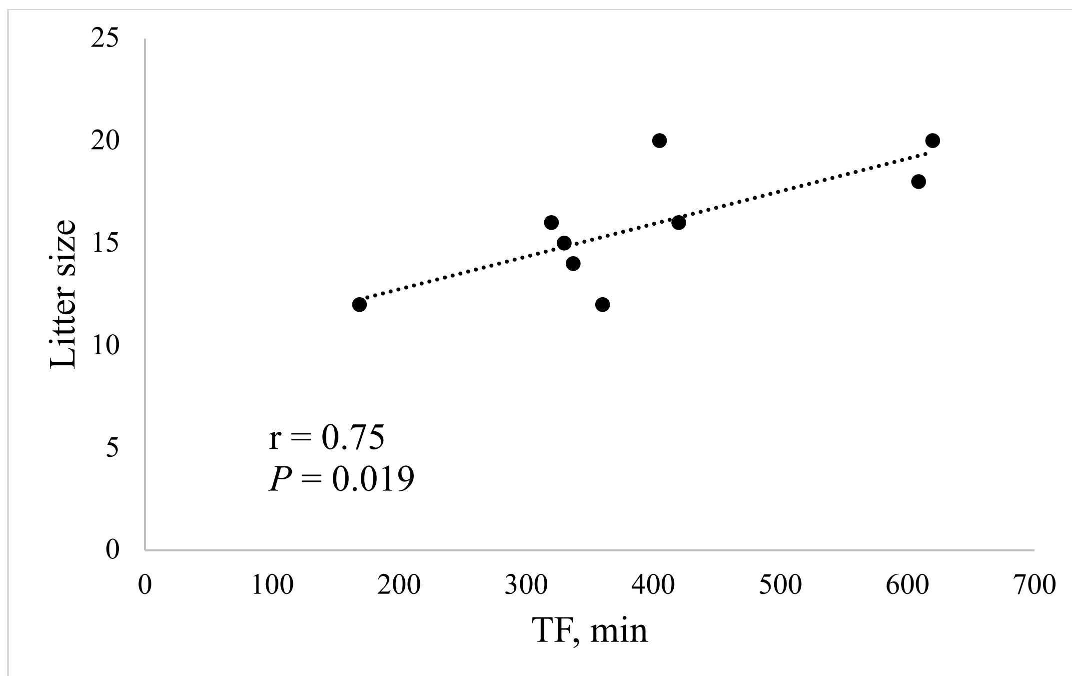

Figure 4.3 Correlation between TF[1] and litter size

[1]Minutes between birth of first piglet until expulsion of last placental part.

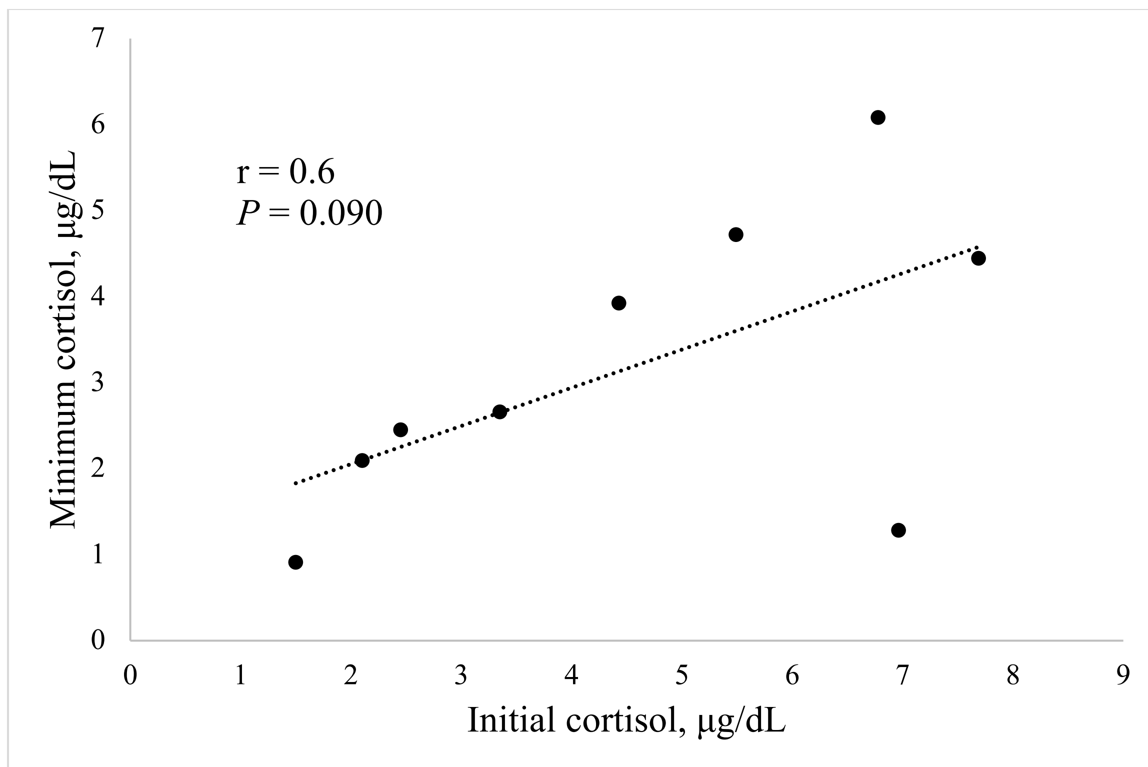

Figure 4.4 Correlation between initial[1] cortisol and minimum cortisol[2]

[1]Sow cortisol at the birth of the first piglet

[2]Minimum sow cortisol from first piglet to expulsion of last placental part

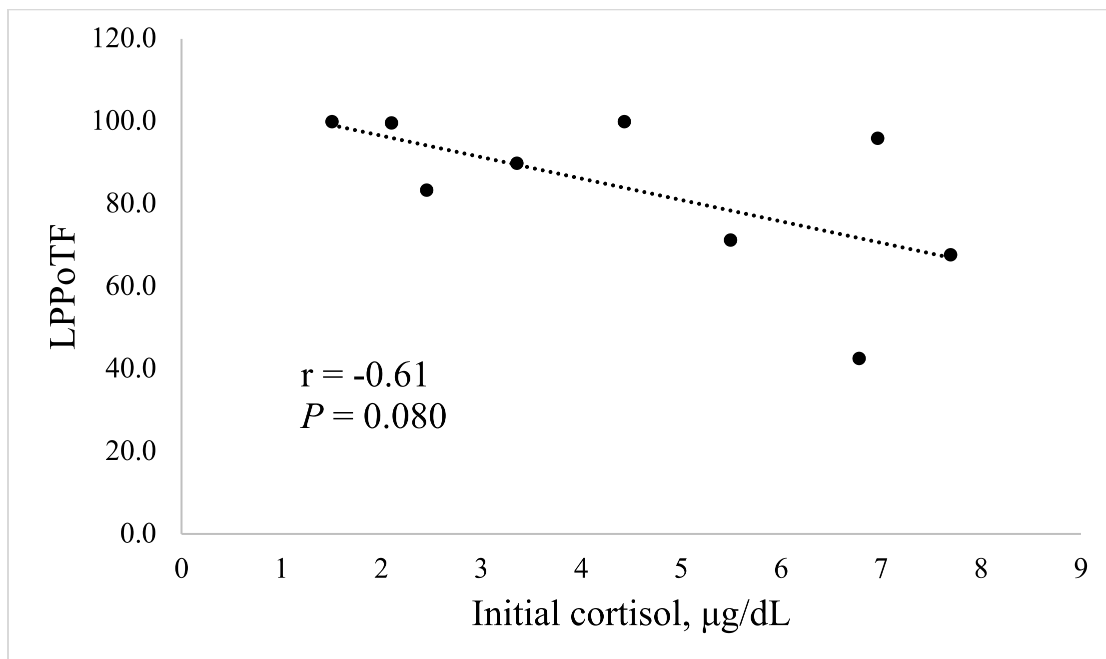

Figure 4.5 Correlations between LPPoTF[1] and initial[2] sow cortisol

[1]Time to last piglet as a percentage of total farrowing time

[2]Sow cortisol at the birth of the first piglet

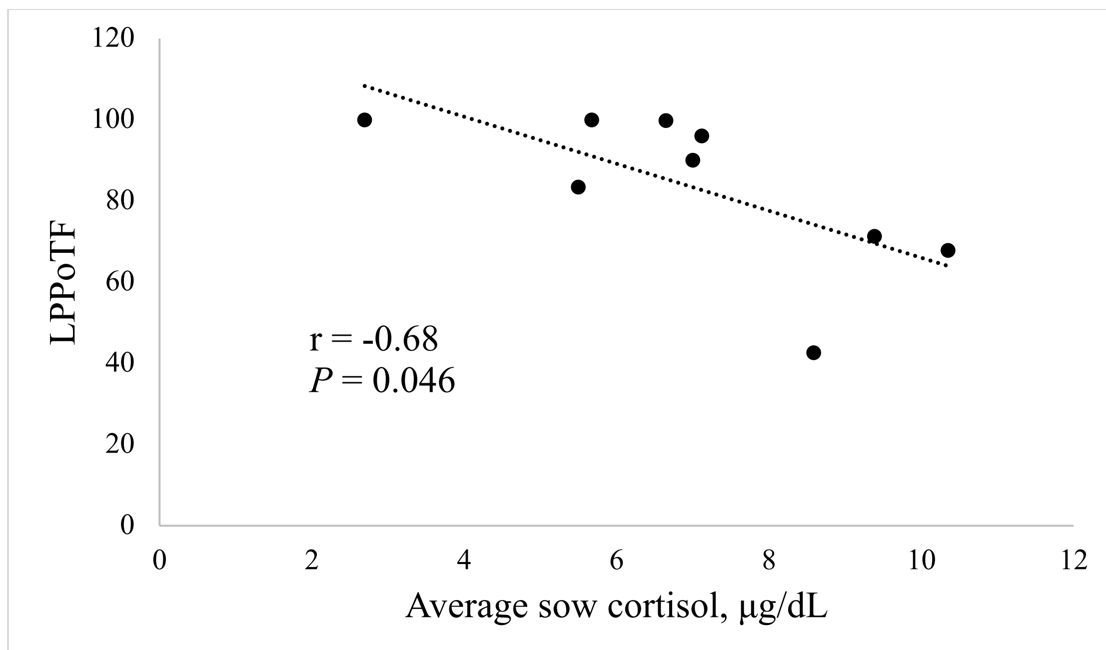

Figure 4.6 Correlation between LPPoTF[1] and average sow cortisol[2]

[1]Time to last piglet as a percentage of total farrowing time

[2]Average sow cortisol from first piglet to last placenta expulsion

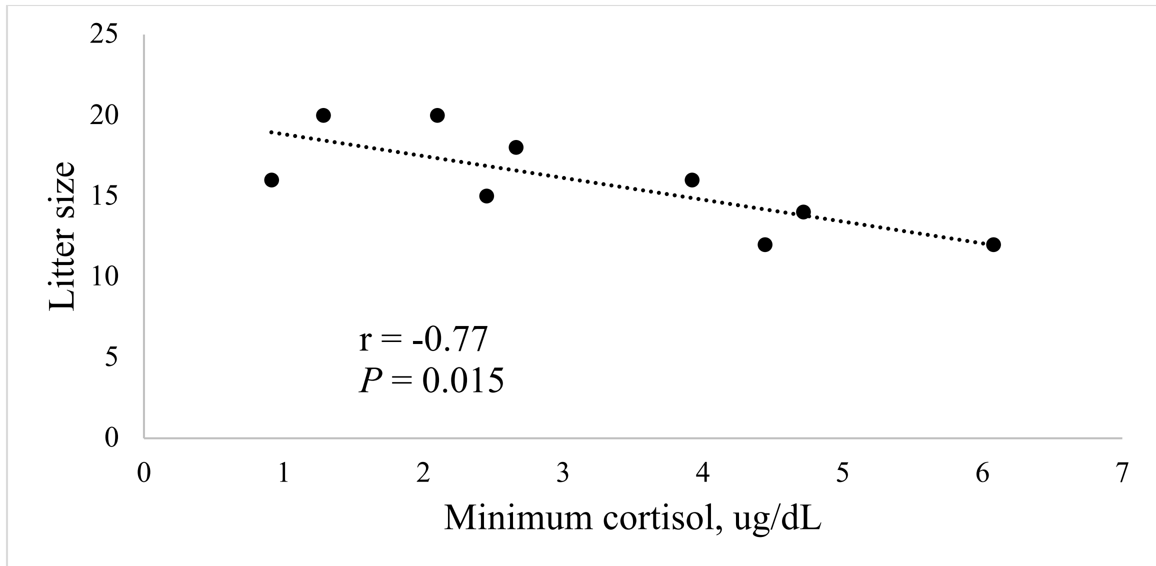

Figure 4.7 Correlation between minimum cortisol[1] and litter size

[1]Minimum sow cortisol from first piglet to last placenta expulsion

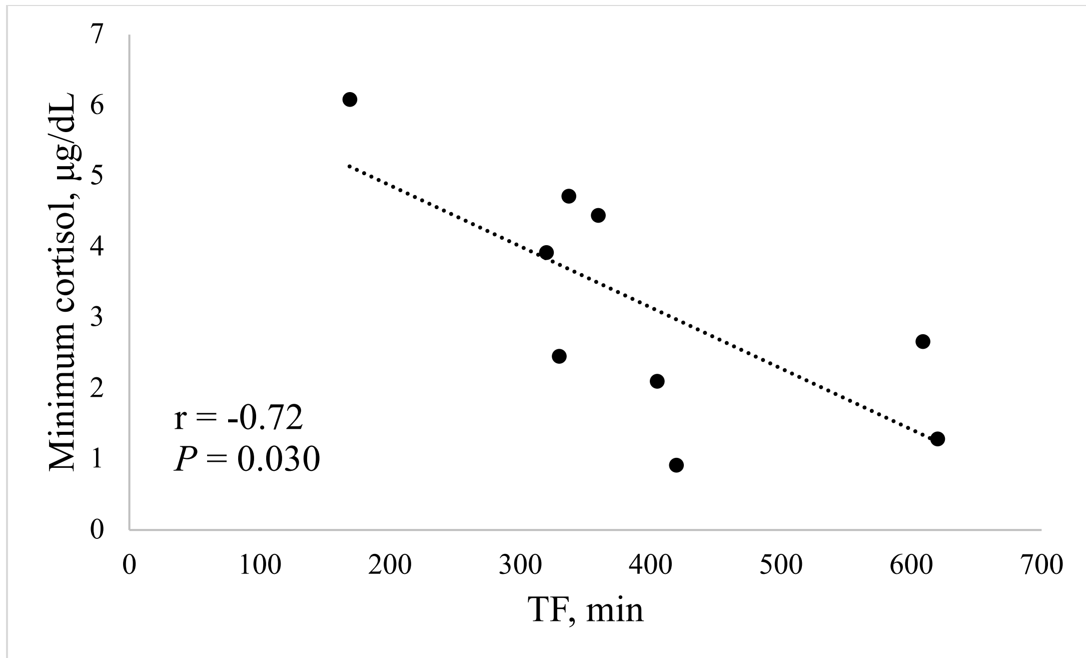

Figure 4.8 Correlation between minimum cortisol[1] and TF[2]

[1]Minimum sow cortisol from birth of first piglet to expulsion of last placental part

[1]Minutes between birth of first piglet until expulsion of last placental part

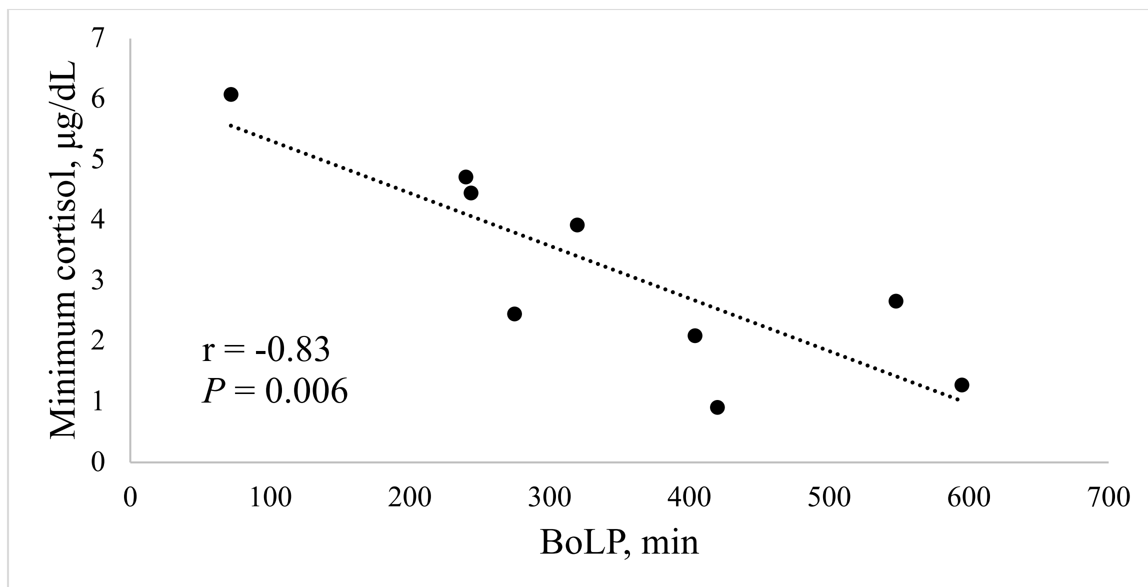

Figure 4.9 Correlation between minimum cortisol[1] and BoLP[2]

[1]Minimum sow cortisol from birth of first piglet to expulsion of last placental part

[1]Minutes between birth of first piglet and last piglet

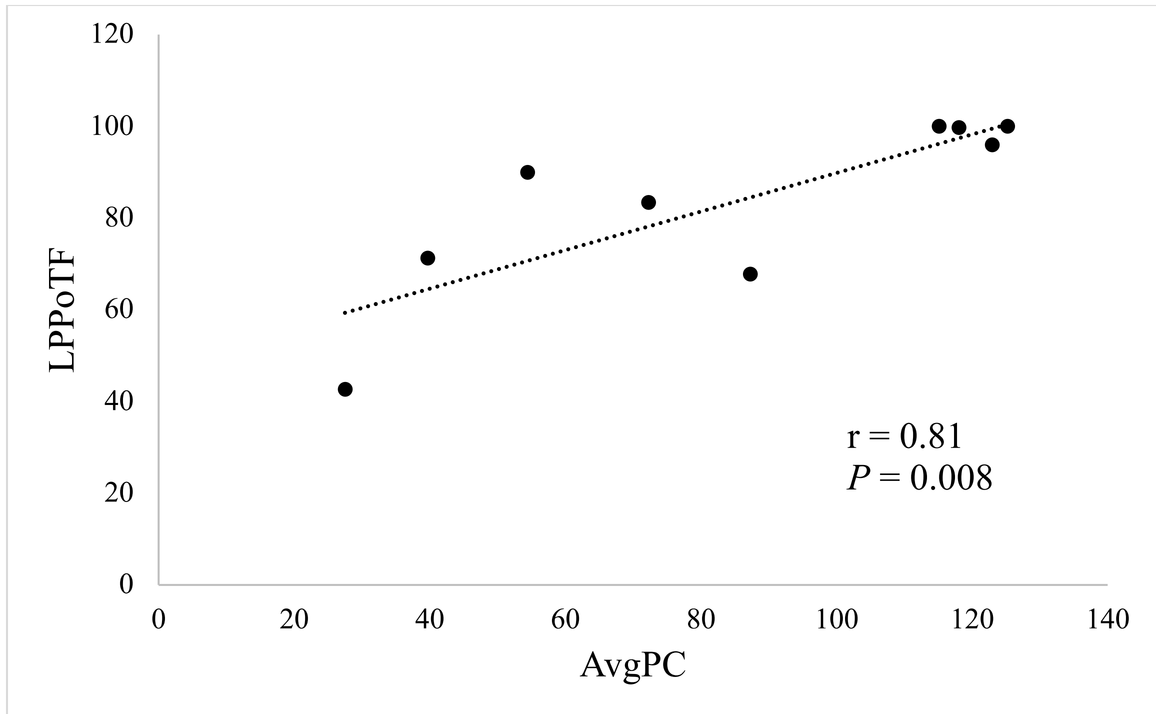

Figure 4.10 Correlation between AvgPC[1] and LPPoTF[2]

[1]Average piglet cortisol in each litter

[2]Time to last piglet as a percentage of total farrowing time

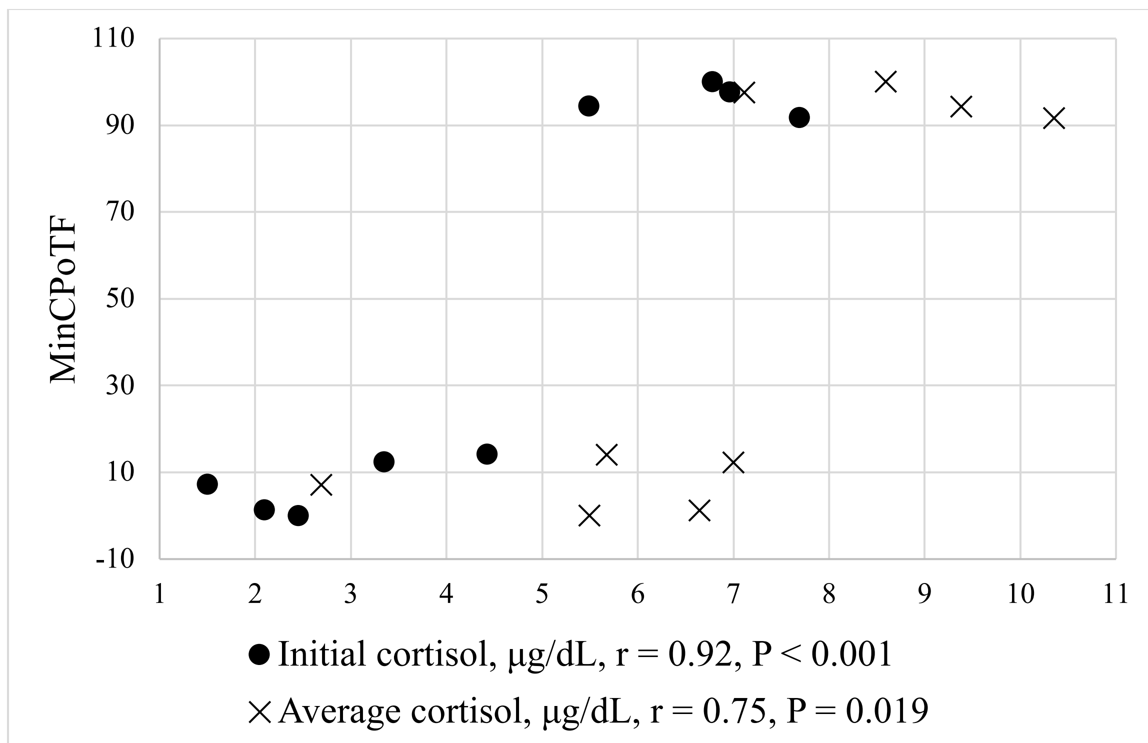

Figure 4.11 Correlations between MinCPoTF[1] and initial[2] and average[3] cortisol

[1]Time to minimum cortisol as a percentage of total farrowing time

[2]Sow cortisol at the birth of the first piglet

[3]Average sow cortisol from first piglet to expulsion of last placental part

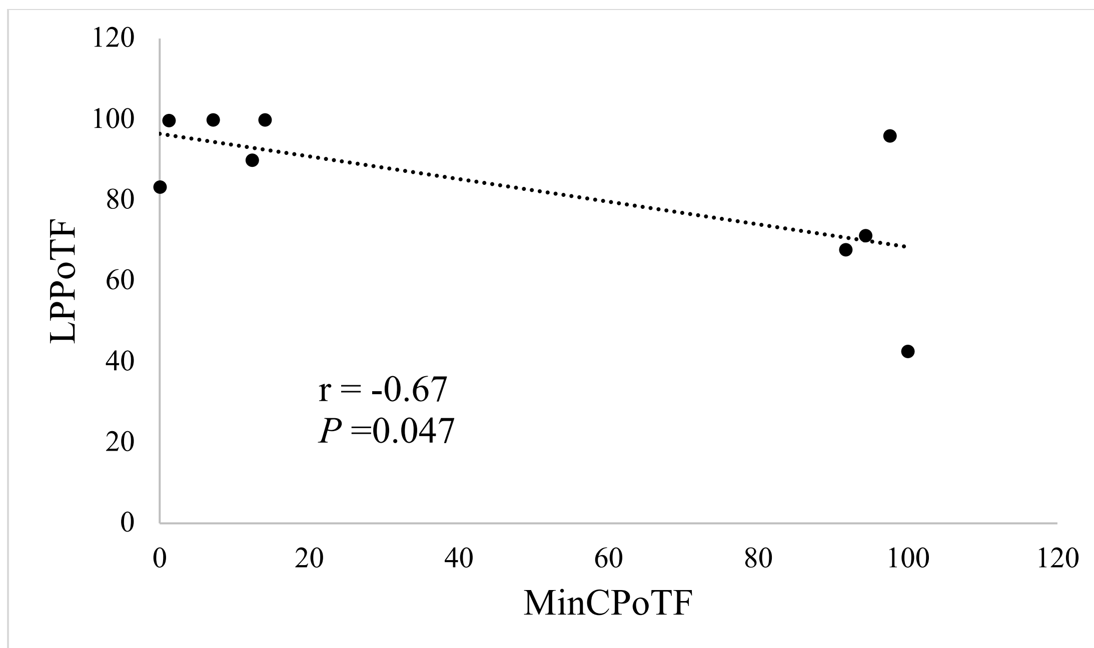

Figure 4.12 Correlation between LPPoTF[1] and MinCPoTF[2]

[1]Time to last piglet as a percentage of total farrowing time

[2]Time to minimum cortisol as a percentage of total farrowing time

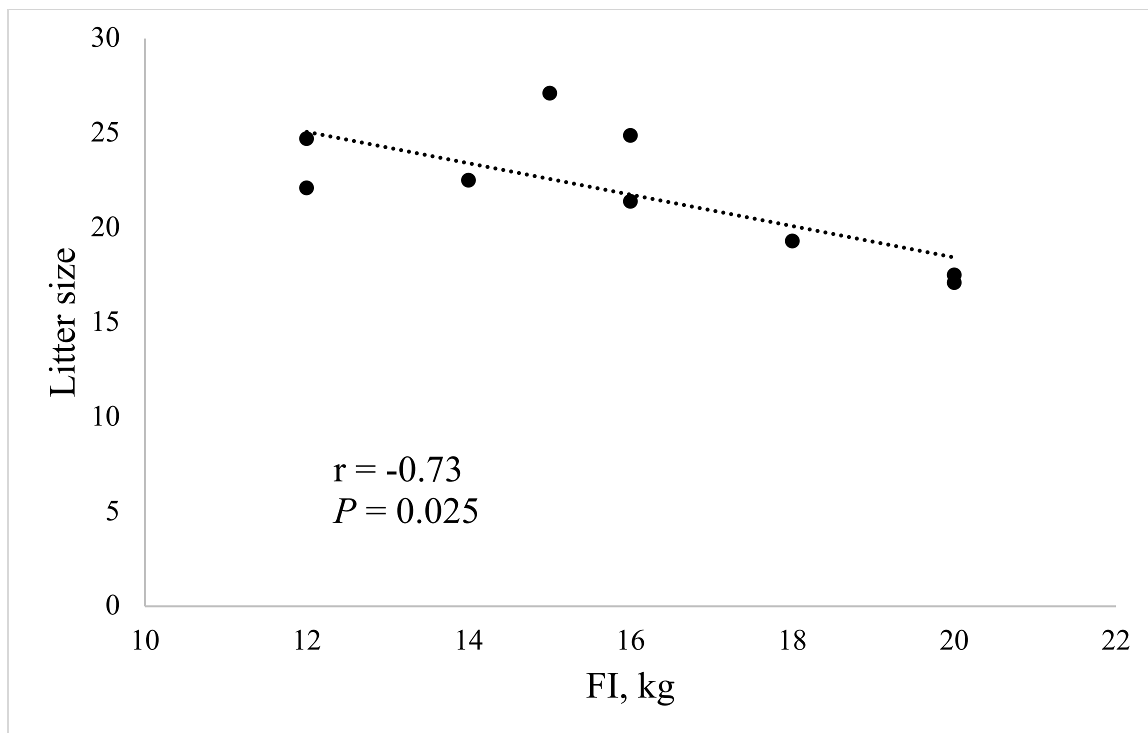

Figure 4.13 Correlation between FI[1] and litter size

[1]Total sow feed intake three days prior to and the day of parturition

5.0 GENERAL DISCUSSION

The objective of this research was to 1) determine the influence of a simulated chronic stress scenario on HCC, 2) determine HCC of sows in two different gestation housing systems as a marker of chronic stress, and 3) examine the pattern of blood cortisol during parturition in the sow.

Maternal cortisol rises as gestation progresses (Hay et al., 2000), although the current understanding of the role of the HPA axis throughout gestation and at parturition is limited. In humans, the HPA response to stressors has been noted to be blunted in late pregnancy (Fliers et al., 2014). However, primiparous women have been noted to have higher cortisol in mid and late gestation than multiparous women (Conde & Figueiredo, 2014; Gillespie et al., 2018), suggesting that cortisol is still released in late gestation in response to external stress or pregnancy-related anxiety. In contrast, Sarkar et al. (2008) reported that maternal anxiety correlated with maternal cortisol for 17 weeks of gestation, but after 18 weeks there was no relationship between anxiety and cortisol.

In our research, we noted that stall-housed gilts and parity 1 sows had elevated HCC compared to all other females throughout gestation; we attribute this to the stress of the housing system, as group-housed gilts and parity 1 sows did not express this elevated HCC in comparison to the older sows. In addition, there was no increase in HCC from early to late gestation in gilts and parity 1 sows in either housing system. This suggests that the expected increase in maternal cortisol caused by progression of pregnancy may not have occurred as it did in older sows. It is possible, however, that HCC in early gestation was associated with the stress of stall housing, and the elevation in late gestation may be a combination of lingering stress from stall housing plus the normal

increase in maternal cortisol that occurs in late gestation. At parturition, we did not observe a parity difference in sow cortisol that would indicate greater distress during the novel experience of farrowing. Similarly, Lawrence et al. (1994) and Oliviero et al. (2008) failed to identify a difference in cortisol during parturition between sows housed with or without enrichment, although differences were detected pre- and post-farrow.

Thus, the HPA axis acts independently of external stress during pregnancy, particularly in late gestation and at parturition. The placenta and endometrium secrete CRH throughout gestation, and the fetus and placenta release ACTH (Fliers et al., 2014), which also confounds a clear relationship between maternal cortisol and anxiety as the conceptus develops during gestation.

Fetal cortisol affects maternal cortisol at parturition but its influence on maternal cortisol in late gestation is not known. Smaller litters were associated with higher minimum sow cortisol during parturition, and minimum cortisol occurring near the end of parturition. The reason for this is not known, but it may be that piglets in smaller litters are more developmentally mature and therefore have a more robust cortisol response at parturition. It is not known whether litter size influences maternal cortisol in late gestation. Roelofs et al. (2019) reported that sows with larger litters also had higher HCC at d112 of gestation. However, their sample size was small (32 sows), and we did not identify an effect of litter size on HCC in our study, so more research is needed to identify any litter effect on maternal cortisol in late gestation. The increase in fetal cortisol in late gestation and at parturition is thought to assist the piglets with organ maturation and the transition from maternal glucose supply to liver glycogen and gluconeogenesis (Fishman et al., 2018; Fowden et al., 1995). Fetal cortisol promotes the

conversion of progesterone to estradiol and the production of prostaglandins at the onset of parturition (Senger, 2012), and fetal cortisol may also weaken the placenta and allow piglets to more easily rupture the fetal membranes during labor (Wang et al., 2020).

Because fetal cortisol plays a role in the initiation of parturition, it has also been suggested that a dysfunctional HPA response may trigger premature parturition or induce parturition. This could be a useful tool in commercial swine production, where inducing sows to farrow during working hours increases piglet survival (Cassar et al., 2005). In human research, Mancuso et al. (2004) noted that women with higher CRH and anxiety at 28 to 30 weeks of gestation went into labor earlier than women with lower CRH and anxiety. In horses, ACTH administration to the fetus shortened gestation length (Ousey et al., 1998). It has been observed that exogenous ACTH administered to piglets from 100-105 d of gestation led to an increase in fetal cortisol similar to levels observed during parturition; however, parturition was not induced (Silver & Fowden, 1989). Randall et al. (1990) injected fetal pigs with ACTH but observed inconsistent changes in maternal hormone profiles. Thus, it is unlikely that elevated sow stress during late gestation or modulating fetal cortisol before parturition would be useful for inducing labor.

Some research has suggested that cortisol influences duration of labor. Glucose is the primary nutrient utilized by the uterus (Steingrímsdóttir et al., 1995), so during parturition cortisol may be necessary for maintaining sufficient energy for myometrial contractions. Additionally, in the uterus cortisol may inhibit prostacyclin, which quiets myometrial contractions, without inhibiting other prostaglandins responsible for accelerating uterine contractility (Casey et al., 1985). It has been observed that women with higher CRH levels during oxytocin induction had shorter labors and greater uterine

contractility (Benfield et al., 2014). In this trial, sows with higher minimum cortisol, occurring later in parturition, had shorter farrowing duration and shorter birth intervals, although the cause of shorter farrowing duration could be smaller litters, increased fetal cortisol, or both. Because initial cortisol was positively correlated with minimum cortisol, and minimum cortisol was negatively correlated with farrowing duration, there is potential, with more research, that cortisol at the onset of parturition may be used to predict farrowing duration. A rapid test similar to a glucometer could be developed to use at the onset of parturition to predict farrowing duration and the potential need for more attention from the farrowing technician. This is relevant for commercial swine production because shorter farrowing durations reduce the risk of stillborn or hypoxic piglets (Van Djik et al., 2005) and reduce the duration of sow pain and fatigue associated with labor (Rutherford et al., 2013).

Generally sow cortisol may be used as a measure of stress, but maternal cortisol during parturition in both swine and humans appears to be unaffected by painkillers and strongly affected by fetal cortisol (Bergant et al., 1998; Gitau et al., 2001; Jarvis et al., 1998), and therefore is not likely to be a good measure of welfare at that time. However, it has been noted that pre-parturient sows (Lawrence et al., 1994) and sows in early lactation (Oliviero et al., 2008) had higher cortisol when housed in environments without enrichment, suggesting that cortisol is an indicator of higher stress when not confounded by the influence of fetal cortisol during parturition.

Cortisol response to psychological stress may also be influenced by individual personality: many animals, including pigs, have been observed to display variable HPA reactivity to stressful situations. High-reactivity pigs, which struggle more during

restraint, have lower HPA reactivity to a novel environment test, handling, and ACTH administration (Koolhaas et al., 1999), and low-reactivity pigs have higher HPA response to the same stressors. Therefore, individual personality plays a large role in cortisol response to stress and is not purely regulated by well-described biological mechanisms. Additionally, maternal stress during gestation is known to modulate piglet HPA reactivity (Brajon, 2017), so there may be some variation in piglet cortisol response to parturition due to maternal stress during gestation. Therefore, it is not recommended to use cortisol as a measure of maternal stress during parturition.

Our results from Chapter 3 indicated that stall-housed gilts and parity 1 sows had higher HCC than all other sows, which is indicative of higher stress during gestation; conversely, the results of Chapter 2 show that mixing gilts with unfamiliar pen-mates causes higher HCC than repeated ACTH injections. Therefore, sow housing during gestation must limit the use of gestation stalls, and also minimize mixing unfamiliar sows to create stressful social scenarios. Stress during gestation may result in lower birth weights, which are associated with higher pre-weaning mortality (Feldpausch et al, 2019). Low body weight at weaning increases the rate of mortalities in the nursery phase (Larriestra et al, 2006), and Kranendonk et al. (2006a) found not only lower birth weight but also lower weaning weights in piglets from sows who were treated with hydrocortisone acetate in gestation. Piglets from sows experiencing elevated glucocorticoids during gestation may be more likely to have poorer welfare, demonstrating more anxiety-related behavior or aggression in novel situations and higher pain score during tail docking (Otten et al., 2015; Kranendonk et al., 2006b). In addition, gilts raised from mothers who had experienced stress during gestation appeared more

restless and uncomfortable in the periparturient period (Jarvis et al., 2006; Rutherford et al., 2014), and in one trial tended to be more aggressive towards piglets (Jarvis et al., 2006); both frequent posture changes and sow aggression can result in newborn piglets being injured or killed, contributing to pre-weaning mortality. Therefore, chronic stress in sows can detrimentally affect the productivity and welfare of their offspring, and sow housing during gestation must be designed to minimize sow stress by limiting the use of gestation stalls, at least in young females, and minimizing mixing unfamiliar pen-mates.

Smaller litter sizes improves both sow and piglet welfare. Smaller litters may reduce sow discomfort during gestation, which may be associated with greater stress in late gestation in stall-housed sows (Anil et al, 2006). Smaller litters reduce farrowing duration and birth interval, reducing the duration of pain the sow experiences at parturition. Prolonged farrowing duration may also be associated with a greater risk of retained placenta, which increases the risk of uterine inflammation and subsequent infertility (Björkman et al., 2017). Larger litters in our data were also associated with lower minimum cortisol, which plays a role in combatting post-partum infections (Nenke et al., 2017). Thus, larger litters increase the risk of infection and may impair the sow's ability to fight the infection.

Large litters also result in intense competition between piglets for limited resources both in utero and after birth. Restricted access to nutrient in utero results in low birth weights or intra-uterine growth-restricted piglets (Edwards & Baxter, 2015). Low birth weight piglets are more likely to die from chilling, being crushed by the sow, or starvation (Rutherford et al., 2013). Piglets compete with littermates using their sharp canine teeth, which cause cuts on other piglets' faces; in order to prevent these injuries,

these teeth are often clipped, which causes concerning welfare implications (Sutherland, 2015).

Two major facets of modern swine production create welfare concerns: sow housing, and litter size. In order to minimize sow stress during gestation, females, particularly gilts and parity 1 sows, should be housed in group pens rather than conventional stall housing. Dynamic group pens may create more stress than static group pens, as repeated and prolonged social stress was shown in our research to be more effective at raising HCC in gilts than injecting ACTH. Hair cortisol is useful for measuring cortisol secretion over prolonged periods of stress. Cortisol at the onset of parturition has not been shown to consistently reflect sow welfare during labor but may be useful for predicting farrowing duration. Large litters result in longer farrowing duration and birth intervals, which may be associated with an attenuated fetal cortisol response by smaller piglets in large litters. This prolonged farrowing duration has welfare and production implications including prolonged sow pain, increased risk of post-partum infection, and increased risk of piglet mortality.

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