Factors associated with puberty onset and reproductive performance of gilts

Kody Lane Graves
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd
Part of the Agriculture Commons, and the Animal Sciences Commons

Recommended Citation
Graves, Kody Lane, "Factors associated with puberty onset and reproductive performance of gilts" (2015). Graduate Theses and Dissertations. 14580.
https://lib.dr.iastate.edu/etd/14580

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Factors associated with puberty onset and reproductive performance of gilts

by

Kody Lane Graves

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Physiology

Program of Study Committee:
Jason W. Ross, Major Professor
Aileen F. Keating
Kenneth J. Stalder

Iowa State University
Ames, Iowa
2015

Copyright © Kody Lane Graves, 2015. All rights reserved
TABLE OF CONTENTS

LIST OF TABLES........................................................................................................ iv

LIST OF FIGURES...................................................................................................... v

LIST OF ABBREVIATIONS......................................................................................... ix

ACKNOWLEDGEMENTS......................................................................................... xi

ABSTRACT............................................................................................................... xii

CHAPTER I: INTRODUCTION.................................................................................... 1

CHAPTER II: LITERATURE REVIEW....................................................................... 3
  Hypothalamic Pituitary Gonadal Axis................................................................. 3
  KNDy Neurons..................................................................................................... 4
  Gonadotropins.................................................................................................... 6
  Steroid Hormones............................................................................................... 6
  Leptin.................................................................................................................. 8
  Puberty Onset..................................................................................................... 9
  Estrous Cycle.................................................................................................... 12
  Maternal Recognition of Pregnancy............................................................... 13
  Estrus Synchronization..................................................................................... 16
  Sow Lifetime Productivity................................................................................ 17
  Factors Influencing Sow Lifetime Productivity............................................ 18
    Physical Condition.......................................................................................... 18
    Puberty Onset.................................................................................................. 19
  Seasonal Infertility.......................................................................................... 20
    Factors Influencing Seasonal Infertility....................................................... 20
    Photoperiod................................................................................................... 20
    Heat Stress...................................................................................................... 21
    Hormones........................................................................................................ 22
  Reproductive Efficiency.................................................................................. 22
    Farrowing Rate.............................................................................................. 23
    Pregnancy Failure......................................................................................... 23
    Wean to Estrus Interval.................................................................................. 24
    Litter Performance......................................................................................... 24
  Summary........................................................................................................... 25

CHAPTER III: IDENTIFICATION OF MEASURES PREDICTIVE OF AGE AT PUBERTY ONSET.................................................................................. 27
  Abstract............................................................................................................ 27
  Introduction....................................................................................................... 29
  Materials and Methods.................................................................................... 31
  Animals.............................................................................................................. 31
CHAPTER IV: EVALUATION OF THE THERMOREGULATORY RESPONSE IN GILTS AND ITS RELATIONSHIP WITH REPRODUCTIVE PERFORMANCE FOLLOWING SYNCHRONIZATION WITH MATRIX

Abstract
Introduction
Materials and Methods
Animals
Acclimation and Synchronization Period
Heat Stress Period
Temperature Measurements
Estrus Detection, Artificial Insemination, and Pregnancy Check
Harvesting and Fetal Analysis
Temperature and Statistical Analysis
Results
Thermoregulatory Response to Heat Stress
Breeding Performance
Reproductive Efficiency
Relationship of TrDelta with Reproductive Performance
Repeatability of Heat Stress Tolerance and Susceptibility
Relationship Between Maximum Rectal Temperatures on Day of Insemination and Reproductive Outcome
Discussion
Conclusion

CHAPTER V: SUMMARY AND CONCLUSIONS

APPENDIX: ADDITIONAL DATA

REFERENCES CITED
LIST OF TABLES

**Table 3.1.** Relationship between body weight during pre-pubertal growth and age at puberty onset................................................................. 44

**Table 3.2.** Relationship between vulva width during pre-pubertal growth and age at puberty onset................................................................. 44

**Table 3.3.** Relationship between vulva length during pre-pubertal growth and age at puberty onset................................................................. 44

**Table 3.4.** Relationship between vulva area during pre-pubertal growth and age at puberty onset................................................................. 45

**Table 3.5:** Relationship between Kp-10 during pre-pubertal growth and age at puberty onset................................................................. 45

**Table 4.1.** Temperature measurement analysis between heat stress response classifications................................................................. 76

**Table 4.2.** Relationship of TrDelta and reproductive performance following heat stress.......... 76

**Table 4.3.** Relationship of bTr and reproductive performance following heat stress............ 77
LIST OF FIGURES

Figure 2.1. KNDy neuropeptide activation of GnRH neurons in the hypothalamus. 5

Figure 2.2. The hypothalamic pituitary gonadal axis. 7

Figure 2.3. The estrous cycle and pregnancy recognition. 15

Figure 3.1. Representative image showing distinct variation in ovarian tertiary follicular development. 46

Figure 3.2. Variation in follicular development in gilts on postnatal days 75, 85, 95, 105, and 115. 47

Figure 3.3. Average uterine weight of sacrificed gilts on postnatal days 75, 85, 95, 105, and 115. 48

Figure 3.4. Distribution of gilts achieving puberty onset by 200 days of age. 49

Figure 3.5. Relationship of body weight at 75 days of age and age at puberty onset. 50

Figure 3.6. Relationship of vulva width at 105 days of age and age at puberty onset. 51

Figure 3.7. Relationship of vulva width at 115 days of age and age at puberty onset. 52

Figure 3.8. Percentage of gilts achieving estrus by 180 days of age on each postnatal day for a given vulva area. 53

Figure 3.9. Percentage of gilts achieving estrus by 200 days of age on each postnatal day for a given vulva area. 54

Figure 3.10. Plasma kisspeptin-10 levels on postnatal days 75, 85, 95, 105 and 115. 55

Figure 3.11. Serum anti-müllerian hormone (AMH) levels on postnatal day 95. 56

Figure 4.1. Distribution of gilts receiving their first service on each day following Matrix® withdrawal. 78

Figure 4.2. Distribution of gilts receiving a given number of artificial insemination services. 79

Figure 4.3. Average fetal weight of pregnant gilts conceived during heat stress that were classified as tolerant or susceptible to heat stress prior to puberty. 80
Figure 4.4. Average fetal crown-rump length (CRL) of pregnant gilts conceived during heat stress that were classified as tolerant or susceptible to heat stress prior to puberty ................................................................. 81

Figure 4.5. Average corpora lutea (CL) diameter of pregnant gilts conceived during heat stress that were classified as tolerant or susceptible to heat stress prior to puberty ........................................................................ 82

Figure 4.6. Relationship between TrDelta and fetal crown-rump length ........................................................................ 83

Figure 4.7. Repeatability of thermal neutral rectal temperature response ........................................................................ 84

Figure 4.8. Repeatability of heat stress rectal temperature response ........................................................................ 85

Figure 4.9. Repeatability of heat stress TrDelta response ......................................................................................... 86

Figure 4.10. Relationship between pre-pubertal thermal neutral rectal temperature and post-pubertal heat stress rectal temperature response ......................................................................................... 87

Figure 4.11. Relationship between the maximum rectal temperature on day of first breeding and fetal weight ................................................................................................................................. 88

Figure 4.12. Relationship between the maximum rectal temperature on day of first breeding and fetal crown-rump length ................................................................................................................................. 89

Figure A.3.1. Relationship of average body weight on postnatal day 75 and age at puberty onset ................................................................................................................................. 96

Figure A.3.2. Relationship of average body weight on postnatal day 85 and age at puberty onset ................................................................................................................................. 96

Figure A.3.3. Relationship of average body weight on postnatal day 95 and age at puberty onset ................................................................................................................................. 97

Figure A.3.4. Relationship of average body weight on postnatal day 105 and age at puberty onset ................................................................................................................................. 97

Figure A.3.5. Relationship of average body weight on postnatal day 115 and age at puberty onset ................................................................................................................................. 98

Figure A.3.6. Relationship of average vulva width on postnatal day 75 and age at puberty onset ................................................................................................................................. 98

Figure A.3.7. Relationship of average vulva width on postnatal day 85 and age at puberty onset ................................................................................................................................. 99
**Figure A.3.8.** Relationship of average vulva width on postnatal day 95 and age at puberty onset................................................................. 99

**Figure A.3.9.** Relationship of average vulva width on postnatal day 105 and age at puberty onset.............................................................................. 100

**Figure A.3.10.** Relationship of average vulva width on postnatal day 115 and age at puberty onset.............................................................................. 100

**Figure A.3.11.** Relationship of average vulva length on postnatal day 75 and age at puberty onset.............................................................................. 101

**Figure A.3.12.** Relationship of average vulva length on postnatal day 85 and age at puberty onset.............................................................................. 101

**Figure A.3.13.** Relationship of average vulva length on postnatal day 95 and age at puberty onset.............................................................................. 102

**Figure A.3.14.** Relationship of average vulva length on postnatal day 105 and age at puberty onset.............................................................................. 102

**Figure A.3.15.** Relationship of average vulva length on postnatal day 115 and age at puberty onset.............................................................................. 103

**Figure A.3.16.** Relationship of average vulva area on postnatal day 75 and age at puberty onset.............................................................................. 103

**Figure A.3.17.** Relationship of average vulva area on postnatal day 85 and age at puberty onset.............................................................................. 104

**Figure A.3.18.** Relationship of average vulva area on postnatal day 95 and age at puberty onset.............................................................................. 104

**Figure A.3.19.** Relationship of average vulva area on postnatal day 105 and age at puberty onset.............................................................................. 105

**Figure A.3.20.** Relationship of average vulva area on postnatal day 115 and age at puberty onset.............................................................................. 105

**Figure A.4.1.** Average uterine weight of the pre-pubertal heat stress tolerant and susceptible gilts.................................................................................. 106

**Figure A.4.2.** Average fetal number of the pre-pubertal heat stress tolerant and susceptible gilts.................................................................................. 106
Figure A.4.3. Average ovary weight of the pre-pubertal heat stress tolerant and susceptible gilts................................................................. 107

Figure A.4.4. Average corpora lutea number of the pre-pubertal heat stress tolerant and susceptible gilts............................................................... 107

Figure A.4.5. Average percentage of embryo survival of the pre-pubertal heat stress tolerant and susceptible gilts............................................................ 108
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.I.</td>
<td>Artificial insemination</td>
</tr>
<tr>
<td>AMH</td>
<td>Anti-müllerian hormone</td>
</tr>
<tr>
<td>ARC</td>
<td>Arcuate nucleus</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>CL</td>
<td>Corpora lutea</td>
</tr>
<tr>
<td>CRL</td>
<td>Crown-rump length</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>E2</td>
<td>Estrogen</td>
</tr>
<tr>
<td>EP</td>
<td>Early puberty</td>
</tr>
<tr>
<td>F</td>
<td>Fahrenheit</td>
</tr>
<tr>
<td>FR</td>
<td>Farrowing rate</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin releasing hormone</td>
</tr>
<tr>
<td>GPR54</td>
<td>G-Protein coupled receptor 54</td>
</tr>
<tr>
<td>HPGx</td>
<td>Hypothalamic pituitary gonadal axis</td>
</tr>
<tr>
<td>IP</td>
<td>Intermediate puberty</td>
</tr>
<tr>
<td>KISS1</td>
<td>Gene encoding the product kisspeptin</td>
</tr>
<tr>
<td>KNDy</td>
<td>Colocalized group of kisspeptin, neurokinin B, and dynorphin neurons</td>
</tr>
<tr>
<td>KOR</td>
<td>Dynorphin receptor</td>
</tr>
<tr>
<td>Kp-10</td>
<td>Kisspeptin-10</td>
</tr>
<tr>
<td>LEP-R</td>
<td>Leptin receptor</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>LP</td>
<td>Late puberty</td>
</tr>
<tr>
<td>MaxRR</td>
<td>Average evening heat stress respiration rate response</td>
</tr>
<tr>
<td>MaxSkin</td>
<td>Average evening heat stress skin temperature response</td>
</tr>
<tr>
<td>MaxTr</td>
<td>Average evening heat stress rectal temperature response</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MUA</td>
<td>Multi-unit activity</td>
</tr>
<tr>
<td>NBA</td>
<td>Number born alive</td>
</tr>
<tr>
<td>NK3R</td>
<td>Neurokinin B receptor</td>
</tr>
<tr>
<td>NR</td>
<td>Non-responsive</td>
</tr>
<tr>
<td>PGF2α</td>
<td>Prostaglandin F2 alpha</td>
</tr>
<tr>
<td>P4</td>
<td>Progesterone</td>
</tr>
<tr>
<td>PND</td>
<td>Postnatal day</td>
</tr>
<tr>
<td>POA</td>
<td>Pre-optic area</td>
</tr>
<tr>
<td>PROC CORR</td>
<td>Correlation analysis of SAS</td>
</tr>
<tr>
<td>PROC FREQ</td>
<td>T-Test analysis of SAS</td>
</tr>
<tr>
<td>SI</td>
<td>Seasonal infertility</td>
</tr>
<tr>
<td>SLP</td>
<td>Sow lifetime productivity</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>TN</td>
<td>Thermal neutral</td>
</tr>
<tr>
<td>Tr</td>
<td>Thermal rectal temperature</td>
</tr>
<tr>
<td>TrDelta</td>
<td>Change in Temperature (heat stress – thermal neutral)</td>
</tr>
</tbody>
</table>
bTr = Maximum rectal temperature on day of estrus onset
VA = Vulva area
VL = Vulva length
VW = Vulva width
X² = Chi-Square
ACKNOWLEDGEMENTS

I would like to thank first and foremost my Masters plan of study committee for their guidance during my time at Iowa State University. It is without a doubt that their knowledge and wisdom provided the foundation for the work conducted in this thesis. To my major professor, Dr. Jason W. Ross, I would like to express my deepest gratitude and appreciation for the opportunity to join his research group and for offering me daily support as I worked on my research. Also for teaching me most of what I know now about pigs. To my committee: Dr. Aileen F. Keating for her assistance in experimental design and knowledge of ovarian function and to Dr. Ken J. Stalder for his guidance and willingness to answer my constant questions on statistics and SAS functions.

In addition to my committee, I would like to thank my lab mates both past and present including Ben Hale, Jake Siebert, Theresa Johnson, Elane Wright, Cai-Xia Yang, and Beth Hines for their unwavering support and assistance in completing these experiments. From our daily office discussions, whether research based or not, to helping with each other’s research without you all my time at ISU would have not been as enjoyable. Also to the countless number of undergraduates that helped with daily data recording and animal care, without them these projects would have not been possible.

I would also like to thank my parents, Keith and Rebecca Graves, for instilling in me a passion for agriculture and the standards of hard work and dedication to be successful in my career. Last, and definitely not least, I would like to thank my wife Sydney for her continuous support and encouragement as I worked towards this goal. I look forward to the next chapter in our lives.
ABSTRACT

Reproductive efficiency is crucial to the sustainability of any swine herd. A female’s ability to contribute to the herd earlier, longer, and more proficiently impacts her individual performance characterizing her sow lifetime productivity. Factors that impact a female’s reproductive performance vary from genetic to environmental influencers such as seasonal infertility. Age at puberty onset represents a useful tool to identify sow lifetime productivity. To determine the positive influence of age at puberty onset, physical and physiological measurements taken on a group of gilts were used to identify the potential correlation with a gilt’s age when puberty is achieved. Vulva development after postnatal day 95 and body weight on postnatal day 75 showed to be correlated to a gilt’s ability to achieve puberty earlier or later than their counterparts. While age of puberty onset can increase reproductive performance, environmental factors such as heat stress can negatively impact a female’s reproductive performance. Heat stress is a major contributing factor to seasonal infertility and is associated with decreased farrowing rates, litter performance, and wean-to-estrus intervals. Mitigating the effects of heat stress is key to increasing a female’s reproductive success. To determine how heat stress affects reproductive performance as well as how gilts adapt to stress periods, females that were either tolerant or susceptible to pre-pubertal heat stress were identified, and their response during post-pubertal heat stress periods were evaluated. A gilt’s response to pre-pubertal heat stress has a positive correlation to her response to post-pubertal heat stress. Reproductive measures of fetal weight and crown-rump length were decreased in gilts susceptible to heat stress while corpora lutea diameter was increased in heat stress tolerant gilts when exposed to heat stress during breeding.
CHAPTER I: INTRODUCTION

In 2013, the United States swine industry produced approximately 117 million pigs from approximately 11.5 million litters farrowed from breeding age sows. This equated to an average of 10.2 number of pigs born alive (NBA) per litter (USDA, 2014). In recent decades pork consumption has increased steadily to become one of the most consumed sources of animal protein, representing 37% of total meat consumption worldwide (FAO, 2014). With an estimated population increase of approximately 34% by 2050, our food supply, including animal protein, will also need to increase to meet the expected increase in demand (United Nations, 2013). For the swine industry to continue meeting demand in pork consumption, an increase in breeding animals will be needed. The dwindling availability of land due to the increases in population, environmental concerns with swine production, and alternative uses for grain, collectively place strains on expanding the size of the swine breeding herd.

Subsequently, the swine industry must continuously strive to improve production efficiency. One way of improving efficiency in the swine breeding herd is by better capturing the investments made in breeding animals by improving the productive lifetime of sows. Sow lifetime productivity (SLP) refers to the number of quality pigs a sow weans from the time she becomes breeding eligible until she leaves the herd. Improving the factors that contribute to SLP can add efficiency to swine production systems. Primary methods of improvement can be through increasing the number of productive sow days while maintaining or improving the number of piglets weaned per litter.

Predicting SLP is difficult due to the many environmental factors that can influence it. One environmental influence that has dramatic effects on swine reproductive performance is heat stress. With continuously changing climate patterns and the selection for growth with
an emphasis on lean tissue accretion, pigs have become more susceptible to heat stress. This increased susceptibility may in part be mediated by the increased metabolic heat production from synthesizing and maintaining skeletal muscle (Hocquette et al., 1998). This increased susceptibility to heat stress has resulted in a decrease in reproductive efficiency, particularly during the summer months. This repeated observation of reduced fecundity in summer months, termed seasonal infertility, is commonly represented by reductions in phenotypic measures of efficiency such as farrowing rate and decreased NBA for inseminations occurring during July, August, and September. By understanding the biology that contributes to seasonal infertility, new management strategies and identifying females tolerant to environmental stressors such as heat stress may enable the ability to mitigate the effects of seasonal infertility.
CHAPTER II: LITERATURE REVIEW

Hypothalamic-Pituitary-Gonadal Axis

The hypothalamic pituitary gonadal axis (HPGx) is a key component of sexual maturation in mammalian species (Pelletier et al., 1981). The development and maintenance of this endocrine axis contributes to the regulation of the reproduction cycle. In both male and female neonates the axis is active, but not yet functional. This was showed in that during pre-pubertal growth and development, reproductive hormones fluctuate until appropriate levels allow for the activation of the HPGx resulting in achievement of puberty (Camous et al., 1985). Gonadotropin releasing hormone (GnRH) is localized within the pre-optic area (POA) and arcuate nucleus (ARC) of the hypothalamus (Wheaton et al., 1975 and Naik, 1976). Secretion occurs in different patterns depending on the feedback mechanisms from gonadal steroid hormones. GnRH is either secreted in a pulsatile, rhythmic or a surge release pattern depending on the mechanism of activation (Marshall et al., 1991). The necessity of multiple mechanisms regulating secretory patterns is important for different biological purposes, such as regulating the luteinizing hormone surge (Karsch et al., 1987).

The neuropeptide kisspeptin has been shown to be a main initiator of GnRH release by binding to its G-protein coupled receptor, GPR54, in the hypothalamus resulting in the release of GnRH (Seminara and Crowley, 2008). Disruptions in kisspeptin signaling, such as mutations in GPR54, are associated with hypogonadotropic hypogonadism and can result in delayed puberty onset (de Roux et al., 2003). The complete relationship between kisspeptin and GnRH neurons are not fully understood and have been a focus of recent study. It is now known there are several additional neuropeptides contributing to hypothalamic control (Lehman et al., 2010). Kisspeptin’s ability to alter normal GnRH secretion patterns by its
absence or defects in associated receptors relay the notion of its direct effect on GnRH pulsatility (Maeda et al., 2007). Similar to kisspeptin, neurokinin B has also been demonstrated to have an effect on GnRH secretion through its association with genetic disorders (Topaloglu et al., 2009). Evidence of neuron colocalization between kisspeptin and neurokinin B suggests their ability to influence GnRH pulses in a similar manner and also confirms observations of neurons being distributed in an interconnected web across the ARC (Burke et al., 2006). Studies using agonists and antagonists to the kisspeptin receptor, GPR54, and the neurokinin B receptor, NK3R, in ewes have shown increases in GnRH secretion as well as increases in both luteinizing hormone (LH) pulses and inter-pulse intervals (Goodman et al., 2013).

Dynorphin neurons have also been proposed as controlling factors on GnRH pulsatility. Administration of an antagonist to the dynorphin receptor, KOR, in ewes resulted in increases in LH pulses and decreases in LH inter-pulse intervals, suggesting an inhibitory effect of dynorphin on GnRH pulsatile secretion (Goodman et al., 2013). The consistent colocalization of the kisspeptin, neurokinin B, and dynorphin neurons have collectively been labeled the KNDy neurons. The identification of corresponding pulses of KNDy peptides and GnRH have directed researchers to investigate multi-unit activity (MUA) within the area of the KNDy neuron cohort. Recordings in goats have shown simultaneous bursts of MUA in the area of the medial basal hypothalamus (Wakabayashi et al., 2013). This along with evidence of LH pulses in goats happening in synchrony with these bursts suggest this area and most notably the caudal region containing the KNDy neurons may be the location of the initiating processes of GnRH release (Ohkura et al., 2009). The administration of agonists of NK3R in goats increased MUA while an agonist to GPR54 had no effect on MUA.
(Wakabayashi et al., 2010). In summary, the utilization of agonist and antagonist, on key aspects of KNDy neuron signals has led to several conclusions regarding KNDy neurons contribution to regulating GnRH pulses: 1) kisspeptin and neurokinin B have a stimulatory effect on GnRH secretion, 2) dynorphin has an inhibitory role on GnRH secretion, and 3) GnRH secretion is mediated by upstream effects of peptides secreted from the KNDy neurons. The conclusions show individual control of GnRH release by the KNDy neurons. Additionally, Navarro et al. (2009) showed evidence of an interconnected network between the three types of neurons suggesting that each peptide provides feedback mechanisms on neighboring neurons (Figure 2.1).

Figure 2.1.
Figure 2.1 (continued). KNDy neuropeptide activation of GnRH neurons in the hypothalamus. After activation from external influencers the KNDy neuron stimulates the release of the three neuropeptides: kisspeptin, neurokinin B, and dynorphin. The secretion occurs in a pulsatile fashion with Neurokinin B providing the positive feedback to stimulate a start signal. Dynorphin is secreted in a negative feedback to the KNDy neuron to create a stop signal. The back and forth of neurokinin B and dynorphin signaling to the respected KNDy neuron creates the pulsatile secretion of kisspeptin which acts on its GPR54 receptor in the GnRH Neuron, ultimately secreting GnRH.

[Adapted from Pinilla et al., 2012]

Follicle stimulating hormone (FSH) and LH are produced in the anterior pituitary and synthesized and released following GnRH binding to receptors on gonadotrophs (Figure 2.2). These gonadotropins partially regulate the development, growth, and maintenance of the theca and granulosa cells within the follicles of the ovary, and during sexual maturation the theca and granulosa cells of developing follicles become dependent on LH and FSH, respectively (Lunenfeld et al., 1975). During pre-pubertal growth and development in pigs, both LH and FSH levels are low until about 40 days of age (Colenbrander et al., 1977), begins to increase in pulse frequency and amplitude up to the beginning of sexual maturation (~80-120 days of age), then declines slightly (Diekman et al., 1983; Diekman and Trout, 1984). Up to the point of the first ovulation LH concentration and LH pulse frequency both increase, however FSH concentrations do not increase after their initial decline (Prunier et al., 1993). Prior to puberty, estrogen (E2) and progesterone (P4) from the ovary are produced in low levels and are not yet capable of exerting positive and negative feedback on the hypothalamus.

Studies have shown kisspeptin facilitates the actions of the positive feedback of E2, and as such, increases in E2 can cause corresponding decreases in GPR54 mRNA expression (Oakley et al., 2009). Negative feedback of P4 on GnRH is mediated through the actions of dynorphin neurons (Goodman et al., 2004). During normal cycles after puberty, negative
feedback of P4 is important to the normal function of inhibiting GnRH release (Goodman et al., 2011). Steroid feedback mechanisms are important in regulating a functional estrous cycle because of their ability to control the timing of GnRH release.

Figure 2.2.
The hypothalamic pituitary gonadal axis. GnRH is produced in the hypothalamus and secreted through the hypothalamic-pituitary stalk binding to the GnRH receptors in the anterior pituitary. Following intracellular signal transduction, LH and FSH are secreted into the endocrine system. After reaching the ovaries in females, they stimulate receptors on the theca and granulosa cells, respectively, of the follicle. During follicular growth and recruitment E2 is released from the growing follicles (green structure on figure) creating negative feedback on the hypothalamus and anterior pituitary, thereby limiting GnRH and gonadotropin secretion. Inhibin and activin are released from granulosa cells and produce a negative and positive feedback, respectively, on the anterior pituitary to inhibit and activate FSH production. An increased level of E2 produced from mature Graafian follicles produces a positive effect on the hypothalamus, creating a downstream surge of LH and causing a cascade of cellular breakdown resulting in the release of oocytes from follicles during the process of ovulation. Following the LH surge, the luteinization of the theca and granulosa cells results in the formation of the corpora lutea (yellow structure on figure) which is capable of synthesizing and releasing P4. Progesterone through negative feedback on the hypothalamus suppresses production and release of GnRH. If no signal from a developing embryo is present, prostaglandin F2α produced in the uterine endometrium reaches the ovary causing regression of the corpora lutea, halting P4 production and enabling follicular recruitment for the next cycle by removing the negative feedback of P4 on the HPGx. If fertilization occurs and two or more viable conceptuses are present in each uterine horn during the implantation window, the maternal recognition of pregnancy signal secreted from the conceptuses, E2, reroutes the prostaglandin F2α from being secreted into the vascular system (endocrine) to being secreted into the uterine lumen (exocrine), thus preventing it from reaching the ovary and regressing the CL.

An important component in livestock production is how body composition relates to sexual maturation and puberty onset. Leptin is a hormone produced by adipocytes and plays an important role in regulating energy balance (Zang et al., 1994). As circulating leptin concentrations increase, it is thought to positively affect the brain by contributing to GnRH pulse regulation through informing the hypothalamus of sufficient energy stores (Ahima et al., 1997). However, until the past decade the actions leptin undergoes to affect GnRH levels were unknown. After the discovery of the KNDy neurons, the mechanisms in the brain that control the pulsatile secretion of GnRH were better understood (Navarro et al., 2007; Sahu and Kalra, 1992; Goodman et al., 2007). KISS1 neurons express the leptin receptor (LEP-R), suggesting leptin has a potential regulatory impact on the secretion of kisspeptin (Ahima,
Leptin is shown to mediate the activation of the circular mechanism of the KNDy neurons to allow stimulation by E2 from the gonads (Sanchez-Garrido and Tena-Sempere, 2013).

**Puberty Onset**

The onset of puberty in gilts is classified as the time point when metabolic factors (i.e. hormone levels and body growth) and age are associated with the achievement of sexual maturation. At this point the first estrous cycle is initiated and the female is capable of conceiving and producing a litter. The reproductive hormonal pathway is a complex system of ligand binding and receptor signaling, ultimately initiating the secretion of GnRH within the hypothalamus. After the discovery of a mutation in GPR54 and the association of it with infertility and delayed puberty onset, it was clear that factors regulating GnRH secretion upstream of the hypothalamus controls the initiation of an animal’s reproductive cycle (Seminara et al., 2003). As previously discussed, the KISS1 gene enables neurons to produce a neuropeptide known as kisspeptin. Along with the other neuropeptides released from KNDy neurons, a feedback hierarchy of inducing and suppressing signals create the pulsatile secretion of GnRH (Figure 2.1), which in turn contributes to the physiological control of puberty onset (Navarro et al., 2007).

Steroid hormones produced from developing gonads during sexual maturation, as well as the hormone leptin produced from adipose tissue, are key factors in KNDy neuron activation and regulation. Estrogen receptors within the KNDy neurons are stimulated through the increased production of hormones from the growing gonads of the female (Lehman and Karsch, 1993). As the female matures she is able to produce sufficient levels of steroid hormones to activate the feedback loop of the KNDy neuropeptides, ultimately
activating the HPGx and reproduction cycle (Goodman et al., 2013). Adipose accretion during growth is also important in the activation of the KNDy neurons. LEP-R within these neurons have shown to regulate kisspeptin secretion (Ahima, 2011). If there is not an adequate amount of stimulation from leptin then the KNDy neurons are unable to initiate the release of kisspeptin (Sanchez-Garrido and Tena-Sempere, 2013). This evidence of physiological and physical determinants provides clues of an animal’s impending puberty onset. However, Patterson et al. (2002) showed that neither age or body weight (BW) alone can be valuable predictors of puberty onset. Puberty onset is highly regulated by the individual animal’s genetics and is variable in the time of developmental onset (Foster et al., 1994). However, there is a relationship between the optimal timing of sexual maturation and growth in order to reach a threshold to achieve puberty onset (Rozeboom et al., 1995). This was also suggested by Le Cozler et al. (1999) that through restriction of feed (and subsequently growth suppression), the timing at which a female achieves puberty can be significantly altered and delayed. In addition, Foxcroft et al. (1996) suggested that puberty onset in a female is reached well after the female has reached the threshold of growth needed to undergo sexual maturation. Patterson et al., (2010) suggested that before a female reaches a specific body condition and weight her growth rate is paralleled with her age of puberty onset; however, once that level of body condition is reached there is no correlation between the two. Kummer et al. (2009) stated gilts with higher growth rates (724 g/day) from birth to approximately 144 days of age are more likely to achieve puberty onset earlier than their counterparts with lower growth rates (577 g/day). Several reports agree in the average age of puberty attainment in gilts. Rozeboom et al. (1995) showed an average age of puberty in a cohort of gilts reared ad libitum was 172.5 days with a standard deviation of 23.4 days. This
study was similar to reports of Young et al. (1990) and Zimmerman et al. (2000) of 167.2 and 168 days, respectively, for gilts to achieve puberty. This evidence points to the potential reasoning that there is not an individual contribution of body composition or age of puberty attainment, but rather a relationship between the two along with an individual threshold.

Follicular growth within the female gonads is evidence of sexual maturation. As the HPGx is activated and the reproduction cycle begins, follicles on the ovary begin to develop. In the follicle, LH and FSH act on theca and granulosa cells to enable the process of recruitment and growth of Graafian follicles (Lunenfeld et al., 1975). A key indicator of follicular reserve is a hormone known as anti-Müllerian hormone (AMH), a dimeric glycol-protein of the transforming growth factor (TGF-β) family (Kevenaar et al., 2006). Although originally recognized as being produced in the sertoli cells of the male fetus, which initiates the regression of the Müllerian ducts (the precursor to female reproductive tract) in the male fetus during sexual differentiation (Lee and Donahoe., 1993), AMH is now known to also be produced within the granulosa cells of small antral follicles in the female ovary. Its role as a follicular reserve predictor is evident as levels of AMH are decreased or not present at all in larger tertiary follicles destined for ovulation (Pellatt et al 2007). The ability of AMH to decrease during the recruitment of Graafian follicles is important for maintaining the follicular pool. Durlinger et al. (2002) showed that in AMH knockout mice an increase in the number of growing follicles occurred, resulting in an early depletion of the follicular pool. This suggests that AMH is important in controlling the rate of follicle recruitment from small antral to tertiary follicles. As more follicles are recruited during sexual maturation, AMH levels rise as a result of an increase in the number of producing granulosa cells from small antral follicles (Monniaux et al., 2012). A substantial increase in the level of AMH may help
identify the sexual maturation timeline since active follicles are a sign of a functional HPGx and KNDy neuron activation.

**Estrous Cycle**

In swine, a female becomes sexually receptive after she undergoes her first successful estrous cycle (5-8 months of age) followed by repeated 21 day estrous cycles. The estrous cycle is broken down into four stages from follicular recruitment to ovulation and eventually luteal tissue death (Figure 2.3). The four stages of the estrous cycle are estrus (~day 0-2), metestrus (~days 2-5), diestrus (~days 5-16), and proestrus (~days 17-21) (Senger, 1997). Each phase is specifically controlled by either a positive or negative feedback of the steroid hormones E2 and P4. The estrous cycle is also made up of two phases, the luteal and follicular phase. The follicular phase is characterized by the proestrus and estrus stages and is the period of time when follicles are recruited for growth and potential ovulation (Senger, 1997). The luteal phase represents the metestrus and diestrus stages, occurring when P4 is secreted from the corpora lutea (CL) (Senger, 1997). The estrus stage is characterized by the physical sexual behaviors performed by the sow or gilt (Hemsworth, 1985). These include “standing” in response to stimulation from a boar, vocalization, and the innate behavior of chewing on enclosures. In addition to the behavioral response exhibited from the female, physical changes such as vulva swelling and reddening as well as discharge of a clear/white mucous are also observed (Johnson, 2007). This behavioral estrus typically lasts for 24-72 hours. Graafian follicles, formed prior to the estrus stage, produce increased amounts of E2 facilitating a positive feedback effect on the release of GnRH (Senger, 1997). The resulting surge in GnRH causes a LH surge resulting in ovulation, occurring about 55-60% of the way through the estrus phase (Soede et al., 2000). As a result of the luteinization of theca and
granulosa cells following ovulation, E2 production drops significantly. The metestrus phase is characterized by the presence of corpora hemorrhagica, which literally refers to “bloody bodies” (Senger, 1997).

During the luteinization of the theca and granulosa cells E2 production decreases and P4 production slowly increases with the development of the CL, characterizing the beginning of the diestrus phase. This is the longest phase of the estrous cycle due to uterine preparation for embryo implantation and maternal recognition of pregnancy (Senger, 1997). The fully developed CL produces P4 resulting in a period of quiescence in the uterus (Christenson and Day, 1971, Bazer et al., 1979). The uterine environment prepares for the embryo by producing histotroph (uterine milk), a substance beneficial in the growth of the implanted embryo (Roberts and Bazer, 1988). During the diestrous phase, if viable embryos are not present the uterine endometrium synthesizes the luteolytic substance PGF2α (Bazer et al., 1979). When released into the bloodstream PGF2α causes the regression of the CL, subsequently resulting in reduced P4 in circulation (Bazer et al., 1979). The regression of the CL eliminates the P4 negative feedback on GnRH allowing for an increase in GnRH secretion and enabling the start of the proestrus phase. The proestrus phase is characterized by the increased pulse frequency and amplitude of GnRH resulting in the release of LH and FSH from the anterior pituitary, enabling follicular recruitment and the growth of tertiary follicles destined to be Graafian follicles (Baird, 1983). As the tertiary follicles form into Graafian follicles, E2 levels reach their peak, reinitiating the 21 day cycle (Senger, 1997).

**Maternal Recognition of Pregnancy**

The presence of embryos in the uterine horns allow for the release of the maternal recognition of pregnancy signal, thus resulting in the maintenance of the CL past the end of a
normal estrous cycle (Bazer et al., 1986). The maintenance of the CL continues production of P4, lengthening the quiescent period of the uterus and the production of endometrial histotroph (Roberts and Bazer, 1988). Bazer (1989) identified the porcine maternal recognition of pregnancy signal synthesized and secreted from the developing conceptuses to be E2. Later it was discovered that the E2 secreted by the conceptuses is released in two phases (Geisert et al., 1990). The first peak being a short release around day 12 and the second a longer sustained release around day 15-18 (Figure 2.3). The end of the 21-day cycle or a successful pregnancy is characterized by the lysis of the CL by PGF2α. For the continuation of pregnancy, PGF2α must be suppressed or rerouted in an effort to maintain the CL (Bazer et al., 1979). The rerouting of PGF2α is an action mediated by the secretion of the maternal recognition of pregnancy signal (Bazer, 1989). In swine, for the maternal recognition of pregnancy signal to be effective, at least two viable conceptuses must be present in each uterine horn on day’s 12-18 of gestation (Dziuk, 1968). The process by which E2 mitigates the luteolytic effect of PGF2α in pregnant females is described by the endocrine/exocrine theory of maternal recognition (Bazer and Thatcher 1977). In that study, it was suggested that opposed to the normal movement of PGF2α through the uterine endometrial stroma and secretion into the vasculature system (endocrine release), during maternal recognition of pregnancy the E2 secretion from conceptuses redirects PGF2α release from the endometrium directly into the uterine lumen (exocrine release). Zavy et al., (1982) supported this theory by showing that during normal pregnancies and E2 induced pseudo-pregnancies, uterine flushing contained increased levels of PGF2α. To establish and maintain a successful pregnancy, a synchronized effort between the conceptuses and uterine endometrium is required. Upon the release of E2 from the conceptuses, as the maternal
recognition of pregnancy signal, PGF2α secretion is redirected from the vasculature system to the uterine lumen protecting the CL from luteolysis (Bazer and Thatcher 1977).

**Figure 2.3. The estrous cycle and pregnancy recognition.** The estrous cycle is comprised of four stages: estrus, metestrus, diestrus, and proestrus. The estrus phase (Day 0-2) is characterized by the period of Graafian follicle E2 production and the LH surge resulting in ovulation. During metestrus corpora hemorrhagica are formed and E2 production decreases. CL are produced at the beginning of the diestrus phase and P4 production increases negatively impacting GnRH-mediated hypothalamus pituitary gonadal axis signaling. If embryo implantation and release of the maternal recognition of pregnancy signal is not released around day 12 from viable conceptuses, successful pregnancy establishment is not feasible. If viable conceptuses are present, a second, more sustained, release (days 15-18) of conceptus E2 is released to help establish and maintain pregnancy. This conceptus signal reroutes PGF2α secretion allowing for the maintenance of the CL. If no signal is present then PGF2α is delivered to the ovary, causing regression of the CL decreasing P4 production.
**Figure 2.3 (continued).** With the removal of the P4 negative feedback, GnRH increases allowing for new follicular growth and recruitment and the start of a new estrous cycle. The follicular phase characterized by follicular growth and recruitment consists of the proestrus and estrus stage. The luteal phase characterized by P4 production from the CL consists of the metestrus and diestrus phase.

*Estrus Synchronization*

Estrus synchronization is a key component to managing reproductive performance in sow and gilt replacement units. Not only is synchronization important for timely breeding, it allows producers to adequately rotate females in and out of gestation, breeding, and farrowing facilities. There are several methods of estrus synchronization used within the swine industry. Timed artificial insemination (A.I.) allows producers to minimize time spent identifying estrus behavior as well as decreased cost in boar collection and/or semen shipment (Horsley et al., 2005).

The most utilized method amongst large swine facilities is to wean a group of sows all at the same time. Due to the suckling inhibition of LH release by prolactin in swine, after parturition a sow will remain in anestrus as long as piglets are nursing regularly (Bevers et al., 1985). This anestrus period subsides the moment the suckling response is removed. The female will then begin her proestrus phase of Graafian follicle growth and ovulate within 3-9 days (Reese et al., 1982). In industry, the use of exogenous hormone agonists and antagonists most commonly occurs within gilt populations. Matrix®, a synthetic progesterone receptor agonist, is a commonly used product that simulates the presence of CL on the ovary (Flowers, 1999). Gilts that are in their diestrus phase when Matrix® is administered will maintain their CL which eventually undergo regression during the Matrix® supplementation period. For gilts that are in any other phase of the cycle, the presence of P4 causes negative feedback on GnRH “pausing” follicular growth and/or recruitment until Matrix® is removed.
The product is fed for a 14 day period preventing formation of new follicles (via negative feedback on the hypothalamus) and allowing regression of existing CL, thereby upon Matrix® removal all females will be within a few days of the same phase allowing for the group to undergo behavioral estrus within 3-9 days (Flowers, 1999).

**Sow Lifetime Productivity**

Longevity of sows is important for the sustainability of a sow herd as well as the profitability of the enterprise (Stalder et al., 2004). With reproductive failure being a common cause for sow removal, the ability to improve a sow’s lifetime performance by managing numerous variables such as length of herd life, number of parities, and litter size at birth and weaning are crucial to maintaining herd profitability (Stalder et al., 2004). However, altering these factors through management is difficult due to the complexity of reproductive traits, controlled through numerous genetic loci, and being subject to many environmental influencers (Serenius and Stalder, 2006). A sow’s lifetime reproductive performance, in terms of litter performance and longevity, is lowly heritable (0.19) with factors such as feet and leg structure, body conformation, number of non-productive days, age of puberty onset, and age at first farrowing impacting SLP, thus creating difficulty for genetic selection (Serenius and Stalder, 2004; Patterson et al. 2010). With genetics being a difficult medium for increasing sow performance, management practices are one strategy for the improvement of SLP. Flowers (2011) demonstrated the influence of pre-weaning environment on SLP as gilts born of normal litter size cross-fostered to small (≤ 7; n = 1100) or large (≥ 10; n = 1157) litters after farrowing showed drastic differences in their lifetime reproductive performance. In this study, farrowing rates (FR) were higher (88.7 ± 1.8% vs. 83.3 ± 2.1%) in gilts from the smaller litter as well as an increased litter size (11.0 ± 0.2 vs.
10.5 ± 0.2) compared to gilts from larger litters. Likewise, litter size in which gilts were reared also correlated to length of herd life, with 38% of gilts from the small litters still remaining in the herd after 6 parities compared to 16% of gilts from the large litters (Flowers, 2011).

Factors influencing sow lifetime productivity

The physical condition of a sow also plays an important role in maintaining reproductive capability. During gilt development, monitoring the ratio of weight and age as well as back fat thickness helps promote successful gilt growth performance and her timely entrance into the breeding herd (Rozeboom et al., 1996). Altering feeding strategies during puberty attainment is important in building body reserves to last through production as gilts switch from an emphasis in protein accretion to adipose accretion and mineral deposition (Stalder et al., 2004). Stalder et al., (1998) showed that through restricting feed intake by decreasing energy in the diet, gilts had a higher chance of reaching their fourth parity, compared to counterparts fed high energy and high protein diets. Prior research has demonstrated that depth of back fat during first mating is correlated with reproductive performance. Gilts having a higher amount of back fat at approximately 100 kg of body weight have a greater chance of demonstrating a shorter wean to estrus interval after their first farrowing as well as an increase in total piglets per litter during their second farrowing when compared to counterparts with a smaller amount of back fat depth (Tummaruk et al., 2000).

Sow body condition also represents a contributing component to reproductive failure, sow culling, and death (Elsley, 1968). If a sow’s body reserves drop below maintenance level during lactation or gestation, her subsequent breeding and farrowing performance is typically
suppressed (Whittemore et al., 1980). Structural conformation is also important in the physical condition of the breeding sow. Feet and leg structure are important factors contributing to long term stayability as well as accounts for an increased number of culled females during their first parity (Douglas and Mackinnon, 1993).

A gilts ability to express puberty earlier in life is a combined result of physical and physiological factors (Rozeboom et al., 1995). An earlier first estrus can lead to greater productivity once in the breeding herd. As an example, gilts exhibiting their first standing estrus early (< 153 days of age) compared to later (154 to 180 days of age) demonstrate significantly fewer non-productive days (Patterson et al. 2010). This study also shows gilts not exhibiting their first estrus by 180 days of age demonstrated lower service rates (73% vs 94%) compared to their counterparts expressing estrus prior to 180 days of age. Decreased age at puberty has also shown to increase a female’s removal parity (Culbertson and Mabry, 1995). Hoge and Bates (2011) demonstrated that as a female’s age at first farrowing increased by 10 days the risk of culling her increased by 2%. After a gilt’s first farrowing, the ability to return to estrus in a timely manner is important to avoid culling and increasing the length of productive life. Sterning et al. (1998) showed that gilts expressing estrus earlier in life had a greater chance of returning to estrus and ovulating within 10 days after weaning compared to counterparts within the same group demonstrating later puberty onset. In terms of nutrition, Miller et al. (2011) showed while utilizing a restricted feed intake program, gilts with a younger first estrus demonstrated a higher likelihood of producing a first parity.

Environmental factors play an important role in SLP. Managing herds starting at gilt development is crucial for herd sustainability and increasing the number of sows reaching higher parities. With reproductive failure accounting for roughly 30% of culling practices,
managing a female’s reproductive ability is important (D’Allaire et al., 1987). With 40-50% of females being culled by the farrowing of a third litter, the profitability of a sow herd can be marginal (Boyle et al., 1998). Therefore increasing a female’s productive lifetime past three parities, the time when a sow becomes profitable, increases the profitability of a sow herd (Stalder et al., 2003).

**Seasonal Infertility**

In the northern hemisphere, many species demonstrate seasonal breeding and will breed during the winter months so that during parturition food availability and weather will be favorable to offspring. Domestic swine, being polyestrous unlike other domestic livestock species (sheep and horses), do not exhibit seasonal anestrus and demonstrate normal length estrous cycles year round. However, one problem plaguing the modern production pig is bouts of seasonal infertility (SI) during the late summer and early fall months (Love, 1978). Many questions with respect to both cause and mitigation surround this phenomenon and its potential contribution to a national reduction in reproductive performance. While SI affects all ranges of production systems (size, environment) in many countries, there lacks consensus on the factors causing SI and the importance of each factor (Hurtgen and Leman, 1980; Peltoniemi et al., 1999). The specific factors influencing SI are shrouded in debate, as there are many influencers from genetics to environment. The most studied factors include photoperiod, heat stress, genetics, and management (Love et al., 1993; Prunier et al., 1994; Auvigne et al., 2010).

*Factors Influencing Seasonal Infertility*

Photoperiod may be an important influencer as this is the mechanism that regulates normal seasonal breeders (Love et al., 1993). The synthesis and secretion of melatonin levels,
produced in the pineal gland, fluctuate based on the amount of available sunlight within a day, ultimately affecting the start and stop of anestrous periods in animals (Revel et al., 2009). Since domestic swine are polyestrous, the effects of photoperiod are debatable. However, the bout of SI in the summer months has lead researchers to study this mechanism in domestic swine to identify a relationship between photoperiod and SI. Melatonin levels have been shown to mimic the circadian rhythm found in wild boars (seasonal breeders) leading to the conclusion that photoperiod could be an important factor of SI (Tast et al., 2001). However, many studies have concluded that SI is not a consistent problem across animals and years (Auvigne et al., 2010). This leads to the question, are there shifting factors that magnify photoperiods bringing about SI? As wild boar are seasonal breeders, genetic parameters are quickly indicted as an influencer due to the evolutionary relationship of wild boars and domestic swine. However, the inconsistency of SI amongst specific animals and the high variability in reproductive performance suggests otherwise (Martinat-Botté et al., 1984)

High ambient temperature (or heat stress) has commonly been implicated as an influencer of SI, largely due to the correlation of SI occurring during the hottest parts of the year (Prunier et al., 1994). Auvigne et al. (2010) showed, across a five year period, the year with the highest number of hot days showed the largest statistical difference in decreased reproductive performance. Collectively, these observations lead to a conclusion that increased ambient temperatures do not alone influence SI, but could exacerbate other factors contributing to reduced fertility during SI. One factor that has been shown to have a larger effect on SI when accompanied with heat stress is nutrition and nutritional management. When a female reaches her critical temperature limit (27-30°C), feed intake decreases
If heat stress occurs during lactation and feed intake decreases, the female’s ability to rebreed can be hindered (Prunier et al., 1996). Likewise, if nutritional challenges occur during gestation (early/late), then litter performance can be affected as well as an increased number of non-pregnant females (Rozeboom et al., 2006). Nutrition during times of SI is important in day to day management to ensure a females nutritional requirements are met and altered to account for the incidence of increased ambient temperatures.

Endocrine control has also been shown to play a role in the onset of SI. The neuropeptide kisspeptin, produced from the KISS1 gene, controls the release of GnRH (Navarro et al., 2009). With GnRH being an initiating hormone of the HPGx, affecting its secretion patterns can alter the steroid hormone responses controlling normal reproductive function. Revel et al., (2006) showed KISS1 expression was decreased in short day breeding hamsters and through administration of kisspeptin reproductive function was restored. Melatonin has been shown to also contribute to the expression and regulation of the hypothalamus in hamsters (Ansel et al., 2010). Although some similarities exist between the breeding patterns of swine and hamsters there has not been evidence of kisspeptin control through photoperiod responses in the pig.

Reproductive Efficiency

SI is detrimental to the economics of the swine industry. The main reproductive performances affected are decreased FR, prolonged wean-to-estrus interval, decreased litter performance, and increased age at first estrus (Peltoniemi et al., 1999; Xue et al., 1994; Hurtgen et al. 1980).
Negative effects on FR have shown to have the biggest impact on reproductive efficiency within swine herds (Peltoniemi et al., 1999). Early pregnancy loss is the main cause of decreased FR and can be affected by nutritional management and/or disruption in endocrinology patterns. Love et al. (1995) showed that sows fed low intake diets (1.6–2.0 kg per sow per day) during gestation compared to their counterparts fed high intake diets (> 3.6 kg per sow per day) had a decreased FR during the summer and autumn months. In the same study when comparing FR during different periods (winter vs summer), lower intake diets that had decreased FR during the summer months were sufficient for sustained FR in the winter months. This data summarizes that FR is not solely affected by nutrition, but may be affected by the additive effect of season and nutrition. Season alone has also shown to affect FR, in that females bred in December and January had a higher FR (79.2% vs 74.1%) than females bred in July and August (Xue et al., 1994).

Interrupted pregnancy signals as a result of disruption in hormone patterns have been shown to affect FR. In normal maternal recognition of pregnancy there are two E2 signals produced from the growing embryo, on day 12 and days 15-18. The interruption of these signals through the regression of the CL causes embryonic death (Geisert et al., 1990). Regression of the CL due to irregular patterns of LH production causes total pregnancy failure if the regression occurs any time during gestation, due to the subsequent decrease in P4 production (Peltoniemi et al., 1995). LH has been shown to demonstrate secretory patterns in a seasonal fashion (Peltoniemi et al., 1997). This seasonal control of LH production has a direct effect on P4 production from the CL. A decrease in P4 can result in decreased suppression of PGF2α causing early loss of pregnancy (Spencer and Bazer, 2002). While it is still not clear on the overall effects of photoperiod on hormone regulation, the evidence of
photoperiod-associated variations in LH and its effect on pregnancy success (Tast et al., 2001) leads to the hypothesis that a seasonal decrease in FR could be a result of an interruption and/or failure of the HPGx.

The wean-to-estrus interval is a crucial time in a sow’s reproductive cycle. A prolonged period can interrupt breeding cycles and affect lifetime performance. The exact events causing an increased wean-to-estrus interval range from interruptions in ovarian activity to decreased feed intake (Prunier et al., 1996). Nutrition during lactation is crucial, as any deficiency can cause an increase in the number of days during the wean-to-estrus interval (Dourmad et al., 1994). Low nutrition availability during the summer months also showed to increase the number of sows with prolonged (25-36 days) wean-to-estrus periods (Love et al., 1995). Hurtgen et al. (1980) showed the seasonality of prolonged wean-to-estrus intervals in sows weaned July through September only 68.6% returned to estrus within 7 days compared to 82% during the remainder of the year. Other studies are in agreement, demonstrating lower percentages of irregular wean-to-estrus intervals in the winter months compared to summer months (Xue et al., 1994).

Decreased litter performance and increased age at first estrus are also factors impacted by SI. The age at which a female achieves puberty can be altered by several factors, be it nutritional or environmental, and when puberty is delayed reproductive performance can be negatively affected (Tummaruk et al., 2007). Bertoldo et al. (2009) showed that age at first breeding was a significant factor in FR amongst gilts, in that gilts bred before 220 days of age had a lesser chance of late pregnancy loss. Peltoniemi et al. (1999) demonstrated that season played a role in age at first breeding by demonstrating that females bred from January to June were at a younger age (231.3 days) compared to those bred from July to December.
(236.9 days). With the association of photoperiod and increased ambient temperature to the instances of increased age at first estrus, the attainment of puberty during these periods suggests that season can prolong the timing of puberty onset (Love et al., 1993, Flowers et al., 1989).

Litter performance is highly affected by environmental influencers. Both ambient temperature and photoperiod have been shown to affect the total litter number as well as litter weight (Prunier et al., 1994, Xue et al., 1994). Xue et al. (1994) showed females bred during the winter months had a higher number of pigs born alive (10.39 vs 9.84) and higher litter birth weights (15.20 vs 14.28 kg) than females bred during the summer months. Likewise, Prunier et al. (1994) showed that live birth weights where higher in piglets born during the summer months compared to winter months.

Seasonal infertility onset is controlled through several influencers and affects many facets of the breeding herd. Nutritional and environmental management are critical to mitigating the effects of SI. The above referenced literature suggests that there is not a specific influencer that brings about the onset of SI, but the association of several factors. It is plausible that photoperiod is a major influencing factor, with increased ambient temperatures intensifying the negative aspects of SI (Auvigne et al., 2010). Further characterizing and understanding the biological basis of SI in pigs is critical for the development of effective mitigation strategies to minimize the negative production consequences and enable improved production efficiency in the US swine herd.

**Summary**

Reproductive performance of a sow herd is crucial for sustainability and profitability. The HPGx is the regulating factor of the reproduction cycle and any interruptions can cause a
variety of performance failures. Specific hormone crosstalk and feedback is important to continue the cycle from puberty attainment to follicular development to ovulation and ultimately pregnancy. Delayed or altered performance of any of these can affect SLP. Altered production efficiency due to environmental factors have shown to increase the instances and severity of SI. To better understand how to prolong and increase SLP as well as what specific environmental influencers impact SI severity, it is necessary to understand: 1) variation among gilt ovarian development and sexual maturation in relation to puberty attainment and 2) the relationship between a gilt’s susceptibility to heat stress and their reproductive success during heat stress periods.

In the following research, the objective of the first study was to identify physical and hormonal characteristics that could be used to identify the variation in ovarian development and sexual maturation within a group of gilts. This variation was used to identify the association between the timing of puberty attainment of the same group of gilts. As decreased age at puberty is associated with increased SLP, identifying pre-pubertal markers of puberty onset would also allow for identifying pubertal age earlier, thereby enabling gilt selection (and exclusion) for replacement sooner. The objectives of the second study were to determine the repeatability of an individual gilt’s susceptibility or tolerance to heat stress and the influence of heat stress during follicular development on gilt reproductive performance.
CHAPTER III: IDENTIFICATION OF MEASURES PREDICTIVE OF AGE AT PUBERTY ONSET

To be submitted to the Journal of Animal Science

K.L. Graves*¹, B. Mordhorst*², E.C. Wright*², B.J. Hale*², K.J. Stalder*³, A.F. Keating*³, J.W. Ross*¹

¹ Department of Animal Sciences, Iowa State University, Ames, Iowa 50010

Abstract

A potential indicator of female lifetime productivity in swine is the age of puberty, or when a gilt achieves her first normal behavioral estrus. Research has determined that follicular activity, as determined by tertiary follicle development, in pre-pubertal gilts begins during postnatal days (PND) 85-115 (Pearce, Ross, Keating, and Baumgard, unpublished results). The central hypothesis of this study is that gilts demonstrating tertiary follicular development earlier in life are more likely to achieve puberty earlier compared to counterparts of a similar age and weight that lack tertiary follicular development. The objectives of this project were to identify a developmental time point when variation in ovarian development exists and to determine if there is relationship between the pre-pubertal ovarian development and the onset of puberty. To accomplish this, 155 gilts of similar age (± 2 days) were weighed, blood collected, and vulva size measured on PND 75, 85, 95, 105 and 115. Circulating kisspeptin-10 and anti-müllerian hormone levels were measured. Vulva

¹ Responsible for experimental design and manuscript preparation.
² Assisted in data and sample collection.
³ Provided assistance with statistical analysis and experimental design.
measurements, including vulva width, length and area were utilized as developmental proxies for follicular activity. At each time point, 10 gilts were sacrificed and ovarian follicular activity was recorded. For the remaining 105 gilts, estrus detection was conducted daily on PND days 126 to 200. Mean vulva area (VA) on PND 75, 85, 95, 105 and 115 was 596 ± 206, 683 ± 190, 864 ± 212, 1014 ± 228 and 1265 ± 252 mm², respectively. Of the gilts demonstrating behavioral estrus, 28 were within PND 140-160, 36 between PND 161-180, 15 between PND 181-200, and 26 did not demonstrate estrus behavior within 200 days of age. All gilts euthanized at PND 75 lacked follicular activity as defined by having a minimum of two antral follicles per ovary, while 6/10, 8/10, 9/10 and 10/10 demonstrated follicular activity on PND 85, 95, 105, and 115, respectively. Body weight at PND 75 and vulva width at PND 115 were both significantly correlated to age at first estrus (P < 0.05). Of the gilts whose VA was less than one standard deviation from the mean on PND 95 (i.e. < 652 mm²), 31% and 50% demonstrated their first behavioral estrus by PND 180 and 200, respectively. However, of those gilts whose VA was within or greater than one standard deviation from the mean (i.e. ≥ 652 mm²), 66% and 79% exhibited estrus prior to PND 180 and 200, respectively. Kisspeptin-10 levels were numerically greater, although not statistically significant, in those gilts achieving puberty prior to 200 days compared to their counterparts, at all postnatal points measured. Anti-müllerian hormone was not different on PND 95 between gilts achieving puberty at different ages. These data suggest that utilization of VA changes between 95 and 115 days of age could be a useful tool to identify replacement gilts prior to puberty for inclusion into the sow herd.

Key words: puberty, follicle development, gilt development
Introduction

Age of first estrus, or puberty onset, has a positive predictive value for future reproductive performance within a breeding herd (Patterson et al., 2010; Culbertson and Mabry, 1995; and Sterning et al., 1998). This is potentially associated with sow lifetime productivity (SLP), or the number of quality pigs a sow produces from the time she becomes breeding eligible until she leaves the herd. Improvement in SLP is difficult as the genetic selection for SLP has low heritability (0.19) due to the trait be controlled by numerous loci (Serenius and Stalder, 2004). Additionally, SLP is influenced by several postnatal factors, such as structural conformation, number of non-productive days, pre- and post-wean environmental factors, and age of puberty onset (Serenius and Stalder, 2006 and Patterson et al., 2010). Age at puberty onset may represent a useful phenotype that can be utilized to select or reject gilts entering the breeding herd. As gilts comprise a large portion of the breeding herd, their reproductive efficiency when they enter the herd can greatly affect the overall performance of the herd as well as individual lifetime performance (Spörke, 2005). As an example, gilts exhibiting their first behavioral estrus early (< 153 days of age) compared to later (154 to 180 days of age) demonstrate significantly fewer non-productive days (Patterson et al. 2010). Of additional importance, Patterson et al. (2010) demonstrated that gilts not exhibiting estrus by 180 days of age had a lower service rate than gilts exhibiting estrus prior to 180 days of age. Of those gilts achieving puberty prior to 180 days of age, 94% were eventually bred compared to only 73% of those achieving puberty after 180 days of age. A younger age at first estrus has also been correlated to the increased likelihood a female will reach her third parity (Engblom et al., 2007).
Physical growth patterns may represent useful markers in identifying gilts that are capable of achieving puberty onset earlier than their counterparts within the same herd. Leaner swine as a result of genetic selection and nutritional management can result in a delay of puberty onset (Evans and O'Doherty, 2001). Therefore managing growth rates may be important in selecting gilts with the ability to achieve puberty at a younger age. Kummer (2009) showed that gilts with heavier growth rates at time of boar exposure achieved puberty earlier than their counterparts. In relation to this, gilts with heavier body weights (BW) at time of boar exposure were younger at puberty onset (King, 1989). Increased BW is connected to puberty onset through elevated levels of important endocrine hormones. Leptin, a hormone secreted from adipose tissue, increases as fat deposition proliferates and as leptin levels increase, the leptin receptors (LEP-R) within the brain are stimulated and become active (Ahima et al., 1997). Kisspeptin neurons, located in areas of the brain associated with the hypothalamus, express LEP-R and, when stimulated, activate the release of the neuropeptide kisspeptin through the circular feedback of KNDy neurons (Ahima, 2011). Kisspeptin is a 145 amino acid neuropeptide produced by the KNDy neurons and is best known for its activation role of the hypothalamic pituitary gonadal axis (HPGx) (Seminara and Crowley, 2008). These relationships suggests the potential for increased leptin, through the progression of body growth, to influence kisspeptin secretion and the subsequent activation of the HPGx leading to puberty onset.

Puberty onset begins before a demonstrated behavioral estrus occurs. Numerous physiological factors, such as activation of the HPGx and initial follicular development preceding puberty onset can be quite variable among a cohort of gilts. Schwarz et al. (2008) showed some of this variation among gilts by identifying the beginning of follicular growth
over a range of 60 to 100 days of age. In a previous study, variation in follicular development of gilts was observed at approximately 98 days of age despite similar weight 35 ± 2 kg BW (Pearce, Ross, Keating, and Baumgard, unpublished results). Of forty-eight gilts, 21 of them possessed undeveloped ovaries compared to the other 27 gilts having ovaries with numerous tertiary follicles (Figure 3.1). This observation led to the hypothesis that gilts demonstrating tertiary follicle development earlier in life are more likely to achieve puberty earlier compared to counterparts of a similar age and weight that lack tertiary follicle development. The objectives of the following study were to identify a developmental time point when variation in ovarian development exists and to determine if there is relationship between the pre-pubertal ovarian development and the onset of puberty.

**Materials and Methods**

This study was conducted at Iowa State University with animal procedures approved by the Iowa State University Animal Care and Use Committee.

**Animals**

One hundred and fifty five crossbred (Yorkshire x Landrace x Duroc) gilts of a similar age (65 ± 2 days) were selected and housed in pens of four and provided *ad libitum* access to feed and water for the project duration.

**Data, Blood, and Tissue Collection**

On postnatal days 75, 85, 95, 105, and 115 of age (PND) each gilt was weighed, blood samples were collected for serum and plasma isolation, and vulva measurements were collected. Vulva length (VL) and vulva width (VW) measures were obtained using Ultra Tech digital calipers (General Tools, Secaucus, NJ) and recorded in millimeters. Vulva area (VA) was later determined by multiplying the vulva length with the vulva width. At each of
the PND time points, 10 gilts were randomly selected and sacrificed. The reproductive tract including the uterus and ovaries were harvested from each sacrificed gilt following euthanasia. The reproductive tract, from the cervix to the oviducts, was weighed and recorded. Ovaries were visually evaluated to get a quantitative assessment of the number and size of tertiary follicles for each PND.

**Puberty Detection**

The remaining 105 gilts received daily boar exposure from 126 to 200 days of age to detect the age at first estrus, which was defined as the age of puberty attainment. Two sexually mature boars were used for daily exposure to all gilts. Gilts received a minimum of 60 minutes of indirect exposure and 10 minutes of direct exposure to the boars each day. During heat detection, gilts were examined for signs that puberty onset did in fact occur such as swollen and red vulva, interest in boars, and response to back pressure concomitant with boar exposure. The age at which gilts reached puberty onset was designated to be the first day behavioral estrus (standing heat) was observed.

**Hormone Assays**

The ligand kisspeptin-10 (Kp-10) was selected for its known conducting role in the activation of the HPGx (Lents et al. 2008). A product of the Kiss-1 gene, Kp-10 is a 10 amino acid residue of the larger 145 amino acid polypeptide, and is important as it is the smallest residue needed to stimulate GPR54 (Bilban et al. 2004). Anti-müllerian hormone, a product of female granulosa cells, was selected as it is a marker of the ovarian follicular reserve (Kevenaar et al. 2006).

Plasma Kp-10 levels were determined using a competitive binding fluorescent enzyme-linked immunosorbent assay (ELISA) for the Kp-10 (Metastin 45-54) polypeptide
(Cat# FEK-048-56, Phoenix Pharmaceuticals, Burlingame, CA, USA). All assays were performed strictly following the manufacturer’s guidelines. Samples were randomized so that each plate had at least one representative of each puberty age group. The sensitivity of the ELISA kit was a minimum detectable level of 6.6 pg/mL and had an average intra-assay coefficient of variation (CV) of 20% and an inter-assay CV of 19%.

Serum AMH levels were measured using a DuoSet ELISA labeled for human MIS/AMH (Cat # DY1737, R&D Systems, Minneapolis, MN, USA). All assays were performed strictly following the manufacturer’s guidelines. For the AMH assays only samples from PND 95 time point were used as this day was where the most variation was observed in vulva and follicular development. Samples were randomized so that each plate had at least one representative of each puberty age group. The microplate reader was set to read light absorbance at 450 nm with a wavelength correction of 540-570 nm. The sensitivity of the ELISA kit was a minimum detectable level of 93.8 pg/mL and had an average intra-assay CV of 8% and an inter-assay CV of 7%.

Statistical Analysis

Statistical Analysis Systems (SAS) University edition (Cary, NC 27513) was used for all statistical analysis. Correlations between physical measurements and age of puberty of each gilt were determined using the PROC CORR function of SAS. Physical measurements were analyzed by puberty age groups as previously described as well as gilts achieving puberty before 200 days and those considered non-responsive (puberty not detected within 200 days of age). Chi-Square ($X^2$) analysis was performed using the PROC FREQ function of SAS to determine the association of physical measurements and ability to achieve puberty.
before or after 180 and 200 days of age. Correlation between hormone concentrations and age of puberty was determined using the PROC CORR function of SAS.

**Results**

*Follicular Development*

A distinct amount of variation in the number of measurable follicles existed in ovaries from the groups of sacrificed gilts (10 each on days 75, 85, 95, 105 and 115). The 10 sacrificed gilts on PND 75 demonstrated a complete lack of follicular activity while all 10 of the sacrificed gilts from PND 115 demonstrated some form of follicular activity (Figure 3.2). Concomitant with increased follicular development, uterine weights from sacrificed gilts increased at each developmental stage. The average uterine weight on the five PND time points were 12.8 ± 2.35, 25.7 ± 3.04, 35.6 ± 2.84, 43.9 ± 3.96, and 54.7 ± 3.91 grams, respectively and were significantly affected by day of age ($P < 0.05$; Figure 3.3).

*Puberty Onset*

Puberty onset was documented as the age when the first behavioral estrus was observed. The earliest behavioral estrus identified was on PND 140 with a total of 79 gilts demonstrating a behavioral estrus while 26 gilts did not achieve estrus by 200 days of age (Figure 3.4). The average age of puberty onset for the 79 gilts that demonstrated estrus was 165 days of age. At the conclusion of boar exposure the gilts were categorized into four groups, for data analysis, based on their age of first estrus for data analysis: early puberty (EP; first estrus by PND 140-160) intermediate puberty (IP; first estrus by PND 161-180), late puberty (LP; first estrus by PND 181-200), and non-responsive (NR; first estrus not observed by PND 200). The groups were divided into 20 day increments to correspond
approximately with one estrous cycle, starting on the day the first gilt demonstrated estrus (140 days of age) and ending when boar exposure ceased.

**Relationship of Puberty Onset with Growth Parameters**

**Body Weight**

The BW of the 105 gilts used for estrus detection increased across the five PND time points ($P < 0.05$). The average BW in kilograms (kg) of gilts was $38.6 \pm 0.5$, $49.1 \pm 0.6$, $57.8 \pm 0.7$, $68.1 \pm 0.7$, and $78.8 \pm 0.8$ kg, at PND 75, 85, 95, 105, and 115, respectively (Table 3.1). X² statistics showed no significant association between BW and a gilts ability to achieve puberty by either 180 or 200 days of age or after ($\chi^2 > 0.05$). However, of the gilts that demonstrated estrus behavior, the correlation between BW and the age of puberty onset was closest to significance at PND 75 ($P = 0.055$), but was not significant at all remaining time points (Figure 3.5). The correlation suggests that of the gilts cycling by 200 days of age, the heavier gilts at PND 75 demonstrated an earlier age of first estrus than their lighter counterparts. To further visualize the data, when broken down into age of puberty groups (EP, IP, LP, and NR) the mean BW was numerically greater for gilts in the earlier puberty groups (EP & IP) than those of the later puberty groups (LP & NR) across all PND (Appendices A.3.1 – A.3.5).

**Vulva Development**

Vulva width (VW), vulva length (VL) and vulva area (VA) all increased over the five PND time points ($P < 0.05$). The average VW of gilts on the five time points were $22.8 \pm 0.5$, $24.7 \pm 0.4$, $27.8 \pm 0.4$, $30.1 \pm 0.4$, and $33.0 \pm 0.4$ mm, respectively (Table 3.2). X² values for VW on PND 75, 85, 95, 105 and 115 to determine any association between VW and a gilts ability to achieve puberty by 200 days of age were 0.56, 0.74, 0.07, 0.24 and 0.84.
respectively, suggesting that VW on PND 95 was marginally predictive of whether or not a gilt would achieve estrus prior to PND 200. However, of the gilts that demonstrated estrus behavior, a correlation between VW and the age at first estrus existed on PND 105 ($P = 0.07$) and 115 ($P = 0.01$) although was not significant at all remaining time points (Figure 3.6 and 3.7). This data suggests that between days 105 and 115 gilts with larger VW have a greater probability to achieve puberty at an earlier age when compared to gilts with smaller VW at the same age. When the average VW were determined for each puberty group, gilts from the earlier puberty groups (EP & IP) showed numerically larger VW when compared to gilts from the later puberty groups (LP & NR) across all PND (Appendices A.3.6 – A.3.10).

The average for VL from the five PND time points were $26.2 \pm 0.6$, $27.7 \pm 0.4$, $31.3 \pm 0.5$, $34.1 \pm 0.5$ and $38.2 \pm 0.5$ mm, respectively. The VL measure lacked significant correlation to age of puberty onset (Table 3.3). The $X^2$ values for VL on PND 75, 85, 95, 105 and 115 to determine any association between VL and a gilts ability to achieve puberty by 200 days of age were 0.97, 0.93, 0.10, 0.69 and 0.47, respectively, suggesting that VL on PND 95 was marginally predictive in determining whether or not a gilt would achieve estrus prior to PND 200. Unlike VW, no correlation with any of the PND that data was collected existed between VL and the age of first estrus for the gilts that achieved puberty by 200 days of age. The lack of correlation in VL to gilt age of first estrus can be further visualized when the average VL were broken down into puberty groups. Gilts from the earlier puberty groups (EP & IP) had numerically larger VL across all PND comapred to the gilts from the later puberty groups (LP & NR) though on some days this data lacked the consistency observed with VW (Appendices A.3.11 – A.3.15).
The VA (VW x VL) average for PND 75, 85, 95, 105 and 115 were 612.6 ± 24.6, 694.0 ± 21.2, 879.9 ± 22.7, 1036.8 ± 26.4 and 1269.5 ± 28.0 mm², respectively (Table 3.4). Chi-square values for VA on PND 75, 85, 95, 105 and 115 to determine an association between VW and a gilts ability to achieve puberty by 200 days of age were 0.56, 0.90, 0.10, 0.39 and 0.64, respectively, suggesting that VA on PND 95 was marginally predictive of whether or not a gilt would achieve estrus prior to PND 200. Unlike VW, and similar to VL, no significant correlation between VA and the age to first estrus existed for the gilts that demonstrated estrus behavior for any of the PNDs that data was collected. Gilts in the earlier puberty groups (EP & IP) had numerically larger VA than those in the later puberty groups (LP & NR) on some days although this data lacked the consistency observed with VW and is likely the influence of VL impacting the VA calculation (Appendices A.3.16 – A.3.20).

To determine the practicality of selecting gilts based on VA prior to puberty, gilts were retrospectively grouped, based on VA, into two groups for each PND of data collection. Groups included those gilts with a VA less than 1 standard deviation from the mean and the remaining gilts (VA within or greater than one standard deviation from the mean). Of the gilts who had a VA less than one standard deviation of the mean at PND 75 and 85 of age, 54% and 53%, respectively, achieved puberty by 180 days of age. However, at PND 95, 105, and 115 days of age only 31, 38 and 36% of gilts with a VA less than one standard deviation from the mean achieved estrus by 180 days of age compared to 66, 66, and 64%, respectively, of all other gilts reaching the same benchmark (Figure 3.8). This result was consistent with determining the ability of gilts grouped by their VA to achieve their first estrus by 200 days of age. On PND 75 and 85, the percentage of gilts achieving their first estrus by 200 days of age was the same between gilts that had small vulvas and normal vulva
size. However, 20-30% less gilts achieved estrus by PND 200 if their VA was below one standard deviation from the mean on days 95, 105 or 115 (Figure 3.9). Collectively, these data suggest that VA could be a useful tool to eliminate gilts from the gilt pool after PND 95 as it is predictive of successful sexual development, but is not useful prior to PND 85.

**Correlation Between Blood Markers and Age at Puberty**

**Kisspeptin -10**

Plasma Kp-10 levels were measured in all samples and mean values per day are presented in Table 3.5. Gilts that achieved puberty within 200 days of age had numerically greater (Figure 3.10) Kp-10 levels on PND 75, 85 and 95 although not statistically significant ($P > 0.05$). Of the gilts that achieved estrus before 200 days of age, other than a weak, and statistically marginal correlation ($P = 0.07$) no significant correlations between plasma Kp-10 and the age of first estrus existed on any of the PNDs that data was collected.

**Anti-Müllerian Hormone**

Anti-müllerian hormone (AMH) was only measured in PND 95 serum samples. This time-point was chosen because of the amount of variation observed in tertiary follicle development from the sacrificed gilts at this time point. The AMH levels were not different across the four puberty groups (EP, IP, LP, NR) (Figure 3.11). No statistical differences were observed between gilts achieving puberty by 200 days of age and those considered non-responsive ($P = 0.68$).

**Discussion**

The onset of puberty is a time point when metabolic, endocrinological, and physical factors are associated with achieving sexual maturation. Gilts make up a substantial portion of the breeding herd, thus the time when they can be bred and their ability to produce a litter
can greatly affect the overall performance of the herd as well as the animal’s individual lifetime performance (Spörke, 2005). Patterson et al. (2010) showed that neither gilt age or (BW) growth alone are valuable predictors of age of puberty onset. Age at puberty onset is highly regulated by the individual animal’s genetics and is variable in the time of developmental onset (Foster et al., 1994). The objectives of this study were to identify a developmental time point when variation in ovarian development exists and to determine if there is a relationship between the pre-pubertal ovarian development and the onset of puberty. Rozeboom et al. (1995) reported there is a relationship in the timing of sexual maturation and growth in reaching a threshold to achieve puberty onset. This is in agreement with work by Le Cozler et al. (1998), that through restriction of feed, and subsequently growth, the timing at which a female reaches puberty can be significantly delayed. Foxcroft et al. (1996) suggested that age of puberty onset in a female is reached well after the female has reached the threshold of growth needed to undergo sexual maturation. In the present study, blood sampling and collection was conducted on PND 75, 85, 95, 105 and 115 in addition to vulva measurements on 155 gilts. Gilts were subsequently euthanized at each time-point (n = 50) to verify follicular activity at each age. This provided the ability to identify a specific timeline when a group of pre-pubertal gilts began follicular development and show distinct variation between gilts. A 40 day timeline of growth and development was observed where variation in follicular activity amongst the gilts progressed from all gilts having no tertiary follicular activity at PND 75 to almost all of the gilts demonstrating increased tertiary follicular activity on PND 115. The goal from the present study was to identify the potential of physical and blood markers to identify gilts from a group with the best probability to demonstrate early
follicular activity and determine if that can be correlated to their ability to undergo their first estrus at an earlier age than their counterparts.

Boar exposure was conducted on the remaining 105 gilts on PND 129 until PND 200 to identify the age at first estrus. The earliest behavioral estrus identified was on PND 140 with a total of 79 gilts demonstrating a behavioral estrus and 26 gilts not achieving estrus by 200 days of age. The average age at estrus onset for the 79 gilts that demonstrated estrus was 165 days of age. These results are consistent with those from Rozeboom et al. (1995), reported the average age at puberty in a group of gilts reared ad libitum was 172.5 days with a standard deviation of 23.4 days. Similar reports from Young et al. (1990) and Zimmerman et al. (2000) demonstrated an average age at puberty was 167.2 and 168 days, respectively.

Kummer et al. (2009) suggested gilts with greater growth rates are more likely to have a decreased age at puberty than their cohorts that have lower growth rates. The present study tested the ability of body weight to predict puberty onset and showed that the utilization of BW on PNDs 75-115 was not useful in predicting whether or not gilts would achieve their first estrus by 200 days of age. However, it was demonstrated that BW on PND 75 did have a statistical correlation with the age of estrus of those that did achieve puberty by 200 days of age, meaning that gilts weighing more on PND 75 have a higher likelihood to achieve estrus earlier than their counterparts at the same age.

Vulva growth was measured and utilized as a sign of increased follicular activity, and subsequently, elevated E2 in circulation. Biologically we expected to see the largest difference amongst measurements between PND 85-105 as most variation observed in follicular activity amongst the sacrificed gilts was on PND 85 and 95. Vulva development from all gilts showed the most variation in VW on PND 95. The variation on this PND
provided the most reliable prediction of whether or not a gilt has the ability to achieve her first estrus by 200 days of age ($X^2 = 0.07$). Vulva width on PND 105 and 115 was predictive in determining how early a gilt will achieve puberty amongst the gilts who demonstrated their first estrus by 200 days of age ($P = 0.07$ and $P = 0.01$ respectively).

With the variation amongst follicular activity and growth parameters it makes physiological sense that some variation would exist in reproductive endocrine hormone levels. The ligand Kp-10 is thought to be an contributing activator of the HPGx through the stimulation of GnRH (Goodman et al., 2007), thus making it a potential upstream regulator of E2 production and initial follicular development. However, Kp-10 was poorly correlated with the parameters evaluated in the present study. Only weak correlations with age at puberty existed and were statistically marginal, despite plasma Kp-10 levels being numerically greater on all PND in those gilts that achieved puberty prior 200 days compared to those who did not.

In the present study, AMH, a product of granulosa cells, was measured as its increase in circulating levels correlates to an increased number of small antral follicles capable of becoming tertiary follicles (Monniaux et al. 2012). In the present study, only this hormone level on PND 95 was measured based on the relationship of vulva growth and puberty being the greatest at PND 95. Similar to Kp-10 results, no statistical significant relationship was observed between pre-pubertal AMH levels with a gilt's ability to achieve estrus by 200 days. The E2 levels were unable to be measured due to pre-pubertal levels being lower than the detectable range of the assays utilized. As circulating E2 indicates a functioning HPGx, being able to measure it has the potential to identify a more accurate timeline of puberty onset. To
detect E2 activity in the present study, VA was measured on all PND since vulva growth during sexual maturation is a direct effect of increased E2 levels.

In summary, follicular activity during pre-pubertal gilt development begins between PND 75 and 85. Subsequently, PND 95-115 represents a useful timeline to better pinpoint when variation in follicular development occurs amongst a cohort of gilts. Variation in follicular activity is most notable when looking at the parameters of growth and development, specifically vulva development. To separate gilts into groups based on achieving puberty within 200 days of age, VW represented a useful predictive tool on PND 95. However, when trying to identify how early a gilt undergoes puberty amongst the cohort that achieved estrus by 200 days of age, VW at PND 115 had the best predictive value. Physiological measurements of Kp-10 and AMH did not provide a reliable predictive value to separate gilts on their ability to achieve puberty by 200 days of age, however, Kp-10 was numerically greater at each of the five PND in the gilts achieving puberty by 200 days of age, compared to those who did not. Altogether this data shows that selection of gilts based on vulva differences before PND 95 is not useful in identifying those gilts who will achieve puberty before 200 days of age. After PND 95, the present study showed that differences in vulva development represented a useful tool in identifying gilts with the ability to demonstrate their first behavioral estrus before 200 days of age.

**Conclusion**

Since the timing of puberty onset is highly variable and is labor intensive to determine through heat detection, it is usually not recorded within the swine industry. Given that animals grow at different rates and that certain physiological measures must be met for puberty to occur, there is likely a time point when animals differ in growth parameters and/or
hormone levels that correlate to puberty onset. Body weight at PND 75 and the change in vulva development spanning PND 75-115 can be useful in making selection decisions focused on increasing the number of gilts capable of achieving puberty onset by 200 days of age. Additionally, Kp-10 and AMH levels represent unlikely tools in identifying gilts that will undergo puberty onset by a certain age. Altogether the physiological development of the vulva may be the best tool to select gilts that have a higher chance of entering the replacement herd and becoming more productive as evident by a decreased number of non-productive days and an increased productive lifetime.

Acknowledgements

The authors would like to acknowledge Jacob Schulte, Natasha Hemann, and Jordan Nankivil for their assistance in collecting data, monitoring daily animal care and maintenance of facilities.

Declaration of Interest

Results described here within were supported by the National Pork Board. Any opinion, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the National Pork Board. No conflicts of interest, financial or otherwise are declared by the author (s).
### Table 3.1. Relationship between body weight during pre-pubertal growth and age at puberty onset.

<table>
<thead>
<tr>
<th>PND&lt;sup&gt;1&lt;/sup&gt;</th>
<th>N&lt;sup&gt;2&lt;/sup&gt;</th>
<th>r-Value&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-Value&lt;sup&gt;3&lt;/sup&gt;</th>
<th>BW ± SEM&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>79</td>
<td>-0.22</td>
<td>0.05</td>
<td>38.6 ± 0.5</td>
</tr>
<tr>
<td>85</td>
<td>79</td>
<td>-0.14</td>
<td>0.23</td>
<td>49.1 ± 0.6</td>
</tr>
<tr>
<td>95</td>
<td>78</td>
<td>-0.14</td>
<td>0.22</td>
<td>57.8 ± 0.7</td>
</tr>
<tr>
<td>105</td>
<td>79</td>
<td>-0.07</td>
<td>0.55</td>
<td>68.1 ± 0.7</td>
</tr>
<tr>
<td>115</td>
<td>79</td>
<td>-0.10</td>
<td>0.37</td>
<td>78.8 ± 0.8</td>
</tr>
</tbody>
</table>

<sup>1</sup>Postnatal day of age.  
<sup>2</sup>Number of crossbred (Yorkshire x Landrace x Duroc) animals in analysis.  
<sup>3</sup>Pearson correlation between body weight and age at puberty onset.  
<sup>4</sup>Body weight mean ± standard error.

### Table 3.2. Relationship between vulva width during pre-pubertal growth and age at puberty onset.

<table>
<thead>
<tr>
<th>PND&lt;sup&gt;1&lt;/sup&gt;</th>
<th>N&lt;sup&gt;2&lt;/sup&gt;</th>
<th>r-Value&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-Value&lt;sup&gt;3&lt;/sup&gt;</th>
<th>VW ± SEM&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>79</td>
<td>-0.08</td>
<td>0.47</td>
<td>22.8 ± 0.5</td>
</tr>
<tr>
<td>85</td>
<td>79</td>
<td>-0.12</td>
<td>0.30</td>
<td>24.7 ± 0.4</td>
</tr>
<tr>
<td>95</td>
<td>78</td>
<td>-0.12</td>
<td>0.31</td>
<td>27.8 ± 0.4</td>
</tr>
<tr>
<td>105</td>
<td>79</td>
<td>-0.20</td>
<td>0.07</td>
<td>30.1 ± 0.4</td>
</tr>
<tr>
<td>115</td>
<td>78</td>
<td>-0.28</td>
<td>0.01</td>
<td>33.0 ± 0.4</td>
</tr>
</tbody>
</table>

<sup>1</sup>Postnatal day of age.  
<sup>2</sup>Number of crossbred (Yorkshire x Landrace x Duroc) animals in analysis.  
<sup>3</sup>Pearson correlation between vulva width (VW; mm) and age at puberty onset.  
<sup>4</sup>Vulva width mean ± standard error (mm).

### Table 3.3. Relationship between vulva length during pre-pubertal growth and age at puberty onset.

<table>
<thead>
<tr>
<th>PND&lt;sup&gt;1&lt;/sup&gt;</th>
<th>N&lt;sup&gt;2&lt;/sup&gt;</th>
<th>r-Value&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-Value&lt;sup&gt;3&lt;/sup&gt;</th>
<th>VL ± SEM&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>79</td>
<td>-0.06</td>
<td>0.58</td>
<td>26.2 ± 0.6</td>
</tr>
<tr>
<td>85</td>
<td>79</td>
<td>-0.01</td>
<td>0.96</td>
<td>27.7 ± 0.4</td>
</tr>
<tr>
<td>95</td>
<td>78</td>
<td>-0.07</td>
<td>0.57</td>
<td>31.3 ± 0.5</td>
</tr>
<tr>
<td>105</td>
<td>79</td>
<td>-0.04</td>
<td>0.70</td>
<td>34.1 ± 0.5</td>
</tr>
<tr>
<td>115</td>
<td>78</td>
<td>-0.04</td>
<td>0.73</td>
<td>38.2 ± 0.5</td>
</tr>
</tbody>
</table>

<sup>1</sup>Postnatal day of age.  
<sup>2</sup>Number of crossbred (Yorkshire x Landrace x Duroc) animals in analysis.  
<sup>3</sup>Pearson correlation between vulva length (VL; mm) and age at puberty onset.  
<sup>4</sup>Vulva length mean ± standard error (mm).
Table 3.4. Relationship between vulva area during pre-pubertal growth and age at puberty onset.

<table>
<thead>
<tr>
<th>PND¹</th>
<th>N²</th>
<th>r-value³</th>
<th>p-value³</th>
<th>VA ± SEM⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>79</td>
<td>-0.08</td>
<td>0.51</td>
<td>612.6 ± 24.6</td>
</tr>
<tr>
<td>85</td>
<td>79</td>
<td>-0.07</td>
<td>0.57</td>
<td>694.0 ± 21.2</td>
</tr>
<tr>
<td>95</td>
<td>78</td>
<td>-0.12</td>
<td>0.30</td>
<td>879.9 ± 22.7</td>
</tr>
<tr>
<td>105</td>
<td>79</td>
<td>-0.14</td>
<td>0.24</td>
<td>1036.8 ± 26.4</td>
</tr>
<tr>
<td>115</td>
<td>78</td>
<td>-0.17</td>
<td>0.14</td>
<td>1269.5 ± 28.0</td>
</tr>
</tbody>
</table>

¹Postnatal day of age.
²Number of crossbred (Yorkshire x Landrace x Duroc) animals in analysis.
³Pearson correlation between vulva area (VA; mm²) and age at puberty onset.
⁴Vulva area mean ± standard error (mm²).

Table 3.5. Relationship between Kp-10⁵ during pre-pubertal growth and age at puberty onset.

<table>
<thead>
<tr>
<th>PND¹</th>
<th>N²</th>
<th>r-value³</th>
<th>p-value³</th>
<th>pg/mL ± SEM⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>71</td>
<td>0.017</td>
<td>0.886</td>
<td>50.0 ± 4.0</td>
</tr>
<tr>
<td>85</td>
<td>70</td>
<td>-0.216</td>
<td>0.072</td>
<td>54.1 ± 3.9</td>
</tr>
<tr>
<td>95</td>
<td>66</td>
<td>-0.130</td>
<td>0.299</td>
<td>59.1 ± 5.6</td>
</tr>
<tr>
<td>105</td>
<td>69</td>
<td>-0.086</td>
<td>0.481</td>
<td>55.3 ± 4.6</td>
</tr>
<tr>
<td>115</td>
<td>70</td>
<td>-0.011</td>
<td>0.931</td>
<td>57.6 ± 5.2</td>
</tr>
</tbody>
</table>

¹Postnatal day of age.
²Number of crossbred (Yorkshire x Landrace x Duroc) animals in analysis.
³Pearson Correlation between kisspeptin-10 (pg/mL) and age of puberty onset.
⁴Kisspeptin-10 (pg/mL) ± standard error for each PND.
⁵Plasma kisspeptin-10 levels.
Figure 3.1. Representative image showing distinct variation in ovarian tertiary follicular development. Ovaries collected from gilts of similar age (98 ± 4 days of age). Left – undeveloped ovary; Right – ovary containing tertiary follicles (Pearce, Ross, Keating, and Baumgard, unpublished results).
Figure 3.2. Variation in follicular development in gilts on postnatal days 75, 85, 95, 105, and 115. As gilts progressed in age follicular development increased. On postnatal day 75 all sacrificed gilts possessed ovaries lacking tertiary follicles. Variation in tertiary follicular development was greatest between postnatal days 85 and 95 in sacrificed gilts. By postnatal day 115, all gilts possessed some form of follicular development with the majority of ovaries having tertiary follicles. The black bars represent the number of gilts sacrificed on each day lacking tertiary follicle development. Gray bars represent the number of gilts sacrificed on each day having a total of 1 to 10 tertiary follicles greater than or equal to two millimeters in size. Striped bars represent the number of gilts having a total of 10 to greater than 100 tertiary follicles greater than or equal to two millimeters in size.
Figure 3.3. Average uterine weight of sacrificed gilts on postnatal days 75, 85, 95, 105, and 115. As gilts progressed in age, uterine weight increased across postnatal days 75-115. The increased weight of the reproductive tract is temporally associated with increased ovarian activity. Different superscripts represent statistical significance ($P < 0.05$).
Figure 3.4. Distribution of gilts achieving puberty onset by 200 days of age. The first gilt achieved puberty on postnatal day 140 and boar exposure ended on postnatal day 200. Boar exposure and daily heat detection was initiated on postnatal day 129.
Figure 3.5. Relationship of body weight at 75 days of age and age at puberty onset. A negative correlation exists between body weight on postnatal day 75 and age at puberty onset from those gilts achieving their first estrus by 200 days of age ($r = -0.22$, $P = 0.05$).
Figure 3.6. Relationship of vulva width at 105 days of age and age at puberty onset. A negative correlation exists between vulva width on postnatal day 105 and age at puberty onset of those gilts achieving their first estrus by 200 days of age ($r = -0.20$, $P = 0.07$).
Figure 3.7. Relationship of vulva width at 115 days of age and age at puberty onset. A negative correlation exists between vulva width on postnatal day 115 and age at puberty onset of those gilts achieving their first estrus by 200 days of age (r = -0.28, P = 0.01).
Figure 3.8. Percentage of gilts achieving estrus by 180 days of age on each postnatal day for a given vulva area. Gilts were grouped based on their calculated vulva area (VA). Black bars represent the percentage of gilts whose VA was less than 1 standard deviation from the mean and achieved estrus prior to 180 days of age. The gray bars represent the percentage of gilts achieving estrus by PND 180 whose VA was within or greater than one standard deviation from the mean. Striped bars represent the percentage of all gilts that achieved estrus by 180 days of age. A greater percentage of gilts with an average or larger vulva area on PND 95, 105, or 115 achieve puberty by 180 days of age than those gilts with a vulva area that is less than one standard deviation from the mean.
Figure 3.9. Percentage of gilts achieving estrus by 200 days of age on each postnatal day for a given vulva area. Gilts were grouped based on the calculated vulva area (VA). Black bars represent the percentage of gilts whose VA was less than 1 standard deviation from the mean and achieved estrus prior to 200 days of age. The gray bars represent the percentage of gilts achieving estrus by postnatal day 200 whose VA was within or greater than one standard deviation from the mean. Striped bars represent the percentage of all gilts that achieved estrus by 200 days of age. A greater percentage of gilts with an average or larger vulva area on postnatal day 95, 105, or 115 achieve puberty by 200 days of age than those gilts with a vulva area that is less than one standard deviation from the mean.
Figure 3.10. Plasma kisspeptin-10 levels on postnatal days 75, 85, 95, 105 and 115. Gilts that achieved their first estrus within 200 days of age (black bars) had levels that were numerically, yet not statistically, higher than in those gilts that did not achieve estrus within 200 days of age (gray bars).
Figure 3.11. Serum anti-müllerian hormone (AMH) levels on postnatal day 95. No differences were observed in serum AMH concentrations between groups of gilts based on when they achieved their first estrus. All gilts not demonstrating a behavioral estrus by 200 days of age were considered non-responsive.
CHAPTER IV: EVALUATION OF THE THERMOREGULATORY RESPONSE IN GILTS AND ITS RELATIONSHIP WITH REPRODUCTIVE PERFORMANCE FOLLOWING SYNCHRONIZATION WITH MATRIX

To be submitted to the Journal of Animal Science


*Department of Animal Sciences, Iowa State University, Ames, Iowa 50010

Abstract

Mitigating heat stress effects in swine breeding stock is crucial as it negatively impacts reproductive performance. The objectives of the study were to determine if a gilt characterized as tolerant or susceptible to a pre-pubertal heat stress challenge can maintain their tolerance or susceptibility post-pubertal and to identify the relationship between a gilt’s thermal regulatory response to heat stress following Matrix® synchronization and reproductive performance. Individual gilts identified as tolerant (n=50) or susceptible (n=50) to pre-pubertal heat stress were selected based on their ability or inability, respectively, to remain euthermic during the peak heat stress period. Gilts were placed in individual stalls and underwent estrus synchronization in a thermal neutral environment (20°C). Rectal temperature (Tr), skin temperature and respiration rate were recorded seven times per day. Rectal temperature during a two-day thermal neutral period and the average of the last three

---

* Responsible for experimental design and manuscript preparation.
* Assisted in data and sample collection.
* Provided assistance with experimental design and analysis and interpretation of results.
time points (MaxTr) on each day of a heat stress period (9 days) were used to create a thermal rectal delta (TrDelta) value for each gilt. The average TrDelta, MaxTr, and thermal neutral Tr of all gilts were 0.6 ± 0.03°C, 38.9 ± 0.02°C, and 38.3 ± 0.02°C, respectively. The time from Matrix® withdrawal to standing estrus averaged 5.8 ± 0.1 days with 84.7% of gilts receiving 2-3 artificial insemination services. For all pregnant gilts the average uterine wet weight, ovary weight, corpora lutea (CL) numbers, and CL diameter was 5.6 ± 0.14 kg, 21.6 ± 0.32 g, 17.8 ± 0.3, and 10.2 ± 0.06 mm, respectively. Fetal measurements of total number, weight, and crown-rump length (CRL) averaged 13.9 ± 0.3 fetuses, 24.5 ± 0.33 g, and 73.8 ± 0.34 mm, respectively. Heat stress tolerant gilts had a significantly longer return to estrus following Matrix® withdrawal and slightly larger CL diameter. Fetal weight and fetal CRL were significantly greater in gilts previously classified as susceptible to heat stress.

**Keywords:** heat stress, reproduction, gilt development, seasonal infertility

**Introduction**

Seasonal infertility is a recurring annual event characterized by decreased reproductive performance in swine manifested as periods of anestrus, increased weaning-to-estrus intervals, decreased farrowing rates (FR), and reductions in litter size (Bertoldo et al., 2009). While it is a global hindrance to swine production and occurs independently of production systems, there is little agreement on all of the factors contributing to seasonal infertility as well as importance of each factor (Hurtgen and Leman, 1980; Peltoniemi et al., 1999). The specific factors influencing seasonal infertility include genetics, environment (i.e. photoperiod and heat stress), nutrition, and management. Increased ambient temperature and associated heat stress represent a plausible mechanism as seasonal infertility occurs during the hottest part of the year (Prunier et al., 1994). In a five year study, Auvigne et al. (2010)
showed that the year with the greatest number of hot days had a statistical difference in severity of seasonal infertility, leading to the hypothesis that heat stress through increased ambient temperature exacerbated seasonal infertility.

Heat stress impacts the economic potential of swine producers through the negative influence of an animal’s growth and development and reproductive efficiency. The ability for an animal to adapt to periods of heat stress during growth impacts their overall performance and can be altered in two ways, through the adaptation of genetics to influence the heat stress phenotype or an animal’s individual ability to acclimate to stressors (Yousef, 1985, Gaughan, 2012). Follicular and early embryonic development in the pig appear to be particularly sensitive biological processes to heat stress. For example, increased ambient temperatures during the follicular stage has been shown to decrease aromatase activity in follicles of heat stressed goats (Ozawa et al., 2005). In the same study, the timing of ovulatory follicle recruitment was significantly delayed by heat stress.

In addition to decreased follicular activity, embryonic development has the potential to be altered by heat stress as well. In a study using sheep, the period of embryo development identified as being the most sensitive to heat stress is blastocyst formation and the beginning stages of cleavage (Dutt, 1963). The timing of heat stress during embryonic development is important, as heat stress occurring later in gestation has a decreased chance of causing reduced embryo viability. This was shown by Ealy et al. (1993) in a study evaluating the effects of heat stress induced on days 1, 3, 5, and 7 following estrus on eight-day old embryos. Embryo development was reduced in cows exposed to heat stress one day following estrus compared to the other days of heat stress exposure. The direct effects of heat stress on the early development of swine embryos has not been identified, however, similar
reports in super ovulated cattle give insight into the potential reduction of follicle and embryo development.

The objectives of this study were to determine if a gilt characterized as tolerant or susceptible to a pre-pubertal heat stress challenge can maintain their tolerance or susceptibility post-pubertal and to identify the relationship between a gilt’s thermal regulatory response to heat stress following Matrix® synchronization and reproductive performance. Additionally, we investigated if the body temperature response during heat stress during the follicular phase and initial embryonic development was predictive of reproductive success.

**Materials and Methods**

This study was conducted at Iowa State University with all animal procedures approved by the Iowa State University Animal Care and Use Committee.

**Animals**

One hundred gilts were selected from a previous study identifying gilts as being tolerant (n= 50) or susceptible (n=50) to heat stress based on their ability or inability, respectively, to maintain a minimal Tr during a 24-hour pre-pubertal heat stress period (Seibert, Baumgard, Ross, unpublished results). All gilts from the previous study underwent estrus detection beginning at 160 days of age and continued until 220 days of age to ensure the selected gilts had demonstrated at least two estrous cycles. At approximately 220 days of age, the gilts were transported to a facility that enabled individual housing and were limit fed six pounds of feed per day. One gilt was removed from the study due to illness.
Acclimation and Synchronization Period

Each gilt was placed in an individual stall in a controlled-environment research facility at Iowa State University. Animals were assigned to individual pens so that each classification (i.e. tolerant and susceptible) were equally represented and evenly spaced in each of two rooms (50 animals each) throughout the entire facility. The acclimation period began 16 days before the beginning of the heat stress period and the room temperature was maintained at approximately 20° Celsius (C). Fans were placed throughout the rooms to ensure equal distribution of heat which was monitored multiple times per day by placing five equidistantly spaced data loggers (EL-USB-2-LCD, Lascar Electronics, Erie, PA, 16505) throughout each of the two rooms in the barn. Fourteen days prior to heat stress, all gilts were placed on an estrus synchronization program utilizing Matrix®. Gilts were fed once daily (6 pounds) in the morning at 0700 and 6.8 mL of Matrix® (15 mg altrenogest) was administered by top dressing the gilts feed in each individual feeder, per manufactures guidelines. Feed consumption was monitored on all animals to ensure the complete dose was effectively consumed by all gilts.

Heat Stress Period

The heat stress period began on the last day of Matrix® feeding treatment and continued for nine consecutive days as the majority of females should reach estrus following Matrix® administration within this time frame (Flowers, 1999). Temperature was controlled in a diurnal pattern with the daily heat stress period beginning at 1000 and turned off at 2200 to simulate natural temperature patterns. The temperature during the 12 hours of heat stress was increased incrementally during the first three days (28.9°C, 31.1°C, and 33.3°C on day
one, two and three, respectively) and then was held to 35°C for the remaining six days. The low temperature for the remaining 12 hours each day was 21.1°C for the entire period.

*Temperature Measurements*

Rectal temperature was measured with a digital thermometer (Welch Allyn SureTemp® Plus 690, Skaneateles Falls, NY 13153), skin temperature was measured using a calibrated infrared thermometer (ST 380A Infrared Thermometer, HDE, Allentown, PA 18104), and respiration rate (RR) was calculated as breaths per minute by counting the number of breaths in 15 seconds and multiplying by four. These measurements were taken on the two days before heat stress to establish a thermal neutral average baseline for each gilt. The same measurements were taken at seven time points each day during the heat stress period at 0800, 1400, 1500, 1600, 1900, 2000 and 2100.

*Estrus Detection, Artificial Insemination and Pregnancy Check*

During the Matrix® withdrawal period each gilt underwent estrus detection for breeding. Four boars were utilized via fence line exposure to enable estrus detection each morning after feeding and prior to heat stress (i.e. between 0700 and 1000). Gilts were bred with a single dose of pooled semen (terminal Duroc) on the first day of standing estrus and received additional insemination each day they continued to exhibit behavioral estrus. By the tenth day all but one of the 99 gilts had demonstrated a standing estrus and had received at least one dose of semen. The remaining 98 gilts inseminated underwent estrus detection 18-22 days later as well as ultrasound checked at ~36 days of pregnancy to identify those returning to estrus or to confirm pregnancy, respectively.
Harvesting and Fetal Analysis

Gilts were harvested at 42-47 days of pregnancy (with day of first service considered day zero) at a sow slaughter facility in a single group. The reproductive tract from each gilt was collected and immediately refrigerated until analysis, which occurred within 48 hours for all reproductive tracts. The reproductive tract from each gilt was weighed using a digital scale for a total tract measurement and then the fetuses and ovaries were removed. Fetuses were counted to determine litter size for each gilt and then individually weighed using a digital scale and measured with Ultra Tech digital calipers (General Tools, Secaucus, NJ) to determine crown-rump length. Ovarian weight of each gilt was measured and the corpora lutea (CL) on each ovary were counted and the diameter of each CL was measured with Ultra Tech digital calipers (General Tools, Secaucus, NJ, 07094). Embryo survival was calculated for each gilt by dividing the number of fetuses in the litter by the number of CL on the gilt’s ovaries. Eight gilts were confirmed to be non-pregnant by the absence of fetuses and/or CL.

Temperature and Statistical Analysis

Average Tr was measured during three-hour periods (twice a day) for each gilt two days prior to heat stress and each day during heat stress beginning four hours after heat stress induction (1400, 1500, and 1600) and during the last three hours of heat stress (1900, 2000, 2100). The Tr change (TrDelta) was determined using each gilts average thermal neutral temperature subtracted from the average Tr collected during the three hour period beginning nine hours after heat stress induction. For each gilt, the thermal neutral Tr, heat stress average Tr and the Tr difference between thermal neutral and heat stress values from the initial study (Seibert, Baumgard, Ross; unpublished) were used to determine if a correlation existed between the pre-pubertal thermoregulatory response and the post-pubertal thermoregulatory
response. The PROC CORR function of SAS was used for this Pearson correlation as well as to determine if any correlation exists between the post-pubertal TrDelta values with each of the reproductive performance measures collected from each gilt. Additionally, gilts were assigned to their initial tolerant and susceptible classifications and t-tests were conducted to determine statistical differences of the reproductive performance measures between each classification.

Results

Thermoregulatory Response to Heat Stress

The average thermal neutral rectal temperature baseline was calculated on the last two days in the thermal neutral climate before the heat stress period. The 50 gilts classified as tolerant prior to puberty had an average thermal neutral Tr of 38.3 ± 0.03°C and the 49 gilts classified susceptible prior to puberty had an average thermal neutral Tr of 38.3 ± 0.02°C. To calculate the maximum heat stress response, the last three time point measurements (MaxTr) on each day were averaged. The tolerant gilts had an average MaxTr of 38.7 ± 0.02°C during heat stress and the susceptible gilts had an average MaxTr of 39.0 ± 0.03°C. The TrDelta was 0.5 ± 0.03°C for tolerant gilts and 0.7 ± 0.04°C for susceptible gilts. On the day that each gilt was initially in behavioral estrus, the maximum Tr was recorded as the breeding Tr (bTr) for each animal. The average bTr of gilts classified as tolerant was 38.9 ± 0.04°C while the bTr for susceptible gilts was 39.3 ± 0.05°C (Table 4.1).

Skin temperature was measured on the rump of each gilt daily and to calculate the maximum heat stress skin temperature response the last three time point measurements (MaxSkin) were averaged. The average MaxSkin for the tolerant gilts was 38.6 ± 0.11 while the susceptible gilts had an average MaxSkin of 38.7 ± 0.09. The tolerant gilts had an
average thermal neutral skin temperature of 28.2 ± 0.18 while the susceptible gilts averaged 27.9 ± 0.19. The skin delta for all gilts was 10.6 ± 0.17 while the tolerant gilts had an average skin delta of 10.4 ± 0.24 and the susceptible gilts had an average skin delta of 10.7 ± 0.24. Between the heat stress tolerant and susceptible gilts there was no significant difference ($P > 0.05$) in MaxSkin, skin thermal neutral, and skin delta temperature measurements.

Respiration rate was taken on each time point when the individual animal was lying down and recorded as breaths per minute. The average RR during thermal neutral conditions for all gilts was 15.5 ± 0.26 and was not different based on classification. The tolerant gilts had an average thermal neutral RR of 15.9 ± 0.38 while the susceptible gilts averaged 15.0 ± 0.37. To determine the maximal heat stress effect on respiration rate the last three time point measurements (MaxRR) were averaged. The tolerant gilts had an average MaxRR of 69.5 ± 2.26 while the susceptible gilts averaged 68.8 ± 2.55 resulting in a MaxRR of 69.2 ± 1.69 for all gilts. No significant difference ($P > 0.05$) was observed in MaxRR, RR thermal neutral, and RR delta temperature measurements between the heat stress tolerant and susceptible gilts.

**Breeding Performance**

Following estrus synchronization using Matrix® during the acclimation period, the average time until behavioral estrus for the 98 gilts demonstrating estrus was 5.8 ± 0.1 days (Figure 4.1). Gilts classified as tolerant had a slightly ($P = 0.006$) greater average time until behavioral estrus (6.1 ± 0.1 days) compared to gilts classified as susceptible (5.5 ± 0.1 days). All gilts that exhibited a standing estrus received one artificial insemination on each day in which they exhibited a standing response with the average number of breeding services for all gilts being 2.4 ± 0.1 (Figure 4.2). There was no significant difference ($P > 0.05$) between
the two classifications of gilts with the tolerant gilts receiving 2.2 ± 0.1 services and the susceptible gilts receiving 2.5 ± 0.1 services.

Reproductive Efficiency

The reproductive efficiency of the group of gilts was determined through analyzing the litter and reproductive tract of each individual animal after harvest (42-47 days of gestation). Of the 98 gilts that were artificially inseminated, eight were determined to be non-pregnant as evident by the absence of fetuses at harvest. Entire whole uterine tracts were weighed to record a total tract weight with the average tract weight of all bred gilts being 5.6 ± 0.14 kg. The tolerant gilts had an average tract weight of 5.5 ± 0.22 kg and lacked significant difference ($P > 0.05$) from the susceptible gilts that had an average tract weight of 5.7 ± 0.18 kg (Appendix A.4.1).

Fetal Measurements

After total reproductive tract weight was recorded, the fetuses were removed and individually analyzed. The average number of fetuses for all gilts was 13.91 ± 0.34 and was not different ($P > 0.05$) between the tolerant (13.6 ± 0.48 fetuses) and susceptible (14.2 ± 0.48 fetuses) gilts (Appendix A.4.2). Each individual fetus was weighed and the average fetal weight for all gilts was 24.5 ± 0.33 g with an average fetal weight of 23.6 ± 0.45 g for the tolerant gilts being significantly lower ($P = 0.007$) than the average of 25.4 ± 0.45 g for the susceptible gilts (Figure 4.3). The crown-rump length (CRL) of each individual fetus was recorded and the average for all gilts was 73.8 ± 0.34 mm. The average CRL was 72.8 ± 0.46 mm for the tolerant gilts and was significantly lower ($P = 0.002$) than the average CRL of 74.8 ± 0.46 mm for the susceptible gilts (Figure 4.4).
Ovarian Measurements

Ovaries were excised from each tract and weighed individually to get a total ovarian weight. The average ovary weight for all bred gilts was 21.6 ± 0.3 g with the tolerant gilts (21.7 ± 0.4 g) not different (P > 0.05) from the susceptible gilts (21.6 ± 0.6 g; Appendix A.4.3). The CL on the ovaries from each gilt were counted to determine total CL number and measured to record an average CL diameter. The average total number of CL for all bred gilts was 17.8 ± 0.34. The tolerant gilts averaged 17.6 ± 0.34 total CL and were not different (P > 0.05) from the susceptible gilts that averaged 18.1 ± 0.62 total CL (Appendix A.4.4). The average CL diameter of all bred gilts was 10.2 ± 0.06 mm and there was a marginal difference (P =0.056) between the tolerant gilts averaging 10.3 ± 0.08 mm and the susceptible gilts averaging 10.1 ± 0.08 mm per CL (Figure 4.5). After total fetus and CL numbers were recorded a percentage was calculated to determine embryo survival, assuming all oocytes were fertilized. The average embryo survival of all the bred gilts was 78.9 ± 1.8%. The tolerant gilts had an average embryo survival of 77.7 ± 2.6% and were not significantly different (P > 0.05) than the susceptible gilts with an embryo survival of 80.4 ± 2.5% (Appendix A.4.5).

Relationship of TrDelta with Reproductive Performance

The TrDelta of each gilt was used to determine if the heat stress response as determined by change in body temperature was associated with reproductive performance and ability to tolerate heat stress during breeding. The average TrDelta had a small positive correlation (R = 0.20 P = 0.059) with fetal CRL, indicating that as TrDelta increased fetal size increased slightly (Figure 4.6). There was no correlation between TrDelta and all other reproductive performance measures (Table 4.2).
Repeatability of Heat Stress Tolerance and Susceptibility

To determine if the pre-pubertal classifications of tolerance or susceptibility were predictive of a future heat stress thermoregulatory response we compared the pre-pubertal and post-pubertal temperature measurements. The pre-pubertal thermal neutral Tr (Figure 4.7), average heat stress Tr (Figure 4.8), and TrDelta (Figure 4.9) was positively correlated to the same indices measured during the post-pubertal heat stress bout \( r = 0.21 \) and \( P = 0.037 \), \( r = 0.63 \) and \( P < 0.001 \), and \( r = 0.40 \) and \( P < 0.001 \), respectively). The pre-pubertal thermal neutral Tr also had a positive correlation \( r = 0.53 \) and \( P < 0.001 \) to the post-pubertal heat stress temperatures (Figure 4.10).

Relationship Between Maximum Rectal Temperature on Day of Insemination and Reproductive Outcome

To examine the effects of heat stress on the specific day each gilt was bred we compared the bTr with each of the reproductive efficiency measurements. The bTr was positively correlated with both the fetal weight \( r = 0.26 \) and \( P = 0.013 \) and the fetal CRL \( r = 0.26 \) and \( P = 0.014 \) of the bred gilts (Figure 4.11 and Figure 4.12, respectively). No correlation between bTr and the remaining reproductive measurements was detected (Table 4.3).

Discussion

Mitigating seasonal infertility is important as the effects of it drastically decrease the economic potential of swine enterprises. Based on a 2012 feed cost analysis, annual economic losses to the swine industry due to lost reproductive performance during seasonal infertility was estimated to be approximately $55 per sow (Pollmann, personal communication with Keating and Ross). Seasonal infertility is a reoccurring problem and
while it could arguably be related to other factors such as photoperiod, seasonal infertility in pigs is often associated with periods of excessive heat. Heat stress has been identified as a primary contributing cause of seasonal infertility due to the associated decrease in reproductive performance occurring during the hotter months of the year (June-September). Due to their lack of functional sweat glands, pigs are poor at dissipating body heat and must rely on other strategies to control body heat such as panting and regulating metabolic heat production through altering their feed intake (Whittow, 1971) which can lead to decreased production performance. Renaudeau et al. (2011) conducted a meta-analysis of data (1970-2009) and reported that increased ambient temperature had a negative impact on average daily feed intake and average daily gain. The same study reported that the effect of temperature was greater in the more recently published data suggesting that as the swine industry selects for an increased efficiency in lean muscle accretion pigs are becoming less tolerant to periods of excessive heat stress. This intolerance may in part be due to increased metabolic heat production from increased synthesis in skeletal muscle and growth (Hocquette et al., 1998). The effects of heat stress vary depending on production stage during exposure to increased ambient temperatures. Since reproductive efficiency is the combined performance of several production stages, mitigating heat stress across all stages is crucial in maintaining production efficiency in the pork industry. The effects that heat stress has on reproductive efficiency range from decreases in litter size, farrowing rate, fetal performance, in addition to increased instances of early embryonic death (Xue et al., 1994; Hurtgen et al. 1980; and Peltoniemi et al., 1999). It can also affect long term performance through an increase in wean-to-estrus interval length and interrupting ovarian follicular development (Prunier et al., 1996).
The objectives of this study were to determine if a gilt characterized as tolerant or susceptible to a pre-pubertal heat stress challenge can maintain their tolerance or susceptibility post-pubertal and to identify the relationship between a gilt’s thermal regulatory response to heat stress following Matrix® synchronization and reproductive performance. Our hypothesis is that gilts demonstrating susceptibility to heat stress during pre-pubertal development will most likely demonstrate susceptibility to heat stress later in life and will have decreased reproductive success during heat stress. Understanding the heat stress response repeatability in pigs, in addition to better defining the relationship between the heat stress response in pigs and reproductive efficiency, is imperative to overcoming the negative effects associated with seasonal infertility.

In this study, 100 gilts, previously identified as tolerant or susceptible to heat stress during pre-pubertal development, were subjected to a diurnal pattern of heat stress during a Matrix® synchronized follicular phase. The average MaxTr values were used for analysis as they represent the period of time when gilts were experiencing the greatest heat load. The normal body temperature range for gilts is 37.8-39.4°C with a normal thermal neutral environment temperature of ≤ 26.7°C (Rix and Ketchum, 2010). The average thermal neutral Tr and MaxTr temperatures in this study were 38.3 ± 0.02°C and 38.9 ± 0.02°C, respectively, which is consistent with the lower and higher end of the above stated normal threshold. The difference in temperature between the tolerant and susceptible gilts was 0.55°C. Liao and Veum (1994) reported a similar Tr temperature change of 0.3°C between gilts in a thermal neutral (23°C) environment and gilts in a heat stress (gradual increase from 25 to 34°C) environment (38.7°C and 39.0°C, respectively).
To determine how heat stress affected reproductive performance the insemination results of gilts previously classified as tolerant and susceptible were compared. The wean-to-estrus interval is the period of follicular development following the period of anestrous a sow undergoes during lactation. After weaning, most sows return to estrus approximately 5-7 days later (Behan and Watson, 2005). For this study we used the time period from Matrix® withdrawal to the onset of behavioral estrus to identify the return to estrus interval. The average number of days until estrus following Matrix® withdrawal was 5.8 ± 0.1 days. Several studies have reported similar return to estrus interval values for sows (Deckert et al., 1997; Killen et al., 1992). A sow’s wean-to-estrus interval is variable and can be influenced by a variety of factors such as parity, season, nutrition, and boar exposure (Dial et al., 1992). In addition to impacting the total number of non-productive days, abnormal wean-to-estrus intervals in sows have been reported to influence reproductive performance. In a study by Wilson and Dewey (1993), sows exhibiting a wean-to-estrus interval of 7-10 days had decreased farrowing rates compared to sows with an interval of 3-6 or 11-14 days. Within the same study, as the wean-to-estrus interval increased from 4 to 8 days, litter size decreased. In the current study, heat stress tolerant gilts had a slightly longer duration to return to estrus following Matrix® withdrawal than those classified as susceptible.

The duration and strength of behavioral estrus is thought to be impacted by heat stress (Bolocan, 2009). In this study, each gilt was artificially inseminated each day it stood in behavioral estrus, which enabled comparison of the number of services each gilt received to serve as a proxy for duration of estrus. However, no significant difference was observe between the heat stress tolerant and heat stress susceptible gilts in the number of services received.
The primary means to characterize the effects of heat stress on reproduction are through evaluation of its effect on pregnancy rate and litter characteristics. The time period in which a female experiences heat stress affects litter performance in different ways. In a study by Xue et al. (1994), females that were bred during the summer months experienced lower number of pigs born alive than the counterparts bred in the winter. Not only is litter size affected during the summer months, but litter weight decreases when breeding occurs during heat stress (Prunier et al., 1994). While we did not see any significant difference in the total number of fetuses there was a significant difference in fetal weight and fetal CRL between the tolerant and susceptible classified gilts. The average total number of fetuses in this study was 13.9 ± 0.34 which is higher than the average total number born of 10.2 (USDA, 2014). This difference can be contributed to the fact that we harvested the fetuses at 45 ± 3 days of gestation and included potential stillborns and reabsorptions. Fetal weight and fetal CRL was significantly greater in the susceptible gilts compared to gilts classified as heat stress tolerant. McNeil et al. (2005) reported an average individual fetus weight of 20.4 ± 0.6g at 45 days of gestation. This is comparable to the present study’s average fetal weight of 24.7 ± 0.33. For this study, the average CRL for all gilts was 73.8 ± 0.34 mm. Mesa et al. (2012) reported similar growth patterns of CRL of > 75mm, but < 90mm for fetuses on day 50 of gestation.

A decrease in ovarian activity can cause early loss of pregnancy and decreased FR which are direct manifestations of seasonal infertility (Love et al., 1993). Lopes et al. (2014) demonstrated reduced ovarian activity during the months associated with seasonal infertility by showing that during ovulation there were less follicles on the ovaries in females ovulating during the summer and fall months compared to females ovulating in the winter and spring months (12.4 ± 0.3 and 13.5 ± 0.3 follicles, respectively). Additionally, there was no
difference in viable CL number at harvesting between the tolerant and susceptible classified gilts. In contrast to these findings, Bertoldo et al. (2011) reported that in a group of sows culled for early pregnancy loss during the summer and spring, the summer culls had fewer CL present on their ovaries compared to the spring culls (11.6 ± 3.3 vs. 9.3 ± 0.99). This suggests that CL number appears to be influenced by environmental factors associated with seasonal infertility. One aspect associated with interrupted ovarian development is altered hormonal patterns. Seasonal effects on irregular LH production can cause sporadic CL death resulting in early pregnancy loss (Peltoniemi et al., 1997). In the current study, a small difference ($P=0.056$) in CL diameter was observed. Grzesiak et al. (2014) showed that androgen deficiencies lead to decreased progesterone production as a result of CL dysfunction. Although no significant pregnancy losses were observed in the present study by day 42-47 of gestation the size difference in the CL could be associated with reduced progesterone levels. The smaller CL, although not affecting the pregnancy maintenance, could represent disruptions in follicular maturation prior to ovulation resulting in decreased progesterone production during pregnancy. Bertoldo et al. (2010) showed that decreased progesterone production in the summer months results in reduced oocyte competence, thus increasing early pregnancy loss occurrences. Managing heat stress during oocyte development, and during CL development is likely a key component in maintaining oocyte and embryonic competence.

The potential value for selecting gilts prior to puberty that will be tolerant to heat stress once they come to breeding age is underscored by the correlation between pre-pubertal heat stress tolerance to post-pubertal heat stress tolerance with respect to thermal regulation. In the current study, the pre-pubertal thermoregulatory response (thermal neutral $Tr$, heat
stress Tr, and TrDelta) was compared to the same post-pubertal measurements. All three measurements showed to have a positive correlation between pre-pubertal and post-pubertal time points. Additionally, the pre-pubertal thermal neutral Tr was positively correlated to the post-pubertal heat stress Tr ($r = 0.53$ and $P < 0.001$). Essentially, females with higher pre-pubertal thermal neutral rectal temperature are more likely to exhibit an increased rectal temperature during heat stress later in life.

**Conclusion**

Heat stress has been identified in multiple reports as a main cause for seasonal infertility. Mitigating heat stress effects is crucial in maintaining reproductive performance. Gilts that are susceptible to heat stress, as described by their thermoregulatory response prior to puberty, have a greater chance of exhibiting susceptibility to heat stress periods following puberty. When evaluating the reproductive performance from gilts, fetal size was different when comparing gilts previously classified as heat stress tolerant and susceptible. However, the heat stress susceptible gilts demonstrated increased fetal weights and fetal CRL compared to the heat stress tolerant gilts. The consistency of the thermoregulatory phenotype during thermal neutral and heat stress conditions, during pre-pubertal and post-pubertal production stages, suggests that this phenotype is likely under some level of genetic regulation and represents an opportunity for future exploration.

**Acknowledgements**

The authors would like to acknowledge Theresa Johnson, Candice Hager, and Ben Hale, for their assistance in collecting data and monitoring daily animal care, and Jacob Myers for assisting in maintenance of facilities.
Declaration of Interest

Results described here within were supported by the National Pork Board. Any opinion, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the National Pork Board. No conflicts of interest, financial or otherwise are declared by the author(s).
Table 4.1. Temperature measurement analysis between heat stress response classifications.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Classification</th>
<th>N</th>
<th>Temperature ± SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN Tr⁶</td>
<td>Tolerant</td>
<td>50</td>
<td>38.28°C ± 0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>TN Tr⁶</td>
<td>Susceptible</td>
<td>49</td>
<td>38.34°C ± 0.02</td>
<td></td>
</tr>
<tr>
<td>MaxTr⁷</td>
<td>Tolerant</td>
<td>50</td>
<td>38.74°C ± 0.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MaxTr⁷</td>
<td>Susceptible</td>
<td>49</td>
<td>39.01°C ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Tr Delta⁸</td>
<td>Tolerant</td>
<td>50</td>
<td>0.452°C ± 0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tr Delta⁸</td>
<td>Susceptible</td>
<td>49</td>
<td>0.663°C ± 0.04</td>
<td></td>
</tr>
<tr>
<td>bTr⁹</td>
<td>Tolerant</td>
<td>50</td>
<td>38.91°C ± 0.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>bTr⁹</td>
<td>Susceptible</td>
<td>49</td>
<td>39.29°C ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

¹Temperature measurement.
²Classification based on pre-pubertal heat stress response.
³Number of crossbred (Yorkshire x Landrace x Duroc) animals used for analysis.
⁴Rectal temperature ± Standard Error of Mean.
⁵P-Value: Pearson correlation of the heat stress classifications with temperature measurements.
⁶Post-pubertal rectal temperature during thermal neutral conditions.
⁷Post-pubertal rectal temperature response during last three hours of heat stress period.
⁸Post-pubertal change in temperature response (heat stress-thermal neutral rectal temperature).
⁹Post-pubertal maximum heat stress rectal temperature on day of estrus onset.

Table 4.2. Relationship of TrDelta and reproductive performance following heat stress.

<table>
<thead>
<tr>
<th>Performance Measure</th>
<th>N²</th>
<th>r-Value</th>
<th>P-Value³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine Weight⁴</td>
<td>90</td>
<td>-0.05</td>
<td>0.64</td>
</tr>
<tr>
<td>Fetal Weight⁵</td>
<td>90</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Fetal CRL⁶</td>
<td>90</td>
<td>0.20</td>
<td>0.06</td>
</tr>
<tr>
<td>Fetal Number⁷</td>
<td>90</td>
<td>0.05</td>
<td>0.63</td>
</tr>
<tr>
<td>Ovary Weight⁸</td>
<td>87</td>
<td>-0.04</td>
<td>0.68</td>
</tr>
<tr>
<td>CL Number⁹</td>
<td>87</td>
<td>-0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>CL Diameter¹⁰</td>
<td>87</td>
<td>-0.13</td>
<td>0.21</td>
</tr>
<tr>
<td>Embryo Survival¹¹</td>
<td>87</td>
<td>0.09</td>
<td>0.42</td>
</tr>
</tbody>
</table>

¹Recorded reproductive performance measures.
²Number of crossbred (Yorkshire x Landrace x Duroc) animals used for analysis.
³P-Value: Pearson correlation of the average TrDelta and performance measures.
⁴Uterine weight measured in kilograms.
⁵Fetal weight measured in grams.
⁶Fetal crown-rump length measured in millimeters.
⁷Number of fetuses per uterine tract.
⁸Total ovary weight measured in grams.
⁹Corpora lutea number from both ovaries.
¹⁰Corpora lutea diameter measured in millimeters.
¹¹Number of fetuses/number of corpora lutea.
Table 4.3. Relationship of bTr<sup>4</sup> and reproductive performance following heat stress.

<table>
<thead>
<tr>
<th>Performance Measure&lt;sup&gt;1&lt;/sup&gt;</th>
<th>N&lt;sup&gt;2&lt;/sup&gt;</th>
<th>r-Value</th>
<th>P-Value&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine Weight&lt;sup&gt;3&lt;/sup&gt;</td>
<td>90</td>
<td>0.06</td>
<td>0.535</td>
</tr>
<tr>
<td>Fetal Weight&lt;sup&gt;6&lt;/sup&gt;</td>
<td>90</td>
<td>0.26</td>
<td>0.013</td>
</tr>
<tr>
<td>Fetal CRL&lt;sup&gt;7&lt;/sup&gt;</td>
<td>90</td>
<td>0.26</td>
<td>0.014</td>
</tr>
<tr>
<td>Fetal Number&lt;sup&gt;8&lt;/sup&gt;</td>
<td>90</td>
<td>0.11</td>
<td>0.319</td>
</tr>
<tr>
<td>Ovary Weight&lt;sup&gt;9&lt;/sup&gt;</td>
<td>87</td>
<td>-0.01</td>
<td>0.894</td>
</tr>
<tr>
<td>CL Number&lt;sup&gt;10&lt;/sup&gt;</td>
<td>87</td>
<td>-0.01</td>
<td>0.956</td>
</tr>
<tr>
<td>CL Diameter&lt;sup&gt;11&lt;/sup&gt;</td>
<td>87</td>
<td>-0.08</td>
<td>0.453</td>
</tr>
<tr>
<td>Embryo Survival&lt;sup&gt;12&lt;/sup&gt;</td>
<td>87</td>
<td>0.11</td>
<td>0.306</td>
</tr>
</tbody>
</table>

<sup>1</sup>Recorded reproductive performance measures.
<sup>2</sup>Number of crossbred (Yorkshire x Landrace x Duroc) animals used for analysis.
<sup>3</sup>P-Value: Pearson correlation of the average TrDelta and performance measures.
<sup>4</sup>Maximum heat stress rectal temperature on day of estrus onset.
<sup>5</sup>Uterine weight measured in kilograms.
<sup>6</sup>Fetal weight measured in grams.
<sup>7</sup>Fetal crown-rump length measured in millimeters.
<sup>8</sup>Fetal number per uterine tract.
<sup>9</sup>Total ovary weight measured in grams.
<sup>10</sup>Corpora lutea number from both ovaries.
<sup>11</sup>Corpora lutea diameter measured in millimeters.
<sup>12</sup>Number of fetuses/number of corpora lutea.
Figure 4.1. Distribution of gilts receiving their first service on each day following Matrix® withdrawal. The interval was determined by recording the day a gilt first demonstrated behavioral estrus relative to the last day of Matrix® feeding. The average interval length to achieve behavioral estrus was $5.8 \pm 0.1$ days.
Figure 4.2. Distribution of gilts receiving a given number of artificial insemination services. Each gilt was inseminated once daily for the duration of behavioral estrus, thus the number of services is representative of the number of days the gilts demonstrated behavioral estrus. The average number of services recorded for each gilt was 2.4 ± 0.1.
Figure 4.3. Average fetal weight of pregnant gilts conceived during heat stress that were classified as tolerant or susceptible to heat stress prior to puberty. Tolerant gilts had an average fetal weight of 23.6 ± 0.45 g while the heat stress susceptible gilts had a significantly higher average fetal weight of 25.4 ± 0.45 g.
Figure 4.4. Average fetal crown-rump length (CRL) of pregnant gilts conceived during heat stress that were classified as tolerant or susceptible to heat stress prior to puberty. The heat stress tolerant gilts had an average CRL of 72.8 ± 0.46 mm while the heat stress susceptible gilts had a significantly higher average CRL of 74.8 ± 0.46 mm.
Figure 4.5. Average corpora lutea (CL) diameter of pregnant gilts conceived during heat stress that were classified as tolerant or susceptible to heat stress prior to puberty. The heat stress tolerant gilts average a CL diameter of 10.3 ± 0.08 mm while the heat stress susceptible gilts had a slightly significantly smaller average CL diameter of 10.1 ± 0.08 mm.
Figure 4.6. **Relationship between TrDelta and fetal crown-rump length.** There is a positive correlation between the fetal crown-rump length measured in millimeters and the TrDelta of each gilt \((r = 0.20, P = 0.058)\). TrDelta was calculated by subtracting the average rectal temperature, in degrees Celsius, during the two day thermal neutral period from the average rectal temperature of the last three time points on each day of the nine day heat stress period for each gilt.
Figure 4.7. **Repeatability of thermal neutral rectal temperature response.** There is a positive correlation between the average post-pubertal and pre-pubertal thermal neutral temperature response of each gilt \( (r = 0.21, \ P = 0.037) \). The pre-pubertal thermal neutral response was assessed by rectal temperature and correlation to the rectal temperature response during the post-pubertal thermal neutral period for each gilt was determined using the PROC CORR function of SAS.
Figure 4.8. Repeatability of heat stress rectal temperature response. There is a positive correlation between the average post-pubertal and pre-pubertal heat stress temperature response of each gilt ($r = 0.63$, $P < 0.001$). The pre-pubertal heat stress response was assessed by rectal temperature and correlation to the rectal temperature response during the post-pubertal heat stress period for each gilt was determined using the PROC CORR function of SAS.
Figure 4.9. **Repeatability of heat stress TrDelta response.** There is a positive correlation between the post-pubertal and pre-pubertal TrDelta response of each gilt ($r = 0.39$, $P < 0.001$). The pre-pubertal thermal rectal temperature change (TrDelta) was determined by subtracting the pre-pubertal thermal neutral rectal temperature response from the pre-pubertal heat stress rectal temperature response, and then the correlation to the post-pubertal TrDelta was determined using the PROC CORR function of SAS.
Figure 4.10. Relationship between pre-pubertal thermal neutral rectal temperature and post-pubertal heat stress rectal temperature response. There is a positive correlation between the average pre-pubertal thermal neutral temperature response and the average post-pubertal heat stress temperature response of each gilt ($r = 0.53, P < 0.001$). The pre-pubertal thermal neutral response was determined through rectal temperature, and the correlation to the rectal temperature response during the post-pubertal heat stress period for each gilt was determined using the PROC CORR function of SAS.
Figure 4.11. Relationship between the maximum rectal temperature on day of first breeding and fetal weight. There is a positive correlation between the maximum rectal temperature on the day of first service and the fetal weight measured in grams for each gilt ($r = 0.26$, $P = 0.013$). The maximum rectal temperature in response to heat stress recorded on the day of first service was correlation to the average fetal weight for each gilt was determined using the PROC CORR function of SAS.
Figure 4.12. Relationship between the maximum rectal temperature on day of first breeding and fetal crown-rump length. There is a positive correlation between the maximum rectal temperature on the day of first service and the fetal crown-rump length measured in millimeters for each gilt ($r = 0.26, P = 0.014$). The maximum rectal temperature in response to heat stress recorded on the day of first service and the correlation to the average fetal crown-rump length for each gilt was determined using the PROC CORR function of SAS.
CHAPTER V: SUMMARY AND CONCLUSIONS

Efficiency in sow reproductive performance is an important component enabling the sustainability and competitive advantage of the US swine industry. In this thesis, scientific investigations into the biological regulation of two factors (age of puberty onset and heat tolerance) that can be crucial in contributing to the reproductive efficiency of a sow and/or sow herd were performed.

Sow lifetime productivity (SLP) refers to the number of quality pigs that a sow weans from the time of breeding eligibility until leaving the herd. Unfortunately, SLP is lowly heritable as it is influenced by numerous traits and can be affected by numerous environmental factors (Serenius and Stalder, 2004). Several factors such as structural conformation, number of non-productive days, age at puberty onset, and pre-wean environment are potential influencers of SLP (Serenius and Stalder, 2006; Patterson et al., 2010). Age at puberty onset has been recognized as having predictive value for SLP as those gilts achieving puberty earlier in life have better reproductive performance after entrance into a breeding herd (Spörke, 2005; and Patterson et al., 2010). Despite its predictive value, selection of gilts for age at puberty onset presents several limitations as it can be highly variable between animals and is a labor intensive trait to identify. According to previous reports the average age of puberty onset for a group of gilts is between 160-170 days of age (Rozeboom et al., 1995; Young et al., 1990; and Zimmerman et al., 2000). In the present study (Chapter 3), the average age of puberty onset was consistent with the literature at approximately 165 days of age.

During pre-pubertal development gilts undergo both physical and physiological changes leading to the onset of puberty. Physically, the body begins to slow skeletal and
muscle growth and initiates a metabolic shift towards adipose tissue deposition. To determine how body weight and vulva development varied during postnatal days 75 through 115 we retrospectively compared differences in body weight and vulva development at specific ages across groups defined by age of puberty. Body weight on PND 75 was predictive of whether or not a gilt would achieve her first estrus prior to 200 days of age ($P = 0.055$). This is consistent with other reports showing that gilts with higher growth rates exhibited earlier puberty onset (Kummer et al., 2009). By selecting gilts with larger body weights during this time, producers could identify and select gilts with a higher chance of demonstrating puberty onset prior to a desired age.

For puberty to occur, there must be a fully functional hypothalamic pituitary gonadal axis (HPGx) (Pelletier et al., 1981; and Camous et al., 1985). This is partially controlled by the increase in steroid hormones from the developing ovaries. Monitoring changes in vulva development represents a potentially useful biological tool to determine and identify gilts initiating pre-pubertal follicular activity as reproductive tract development is particularly sensitive to even small increases in circulating estrogen. In the present study, changes in vulva development during pre-pubertal growth in gilts was characterized and demonstrated that vulva widths at 105 and 115 days of age were significantly correlated with age at puberty onset. Thus, gilts with wider vulvas at these time points ($P = 0.071$ and $P = 0.012$) tended to achieve puberty at a earlier age.

In the present study, the time period when ovarian maturation is initiated as evident by the appearance of the initial tertiary follicles was identified. Postnatal days of age 75 to 115 represent a time frame when initial tertiary follicle development begins and variation in the age when individual gilts demonstrate physical developmental changes is the greatest.
Specifically, vulva development has the potential to be used as a predictive measure to identify gilts with advanced follicular development within a cohort. This study demonstrated the most variation in follicular development exists at 95 days of age, as determined in the subset of gilts which were sacrificed from the present study and ovaries examined, suggesting that variation in the activation of the hypothalamic-pituitary-gonadal axis may be occurring around this age of development.

Because of its established role in activation of the HPGx, we measured kisspeptin-10 during the time period of variation in follicular development in gilts. This study showed, during days 75 to 115 of age there was no significant difference in kisspeptin-10 levels between gilts with different puberty onset ages. It is possible that kisspeptin-10 signaling may already be fully activated prior to day 75 and variation between puberty groups was no longer detectable.

Another potential endocrine marker of sexual development is anti-müllerian hormone (AMH). It has been identified as a potential follicular pool size indicator and is a granulosa cell product (Monniaux et al. 2012). For this study it was hypothesized that circulating AMH levels at 95 days of age would be associated with the variation in follicular development. However, in the present study detecting significant differences in circulating AMH concentrations between gilts achieving puberty onset by 200 days of age and those considered non-responsive was not possible.

Physical and physiological changes (e.g. body weight and vulva changes) in gilts during pre-pubertal development are associated with their age of puberty onset and may be useful in identifying potential replacement gilts prior to puberty. Selecting gilts with a propensity to achieve puberty earlier in life may be useful for improving SLP. By entering
the breeding herd having experienced multiple estrous cycles, it is expected that fewer non-productive days will be accumulated from the time the gilt enters the breeding herd and they will have a higher probability of producing multiple parities. The data in this thesis suggest that body weight at 75 days of age and vulva size on days 105 to 115 of age represent useful tools for selecting gilts with an improved opportunity for earlier puberty onset.

Efforts to identify and utilize replacement females in the swine industry to maximize SLP will continue to have economic value to the US swine industry, however, environmental factors represent hurdles to reproductive success. One repeatable phenomenon that compromises reproductive efficiency in swine every year is seasonal infertility. This seasonal period of reduced reproductive performance in the swine industry occurs in the United States during July-September and is a repeatable yet unavoidable occurrence. Multiple reproductive performance indicators are affected by seasonal infertility ranging from decreased farrowing rates, decreased litter size, increased wean-to-estrus intervals, and decreased ovarian activity. While the negative impact of seasonal infertility is well characterized, identification of the biological mechanisms through which seasonal reductions in reproduction occur remain widely under investigation. Multiple environmental factors including photoperiod and heat stress have the potential to influence seasonal infertility. Previous studies have shown heat stress as a predominant factor causing seasonal infertility as the reported decrease in performance occurs during the hottest parts of the year (Prunier et al., 1994; and Auvigne et al. 2010). In this thesis (Chapter 4), effects of heat stress on breeding and reproductive performance were investigated in addition to determining if susceptibility and tolerance of gilts to heat stress (as defined by their thermoregulatory response) during pre-pubertal development is predictive of their thermoregulatory response to heat stress after puberty. The
hypothesis was that gilts demonstrating a susceptible thermoregulatory response to heat stress prior to puberty would be most susceptible post-pubertally and would have a greater incidence of heat stress induced reductions in reproductive performance. Interestingly, and as hypothesized, a positive correlation existed between the heat stress response prior to puberty with the heat stress response after puberty. When comparing reproductive performance between the heat stress tolerant and susceptible gilts only a few parameters (fetal weight, fetal crown-rump length, and CL diameter) were significantly different in the present study. The fetal weight and crown-rump length were higher in the heat stress susceptible gilts. Some literature reports that \textit{in-utero} heat stress can imprint thermotolerance to offspring in insects and birds (Sorensen et al., 2001; and Tzschentke, 2007 respectively). However, for the present study heat stress was only experienced during the follicular phase and up to approximately four days after behavioural estrus. Based on the present study, susceptibility to heat stress in gilts as defined by their thermoregulatory response did not demonstrate a decrease in reproductive performance although gilts classified as tolerant to heat stress had greater CL diameter. Since the heat stress occurred during the period of ovulation, decreased corpora lutea diameter suggests that heat stress during follicular development and ovulation may subsequently affect corpora lutea development and steroid production. Previous research has shown that ovarian activity is interrupted during heat stress including failure to ovulate and early pregnancy loss (Love et al., 1993; and Lopes et al., 2014). One potential reason we did not observe a negative impact of heat stress on reproductive performance is that the heat stress period was focused strictly on the follicular phase and early pregnancy (9 days following Matrix\textsuperscript{®} withdrawal). Other studies that have demonstrated decreased
performance included longer exposure times of heat stress during different reproductive phases.

Reproductive efficiency of gilts entering the sow herd is crucial for the sustainability of the swine industry and efficient utilization of natural resources. Many factors, such as biological, environmental, and management practices can affect sow reproductive performance. Identification and examination of these factors and the biological footings that contribute to their ability to influence reproductive performance will benefit swine producers, through identification and selection of gilts that will have the highest chance of increasing reproductive lifetime performance in the swine industry.

To continue building scientific data in this area, future investigations should be focused on 1) identifying useful blood markers that may have predictive potential to serve as tools in the identification of gilts with a higher capability of achieving puberty onset prior to 180 days of age and 2) evaluate the affects of post-pubertal heat stress occurring during the follicular phase and early embryo development on CL progesterone production.
APPENDIX: ADDITIONAL DATA

Figure A.3.1. Relationship of average body weight on postnatal day 75 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in body weight between puberty age groups ($P > 0.05$).

Figure A.3.2. Relationship of average body weight on postnatal day 85 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in body weight between puberty age groups ($P > 0.05$).
Figure A.3.3. Relationship of average body weight on postnatal day 95 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in body weight between puberty age groups ($P > 0.05$).

Figure A.3.4. Relationship of average body weight on postnatal day 105 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in body weight between puberty age groups ($P > 0.05$).
Figure A.3.5. Relationship of average body weight on postnatal day 115 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in body weight between puberty age groups ($P > 0.05$).

Figure A.3.6. Relationship of average vulva width on postnatal day 75 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva width between puberty age groups ($P > 0.05$).
Figure A.3.7. Relationship of average vulva width on postnatal day 85 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva width between puberty age groups ($P > 0.05$).

Figure A.3.8. Relationship of average vulva width on postnatal day 95 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva width between puberty age groups ($P > 0.05$).
Figure A.3.9. Relationship of average vulva width on postnatal day 105 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva width between puberty age groups ($P > 0.05$).

Figure A.3.10. Relationship of average vulva width on postnatal day 115 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva width between puberty age groups ($P > 0.05$).
Figure A.3.11. Relationship of average vulva length on postnatal day 75 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva length between puberty age groups ($P > 0.05$).

Figure A.3.12. Relationship of average vulva length on postnatal day 85 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva length between puberty age groups ($P > 0.05$).
Figure A.3.13. Relationship of average vulva length on postnatal day 95 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva length between puberty age groups ($P > 0.05$).

Figure A.3.14. Relationship of average vulva length on postnatal day 105 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva length between puberty age groups ($P > 0.05$).
Figure A.3.15. **Relationship of average vulva length on postnatal day 115 and age at puberty onset.** Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva length between puberty age groups ($P > 0.05$).

Figure A.3.16. **Relationship of average vulva area on postnatal day 75 and age at puberty onset.** Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva area between puberty age groups ($P > 0.05$).
Figure A.3.17. Relationship of average vulva area on postnatal day 85 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva area between puberty age groups ($P > 0.05$).

Figure A.3.18. Relationship of average vulva area on postnatal day 95 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva area between puberty age groups ($P > 0.05$).
Figure A.3.19. Relationship of average vulva area on postnatal day 105 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva area between puberty age groups ($P > 0.05$).

Figure A.3.20. Relationship of average vulva area on postnatal day 115 and age at puberty onset. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days). There was no significant difference in vulva area between puberty age groups ($P > 0.05$).
Figure A.4.1. Average uterine weight of the pre-pubertal heat stress tolerant and susceptible gilts. There was no significant difference ($P > 0.05$) in uterine weight between the pre-pubertal heat stress tolerant and heat stress susceptible gilts. Classification of tolerant or susceptible to heat stress was determined by a gilts ability or inability to maintain low rectal temperatures during pre-pubertal heat stress periods.

Figure A.4.2. Average fetal number of the pre-pubertal heat stress tolerant and susceptible gilts. There was no significant difference ($P > 0.05$) in fetal number between the pre-pubertal heat stress tolerant and heat stress susceptible gilts. Classification of tolerant or susceptible to heat stress was determined by a gilts ability or inability to maintain low rectal temperatures during pre-pubertal heat stress periods.
Figure A.4.3. *Average ovary weight of the pre-pubertal heat stress tolerant and susceptible gilts.* There was no significant difference ($P > 0.05$) in ovary weight between the pre-pubertal heat stress tolerant and heat stress susceptible gilts. Classification of tolerant or susceptible to heat stress was determined by a gilts ability or inability to maintain low rectal temperatures during pre-pubertal heat stress periods.

Figure A.4.4. *Average corpora lutea number of the pre-pubertal heat stress tolerant and susceptible gilts.* There was no significant difference ($P > 0.05$) in corpora lutea between the pre-pubertal heat stress tolerant and heat stress susceptible gilts. Classification of tolerant or susceptible to heat stress was determined by a gilts ability or inability to maintain low rectal temperatures during pre-pubertal heat stress periods.
Figure A.4.5. Average percentage of embryo survival of the pre-pubertal heat stress tolerant and susceptible gilts. There was no significant difference ($P > 0.05$) in embryo survival between the pre-pubertal heat stress tolerant and heat stress susceptible gilts. Classification of tolerant or susceptible to heat stress was determined by a gilts ability or inability to maintain low rectal temperatures during pre-pubertal heat stress periods.
REFERENCES CITED

Ahima, R.S., J. Dushay, S.N. Flier, and D. Prabakaran, J.S. Flier. 1997. Leptin accelerates


Differential regulation of kiss1 expression by melatonin and gonadal hormones in

in sows: a five year field study to analyze the relative roles of heat stress and
photoperiod. Theriogenology 74, 60–66.

Baird, D.T. 1983. Factors regulating the growth of the pre-ovulatory follicle in the sheep and
human. J. Reprod. Fert. 69:343-52

swine based on estrogen controlled endocrine versus exocrine secretion of


conceptus secretory products in establishment of pregnancy. J. Reprod. Fert. 76:841-
850


319-24.

Identification of sow-specific risk factors for late pregnancy loss during the seasonal
infertility period in pigs. Theriogenology 72 393–400.

competence is reduced in sows during the seasonal infertility period. Reproduction,
Fertility, and Development. 22:1222–1229

Bertoldo, M.J., P.K. Holyoake, G. Evans, and C.G. Grupen. 2011. Seasonal effects on oocyte
developmental competence in sows experiencing pregnancy loss. Animal


Flowers, W. L. 1999. "Dose confirmation study in sexually mature gilts orally administered altrenogest (Regu-Mate solution 0.22%) to suppress and synchronize estrus."


Gaughan, J.B. 2012. Basic principles involved in adaption of livestock to climate change. In: Environmental Stress and Amelioration in Livestock Production. Springer-Verlag GmbH Publisher, Germany. 245-261


