

**Strategies for improving reproductive performance in gilts**

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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## **DEDICATION**

**LOYAL • FOREVER • TRUE**

I dedicate this thesis to the faculty in the Animal Science Department at Iowa State, both current and past, who provided me with encouragement and guidance in pursuit of my future.

To the friends and family, especially Mom and Dad, whose unwavering support during my academic tenure never went unnoticed nor unappreciated. Thank You.

Go Cyclones!

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**NOMENCLATURE**

<b>ALT</b>	Altrenogest
<b>BA</b>	Born Alive
<b>BW</b>	Body Weight
<b>C</b>	Celsius
<b>CL</b>	Corpora Lutea
<b>CON</b>	Control
<b>CYP19A1</b>	Aromatase
<b>DPE</b>	Days Post Estrus
<b>D</b>	Day
<b>eCG</b>	Equine Chorionic Gonadotropin
<b>E2</b>	Estrogen
<b>ERS1</b>	Estrogen Receptor 1
<b>FI</b>	Feed Intake
<b>FS</b>	Farm Score
<b>FSH</b>	Follicle Stimulating Hormone
<b>GE</b>	Glandular Epithelium
<b>GnRH</b>	Gonadotropin Releasing Hormone
<b>hCG</b>	Human Chorionic Gonadotropin
<b>HPGx</b>	Hypothalamic Pituitary Gonadal axis
<b>HS</b>	Heat Stress
<b>IL1B</b>	Interleukin-1 $\beta$
<b>KNDy</b>	Kisspeptin, Neurokinin B, and Dynorphin neurons
<b>L</b>	Large Vulva score – VSA
<b>LE</b>	Luminal Epithelium
<b>LH</b>	Luteinizing Hormone
<b>LLC</b>	Large Luteal Cells
<b>M</b>	Medium Vulva Score - VSA
<b>MM</b>	Mummified Fetuses
<b>mRNA</b>	Messenger RNA
<b>P4</b>	Progesterone
<b>PGF2<math>\alpha</math></b>	Prostaglandin F2 alpha
<b>PMSG</b>	Pregnant Mare Serum Gonadotropin
<b>PND</b>	Postnatal Day
<b>PROC CORR</b>	SAS correlation analysis
<b>PROC FREQ</b>	SAS frequency analysis
<b>PROC MIXED</b>	SAS mixed procedure
<b>PR</b>	Progesterone Receptor
<b>RR</b>	Respiration Rate
<b>S</b>	Small Vulva Score VSA
<b>SB</b>	Stillborn
<b>SI</b>	Seasonal Infertility
<b>SLC</b>	Small Luteal Cells
<b>SLP</b>	Sow Lifetime Productivity
<b>TB</b>	Total Born



<b>TR</b>	Rectal Temperature
<b>TN</b>	Thermal Neutral
<b>VSA</b>	Vulva Score Method A
<b>VS<sub>B</sub></b>	Vulva Score Method B
<b>VS<sub>1</sub></b>	Vulva Score 1
<b>VS<sub>2</sub></b>	Vulva Score 2
<b>VS<sub>3</sub></b>	Vulva Score 3
<b>VW</b>	Vulva Width
<b>WEI</b>	Wean-to-Estrus Interval

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## ABSTRACT

Efficient pork production is largely dictated by the reproductive success of the sow. Identifying gilts with the ability to produce an optimal number of pigs over successive parities, and do so in as few days as possible is the fundamental key to improving sow lifetime productivity. Low heritability and effect of external factors such as heat stress, makes selecting for reproductive success problematic. Age at puberty is predictive of sow longevity and has been associated with vulva development at approximately 100 days of age. To determine the effectiveness of scoring gilts according to prepubertal vulva width as a means of identifying reproductive superior replacements, physical measurements were recorded at 15 weeks of age and performance was tracked through production. Gilts assigned lowest possible scores based on prepubertal vulva development proved to be less likely to farrow a litter, had an increased age at first farrowing, and showed poorer productivity through their second parity. In addition to maximizing productivity, mitigating negative influences on reproduction is also vital for sow farm sustainability. Heat stress contributes to significant economic losses annually in the swine industry, and results in decreased farrowing rates, smaller litters, and greater wean-to-estrus intervals. To determine the effect of heat stress (HS) on corpus luteum function and the effect on early conceptus development, gilts were assigned to HS or thermal neutral (TN) conditions and either received altrenogest (a progesterone receptor agonist) supplementation or a control group that was not supplemented. Corpus luteum weight was affected by environment and supplementation, however, this change was not reflected in luteal or circulating progesterone concentrations. Conceptus development was accelerated in those supplemented with altrenogest with a higher percentage of conceptuses transitioned into a filamentous

conformation. Conceptus stage of development remained unaffected when environmental assignments were compared. Collectively, these findings provide new insight into the areas of gilt development and selection, and the possible effects heat stress has on early pregnancy, and provide potential management strategies that can be implemented by pork producers.

## **CHAPTER 1: INTRODUCTION**

The continual rise in global food demand coupled the push for higher quality protein worldwide presents challenges as well as opportunities for the U.S. livestock producer and food industries alike (Henchion et al., 2014; Keating et al., 2014). Representing 37% of total meat consumption worldwide, pork serves as one of the primary sources of animal protein (FAO, 2014). Considering the 34% growth in population expected to occur by year 2050, our food supply is pressured to increase to meet the predicted demand (United Nations, 2013). With this call for greater food production, U.S. swine producers must continually strive for improved productivity.

Maximizing sow lifetime productivity is critical for the sustainability and profitability of a sow herd (Stalder et al., 2004); with the objective of having females produce multiple parities while providing adequate nourishment to wean a maximum number of full value pigs with as few non-productive days as possible. When insufficient litters or an inadequate number of full value pigs are weaned per litter, the opportunity for a sow to offset the initial investment and contribute profit to an enterprise is reduced. Based on a variety of reasons, different culling strategies may be utilized on a sow farm to ensure the most productive and genetically current females remain in production. Recent estimates of the average sow turnover rate in the U.S. swine industry are approximately 45% (PigChamp year-end summary 2000-2015). Of sows culled, younger females make up a disproportionate percentage (Dallaire et al., 1987; Boyle et al., 1998; Lucia et al., 2000). This scenario presents many challenges to an enterprise, specifically from a parity structure standpoint. With young females, producers experience increased costs associated with initial purchasing, development, and acclimation of new

replacement gilt candidates (Stalder et al., 2003), as well as increased opportunity cost due to decreased productivity through the first parity (Lucia et al., 1999). In today's industry, reproductive failure accounts for approximately 35% of females culled from breeding herds and is the primary reason for female removal (Koketsu et al., 1997; Mote et al., 2009). Selecting for reproductive performance, however, is difficult due to the complexity of traits associated with reproductive success and the large influence environmental factors may have (Serenius and Stalder, 2006). While selection is possible, the low heritability of related traits results in slow genetic progress.

Additionally, seasonal stressors negatively impact reproduction in swine as denoted by the seasonally-induced reductions in productivity (Love, 1978) imposing further economic strain on the industry (Pollmann, 2010). This predictable dip in reduced fecundity is commonly manifested by reduced farrowing rate, litter size, and extended wean-to-estrus intervals. Understanding the underlying biology associated with heat stress, seasonal infertility and factors associated with early gilt development can allow for the creation and implementation of management practices, ultimately to improve sow lifetime productivity in the U.S. swine industry.

## CHAPTER 2: LITERATURE REVIEW

### Developmental Impacts on Reproduction Performance

*In utero*, the ovarian follicular pool develops through both meiotic and mitotic division (Hirshfield, 1991). Once complete, the finite number of primordial follicles present comprises the entire ovarian reserve and serves as the lone source for all oocytes during a female's reproductive life. As a result, factors during postnatal development have the potential to negatively impact this ovarian reserve and reduce subsequent fertility.

#### *Neonatal Environment*

Adequate gilt development is an important determinant of a sow's reproductive ability, and effective development strategies for gilts throughout their prepubertal life are necessary for breeding herd efficiency. Morbeck et al., (1993) showed that egg nest form ovarian follicles and begin to acquire their functional competence and during the first few weeks of life, suggesting management has the potential to impact the ovarian reserve and subsequent fertility as a mature adult. Additionally, differentiation of uterine glandular epithelium (GE) from luminal epithelium (LE) and expression of estrogen receptor 1 (ESR1) has been shown to be impacted by colostrum consumption, indicating uterine development and function can potentially be influenced during the first few days of life (Spencer et al., 1993; Tarleton et al., 1998; Bartol et al., 2006; Bartol et al., 2013).

Colostrum consumption within the first 24 hours of a gilt's life has also been shown to be beneficial for gilt development with implications for reproductive performance (Vallet et al., 2015). Gilts with inadequate colostrum consumption on postnatal day 1 had lower growth rates, increased age at puberty and reduced number born alive in subsequent

pregnancies. Furthermore, the pigs produced in the first litter from gilts receiving insufficient colostrum had reduced serum immunoglobulin levels, a measurement correlated with day 1 colostrum consumption, and decreased preweaning growth.

Nelson and Robinson (1976) showed that gilts reared in small litters (6 piglets) had increased ovulation rate, number of embryos, and number of live-born piglets in their first litter when compared to gilts reared in large litters (14 piglets). Likewise, Flowers (2009) more recently validated that pre-weaning competition influences sow lifetime productivity by cross-fostering gilts to small ( $\leq 7$ ) or large ( $\geq 10$ ) litters. It was concluded that gilts raised in smaller litters compared to large litters had higher farrowing rates ( $88.7 \pm 1.8\%$  vs.  $83.3 \pm 2.1\%$ ), increased litter size ( $11.0 \pm 0.2$  vs.  $10.5 \pm 0.2$ ), and had a higher percentage of females still in production after six parities (38% vs 16%).

With considerable evidence suggesting key aspects of the preweaning environment can impact reproductive competence, litter of origin effects on puberty in gilts has also been investigated. Interestingly, preweaning growth rate is negatively associated with age at puberty as was total mammary gland protein, which served as a measure of duct cellularity and gland development (Vallet et al, 2016). A negative association was also observed between preweaning growth rate and age at puberty. A relationship between birth weight and total uterine length was also observed reinforcing the idea that a female's reproductive potential is highest at birth, and optimal management and environment are paramount in preserving her future value as a replacement gilt. The idea that reproductive potential of gilts could be manipulated early in life poses some new opportunities and challenges to sow herds.



### *Post-weaning Environment*

In modern confinement systems, gilts are subjected to a variety of unnatural circumstances including artificial lighting, confined pen sizes, and anaerobic byproducts from manure such as ammonia ( $\text{NH}_3$ ) and hydrogen sulfide ( $\text{H}_2\text{S}$ ). These factors during the growing phase have the potential to impact fertility of replacement gilts. When gilts are exposed to elevated ammonia concentrations (21 ppm) from 10 to 40 weeks of age, a decreased proportion are able to attain puberty by 26 weeks of age (Malayer et al., 1987). Similarly, gilts exposed to increased ammonia levels (21 ppm) had a delayed response to boar exposure indicating an increased age at puberty onset (Malayer et al., 1988).

Kuhlers et al., (1985) investigated the effect of space allowance on subsequent reproductive performance. Gilts were housed in groups of 8 or 16, allowing for 1.06 m<sup>2</sup> or 0.53 m<sup>2</sup> from 30 to 65kg and 1.25 m<sup>2</sup> and 0.63 m<sup>2</sup> to 100 kg of body weight, respectively. A higher percentage of gilts ovulated by 100 kg when reared in a low density/higher square footage environment (32.4 %) compared to high density/low square footage (13.5 %). Additionally, parity 1 total born was increased in gilts raised in groups of 8 compared to 16 (10.8 vs. 9.8), along with an improvement in number born alive (10.1 vs. 9.3). A study using the same experimental design investigated the effect of crowding on endocrine development (Rahe et al., 1987). Interestingly, decreased stocking density tended to result in the development of heavier pituitary glands, ovaries, and uteri compared to those reared in pens of 16. Not all studies evaluating the effect of space allowance appear to be in congruence. In a study conducted by Young et al., (2008) 75 day old gilts were assigned to 1.13 m<sup>2</sup>/gilt or 0.77 m<sup>2</sup>/gilt and were raised to 200 days of age when they were moved to the breeding farm. Increased space allowance during

development resulted in puberty achievement at a younger age ( $182 \pm 1.1$  vs  $184 \pm .8$ ) as well as a greater proportion of gilts achieving puberty prior to moving to the sow farm (37.2% vs. 30.3%). However, contrary to Kuhlers et al., (1985) an effect of space allowance on litter size through parity 1 was not observed nor did it influence total pigs born through the first three parities. Although the effect of age at puberty on subsequent reproductive performance was apparent in that gilts reaching puberty earlier (<185 days) compared to later (>185 days), resulting in more pigs produced over the first three parities. Additionally, gilts first artificially inseminated between 240 and 260 d of age produced more pigs over 3 parities compared to those inseminated after 260 days of age, with an increased percentage of females culled by parity 3 for those first inseminated after 280 d of age (Young et al., 2008). Collectively, these studies indicate the importance, and sometimes substantial impact, of prepubertal development environment for maximizing a gilt's genetic potential for reproduction.

#### *Nutrition during Development*

In addition to environmental management, providing adequate nutrition during the prepubertal phase has important implications on sow lifetime productivity. During development, feeding programs that allow for accumulation of adequate body reserves to last into the productive lifetime for females should be implemented. Early literature suggests that when energy stores become too depleted reproductive function can be disrupted (King and Williams, 1984). Anderson and Melampy (1972) investigated the effects of restricted energy intake during the growing phase and reported a delay in the onset of puberty. They concluded that increased energy prior to estrus resulted in an

increased ovulation rate. Additionally, Hughes et al., (1984) observed an increase in embryo survival by ‘bumping’ feed intake prior to estrus. The relationship between growth rate from birth to age at puberty has also been investigated and gilts achieving a lower growth rate because of feed restriction (2 kg/d) from 47 kg to first estrus had a delay in puberty (Beltranena et al., 1991). Interestingly, no differences in parity 1 performance were observed when gilts were subjected to dietary energy restriction from 82 kg to 100 kg (Stalder et al., 2000). However, results from that same group of gilts revealed that limiting energy intake increased the proportion of gilts to achieve parity 1 and showed an increase in a gilt’s ability to reach parity 4 (Stalder et al., 1998).

By limiting energy intake during the developmental phase of a gilt’s life, unnecessary and excessive weight gain can be limited which is beneficial in helping reduce subsequent lameness and locomotion issues (Jorgensen and Sorensen, 1998). The metabolic demands of the gilts used through the early research on nutritional impacts on gilt development are dramatically different from modern maternal lines used in today’s industry, due to selection of lean tissue growth. However, during prepubertal growth and maturation, a gilt’s metabolic demands still shift from protein accretion towards adipose accretion and bone mineralization as they begin to achieve sexual maturity (Stalder et al., 2004). One strategy to promote successful gilt development is through monitoring the weight:age ratio to enable timely entrance into the sow herd, as gilts heavier at first breeding remain heavier to later parities (Rozeboom et al., 1996). Based on modern maternal lines, a replacement gilt should be of moderate weight (135-150 kg) at first breeding and allowed continued growth through the first parity to maximize sow lifetime productivity (Williams et al., 2005).

### **Puberty Onset**

Puberty refers to the achievement of a developmental state in which a female becomes capable of reproducing. In pigs, this is denoted by the onset of the first behavioral estrus. For puberty onset, a gilt must be of adequate maturity, possessing an appropriate body composition and an endocrine capacity capable of activating the hypothalamic-pituitary-ovarian axis (HPGx). This endocrine activation is necessary to facilitate follicular development and eventual ovulation of dominant follicles.

The idea that gilts achieving puberty at an early age is positively associated with improved lifetime reproductive performance is well-established in the literature (Chapman et al., 1978; Koketsu et al., 1999). Patterson et al., (2010) concluded that gilts achieving puberty prior to 180 d of age had an improved retention to parity 3. It has also been shown that gilts expressing early puberty had an increased ability to return to estrus and ovulate within 10 d after weaning their first parity (Sterning et al., 1998).

As mentioned, several factors including both genetic and environment can influence a gilt's ability to achieve puberty. As a result, this can create significant variability in a gilt's age when first estrus is achieved. Successful implementation of management practices including boar exposure, gonadotropin administration, and altering the stress levels by mixing and relocation gilts can all influence attainment of puberty (Brooks and Cole, 1970; Patterson et al., 2016; Hughes et al., 1997).

### ***Puberty Induction***

One of the most effective practices for inducing an initial estrus in prepubertal gilts is exposing them to mature boars (Brooks and Cole, 1970; Kirkwood and Hughes,

1982; Patterson et al., 2002). Several factors, including auditory and visual stimuli in the presence of a boar facilitates puberty induction (Hughes et al., 1990). However, pheromone release from mature boars exerts the greatest contribution to the onset of estrus as demonstrated by Kirkwood et al., (1981) where the removal of the olfactory bulb resulting in no response to boar stimulation. This supports the plausibility that a boar of sufficient maturity, libido and pheromone production could have positive impacts in facilitating induction of puberty. Affirming this postulate, gilts exposed to boars with high libido reached puberty earlier than gilts exposed to low libido boars (Hughes, 1994). Duration and frequency of boar contact also positively influences puberty attainment, as well as the type of exposure (i.e. direct vs. fence-line) administered (Caton et al., 1986; Paterson et al., 1989). Further, boar exposure at an early age accelerates puberty induction (Filha et al., 2009), however the interval from initial boar exposure to puberty decreases with increasing gilt age at first boar contact (van Wetters et al., 2006; Filha et al., 2009) resulting in a tighter synchrony of puberty onset in a group of gilts. Thus, proper boar exposure should be a primary method used for puberty induction in a commercial system gilt pool.

Despite boar exposure being highly effective at inducing estrus, the range of which a cohort of gilts exhibits puberty can be variable. Thus, a common swine industry practice is to initiate cyclicity in gilts by administering a gonadotropin cocktail of equine chorionic gonadotropin (eCG) combined with human chorionic gonadotropin (hCG), functioning analogous to follicle stimulating hormone (FSH) and luteinizing hormone (LH), respectively. Gonadotropins act directly on the follicles of the ovary, ultimately inducing ovulation (Vargas et al., 2006). Gilts injected with P.G. 600<sup>®</sup> (400 i.u. eCG and

200 i.u. hCG) while also receiving routine boar exposure have been shown to decrease the age at puberty as well as decrease the interval from initial treatment to the onset of estrus (Burnett et al., 1988). In this study, ovulation rate was increased from 12.1 to 16.8 in gonadotropin treated groups although embryo survival rate by day 35 was decreased. Britt et al., (1989) showed a single injection of P.G. 600 was beneficial in inducing a fertile estrus in gilts 5.5 to 7.5 months of age and 86 to 163 kg, where number of pigs born alive and number of stillborn piglets remained unaffected compared to non-treated controls. Gonadotropin injections have been shown to induce ovulation in approximately 90% of gilts, however, behavioral estrus is not detected in approximately one third of gilts treated (Knox et al., 2000). Interestingly, maintenance of cyclicity has shown to be negatively affected by gonadotropin induced puberty, with only approximately 50% demonstrating behavioral estrus and ovulating (Paterson and Lindsay, 1981). However, this same study demonstrated when gonadotropin treated gilts received routine boar exposure, an increase in the number of gilts expressing a second estrus and ovulating was observed. This suggests that boar exposure plays an influential role in sexual maturation, facilitating necessary biological and endocrinological function. Despite of the some of the negative implications of estrus induction using gonadotropins, improved synchrony of heat for a gilt pool has the potential to improve efficiency of a sow farm. Additionally, recent work has shown that the use of exogenous gonadotropins for the induction of puberty does not adversely impact sow lifetime productivity (Kirkwood et al., 2000; Patterson et al., 2016)

### *Hypothalamic Neurons and Neuropeptides*

For puberty onset and subsequent maintenance of cyclicity, activation of the hypothalamus must transpire. Within the hypothalamus resides a cohort of neurons that work synergistically to regulate its action through the release of neuropeptides. The idea of neuroendocrine control between hypothalamus and pituitary was originally fostered by (Harris, 1955). Knobil et al., (1980) determined that pulsatile gonadotropin releasing hormone (GnRH) releases were necessary for LH and FSH release. From this it was determined the GnRH release was driven by neural mechanisms which became known as the pulse generator (Knobil, 1980). The concept of neuropeptides controlling the hypothalamus gained increasing support when it was discovered that mutations in the G-protein couple receptor, GPR54, resulted in infertility in men and women (Seminara et al., 2003). GPR54 is expressed in GnRH-secreting neurons and is the receptor for Kisspeptin. Effective binding of Kisspeptin to GPR54 stimulates release of GnRH (Messager et al., 2005), though it is now known that additional neuropeptides also control hypothalamic activity (Lehman et al., 2010). As with Kisspeptin, mutations to the genes encoding neurokinin B and its receptor leads to impaired control of GnRH release and subsequent hypogonadism (Topaloglu et al., 2009). The presence of Kisspeptin and neurokinin B in the arcuate nuclei of the hypothalamus was first reported in the ewe by Goodman et al., (2007) who also discovered a third peptide expressed in the ewe, dynorphin, that exerts inhibitory effects on GnRH release. The inhibitory action of dynorphin on GnRH release was investigated through administration of a dynorphin receptor antagonist, which resulted in increased LH pulse frequency (Goodman et al., 2013). The peptides Kisspeptin, neurokinin B, and dynorphin, also known as the KNDy

neurons (Cheng et al., 2010), are responsible for the production of peptides presumed to be generators of GnRH pulses. The proposed model for GnRH pulse generation involves a reciprocating feedback mechanism between neurokinin B exerting positive feedback and dynorphin exerting a negative feedback on their receptors (NK3R and KOR, respectively), resulting in a pulsatile release of Kisspeptin to act on GPR54 (Rance et al., 2010; Goodman et al., 2013). Based on this model, Kisspeptin is thought to be the primary GnRH pulse generating peptide (Terasawa et al., 2013).

#### *Hypothalamic Pituitary Gonadal Axis*

The HPGx is a complex, multifaceted system of specific ligand binding and receptor signaling resulting in positive and negative feedback between reproductive organs and the brain.

Starting at the hypothalamus, the peptide GnRH is localized in the arcuate nucleus and pre-optic area. GnRH is released in a pulsatile manner and travels via the hypothalamic-hypophyseal portal vein to the anterior pituitary (Clarke and Cummins, 1982). At the anterior pituitary, GnRH binds with a G protein-coupled receptor on the surface of the gonadotrophs, resulting in biosynthesis and secretion of gonadotropins, LH and FSH (Schally et al., 1971; Belchetz et al., 1978). These gonadotropins then enter systemic circulation, where they interact with luteinizing hormone receptor (LHr) on the theca cells and follicle stimulating hormone receptor (FSHr) on the granulosa cells (Peng et al., 1991). LHr is also expressed on the granulosa cells in the days leading up to ovulation to prepare the follicle for the subsequent LH surge, ovulation and eventual luteinization for corpus luteum (CL) formation (Erickson et al., 1979).



Low basal levels of FSH and LH persist throughout the follicular phase of spontaneously ovulating mammalian species, which in turn drives folliculogenesis (Beattie et al., 1973). During early and mid-follicular development phases, the pulsatility of gonadotropins remains low only increasing in frequency and amplitude toward the later stages due to increased gonadotroph sensitivity to GnRH. Upon gonadotropin binding to their specific receptor on respective ovarian cell types, cholesterol is utilized to initiate the process of steroidogenesis. The granulosa and theca cells of the growing dominant follicle work synergistically through a series of enzymatic reactions to convert cholesterol to androgens and estrogens (Fortune and Armstrong, 1977; Fortune, 1986). Throughout early folliculogenesis, the estrogen (E2) produced from the growing follicles exerts a negative feedback on the tonic center of the hypothalamus (Fink, 1979). Additionally, granulosa cells also produce glycoproteins, inhibin and activin, to regulate FSH production from the anterior pituitary, without changing LH production. These feedback mechanisms change at the end of the follicular phase, wherein E2 produced from dominant follicle exerts a positive feedback on the pre-optic area of the hypothalamus and the anterior pituitary to induce a surge release of GnRH and an ensuing LH surge to trigger ovulation (Goding et al., 1969; Scaramuzzi et al., 1971; Chenault et al., 1975; Diskin et al., 2003).

Following ovulation, the ovarian cell types undergo luteinization to form the CL. This differentiation from granulosa cells to luteal cells happen soon after the LH surge and ovulation (Richards et al., 2002). The CL is responsible for producing the hormone progesterone (P4) that exerts a negative feedback on hypothalamus (Goodman et al., 2004). When the ovulated oocyte is not fertilized, the endometrium secretes

Prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) into the uterine vasculature exerting a luteolytic effect on the CL (Moeljono et al., 1977; Shille et al., 1979). Upon regression of the CL, the loss of  $P_4$  induced negative feedback on the hypothalamus and anterior pituitary enables the resumption of GnRH pulsatility and reentry into the follicular phase of the estrous cycle.

### **Estrous Cycle in Swine**

Compared to other domesticated farm animals, pigs exhibit high fecundity, being a litter-bearing species that achieves sexual maturity at a comparatively younger age (5-8 months). Spontaneous ovulation and polyestrous cycles allow pigs to be sexual receptive and conceive during all times of the year (Anderson, 1993). As with other domestic animals, the estrous cycle in pigs (Figure 2.1) can be classified into two phases, the luteal and follicular phases. In the days preceding ovulation, distinct physical and behavioral changes occur indicating sexual receptivity (Hemsworth, 1985). The behavior most characteristic of sows and gilts in estrus is the standing response to back pressure in the presence of a boar (Signoret, 1971). The standing response can last from 24-72 hours in pigs with ovulation occurring 36-42 hours after onset of estrus (Soede et al., 1994). Additional behavioral changes include increased vocalization, attempted mounting of pen mates and reduction in feed intake (Anderson, 1993). Physical characteristics include reddening and swelling of the vulva (Burger, 1952; Holt, 1959; Schenk, 1967) due to increased blood flow to the reproductive tract and expulsion of vaginal discharge (Haynes, 1971).

### *Follicular Phase*

The duration of the follicular phase in swine lasts 4-6 days and is represented by two stages of the estrous cycle; proestrus (approximately days 17-21) and estrus (days 0-2). In pigs, the follicular phase is suppressed during the luteal phase (Goodman and Karsch, 1980 ) due to CL P4 secretion, exerting a negative feedback on the hypothalamus. Following CL regression or lactation, GnRH and gonadotropin pulsatility resumes, allowing the antral follicle pool to develop. The initial GnRH pulsatility is critical for the resumption of the follicular phase, as shown by Esbenshade and Britt (1985) in which immunization against GnRH in gilts halted gonadotropin circulation and subsequent follicular development. When hormonal conditions are appropriate for follicle recruitment to occur, the pulsatile patterns of GnRH and gonadotropins transition to increased frequency and decreased amplitude release pattern, which has been demonstrated in both weaned sows (Shaw and Foxcroft, 1985) and estrus induced females (Flowers et al., 1991). Guthrie et al., (1990) looked further into influences FSH and LH had on the recruitment and development of growing follicles. To do so, gilts were assigned to one of three treatment groups where they were administered FSH, LH, or eCG. When compared to the saline injected control, FSH treated gilts had an increased recruited antral follicle number, whereas the antral follicle pool was decreased in LH and eCG groups. Follicular size and development of dominant follicles was improved when gilts were injected with LH and pregnancy mare serum gonadotropin (PMSG). Additionally, LH and PMSG also increased plasma E2 levels and granulosa cell aromatase activity, further exemplifying the dependency of the developing follicle on gonadotropin activity. Knox et al., (2003) examined the relationship between circulating

gonadotropin levels and ovarian hormones during the porcine estrous cycle for gilts classified as having low and high ovulation rate. Gilts with high ovulation rates (17-26 corpora lutea) had increased FSH and LH levels at ovulation and FSH throughout the luteal phase compared to low ovulation rate gilts (12-16 corpora lutea). Further work was conducted by (Estienne and Crawford, 2015) to investigate effects of gonadotropins on cyclicity after gilts had achieved puberty and after treatment with a P4 analogue. Their work concluded that gonadotropin treatment during the luteal phase altered estrous cycle lengths compared to gonadotropin administration at the follicular phase, which had a greater proportion of gilts with normal estrous cycle lengths (18-24 d). Furthermore, when gilts were synchronized using a progesterone analogue for 14 days followed by a gonadotropin injection, a greater percentage showed estrus within 7 days with a shorter duration from injection to estrus compared to non-injected controls. The importance of the initial pulsatility of GnRH and gonadotropin is validated in the literature and is critical for proper recruitment and development of the follicle leading up to the LH surge.

### *Ovulation*

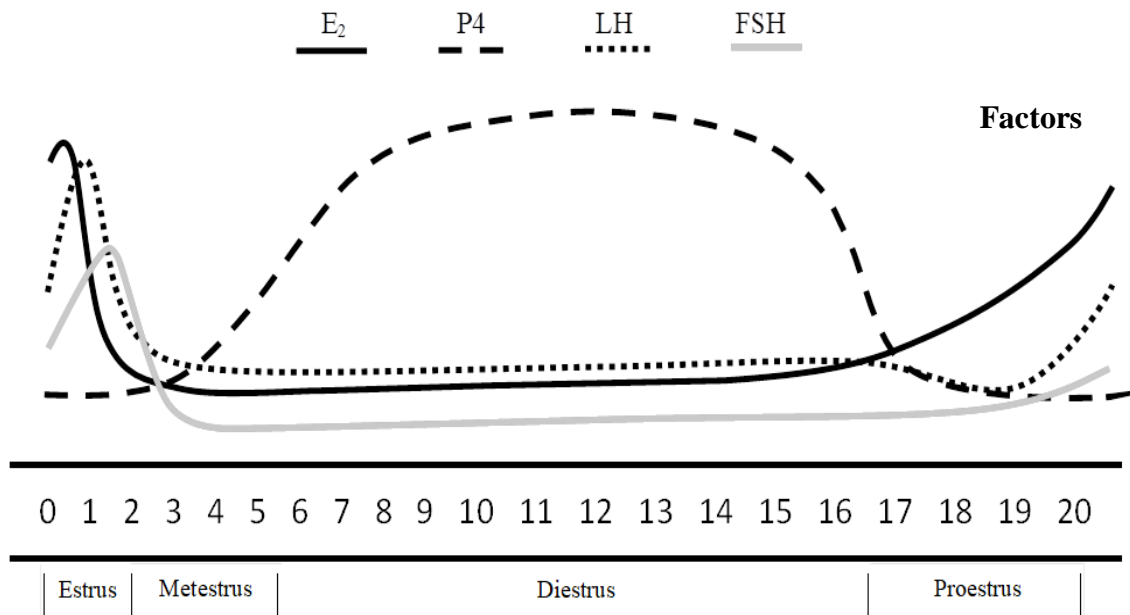
At the time of estrus, a sow or gilt will experience notable behavioral changes indicating sexual receptivity, specifically the standing response to the presence of a boar or back pressure (Signoret, 1970). In the days leading up to ovulation, the conversion of cholesterol to androgens and estrogens in follicular cell increases rapidly with the loss of P4 inhibition from the CL. Circulating E2 levels continues to rise, eventually exerting a positive feedback on the pre-optic area of the hypothalamus to generate a surge of GnRH and ensuing LH surge. Tilton et al., (1982) concluded that the LH surge occurs in concert

with the onset of the standing response, with ovulation occurring approximately 30 hours after the LH peak (Soede et al., 1994). The LH surge propagates the changes that result in rupture of the follicles and ovulation, as well as the oocyte's resumption of meiosis, denoted by the extrusion of the polar body. Immediately following ovulation, LH also initiates luteinization and the differentiation of cell types from follicular to luteal.

### *Luteal Phase*

The luteal phase and the process of CL formation can be characterized by the shift from E2 production by the coordinated efforts of granulosa and thecal cells, to P4 production from luteal cells (Juengel and Niswender, 1999). Following ovulation, dramatic reorganization of the theca and granulosa cells occurs to form the small luteal cells (SLC) and large luteal cells (LLC), respectively (Niswender, 2002). During metestrus (~days 2-5), the mitotic rate of SLC increases rapidly while LLC increase in size (Niswender et al., 2000). As formation of the luteal tissue progresses, LH induces several angiogenic processes (Garrido et al., 1993), where factors such as vascular endothelial growth factor (VEGF) facilitate formation of the capillary system of the early luteal structure, the corpus hemorrhagicum (Senger et al., 1997; Schams and Berisha, 2004). In addition to angiogenic factors, luteotropins including growth hormone (GH) and insulin-like growth factor 1 (IGF-1) increase P4 synthesis (Ptak et al., 2003). At diestrus (days 5-16 of the cycle) the CL is the dominant ovarian structure. By day 8-12 the CL reach peak P4 production, with circulating plasma levels ranging from ~20 ng/mL (Soede et al., 2011) to ~40 ng/mL (Anderson, 2009).

During the diestrus phase, the uterus enters a state of preparation for the event of a pregnancy and potential implantation. The uterine environment provides nourishment for the embryo by producing histotroph, a substance advantageous in the growth and development of implanted embryos (Roberts and Bazer, 1988). If fertilization and growth of viable embryos do not occur, the luteolytic agent,  $\text{PGF2}\alpha$ , is released from the uterine endometrium into the uterine vasculature (Anderson et al., 1961; Moeljono et al., 1977; Shille et al., 1979), initiating luteal regression around day 15 of the estrous cycle. Interestingly, in swine, the corpus luteum is not responsive to  $\text{PGF2}\alpha$  prior to day 12 of the estrous cycle, as shown by Gadsby et al. (2006), demonstrated that administration of  $\text{PGF2}\alpha$  earlier than day 12 did not result in luteolysis due to the low presence of  $\text{PGF2}\alpha$  receptors on the luteal tissue. The lack of responsiveness earlier in the estrous cycle makes luteolytic analogue injections a non-effective method of estrous synchronization in swine. With the lysing of the CL, the negative feedback from P4 on GnRH secretion is reduced, enabling the initial GnRH pulsatility to promote the proestrus stage and resumption of the follicular phase.



**Figure 2.1 Hormone profile of estrous cycle in pigs.** At the start of the luteal phase (metestrus), P<sub>4</sub> levels increase as E<sub>2</sub> levels decline following ovulation and luteinization of the follicular cells. At this point, the corpus hemorrhagicum is the dominant structure on the ovary, eventually becoming a fully functional corpus luteum in diestrus. During diestrus, P<sub>4</sub> initiates negative feedback on the hypothalamus and anterior pituitary, inhibiting gonadotropin release and suppressing follicular development. When pregnancy is not recognized by the uterus, prostaglandin F<sub>2</sub> alpha is released from the endometrium, lysing the corpus luteum. Upon luteal regression, the start of the follicular phase begins at proestrus and antral follicle development is initiated. Rising E<sub>2</sub> levels from the developing follicle provide negative feedback on the tonic center. Estrus onset occurs when follicular produced E<sub>2</sub> induces sexual receptivity of the female and exerts positive feedback on the surge center causing a surge release of GnRH. The GnRH surge results in the LH surge from the anterior pituitary, causing the ovulation of dominant follicles.

### **Factors Associated with Litter Size and Reproductive Performance**

Selection for increased prolificacy has been a primary focus in the U.S. swine industry resulting in steady increases in litter size over the last 20 years (USDA, 2015). Ovulation rate is an initial limiting factor of litter size and has been influenced because of selecting for increased pigs born (Johnson et al., 1999). Fertilization success of ovulated oocytes is less of a limitation for reproductive efficiency. Even artificial insemination, which is highly dependent on timing, is very effective in swine with typical fertilization rates exceeding 90% (Soede et al., 1995).

In the early work investigating factors contributing to litter size, gilts were superovulated to increase the number of viable embryos and subsequently improve litter size (Longenecker and Day, 1968). Interestingly, the number of viable fetuses at day 40 of gestation was not improved by superovulation, suggesting additional limiting factors such as uterine capacity could also impose a biological limit on total pigs born. Selecting solely for ovulation rate has limitations, however, as it is inversely related to birth weight (Johnson et al., 1999) and can potentially contribute to smaller pigs with decreased colostrum intake and increased mortality (Declerck et al., 2016).

Ensuring proper embryonic development is critical for pregnancy establishment. Polge, (1982) demonstrated that the transfer of advanced embryos (24-48 hr. asynchronous from the recipient) could achieve similar pregnancy rates to embryos transferred at the same developmental day as the estrous cycle for the recipient. In the event of asynchrony more than 48 hours between the uterus and the developing conceptus, the uterine environment will not be suitable for implantation and embryo mortality will occur (Geisert et al., 1991). It is estimated that 17-46% of the conceptuses



are lost during the peri-implantation period (Anderson, 1978;Pope et al., 1990). Along with an incompatible uterine environment, variation in development between littermates may occur (Anderson, 1978) which is also thought to contribute to early embryo loss. Considering the competitive environment around the window of implantation, embryos delayed in trophoblastic elongation have insufficient contact with the uterine endometrium, ultimately impairing placentation.

Synonymous with impaired embryo implantation, uterine capacity largely limits potential litter size in pigs. Christenson et al., (1987) defined uterine capacity as the maximum number of piglets carried to term when potentially viable conceptuses are not limiting. Uterine crowding models have shown that when uterine space is limited, an increase in conceptus mortality occurs between days 20 and 50 (Wu et al., 1989). The ability to select for gilts with increased uterine capacity could prove beneficial in improving litter size without negatively affecting piglet birth weight or *in utero* development.

In attempt to assess the effect of reproductive tract size on litter performance, Tarocco and Kirkwood (2002), took vaginal length measurements at the time of artificial insemination following estrus expression. This study concluded that vaginal length is not correlated with litter size in gilts. A better assessment of uterine capacity on potential litter size was reported by Freking et al., (2016) using of a specific genetic line from a population selected for increased uterine capacity compared to a standard control line. This study demonstrated that gilts with increased uterine capacity on average endure more parities, produce more total born, born alive, fewer stillbirths and weaned pigs in their lifetime. Additionally, sows with increased uterine capacity had increased birth

weight and weaning weight over four parities when compared to control sows. These results strongly suggest that uterine capacity is positively associated with many aspects of sow lifetime productivity, and that a method to distinguish gilts with such advantage could prove beneficial.

Selection and retention of highly prolific individuals based on parity 1 performance could be predictive of lifetime performance (Iida et al., 2015). Gilts giving birth to >15 live pigs at parity 1 produced an increased number of live born piglets through subsequent parities when compared to gilts having fewer live piglets in their first litter. While sows that birth 15 live-born piglets in their first litter account for a small percentage of most sow herds, preservation of these females has the potential to improve sow farm output and lifetime production.

### **Maternal Recognition and Establishment of Pregnancy**

Maternal recognition of pregnancy, a term first coined by Short in 1969, is the tightly regulated process through which mammalian embryos signal their presence to the dam, in effort to extend the lifespan of the CL, and support pregnancy establishment. Synthesis of specific signaling molecules results in CL maintenance and subsequent production P4. CL maintenance is critical for the entirety of gestation in pigs, as loss of this P4 source at any time during gestation results in abortion (Diehl et al., 1974; Nara et al., 1981)

### *Estrogen*

In mammalian species, sufficient communication between conceptus and the maternal uterine environment must occur to facilitate successful implantation and gestation. The pig embryo's initial mechanism for signaling pregnancy to the dam is through the production of estrogens. Early studies initially examined the effects of non-steroidal estrogens (diethylstilbestrol) injections during the estrous cycle (Kidder et al., 1955), and demonstrated that E2 exposure at day 11 extended cycle length to ~25 d. The presence of viable embryos after day 11 is critical for preserving the CL (Dhindsa and Dziuk, 1968), who also concluded that removal of embryos prior to day 11 resulted in resumption of normal cyclicity and estrus expression by day 21. Perry et al., (1973) was the first to validate that porcine conceptuses had the capacity to produce E2 from days 11-18 leading to the acceptance that E2 is the signal for maternal recognition of pregnancy in swine. E2 release from the embryo is biphasic, with the first surge occurring concomitant with embryo elongation (day 11-12) and the second surge at trophoblast attachment around days 15 to 18 (Geisert et al., 1987).

At the time of rapid elongation when the conceptus transitions from a spherical form (~10 mm diameter) to a filamentous conformation (>150 mm in length), the trophoblast dramatically increases in steroidogenic acute regulatory protein (StAR) abundance as well as expression of aromatase (CYP19A1), enabling E2 synthesis (Conley et al., 1994). However, E2 is not thought to initiate conceptus elongation, where instead, E2 production at these time points is critical for maintenance of the CL (Morgan et al., 1987a; Morgan et al., 1987b). This was demonstrated by Frank et al. (1977), where

exogenous E2 exposure provided during the entire peri-implantation window extended functional CL life to 60 days.

From a production perspective, it is important to recognize that environmental exposure to estrogenic compounds prior to or during the implantation window can have negative implications. In pigs, exposure to mycotoxins such as zearalenone can cause endocrine disruption, leading to early embryonic death (Long et al., 1983) and CL preservation resulting in a pseudopregnancy. Additionally, during an established pregnancy, exogenous estrogenic compounds have been shown to reduce uterine and fetal weight (Etienne and Jemmali, 1982).

### *Progesterone*

For pregnancy to be carried to term, a continuous source of P4 is required. In the pig, P4 is synthesized and released by the CL for the duration of gestation, and will undergo regression if exposed to PGF2 $\alpha$  any time after day 12 of pregnancy (Moeljono et al., 1977). At the time of maternal recognition, conceptus produced E2 exerts a direct luteotropic effect on the CL (Conley and Ford, 1989), in addition to initiating endometrial changes to prevent luteolysis and maintain P4 production (Geisert et al., 1990). For pregnancy to be established, down regulation of P4 receptor must occur in both the LE and GE. Simultaneous with the down regulation of P4 receptor, alteration in uterine gene expression and receptivity occur. P4 action and suppression of its receptor allows for progestamedins and estrogens to stimulate the production of different growth factors associated with trophoblastic growth and implantation (Ka et al., 2007).

With the significant role P4 has in preparing for implantation and maintaining pregnancy, much research has been done to investigate the potential benefits of supplementing P4 in early pregnancy. Initial work done by Ashworth (1991), investigated the effect of supplementing P4 after mating to gilts with known differences in nutritional status. This study concluded that when gilts were provided *ad libitum* access to feed, ovulation rates increased, however only  $12.4 \pm 1.7$  of the  $19.2 \pm 1.2$  ovulated oocytes were considered viable fetuses at day 30 (66.4%). The number of viable fetuses in this study was increased when *ad libitum* fed gilts received daily injections of P4 from day 4 to day 30 of pregnancy to  $15.9 \pm 1.6$  out of  $19.2 \pm 1.2$  ovulated oocytes (82.8%). Interestingly, positive results were not found by Mao and Foxcroft, (1998), when P4 supplementation strategies were applied to weaned sows following lactation. In this study, gilts receiving P4 injections experienced decreased total embryo number, percent viable embryos, and embryo survival rate to day 28 of pregnancy. It is important to note the P4 administration protocol differed between the Ashworth (1991) and Mao and Foxcroft (1998) studies. Mao and Foxcroft started P4 treatment 36 hours after estrus onset, and was continued over a series of six injections 12 hours apart. When a P4 analogue, altrenogest (ALT), is given in the initial days following ovulation, similar results on fetus development have been reported as well as litter size (Soede et al., 2012). Effects of oral ALT treatment from days 1-4 (n=24) resulted in decreased pregnancy rate, 38%, compared to ALT treatment days 2-4 (n=29), 83%, and untreated controls (n=23) (100% pregnancy rate). Reduction in fetal count and survival rate was also noted for both ALT groups compared to the control. In a second experiment involving weaned sows, Soede and others investigated administering ALT at both days 4 and 6 after estrus onset,

group one receiving 10 mg (ALT10) and group two receiving 20 mg (ALT20). When litter data was analyzed across treatments groups, no differences were detected for farrowing rate or number born alive. However, a tendency for increased total born in untreated control sows compared to ALT20 sows was observed.

From this work, the time and concentration of which P4 or progestens are present following ovulation clearly has important implications on early embryo development. Recent work has investigated exogenous P4 effects on gene expression that is reflective of endometrial receptivity and conceptus development during the peri-implantation window (Szymanska and Blitek, 2016). Beginning 3 days after the last insemination, gilts received 25 mg/100kg BW of P4 until day 4 and an additional 50mg/100kg BW days 5-10. From this study, the investigators concluded P4 treatment resulted in an increase in 6-keto PGF1 $\alpha$  and a tendency for increased E2 in uterine flush content. When mRNA for endometrial tissue was analyzed, increased expression of prostaglandin-endoperoxide synthase 2 (PTGS2), microsomal prostaglandin E<sub>2</sub> synthase (mPGES1), and VEGF was increased in P4 supplemented groups compared to controls. It is clear the P4 is necessary for conceptus survival as well as facilitating the maternal actions needed to support a pregnancy. It is also apparent that many more questions are still to be answered regarding the specific role P4 has during the peri-implantation window, and the timing at which P4 exerts its effects. To understand this, the use of P4 as a treatment to reduce embryonic mortality could prove beneficial in the swine industry.

### *Endocrine-Exocrine Theory*

The mechanism of preventing luteolysis by PGF<sub>2</sub> $\alpha$  on the CL is unique in the pig compared to other domestic animal species. Originally proposed as the “endocrine-exocrine theory” (Bazer and Thatcher, 1977), the pig uterus undergoes protective processes at the time of maternal recognition of pregnancy to prevent PGF<sub>2</sub> $\alpha$  from executing its luteolytic action, ultimately prolonging CL lifetime. The endocrine-exocrine theory is based off evidence that has shown (1) PGF<sub>2</sub> $\alpha$  circulating in the uterine vasculature is reduced from days 12-18 in pregnant gilts (Moeljono et al, 1977). (2) PGF<sub>2</sub> $\alpha$  is increased in the uterine content from days 11-15 in pregnant gilts compared to non-pregnant (Zavy et al., 1980). (3) Estrogens produced from growing blastocysts are the cause for the redirection and sequestering of prostaglandins into the uterine lumen around days 11-15 (Frank et al., 1977; Bazer et al., 1982). In the uterine lumen, PGF<sub>2</sub> $\alpha$  is metabolized to an inactive form before reaching the ovaries via circulation (Ziecik et al., 2011), and can no longer cause the structural or functional regression of the CL. Further support for the endocrine-exocrine theory came from Gross et al. (1988), demonstrating luminal production of PGF<sub>2</sub> $\alpha$  and PGE<sub>2</sub> at days 12-14 of pregnant, and d 14 of pseudopregnant was observed compared to d 14 of the estrous cycle in non-pregnant gilts. Conversely, myometrial production of prostaglandins was increased on d 14 of cyclic gilts compared to pregnant and pseudopregnant gilts. Thus, validating that around the time of maternal recognition, the directionality of prostaglandin synthesis appears shifted and as a result decreases endocrine released PGF<sub>2</sub> $\alpha$  capable of entering circulation and exerting luteolytic effects.

### *Conceptus Elongation*

Heuser and Streeter, (1929) were the first to recognize the different morphological stages of the porcine conceptus showing that some conceptuses could be rapidly elongating (> 150 mm) in the presence of small (<10 mm diameter) spherical littermates. This phenomenon of rapid conceptus elongation begins after the embryo hatches from the zona pellucida (day 6-7), and progresses to 9-10 mm in diameter by day 10-11 (Stroband and Van der Lende, 1990; Geisert et al., 2014). From days 11-12, the porcine conceptus undergoes significant cellular remodeling, transitioning from a tubular conformation to a filamentous structure (Geisert et al., 1982). This transition to a filamentous conformation occurs rapidly (Anderson, 1978; Geisert et al., 1982), and is critical for effective expansion across the uterine surface. With increased surface area in conceptus/uterine interface, E2 is better able to minimize the endocrine release of PGF2 $\alpha$  into utero-ovarian circulation in addition to establishing and maximizing nutrient transfer for the trophoctoderm, and eventually the diffuse, epitheliochorial placenta (Giesert and Bazer, 2015). Anderson (1978) determined that these conceptus modifications from spherical to filamentous do not occur simultaneous within a litter, as indicated by various morphological forms that can be found in the uterus at one time. As aforementioned, it is thought that variation in the timing and rate of elongation could contribute to embryonic loss, as the competitive environment for establishment of uterine location and surface area may favor those that undergo transformation sooner and more rapidly (Pope, 1988). The specific factors that activate these transformations from ovoid to filamentous shape are not fully understood (Geisert et al., 2014).



Uterine environment can be influenced by steroid hormones as demonstrated by Vallet et al., (1998), where P4 administered on days 2 and 3 post-estrus resulted in increased uterine flush total protein. However, as mentioned, the production of conceptus E2 seems unrelated to the initiation of elongation (Morgan et al., 1987b). Additionally, CYP19A1 null embryos can still develop into spherical and filamentous morphologies (Meyer, Geisert, Spencer, Prather unpublished data) despite the lack of CYP19A1 activity. *In vitro* attempts to facilitate conceptus elongation have resulted in successful embryo development but failure to form filamentous conceptuses (Vejlsted et al., 2006), further exemplifying the importance of the uterine environment, architecture, and other factors associated with this critical time point.

Pig conceptus elongation is not the result of increased hyperplasia, but rather an alteration of the shape and morphology of the trophoctoderm and endoderm (Perry, 1981; Geisert et al., 1982). Beginning at the ends of the epiblast, weakening in the trophoctoderm cell-junctional complexes allows for the migration, increase in intracellular tension and subsequent alteration in the shape of the endodermal cells to more of a columnar shape (Geisert et al., 1982). This modification in cell morphology is thought to be caused by the changes in localization of microfilaments in the cell (Mattson et al., 1990). From the epiblast, these structural changes extend down from the dense embryonic region termed the “elongation zone” toward the ends of the tubular conceptus. It has been proposed that the process of elongation and the cellular changes that occur are the result of a paracrine effect from the developing mesoderm (Geisert and Bazer, 2015). Epiblast production of fibroblast growth factor 4 (FGF4) is presumed to activate the mitogen-activated protein kinase (MAPK) cascade via interaction with fibroblast growth

factor receptor 2 (FGFR2) expressed on the trophectoderm, stimulating the production of bone morphogenetic protein 4 (BMP4) (Magana et al., 2014). Synthesis of BMP4 instigates the differential changes of the mesoderm cells. Simultaneously, the underlying endoderm takes on a spindle shape formation and condenses at the elongation zone. The formation of filopodia allow the endodermal cells to pull the adjacent trophectoderm to the elongation zone, where the paracrine effects and cellular mechanisms can be initiated, eventually resulting in the filamentous structure seen on day 12.

### *Interleukin-1 Beta*

Interleukin-1 Beta (IL1B), a pro-inflammatory cytokine, increases dramatically during the transition from tubular to filamentous (Tuo et al., 1996; Ross et al., 2003a). Immediately following the elongation phase, however, *IL1B* mRNA expression returns to its nadir, indicating IL1B is an important component in the cascade of events related to elongation. Unique to the pig, an embryonic form of interleukin 1 beta, IL1B2, exists that is only detected in conceptus prior to implantation (Groenen et al., 2012). During rapid elongation, IL1B2 is thought to have significant contribution by increasing the membrane fluidity of trophectoderm through activation on phospholipase A2 and subsequent upregulation of arachidonic acid in the phospholipid bilayer (Geisert et al., 2012). While *IL1B2* mRNA is only increased for the short duration of the elongation window, IL1B2 protein can still be detected in the uterine lumen until day 15 (Ross et al., 2003a) and plays integral roles in the cross talk between the developing conceptus and the endometrium in the establishment of pregnancy.

From the onset of the elongation process to implantation, IL1B is thought play a critical role functioning as an inflammatory mediator, and an activator of the nuclear factor kappa-B (NFKB) cascade (Ross et al., 2010). Activation of inflammatory pathways, however, poses risk for the developing embryo, being semi-allogeneic (Warning et al., 2011). Thus, the conceptus must evade rejection from the maternal immune system. Simultaneous with the release with IL1B and activation of the NFKB pathway, is the release of conceptus E2. The activation of estrogen receptor (ESR) has been proposed to aid in regulating the inflammatory response (Ross et al., 2010), thereby suppressing the inflammatory reaction that could negatively affect embryo survival. By IL1B activating NFKB, prostaglandin synthesis is also stimulated from days 10-13 of pregnancy resulting in increased prostaglandin E<sub>2</sub> (Waclawik et al., 2009; Franczak et al., 2010), which has protective effects on prolonging the life of the CL. Indeed, the upregulation of IL1B is significant at the time of elongation and aids in alteration of the cellular morphology and induces the changes necessary for embryonic survival and implantation.

#### *Fibroblast Growth Factor 7 (FGF7)*

*FGF7* mRNA expression in the pigs differs from other species, as it is initiated in the LE, as opposed to the stromal cells, starting at day 9 of the estrous cycle with maximum levels being reached by day 12 of pregnancy. This increased expression results in increased FGF7 protein abundance and is prevalent up to day 20 of pregnancy (Ka et al., 2000). Steroid hormones, E2 and P4, work in coordination to regulate the expression of FGF7. For *FGF7* mRNA expression to occur, it seems that P4 must first induce

progesterone receptor (PR) downregulation in the uterine epithelium. This action by P4 seems to be permissive in allowing E2 to interact with estrogen receptor 1 (ERS1) in the endometrium, resulting in upregulation of *FGF7* mRNA (Ka et al., 2007). FGF7 receptor, FGFR2IIIb, is present on the endometrial epithelium as well as the conceptus trophoctoderm (Ka et al., 2000) and can induce cell proliferation, differentiation and migration (Szebenyi and Fallon, 1999). FGF7 stimulates the trophoctoderm to proliferate, suggesting a paracrine effect to mediate conceptus elongation, serving as an important instigator of critical events preceding implantation.

### **Seasonal Infertility**

Seasonal infertility (SI) is a complex, multifaceted concept likely influenced by several factors including photoperiod, temperature, genetics, and management (Love et al., 1993; Prunier et al., 1994; Auvigne et al., 2010). With swine being a polyestrous species, they are routinely exposed to seasonal variations having the potential to influence reproductive ability. Seasonal infertility is characterized by an increased percentage of sows and gilts that remain anestrous or fail to conceive after insemination. Seasonal dips in production are witnessed across the industry, primarily during late summer and early fall months, correlating with the months of increased ambient temperatures (Love, 1978). The economic impact of heat stress is not easily quantified; however, it is estimated that decreased sow reproductive performance alone accounts for approximately \$450 million lost for the U.S. Swine industry (Pollmann, 2010). Additional costs associated with decreased offspring growth efficiency and carcass yield add to the losses the industry experiences, further exemplifying the severity of SI and the

importance of development of mitigation strategies to reduce the negative effects of heat stress.

### *Factors Influencing Seasonal Infertility*

While what causes SI is not clear, changes in efficacy of the gonadotropin stimulation and release are a potential driving factor. Characteristic behavior of swine during times of heat stress is a reduction in feed intake, and while highly correlated it has not been confirmed to be the root cause of SI. Much work has suggested that reduced nutrient intake is a possible link to lower sow fertility (Kirkwood et al., 1987; Kirkwood et al., 1990), with a negative energy balance impacting the pre-ovulatory LH surge (Baidoo et al., 1992) and decreased regular LH pulsatility in early pregnant gilts (Peltoniemi et al., 1997). The importance of adequate nutrient intake becomes increasingly critical during and immediately following a lactation, where mobilization of energy stores would be increased. Increased circulating leptin has been shown to occur in concert with increased incidence of anestrus and lower pregnancy rates, likely due to a lessened nutrient intake (De Rensis et al., 2005). While it is not clear what effect leptin has on the HPGx in the pig, there is a potential connection between leptin and Kisspeptin neuron function as shown in the mouse (Smith et al., 2006). With Kisspeptin being a primary upstream activator of GnRH release, it has been a recent area of focus for SI. The effects of restricted feed intake and higher melatonin levels during the summer months been shown to reduce Kisspeptin production (Castellano et al., 2005; Zhou et al., 2014). While the impact of heat stress on endocrinology is not fully understood, work in the area suggests it could be a contributing factor to seasonal dips in production.

### *Heat Stress Effects on Reproduction*

The effects of increased temperatures on gilt reproductive ability have been studied extensively for many years and have the potential to impact fertility at any point during a sow's lifetime. In a five-year study, Auvigne et al., (2010) demonstrated a reduction in reproductive performance most prevalent during summer months, with the greatest reduction being in the years with a greater number of days classified as being hot ( $>25^{\circ}\text{C}$ ).

As stated above, a gilts ability to achieve puberty in a timely manner is critical for sow farm efficiency and predictive of lifetime reproductive potential (Patterson et al., 2010). The ability for a gilt to achieve sexual maturity can be challenged by multiple stressors, including elevated ambient temperatures. Previous work has shown a significant reduction in a percentage of females achieving puberty by 230 days when reared in heat stress conditions compared to gilts reared in control conditions indicating chronic exposure to increased ambient temperatures delays sexual maturation (Wettemann et al., 1988; Flowers et al., 1989).

In early work investigating heat stress on swine reproduction, Edwards et al., (1968) determined that prior to breeding, reproductive behavior did not seem to be severely compromised, although duration of the estrous cycle was numerically increased. In addition, they determined that extreme temperatures ( $39^{\circ}\text{C}$ ) for the first 15 days of gestation resulted in significant reduction in embryo viability. Wettemann et al., (1988) also reported a heat stress induced increase in conceptus fragmentation at day 16 of gestation in gilts, illustrating that the time of peri-implantation is potentially sensitive to heat stress.

The wean-to-estrus interval (WEI) is also pivotal aspect of assessing sow reproductive efficiency as it represents a production phase when accumulation of non-productive sow days is inevitable but highly variable. A prolonged WEI adds additional costs to sow farms through non-productive day accumulation, interrupted breeding cycles, missing the targeted number of matings, and potentially lessens lifetime performance of individual sows. During times of elevated ambient temperatures, WEI and estrus-ovulation-interval can be increased in length and variability. Armstrong et al., (1986) attributed the extended WEI to reduced activity of the hypothalamus during the summer months, which resulted in decreased LH synthesis and decreased follicle diameter in weaned primiparous sows. Lopes et al., (2014) confirmed these claims, reporting a negative relationship between follicle diameter at weaning and an increase in days to estrus and ovulation.

To understand the effects of heat stress during early pregnancy, its influence during various times of gestation was investigated by Omtvedt et al., (1971). In agreement with the other studies, early gestational heat stress decreased embryo viability and resulted in fewer pregnancies at 30 days post-breeding. In the same study, heat stress during late gestation (102-110 days) was also shown to negatively impact litter performance, with a reduction in number of pigs born alive and an increase in dead pigs when compared to gilts from control conditions.

Reduced farrowing rates are responsible for the greatest losses to the swine industry (Peltoniemi et al., 1999; Bloemhof et al., 2008), of which early pregnancy loss likely accounts for the reduction in farrowing rate that is commonly seen with SI. When sows farrowing in summer and autumn months were compared to sows farrowing in the

winter and spring months, a 15.8% reduction in farrowing rate was observed when sows were farrowed in the summer and autumn months (Lopes et al., 2014). This same study also reported an additional 1.5 piglet advantage in litter size for those that farrowing the winter and spring months. Interestingly, sows that exhibited a WEI between 3-6 days had higher farrowing rates compared to those with a delayed return to estrus, regardless of season.

#### *Mitigation Strategies of Seasonal Infertility*

As already discussed, one potential contributor to SI is the variation in activity of HPGx, thus, use of exogenous hormones offer a potential strategy to aid in a timelier resumption to cyclicity or synchronizing ovulation for accurate timing of semen deposition (Kirkwood and Kauffold, 2015).

Stimulation of ovarian follicular development using exogenous gonadotropins is a common swine industry practice. Administration of eCG and hCG at weaning during the summer months has been effective in getting a greater number of sows, parity 1 and 2, to return to estrus sooner (Bates et al., 1991; Manjarin et al., 2010). Therefore, in the instance where ovarian activity is delayed, intervention with gonadotropins is an option to promote estrus. Administration of the progesterone analogue, ALT, blocks LH secretion from the pituitary gland preventing follicle growth beyond 4-6 mm, in turn limiting E2 production and estrus expression (Redmer and Day, 1981). If negative energy balance is indeed a major contributor to SI, and a prolonged WEI is the biological attempt to enable metabolic recovery, the use of ALT could serve as a tool to prolong and tighten the window of estrus expression in a group of females. The efficacy of using ALT during



the summer months is not well studied, however, administering ALT to gilts and sows at weaning has been linked with increased ovulation rates and litter size (Martinatbotte et al., 1995; Koutsotheodoros et al., 1998; Martinat-Botte et al., 2010). Clearly, the primary approach to counter the negative effects of increased temperatures and humidity is to implement cooling strategies. The intervention with the use of hormonal treatment for stimulating/synchronizing estrus could facilitate and improve the reproductive function during different seasons.

### **Summary**

As highlighted, reproductive performance is critical for the sustainability of sow farms, and implementing management and development practices is paramount to maximize lifetime productivity. Much of the recent work suggests that reproductive capabilities of replacement gilts can be influenced very early in life by extrinsic factors (i.e. neonatal environment, disease, nutrition, stocking density, boar exposure). Developing a gilt pool with quality replacement females requires rearing in an environment conducive to achieving adequate growth, early puberty onset, successful conception and lactation. Accurately selecting females with the greatest reproductive potential is necessary to improving sow retention rates and sow lifetime productivity. Seasonal effects exert costly impacts on reproduction and mitigation strategies to combat these effects are important to improving the efficiency of the industry.

In the following research, the objective of the first study was to test the accuracy of different vulva scoring methods on a group of prepubertal gilts, and determine if distinguishing gilts with differences in reproductive potential is possible. The second

study was conducted in parallel with the first, with the objective being to test the effectiveness of visual appraisal of vulva size by farm personnel as a minimally invasive method to identify gilts at a prepubertal stage with decreased reproductive potential. The final study was conducted to investigate the effects if increased ambient temperatures during early pregnancy establishment, and whether supplementation with ALT during this time was beneficial to embryo development or mitigated any negative impacts of heat stress with respect to luteal function.

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**CHAPTER 3:  
METHODS FOR REPRODUCTIVE TRACT SCORING AS A TOOL FOR  
IMPROVING SOW PRODUCTIVITY**

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Contribution Statement

MRR coordinated data collection, statistical analysis and interpretation, and wrote the manuscript. BJH and JTS contributed to data collection. TG represented TriOak Foods, was primary collaborator and coordinated data collection. LHB, AFK, CJR and JWR advised on experimental design and statistical analysis. JWR served as primary investigator and corresponding author.

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### Abstract

Improving sow lifetime productivity (SLP) is essential for maximizing sow farm profitability. Study objectives were to determine the accuracy of different vulva scoring methods in a commercial production system and to assess whether gilt reproductive tract scoring evaluated by vulva width (VW) prior to puberty could serve as a useful selection criteria. To accomplish this, 958 prepubertal replacement gilts in a commercial system were evaluated at approximately 15 wk of age. Body weight for each gilt was recorded in addition to four different methods to evaluate VW. Methods for VW assessment included digital caliper measurement (mm), visual evaluation and scoring by trained farm personnel (Farm Score; FS), and two methods using scoring tools (Vulva Score Method A and B; VSA and VSB, respectively) specifically calibrated from the distribution of VW measurements of gilts from previous studies. VSA and FS methods assigned gilts to one of three categories (S, M, L and 1, 2, 3, respectively) whereas VSB classified gilts into a five-point system (1 to 5). At 15 wk of age, a low proportion of variability in vulva size ( $27.8 \pm 0.1$  mm) could be explained by BW ( $62.2 \pm 0.2$  kg;  $R^2 = 0.05$ ). All three scoring methods were effective in differentiating gilts, as mean VW measurements for each score across methods differed ( $P < 0.01$ ). Percentage of gilts achieving their first parity increased with score for VSA (64.7, 73.2, and 84.4%;  $P = 0.02$ ), VSB (66.0, 71.7, 79.2, 76.4, and 84.2%;  $P = 0.02$ ), FS (67.2, 75.0, and 88.8%;  $P = 0.03$ ). VSA, VSB, and FS did not influence percentage of gilts achieving their second parity ( $P = 0.32, 0.29$ , and  $0.30$ , respectively). Litter performance of gilts scored as M or L using VSA showed an increased total born over two parities compared to those scored as S (23.96 vs. 26.38 pigs;  $P < 0.01$ ) as well as born alive (21.13 vs. 23.05 pigs;  $P < 0.05$ ). Results were similar

for VSB, where scores 2-5 had greater total born (23.97 vs. 26.33 pigs;  $P < 0.01$ ) and born alive (21.11 vs. 23.02 pigs;  $P < 0.05$ ) through two parities compared to gilts scored 1. Using the FS, total born pigs tended to be greater ( $P = 0.06$ ) through two parities for gilts scored a 2 or 3 compared to those scored as a 1. Collectively, assessing VW at 15 wk of age appears to have advantages for prepubertal identification of sows with improved productivity through two parities.

**Keywords:** gilt, vulva width, sow lifetime productivity, litter size

## Introduction

Accurate selection and retention of replacement females with the reproductive ability to wean a maximum number of quality pigs over recurrent parities is imperative for maximizing sow lifetime productivity (SLP) (Stalder et al., 2003). However, selecting for SLP is arduous as reproductive performance is under the control of numerous genetic loci and largely impacted by environmental factors (Serenius and Stalder, 2006). Currently, the most predictive trait for identifying young females with the greatest potential for SLP is age at puberty. Puberty, or age at first estrus, is predictive of a sow's ability to produce at least 3 parities (Patterson et al., 2010). Furthermore, gilts reaching puberty earlier are more likely to show estrus and ovulate within ten days of weaning (Sterning et al., 1998), which reduces non-productive sow days in the breeding herd.

Gilt management practices prior to introduction into the sow herd can ultimately impact female reproductive potential. During development, a gilt's reproductive tract becomes responsive to hormonal changes and is associated with increased follicular

development and total tract size around 70 d of age (Dyck and Swierstra, 1983). This change in reproductive tract size is presumably the result of endogenous estrogen production from the initial follicular growth during the prepubertal period. Our group has demonstrated that the initiation of tertiary follicle development is highly variable within a cohort of gilts but begins after postnatal d (PND) 75 (Graves et al., 2015). Additionally, reproductive tract growth when assessed by vulva width (VW) at PND 95-115, has predictive value with a gilt's ability to achieve puberty by 200 d of age (Graves et al., 2015). Collectively, this led to our hypothesis that reproductive tract scoring in a commercial pork production system, as assessed by VW at approximately 15 wk of age could effectively identify gilts with different reproductive potential. The objective of this study was to evaluate different prepubertal vulva scoring methods on a commercial farm to determine the method's effectiveness for identifying gilts differing in reproductive potential.

## **Materials and Methods**

This study was conducted in cooperation with TriOak Foods, Inc. with animal procedures approved by the Iowa State University Animal Care and Use Committee.

### ***Animals***

Gilts (n = 958) across three birth wk were used for this study. At approximately six wk of age, gilts were transferred from the parent multiplier to the initial receiving gilt development unit (GDU). At approximately 15 wk of age, gilts were subjected to routine culling criteria (lameness, poor structural conformation, abdominal hernias). Following

initial culling, the remaining gilts were transported to two different GDUs specific to the destination sow farm. The number shipped from each birth wk depended on replacement gilt demand at the respective sow farm. As a result, the number of potential replacement gilts that had the opportunity to enter the sow herd was reduced to 731.

### ***Gilt Development***

At the receiving GDU, gilts were allotted approximately 0.84 m<sup>2</sup> each and housed in groups of 25 on fully slatted floors. Upon arrival, gilts received exposure to porcine epidemic diarrhea virus (PEDv), as well as vaccinations for ileitis (Enterisol<sup>®</sup> Ileitis, Boehringer Ingelheim) and erysipelas (Ery Vac<sup>®</sup>, ARKO Laboratories). Once transported to the sow farm specific GDU, all gilts were inoculated with a site specific PRRSv strain. At the sow farm gilts were again allowed approximately 0.84 m<sup>2</sup> per head. At 20 wk of age gilts were again subjected to culling criteria unrelated to reproductive performance. Boar exposure (approximately 10 min of contact/pen) for puberty stimulation and heat detection began at approximately 180 d of age. Beginning at approximately 26 wk of age, selected gilts entered the designated sow farm. At the time of first mating, a sow farm specific ID was given, that enabled individual gilt production data to be extracted from the production system database. Gilts not demonstrating behavioral estrus by 36 wk of age were culled, and noted for failure to display estrus.

### ***Data Collection***

Data collection took place at the receiving GDU in March 2016. At approximately 15 wk of age, gilts were given an ear tag for identification (Hog Max<sup>®</sup>, Destron Fearing)

and BW were recorded. VW was recorded in millimeters using Ultra Tech digital calipers (General Tools, Secaucus, NJ). At the time of measurement, the guides of the digital calipers were positioned at the widest part of the vulva. Vulva scores (VS) were also assigned for each gilt using three different vulva scoring methods. Vulva Score Method A (VSA) used a three-score strategy designed using previous data (Graves et al., 2015) and gilts were stratified into the following categories; Small (S;  $VW < 27$  mm), Medium (M;  $VW 27-34$  mm), and Large (L;  $VW > 34$  mm). Vulva Score Method B (VSB) used a similar tool to categorize gilts into five groups based on VW: 1 ( $VW < 27$  mm), 2 ( $VW 27 - 29$  mm), 3 ( $VW 29 - 31$  mm), 4 ( $VW 31 - 33$  mm), and 5 ( $VW > 33$  mm). The design of the tool used for VSA and VSB was a laminated card with a series of sections of precise sizes removed to accurately assign a VS based on the above described dimensions (Figure 3.1). Farm Score (FS) was a subjective assessment of vulva size (conducted visually by trained farm staff) which stratified gilts into three categories (1, 2 or 3), with score 1 intended to identify gilts whose vulva size representative of the bottom 15%, score 2 targeting intermediate 70%, and score 3 the upper 15%. The FS was conducted independently of all other measurements to avoid bias in the subjective score. At the sow farm, production data was recorded by farm staff, documented and stored in an online swine data base (PigKnows LLC.).

### ***Reproductive Performance***

Production records of selected gilts were monitored and data recorded for any event occurring prior to achieving their first parity (P1), including return to estrus events, failed pregnancy checks, abortion, and other reasons for removal. For those that



successfully farrowed, litter data recorded included total piglets born (TB), number born alive (BA), stillborn (SB), and mummified fetuses (MM). Following P1 weaning, subsequent reproductive performance data was collected through the second parity (P2).

### ***Statistical Analysis***

Statistical Analysis Systems University Edition, version 9.4 (Cary, NC) was used for all statistical analysis. Relationship between BW and VW was analyzed using PROC REG to generate coefficient of determination values. Group means for each classification method were compared using PROC TTEST. Chi-Square ( $\chi^2$ ) analysis was performed using the PROC FREQ function to determine the association between VS classification and ability to achieve P1 and P2. Additionally, for each vulva scoring method (VSA, VSB, or FS) PROC MIXED was used to analyze the litter performance data, with the model including VS, sow farm, birth wk, and the associated interactions. Prior to analyzing litter performance data, data points extending beyond 2.5 standard deviations from the mean of TB, BA, SB, and MM were considered outliers and were removed from analysis.

## **Results**

### ***Vulva scoring methods were effective in separating groups of gilts based on vulva width***

At approximately 15 wk of age, a weak linear association was observed between VW and BW ( $R^2 = 0.05$ ;  $P < 0.01$ ; Figure 3.2A). Average VW measurement for categories within scoring method VSA, VSB and FS differed ( $P < 0.01$ , Figures 3.2B,

3.2C, and 3.2D, respectively). Across birth wk, VW was decreased in birth wk 1 relative to birth wk 2 or 3 (0.99 and 1.33 mm, respectively;  $P < 0.05$ ; Table 1). Additionally, BW at 15 wk was increased for birth wk 2 compared to birth wk 1 or 3 (2.04 and 1.96 kg, respectively;  $P < 0.05$ ; Table 1). Using VSA method, 23.5, 70.3 and 6.2% of gilts were distributed into S, M, and L classifications, respectively (Table 2). Similarly, for the VSB method, 22.7, 25.5, 34.3, 12.3, and 5.3% of gilts were represented in scores 1 to 5, respectively (Table 2). Additionally, the FS method allocated 22.8, 69.7 and 7.6% of gilts into VS 1 to 3, respectively (Table 2).

### ***Gilts with increased vulva score have improved ability to achieve parity 1***

Across all scoring methods, gilts scored as S for VSA, 1 for VSB, and 1 for FS all had decreased likelihood of reaching P1 when compared to remaining cohorts within the same scoring method ( $P = 0.02, 0.02, 0.03$ , respectively). No difference was observed for the percentage of gilts that achieved parity 2 (P2) across all scoring methods ( $P = 0.32, 0.29, 0.25$ ). For all scoring methods, no difference was detected in farrowing interval, the duration from time at scoring to P1 interval, 1<sup>st</sup> service to P1 interval, and 1<sup>st</sup> service to P2 interval (Tables 3-5).

### ***P1 and P2 litter performance is affected by prepubertal vulva score classification***

#### ***Vulva Score Method A***

First parity TB was increased 1.2 pigs ( $P < 0.01$ ) for gilts scored as M compared to S (Table 6). In contrast to S or M, gilts scored as L were not different, although TB for M and L combined (M+L) was increased 1.1 pigs ( $P < 0.01$ ) relative to S scored gilts.

Differences in P2 performance were detected with fewer ( $P = 0.04$ ) TB between S (12.3) and M (13.6) scored gilts. Furthermore, gilts scored as S produced approximately 1.3 fewer piglets ( $P = 0.02$ ) compared to the rest of the cohort (M+L). Similarly, P2 BA piglets was 1.3 fewer ( $P = 0.02$ ) for gilts whose vulvas scored S compared to counterparts. SB was not affected by VS ( $P = 0.24$ ). Expectedly, when total production through P2 was analyzed, TB for M (26.4) and M+L (26.4) combined was greater ( $P < 0.01$ ) compared to S (24.0) gilts. BA through P2 for S gilts was 2.0 and 1.9 pigs fewer ( $P = 0.03$ ) compared to M and M+L, respectively.

#### *Vulva Score Method B*

Using VSB to distinguish prepubertal differences in vulva size, P1 TB was increased for gilts receiving VS of 3 (1.4 pigs) and VS of 4 (1.5 pigs) compared to gilts with a VS of 1 ( $P < 0.01$ , Table 7). P1 TB for all gilts scored 2 to 5 combined (2-5) was increased 1.1 pigs compared to gilts scored as 1 ( $P < 0.01$ ). P1 BA tended ( $P = 0.08$ ) to be increased (1.01 pigs) in gilts scored 3 compared to 1. Although other VS were not different, P1 BA was increased (1.1 pig;  $P < 0.01$ ) for gilts scored 2 to 5 compared to gilts assigned a VS of 1. No effect of VS was detected for P1 SB ( $P = 0.1$ ) or mummified fetuses ( $P = 0.22$ ). Likewise, SB and MM were not affected between gilts with a VS of 1 compared to the combined of 2-5. Gilts achieving P2 with a VS of 1 produced 1.0 fewer ( $P = 0.02$ ) TB piglets and 1.2 fewer ( $P = 0.03$ ) BA compared to the remaining gilts that scored 2 to 5 (Table 7). Combined TB for P1 and P2 was affected ( $P < 0.03$ ) by VS while total BA through P2 was not ( $P = 0.20$ ). However, total TB and BA

for P1 and P2 were increased 2.3 ( $P < 0.01$ ) and 1.9 ( $P = 0.03$ ), respectively, in gilts with a VS of 2 to 5 compared to gilts scored 1.

### *Farm Score*

Interestingly, no differences were observed across P1 litter data for gilts scored using the farm score method (Table 8.). For P2, TB tended to be increased between scores 1 and 3 (1.8 pigs;  $P = 0.06$ ) and between scores 1 and 2 for BA (1.1,  $P = 0.09$ ). When the combined totals for scores 2 and 3 were compared to 1, a tendency for increased TB and BA (1.0, 1.2 pigs;  $P < 0.09$ , respectively) was observed. When total P1 and P2 production was considered, TB for gilts scored 2 to 3 tended to increase compared to gilts with a score of 1 (1.6,  $P = 0.06$ ).

## **Discussion**

Maximizing sow farm inclusion and reproductive efficiency of selected replacement gilts is essential for sow farm productivity. However, multiple genetic elements contributing to reproductive success coupled with a large environmental influence can make selecting replacement gilts with the greatest reproductive potential challenging (Serenius and Stalder, 2006). Age at which a gilt achieves first estrus can be predictive of longevity and a gilt's ability to reach later parities (Patterson et al., 2010) and age at first farrowing has also shown to be a favorable indicator of sow longevity (Hoge and Bates, 2011). Furthermore, gilts who reach puberty sooner have an increased ability of returning to estrus and ovulating within 10 d after weaning their first parity (Sterning et al., 1998). This evidence suggests that gilts achieving specific reproductive

checkpoints (i.e. puberty and parturition) earlier in life are more reproductively capable compared to counterparts achieving the same milestones later in life.

A prerequisite for puberty attainment is the activation of the hypothalamic-pituitary-gonadal axis (HPGx), which is reliant on hypothalamic neurons, known as KNDy neurons (Cheng et al., 2010). Upon stimulation, the KNDy neurons produce and secrete Kisspeptin to initiate GnRH pulsatility via its receptor GPR54 (Goodman et al., 2013), subsequently, GnRH then induces gonadotropin release from the pituitary gland. The early presence of LH pulses results in initial follicular development and reproductive tract growth from 80 -120 d of age (Dyck and Swierstra, 1983; Evans and O'Doherty, 2001). The timing of this initial reproductive tract development is variable amongst individual animals, resulting in the absence or presence of tertiary follicles at specific ages (Christenson et al., 1985).

Previously, differences in VW beginning at approximately 95 to 115 d of age was predictive of a gilts ability to achieve behavioral estrus by 200 d of age (Graves et al., 2015). Puberty is a critical time-sensitive checkpoint as a gilt's inability to express standing estrus is a primary cause for failure to enter the breeding herd (Mote et al., 2009). While the relationship between prepubertal reproductive tract development and subsequent reproductive performance is not well-documented in the literature, a consensus exists that early reproductive maturation and puberty onset is associated with improved lifetime productivity (Chapman et al., 1978; Koketsu et al., 1999). The present study objective was to determine the effectiveness of prepubertal VW scoring as a marker of reproductive tract development, with intent to identify gilts with increased reproductive ability. Regardless of scoring method used, gilts classified with a VS in the

lowest ranking category (approximately 20%) had a reduced ability to successfully achieve P1.

Although, litter size can be affected by numerous aspects, ovulation rate, fertilization rate, early embryo survival, and uterine capacity are ostensibly all major influencers of progeny number. However, ovulation rate, while being the initial limiting factor, has limited influence on litter size when intensely selected for (Cunningham et al., 1979; Lamberson et al., 1991). Furthermore, fertilization rate is very efficient, at approximately 95% (Polge, 1978; Steverink et al., 1997), suggesting that litter size is primarily affected by embryo survival and uterine capacity. While we did not measure uterine capacity *per se*, this study revealed that gilts with larger vulvas at 15 wk of age had increased TB and BA through two parities.

The relationship between early reproductive tract growth and fertility is not clearly defined, however, correlations between the two traits have been shown to exist in specific porcine breeds. In Meishan pigs, a breed known for attaining puberty early and producing large litters, follicular development can start as early as 45 d of age (Miyano et al., 1990), indicating HPGx activity may begin sooner than in other domestic pig breeds. In the current body of work, gilts were distinguished by variation in prepubertal VW at approximately 15 wk of age, presumably the result of differential follicular activity and resultant estrogen synthesis and release. Speculatively, it seems logical that early reproductive tract development would contribute to greater fecundity. It is also possible that selecting for gilts with increased reproductive tract development early in life consequently identifies those with improved uterine capacity, which is associated with

decreased embryonic loss (Bolet et al., 1986) and increased pigs produced though four parities (Freking et al., 2016).

Based on the results herein, it seems plausible that variation in vulva size at 15 wk of age is the result of endogenous estrogen production from tertiary follicle development (Graves et al., 2015). Estrogen activity, mediated through its multiple receptors, is essential for regulating reproductive function in the pig; providing regulation of the HPGx as well as the signal for pregnancy recognition (Dhindsa and Dziuk, 1968; Perry et al., 1973; Bazer and Thatcher, 1977; Geisert et al., 1982). Underscoring the importance of estrogen signaling in porcine reproduction is that the estrogen receptor locus is a major contributor to litter size in pigs (Rothschild et al., 1996). In pigs, expression of estrogen receptor in the glandular epithelium can be influenced in neonatal life via colostrum, and is associated with improved reproductive performance in gilts (Bartol et al., 2006; Vallet et al., 2015). Additionally, the pig uterus starts to increase in size and growth rate starting at approximately 80 d of age in response to ovarian produced estrogens (Dyck and Swierstra, 1983). In the present study VW (as a biomarker to distinguish gilts with earlier reproductive tract development) is presumably responsive to estrogen production. This response potentially indicates earlier HPGx activation in addition to the uterus potentially having increased sensitivity to endogenously produced estrogen. Based on the previous work demonstrating the importance of estrogen and its receptors (Bazer and Thatcher, 1977; Rothschild et al., 1996), particularly with respect to establishing the cross communication between the dam and conceptus (Geisert et al., 1982; Morgan et al., 1987), it seems plausible that gilts with increased sensitivity to estrogen may have a

reproductive advantage. This speculation warrants further investigation to understand the relationship between reproductive tract responses to estrogen and its effects on fertility.

### **Conclusion**

Selection for reproductively superior replacement gilts is challenging. Gilts with reduced reproductive tract development at 15 wk of age, as assessed by vulva size, exhibited a lower inclusion rate into the breeding herd and produced fewer pigs through two parities. Activation of the HPGx precedes the onset of puberty and these data suggest that relative differences in vulva size, as a proxy for ovarian estrogen synthesis, prior to puberty onset at 15 wk of age is predictive of reproductive ability and could be a valuable approach for identification of gilts for inclusion and/or exclusion as potential replacements. The underlying biological cause for litter size improvement, and the effectiveness of vulva scoring across different genetic lines presents opportunities for further investigation.

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### **Declaration of Interest**

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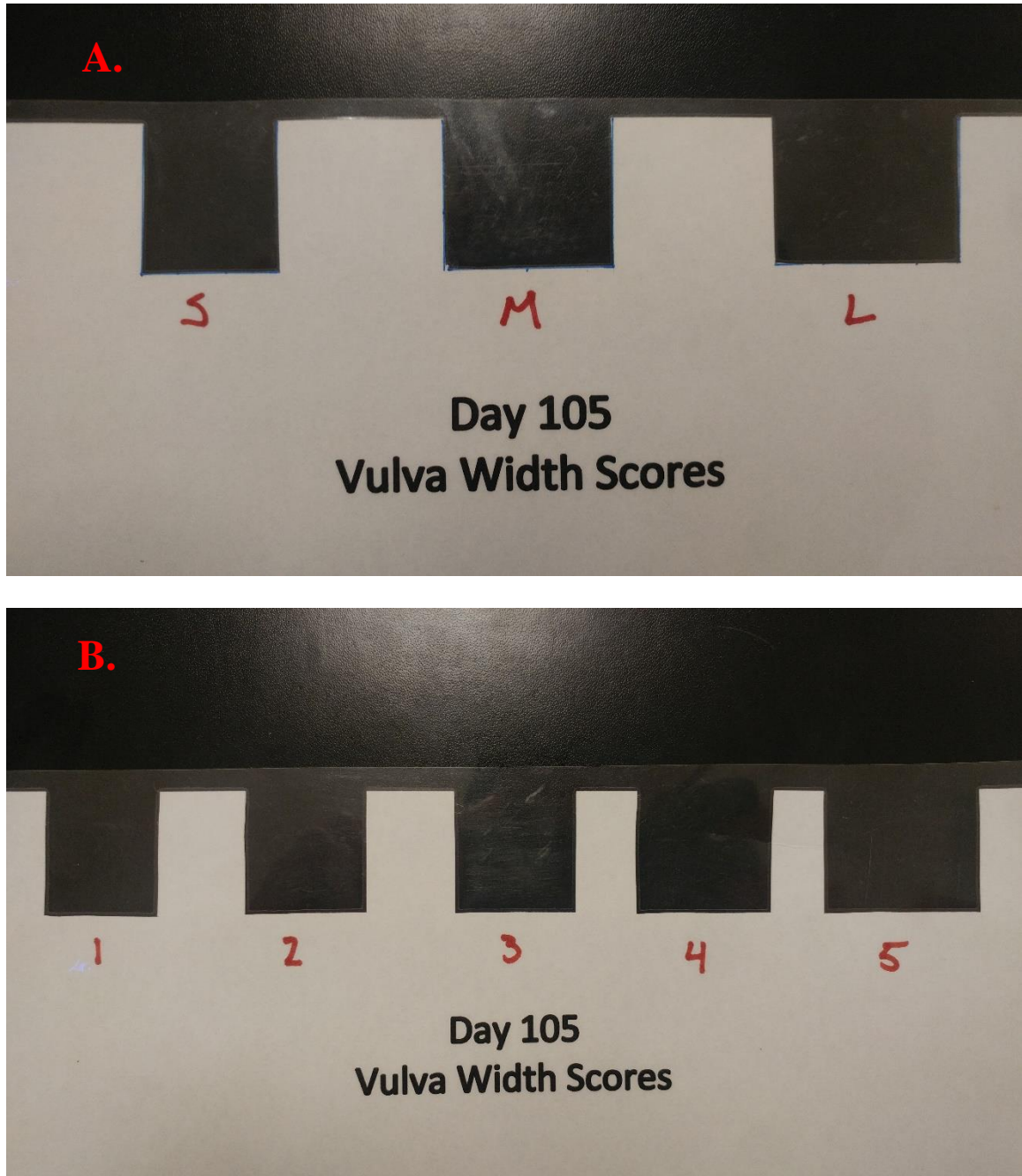
publication are those of the authors and do not necessarily reflect the views of TriOak foods Inc. or Iowa Pork Producers Association. No conflicts of interest, financial or otherwise are declared by the authors.

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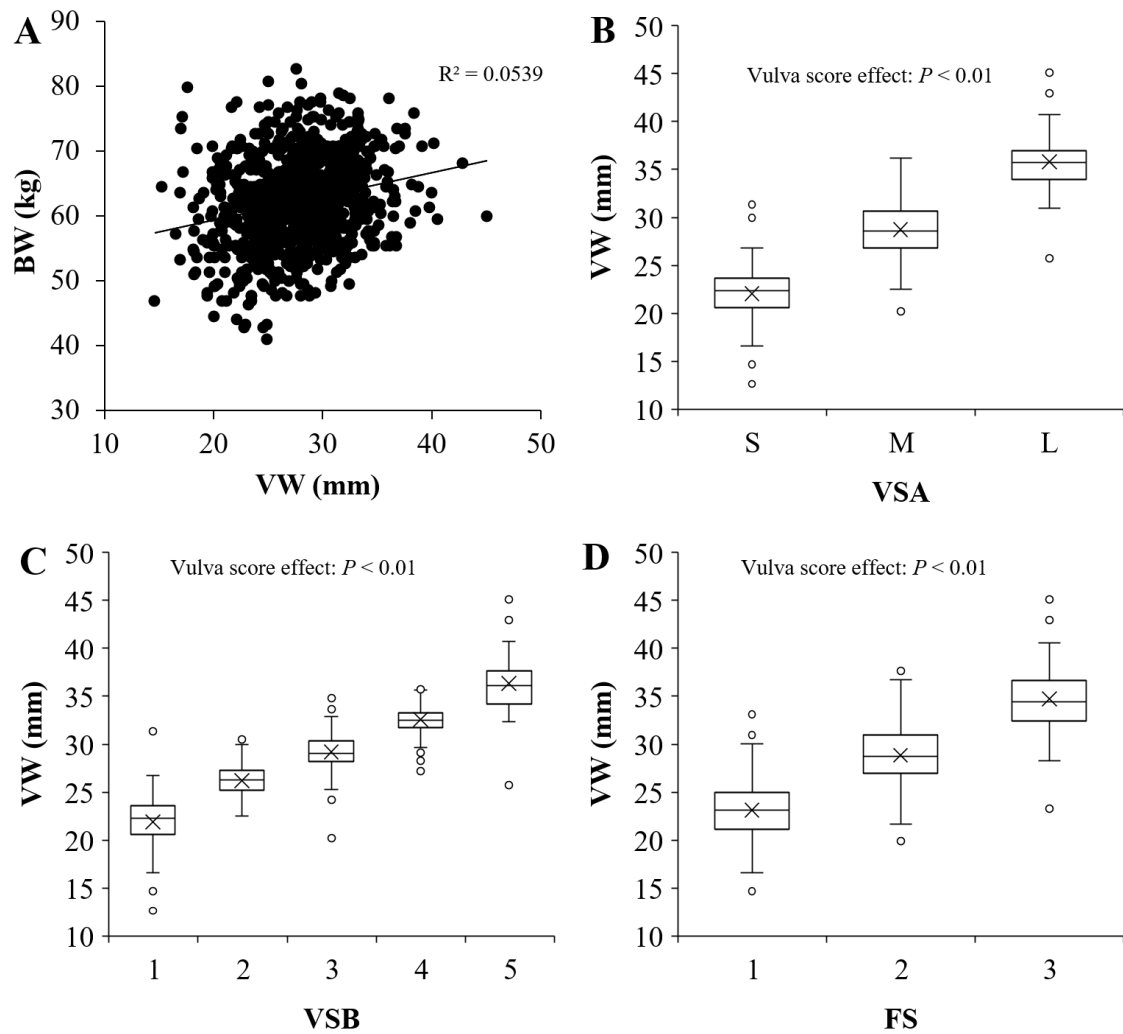
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subsequent growth, puberty attainment, litter size, and lactation performance. J. Anim. Sci. 93(6):2722-2729.



**Figure 3.1 Vulva Score A and B Calibrated Tools.** **A.** Vulva Score Method A (VSA) tool used to score gilts based on vulva width (VW): small (S; VW < 27 mm), medium (M; VW 27-34 mm), and large (L; VW > 34 mm). **B.** Vulva Score Method B (VSB) tool used to score gilts based on vulva width: 1 (VW < 27 mm), 2 (VW 27-29 mm), 3 (29-31 mm), 4 (VW 31-33 mm), and 5 (VW > 33 mm).



**Figure 3.2 Relationship of Vulva Width (VW) with Body Weight (BW) and Effectiveness of Different Vulva Scoring Methods to Accurately Partition Gilts.**

**A.** BW and VW were assessed at approximately 15 wk of age. A weak positive correlation exists between the two traits, with an  $R^2$  value of 0.05 ( $P < 0.01$ ). **B-D.** Box and whisker plots of distribution for VW measurements at 15 wk of age based on Vulva Score Method A (VSA; **B.**), Vulva Score Method B (VSB; **C.**), and Farm Score (FS; **D.**). Each method, while variable, was effective in partitioning gilts into separate groups based on vulva size. Points shown beyond the whiskers represent outliers for each score within the scoring method. Whiskers denote the minimum and maximum value for each score while top and bottom boundaries represent the upper and lower quartiles, respectively, with the middle line indicating the median for each score. The X near the median line signifies the mean VW measurement for each category. Statistical significance exists for each vulva score mean across methods ( $P < 0.01$ ).

**Table 3.1. Vulva Width (VW) and Body Weight (BW) across Birth wk.**

Birth wk <sup>1</sup>	1		2		3		Total
	n	%	n	%	n	%	n
	194	16.2	490	40.9	274	22.9	958
Parameter							
VW (mm) <sup>1</sup>							
Mean	28.72 <sup>a</sup>		27.73 <sup>b</sup>		27.39 <sup>b</sup>		27.58
SEM	0.34		0.18		0.25		0.13
BW (kg) <sup>2</sup>							
Mean	61.18 <sup>a</sup>		63.22 <sup>b</sup>		61.26 <sup>a</sup>		61.60
SEM	0.93		0.66		0.97		0.44

<sup>1</sup>Birth wk refers to the specific calendar wk during which the gilt was born

<sup>2</sup>Vulva Width (VW) was measured to the nearest millimeter using digital calipers

<sup>3</sup> Body Weight (BW) was recorded using an individual scale provided at the cooperating GDU

<sup>a-b</sup> Differences in letter denote significance level ( $P < 0.05$ )

**Table 3.2. Distribution of Gilts by Vulva Score.**

Score	n	%
VSA <sup>1</sup>		
S	281	23.5
M	842	70.3
L	74	6.2
VSB <sup>2</sup>		
1	272	22.7
2	305	25.5
3	410	34.3
4	147	12.3
5	63	5.3
FS <sup>3</sup>		
1	162	22.8
2	496	69.7
3	54	7.6

<sup>1</sup>Three scores, small (S), medium (M), and large (L) were assigned using the VSA method

<sup>2</sup>Five scores, 1-5 were assigned using VSB method

<sup>3</sup>Three scores, 1-3, were assigned using FS method

**Table 3.3. Gilt Inclusion Rate and comparison of Sow Productive Says using VSA<sup>1</sup>.**

	Classification			SEM	<i>P</i>
	S	M	L		
Breeding Parameter					
Selected Gilts <sup>2</sup>	156	530	45		
Gilts Achieving Parity 1 <sup>3</sup>	101	388	38		
% Achieve Parity 1 <sup>4</sup>	64.7	73.2	84.4		0.02
Gilts Achieving Parity 2 <sup>5</sup>	69	263	25		
% Achieve Parity 2 <sup>6</sup>	44.2	49.6	55.6		0.32
Score to Farrow Interval <sup>7</sup>	251.4	252.2	243.3	2.3	0.43
1 <sup>st</sup> Service to P1 Interval <sup>8</sup>	121.5	121.8	116.9	3.1	0.20
1 <sup>st</sup> Service to P2 interval <sup>9</sup>	269.7	268.8	261.8	3.0	0.32

<sup>1</sup>Vulva Score Method A<sup>2</sup>Gilts that arrived at the designated sow farm gilt development unit (GDU)<sup>3</sup>Number of gilts successfully producing a first parity<sup>4</sup>P-value calculated using Chi-square comparison<sup>5</sup>Number of gilts who farrowed a second litter<sup>6</sup>P-value calculated using Chi-square comparison<sup>7</sup>Number of d from vulva scoring at the receiving GDU until achieving first parity<sup>8</sup>Number of d from first service until first litter farrowing<sup>9</sup>Number of d from first service until second litter farrowing



**Table 3.4. Gilt Inclusion Rate and comparison of Sow Productive Days using VSB<sup>1</sup>.**

	Classification					SEM	<i>P</i>
	1	2	3	4	5		
Breeding Parameter							
Selected Gilts <sup>2</sup>	150	173	264	106	38		
Gilts Achieving Parity 1 <sup>3</sup>	99	124	209	81	32		
% Achieve Parity 1 <sup>4</sup>	66.0	71.7	79.2	76.4	84.2		0.02
Gilts Achieving Parity 2 <sup>5</sup>	66	77	138	55	21		
% Achieve Parity 2 <sup>6</sup>	44.0	44.5	52.3	51.9	55.3		0.29
Score to Farrow Interval <sup>7</sup>	252.0	252.0	252.4	250.0	244.2	3.2	0.46
1 <sup>st</sup> Service to P1 Interval <sup>8</sup>	121.7	119.7	123.2	120.2	117.3	2.4	0.66
1 <sup>st</sup> Service to P2 interval <sup>9</sup>	269.1	266.0	268.8	272.3	262.4	3.2	0.43

<sup>1</sup>Vulva Score Method B<sup>2</sup>Gilts that arrived at the designated sow farm gilt development unit (GDU)<sup>3</sup>Number of gilts successfully producing a first parity<sup>4</sup>P-value calculated using Chi-square comparison<sup>5</sup>Number of gilts who farrowed a second litter<sup>6</sup>P-value calculated using Chi-square comparison<sup>7</sup>Number of d from vulva scoring at the receiving GDU until achieving first parity<sup>8</sup>Number of d from first service until first litter farrowing<sup>9</sup>Number of d from first service until second litter farrowing

**Table 3.5. Gilt Inclusion Rate and comparison of Sow Productive Days using FS<sup>1</sup>.**

	Classification			SEM	<i>P</i>
	1	2	3		
Breeding Parameter					
Selected Gilts <sup>2</sup>	125	384	36		
Gilts Achieving Parity 1 <sup>3</sup>	84	288	32		
% Achieve Parity 1 <sup>4</sup>	67.2	75.0	88.8		0.03
Gilts Achieving Parity 2 <sup>5</sup>	50	173	21		
% Achieve Parity 2 <sup>6</sup>	40.0	45.1	58.3		0.25
Score to Farrow Interval <sup>7</sup>	254.1	252.2	243.3	2.7	0.34
1 <sup>st</sup> Service to P1 Interval <sup>8</sup>	121.6	123.3	117.0	3.5	0.24
1 <sup>st</sup> Service to P2 interval <sup>9</sup>	266.6	270.4	265.8	3.4	0.46

<sup>1</sup>Farm score method where trained personnel assigned vulva score

<sup>2</sup>Gilts that arrived at the designated sow farm gilt development unit (GDU)

<sup>3</sup>Number of gilts successfully producing a first parity

<sup>4</sup>P-value calculated using Chi-square comparison

<sup>5</sup>Number of gilts who farrowed a second litter

<sup>6</sup>P-value calculated using Chi-square comparison

<sup>7</sup>Number of d from vulva scoring at the receiving GDU until achieving first parity

<sup>8</sup>Number of d from first service until first litter farrowing

<sup>9</sup>Number of d from first service until second litter farrowing

**Table 3.6. Parity 1 and 2 Performance for Gilts classified using VSA.**

Parity 1	Classification <sup>1</sup>				S vs. M+L			
	Index	S	M	L	SEM	P	M+L	P
		(n=100)	(n=397)	(n=37)			(n=434)	
Total Born		11.79 <sup>a</sup>	12.96 <sup>b</sup>	12.42 <sup>ab</sup>	0.39	0.02	12.91	<0.01
Born Alive		10.11	10.85	10.57	0.48	0.35	10.82	0.16
Stillborn		0.43	0.55	0.58	0.10	0.48	0.55	0.24
Mummified		1.09	0.84	0.81	0.19	0.48	0.83	0.20
Parity 2								
		(n=69)	(n=260)	(n=24)			(n=284)	
Total Born		12.25 <sup>a</sup>	13.62 <sup>b</sup>	13.34 <sup>ab</sup>	0.50	0.04	13.59	0.02
Born Alive		10.86 <sup>a</sup>	12.19 <sup>b</sup>	11.59 <sup>ab</sup>	0.52	0.06	12.14	0.02
Stillborn		0.62	0.73	1.17	0.19	0.24	0.78	0.42
Mummified		0.41	0.37	0.45	0.11	0.88	0.37	0.78
P1 + P2								
		(n=69)	(n=259)	(n=24)			(n=283)	
Total Born		23.96 <sup>a</sup>	26.44 <sup>b</sup>	25.81 <sup>ab</sup>	0.74	<0.01	26.38	<0.01
Born Alive		21.13 <sup>a</sup>	23.11 <sup>b</sup>	22.43 <sup>ab</sup>	0.81	0.09	23.05	0.03
Stillborn		0.98	1.26	1.65	0.20	0.16	1.30	0.14
Mummified		1.41	1.29	1.16	0.32	0.90	1.27	0.70

<sup>1</sup>Classification assigned using Vulva Score Method A (VSA). Scores Small (S), Medium (M), Large (L) were assigned based on best fit using VSA scoring tool. M+L represents the combined result of all gilts scored > S.

<sup>a-b</sup> Differences in means denoted with different letters are statistically significant ( $P \leq 0.05$ )

**Table 3.7. Parity 1 and 2 Performance for Gilts classified using VSB.**

Parity 1	Classification <sup>1</sup>					1 vs. 2-5			
	1	2	3	4	5	SEM	P	2-5	P
	(n=99)	(n=124)	(n=209)	(n=81)	(n=32)			(n=446)	
Total Born	11.75 <sup>a</sup>	12.54 <sup>ab</sup>	13.14 <sup>b</sup>	13.27 <sup>b</sup>	11.83 <sup>ab</sup>	0.42	0.01	12.85	<0.01
Born Alive	10.04	10.47	11.05	11.20	10.33	0.52	0.36	10.83	0.13
Stillborn	0.43 <sup>a</sup>	0.43 <sup>a</sup>	0.59 <sup>abc</sup>	0.34 <sup>abd</sup>	0.82 <sup>bc</sup>	0.10	0.06	0.51	0.45
Mummified	1.11	0.81	0.79	0.97	0.96	0.22	0.73	0.84	0.22
<b>Parity 2</b>									
	(n=66)	(n=76)	(n=134)	(n=54)	(n=21)			(n=351)	
Total Born	12.35	13.40	13.65	13.67	13.83	0.55	0.22	13.30	0.02
Born Alive	10.94	12.01	12.16	12.34	12.22	0.58	0.31	12.17	0.03
Stillborn	0.60 <sup>a</sup>	0.86 <sup>ab</sup>	0.48 <sup>a</sup>	0.68 <sup>ab</sup>	1.18 <sup>b</sup>	0.17	0.07	0.70	0.55
Mummified	0.42	0.49	0.35	0.25	0.51	0.12	0.63	0.37	0.66
<b>P1 + P2</b>									
	(n=65)	(n=76)	(n=136)	(n=54)	(n=21)			(n=352)	
Total Born	23.97 <sup>a</sup>	25.57 <sup>ab</sup>	26.63 <sup>b</sup>	26.91 <sup>b</sup>	26.36 <sup>ab</sup>	0.83	0.03	26.33	<0.01
Born Alive	21.11	22.39	23.26	23.75	22.83	0.92	0.20	23.02	0.03
Stillborn	0.96	1.30	1.27	1.11	1.73	0.23	0.35	1.30	0.14
Mummified	1.43	1.10	1.23	1.63	1.30	0.37	0.86	1.27	0.65

<sup>1</sup>Classification assigned using Vulva Score Method B (VSB). Numerical scores (1-5) were assigned based on best fit using VSB scoring tool, with increasing score corresponding to increase in vulva with. 2-5 represents the combined result of all gilts scored > 1

<sup>a-d</sup> Differences in means denoted with different letters are statistically significant ( $P \leq 0.05$ )

**Table 3.8. Parity 1 and 2 Performances for Gilts classified using FS.**

Parity 1	Classification <sup>1</sup>			SEM	<i>P</i>	1 vs. 2-3	
	Index	1 (n=84)	2 (n=288)	3 (n=33)		2-3 (n=321)	<i>P</i>
Total Born		12.76	12.91	12.83	0.40	0.94	12.90 0.76
Born Alive		11.06	10.76	10.93	0.48	0.85	10.79 0.60
Stillborn		0.42	0.55	0.59	0.11	0.47	0.55 0.22
Mummified		0.89	0.87	0.85	0.21	0.99	0.89 0.98
<b>Parity 2</b>							
		(n=50)	(n=174)	(n=23)			(n=197)
Total Born		12.82	13.66	14.60	0.52	0.13	13.78 0.09
Born Alive		11.09	12.14	12.64	0.56	0.16	12.24 0.06
Stillborn		0.69	0.70	0.71	0.17	1.00	0.70 0.93
Mummified		0.42	0.36	0.16	0.12	0.49	.32 0.49
<b>P1 + P2</b>							
		(n=50)	(n=174)	(n=23)			(n=197)
Total Born		24.88	26.38	26.81	0.82	0.20	26.51 0.06
Born Alive		22.01	22.92	23.75	0.89	.49	23.10 0.25
Stillborn		1.07	1.30	1.51	0.23	0.52	1.32 0.32
Mummified		1.47	1.31	1.25	0.32	0.88	1.29 0.60

<sup>1</sup>Classification assigned using the Farm Score (FS) vulva scoring method. Numerical scores were assigned based on visual appraisal of vulva size with 1 being the smallest, 2 being average and 3 representing larger vulva size within a contemporary group. 2+3 is the combined results of all gilts scoring > 1.

<sup>a-d</sup> Differences in means denoted with different letters are statistically significant ( $P \leq 0.05$ )

**CHAPTER 4:  
EFFICACY OF VISUALLY ASSESSING PREPUBERTAL REPRODUCTIVE  
TRACT DEVELOPMENT AS A TOOL FOR IMPROVING SOW  
PRODUCTIVITY**

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Contribution Statement

MRR conducted statistical analysis and interpretation, and wrote the manuscript. TG represented TriOak Foods, supplied data, and was the primary collaborator on the project. LHB, AFK, and JWR advised statistical analysis. JWR served as primary investigator and corresponding author.

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### Abstract

Maximizing the number of quality pigs produced from the time a gilt becomes breeding eligible until leaving the herd is essential for sow farm sustainability. The complexity and low heritability of reproductive traits associated with sow lifetime productivity (SLP) makes selecting for it a challenge for pork producers. Age at first estrus is one of the most predictive traits for SLP. Our group has shown that gilts average or above for vulva size from 95 to 115 d of age, have greater likelihood of reaching puberty by 180 d of age compared to those below average at that time. The objective of the current study was to assess whether distinguishing potential replacement gilts based on prepubertal reproductive tract development is associated with productivity as a sow in a commercial setting. To do so, gilts were classified based on prepubertal vulva development at approximately 100 d of age at a collaborating nucleus herd. Gilts (n=1520) were subjectively scored by trained personnel, assigning gilts in to one of three categories; vulva score 1 (VS1), vulva score 2 (VS2) and vulva score 3 (VS3). With increased selection intensity at the nucleus level, a subset of the originally scored gilts were selected into the breeding herd (n=867). Gilt reproductive data was extracted and analyzed from a company database. Age at Parity 1 (P1) was reduced ( $P \leq 0.01$ ) for VS1 gilts ( $383.9 \pm 3.2$ ) compared to VS2 gilts ( $376.0 \pm 1.7$ ). Likewise, age at P1 was also reduced ( $P \leq 0.01$ ) from VS2 gilts to VS3 gilts ( $365.5 \pm 3.5$ ). Similarly, age at Parity 2 (P2) was younger ( $P \leq 0.01$ ) for gilts scored VS2 and VS3 ( $497.3 \pm 2.3$  and  $504.4 \pm 5.5$ , respectively) compared to VS1 gilts ( $522.4 \pm 4.5$ ). When analyzing litter data, P1 total born for gilts scored as VS2 ( $11.0 \pm 0.2$ ), VS3 ( $11.0 \pm 0.4$ ) and VS2 and VS3 combined ( $11.0 \pm 0.1$ ) was greater ( $P \leq 0.05$ ) compared to VS1 scored gilts ( $10.1 \pm 0.3$ ). Number

born alive was similar with VS1 ( $9.3 \pm 0.3$ ) being lower ( $P \leq 0.05$ ) compared to VS2, VS3 and combined VS2 and VS3 ( $10.3 \pm 0.2$ ;  $10.3 \pm 0.4$ ;  $10.3 \pm 0.1$ , respectively). Stillborn piglets did not differ across vulva scores. Mummified fetuses tended to be greater ( $P = 0.07$ ) for gilts scored VS3 (0.2) compared to VS1 (0.1). No differences in litter performance were observed for parity 2 according to vulva score, although total production through parity 2 remained greater in VS2 and VS3 scored gilts compared to VS1. Visual assessment of vulva development around 100 day of age appears to be beneficial in identification of females who are more likely to farrow at a younger age with improved litter performance in parity 1.

**Keywords:** sow lifetime productivity, vulva size, litter size, age at farrowing

## Introduction

The reproductive success and longevity of a sow largely determines whether she will contribute to sow farm profit (Stalder et al., 2003). However, selecting for longevity and reproductive performance in production systems is challenging due to the influence of environmental factors, along with numerous traits associated with reproduction (Rydhmer, 2000; Serenius and Stalder, 2006). Currently, one of the best predictors of sow lifetime productivity (SLP) is age at which a gilt achieves first estrus (i.e. puberty). Gilts reaching puberty earlier tend to produce more pigs over their lifetime (Nelson et al., 1990), have decreased  $d$  from weaning to estrus (Sterning et al., 1998), and increased ability to reach parity three (Patterson et al., 2010). Likewise, younger age at first service has shown to be beneficial for parity 1 farrowing rate, increased parity at



removal from the herd, and increased lifetime piglets produced (Koketsu et al., 1999). However, identifying gilts with different reproductive potential prior to a puberty remains difficult.

Previously, we have shown gilts below average for VW at 95-115 d of age have decreased ability to reach puberty by 200 d of age (Graves et al., 2015) and that gilts below average for vulva size at 15 wk of age can effectively be distinguished in commercial production systems and experience a reduction in reproductive performance (Romoser et al, 2017). The objective of this study was to determine the efficacy of subjective visual assessment of prepubertal vulva size within a gilt contemporary group and its effect on subsequent reproductive performance. This experiment tested the hypothesis that gilts with a subjectively scored small vulva size at approximately 100 d of age would produce fewer piglets compared to contemporaries subjectively scored as average or above average for vulva size.

## **Materials and Methods**

Data and animal records were provided by an industry collaborator (TriOak Foods, Inc.) from commercial swine herd.

### ***Animals***

Beginning in May 2015, 1520 purebred (Fast Yorkshire) gilts (Fast Genetics, Saskatoon, SK) approximately 100 d of age were visually assessed for prepubertal vulva development. The evaluation was conducted at a cooperating nucleus sow herd (TriOak Foods, Inc.). Vulva scoring was conducted by farm staff by visually classifying gilts

according to vulva size relative to their contemporaries. Gilts received a vulva score 1 (VS1) if subjectively estimated to be below average for vulva size within a contemporary group (approximate bottom 15%). Gilts receiving a vulva score 2 (VS2) were considered average for their contemporary group. Likewise, gilts receiving vulva score 3 (VS3) were estimated to be above average for vulva size within a contemporary group (approximate upper 15%).

### ***Gilt Development***

At the nucleus gilt development unite (GDU) gilts were housed in groups of twenty-five on fully slatted floors with approximately 0.74 m<sup>2</sup> provided per individual. Gilts were subjected to routine boar exposure (approximately 10 min of contact/pen) beginning around 190 d of age, for puberty stimulation and estrus detection. Of those gilts receiving a vulva score (VS), 867 were eventually serviced and entered the breeding herd.

### ***Reproductive Performance***

Reproductive performance of gilts incorporated into the breeding herd were tracked through production. For gilts who successfully farrowed a litter, farrowing date and litter data was recorded. Data including total born (TB), born alive (BA), stillborn (SB), and mummified fetuses (MM) was recorded at the time of parturition. Records were documented and stored in an online swine data base (PigKnows, LLC.).

### ***Statistical Analysis***

Statistical Analysis Systems (SAS) University Edition, version 9.4 (Cary, NC 27513) was used for all statistical analysis. PROC MIXED was used to analyze litter and farrowing age data. Model tested the fixed effect of VS on reproductive performance.

### **Results**

#### ***Prepubertal vulva score was associated with reduced age at first and second farrowing***

Of the 867 gilts scored and retained, 18.1, 69.0 and 12.9% were assigned to VS1, VS2 and VS3, respectively. Gilts with a VS1 had an increased age at first farrowing (383.9 d) compared to VS2 (376.0 d;  $P = 0.03$ ) or VS3 (365.5 d;  $P \leq 0.01$ ). When VS2 gilts were compared to VS3 gilts, age at first farrowing was also increased ( $P \leq 0.01$ ). When VS2 and VS3 groups were combined (VS2-3), age at first farrowing was decreased ( $P \leq 0.01$ ) compared to gilts scored VS1 (Figure 4.1A).

VS1 gilts were older ( $P \leq 0.01$ ) at second farrowing when compared to VS2 and VS3 gilts (522.4 d vs. 497.3, 504.4 d, respectively). When comparing VS2 and VS3 combined (498.3d) to VS1 (522.4), a similar reduction ( $P \leq 0.01$ ) in age at second farrowing was observed (Figure 4.1B).

#### ***Prepubertal vulva score affects parity 1 litter size***

Parity 1 (P1) TB was affected by VS ( $P = 0.03$ ), specifically TB was increased 1.2 pigs ( $P \leq 0.01$ ) when VS2 (11.03) and VS2-3 (11.03) gilts were compared to VS1 gilts (10.1). When TB for VS1 was compared to VS3 (11.05), a difference was again observed ( $P = 0.05$ ). Similarly, P1 BA was affected ( $P = 0.01$ ), with BA increased ( $P \leq$

0.01) for VS2 (10.3) and VS2-3 (10.3) compared to VS1 (9.3). VS3 (10.3) was also increased ( $P = 0.03$ ) when compared to VS1 (Figure 4.2A). No differences in SB piglets were observed across VS ( $P = 0.68$ ). An effect of VS was not observed for MM ( $P = 0.18$ ), although a tendency for increased ( $P = 0.07$ ) MM was observed for VS1 (0.1) compared to VS3 (0.2) (Figure 4.2B).

When parity 2 (P2) data was analyzed, no significant difference was detected across VS for TB, BA, SB or MM ( $P > 0.05$ ) (Figs. 4.3 A-B)

***Effect of prepubertal vulva score classification on total production through 2 parities.***

When total production over two parities was assessed for gilts that farrowed a second litter, VS did not influence TB or BA ( $P > 0.14$ ). When directly comparing VS groups, TB tended to decrease ( $P \leq 0.08$ ) in VS1 (21.6) compared to VS2 (22.6) and VS2-3 (22.5). BA yielded similar results with an increase ( $P = 0.05$ ) for VS2 (21.2) compared to VS1 (20.2). Additionally, BA for VS2-3 (21.2) tended to be greater ( $P = 0.06$ ) compared to VS1 (Figure 4.4A). VS did not affect SB over two parities ( $P = 0.36$ ). There were no detectable effects of S on MM ( $P = 0.24$ ), although MM tended to be greater ( $P = 0.1$ ) for VS3 compared to VS1 (Figure 4.4B).

## **Discussion**

Sow lifetime productivity refers to the number of quality piglets produced during a sow's life, and is largely impacted by the number of parities a sow remains in the herd (Serenius and Stalder, 2006). A gilt's ability to remain productive over multiple parities is associated with age at which she reaches reproductive maturity and is incorporated into

the breeding herd (Patterson et al., 2010; Hoge and Bates, 2011). Additionally, gilts who reach puberty at a younger age are more likely to show estrus and ovulate within 10 d after weaning their first litter (Sterning et al., 1998).

While age at first estrus is a valuable phenotype for distinguishing gilts with increased SLP potential (Patterson et al., 2010), events influencing puberty onset begin well before estrus is observed. Beginning as early as 70 d of age, initial LH pulses from the anterior pituitary result in early follicular development and subsequent estrogen production (Dyck and Swierstra, 1983). This initial estrogen production is associated with increased reproductive tract size (Dyck and Swierstra, 1983; Evans and O'Doherty, 2001). Meishan pigs, a breed known for its extreme prolificacy, initiate follicular development and reach puberty very early in life (Miyano et al., 1990). Considering the association between early reproductive tract development and greater fecundity in Meishan pigs, we proposed this same concept may be true across individuals in commercially used European breeds as well.

Graves et al., (2015) confirmed that prepubertal follicular development begins between 75 and 115 d of age. Presumably, the variation in vulva size at this timepoint serves as a proxy for the variation in the timing of prepubertal follicular development and resultant estrogen synthesis. This distinction between VW around 95-115 d of age was associated with a gilt's ability to reach puberty by 200 d of age. In agreeance with the current study, when prepubertal gilts were classified based on VW, improved litter size through P1 was observed in addition to increased inclusion rate into the breeding herd (Romoser et al., 2017).

In the current literature, the association between early reproductive tract development and reproductive performance as sows is vague. It has been shown that selection for increased uterine capacity is associated with decreased embryonic loss (Bolet et al., 1986) and improved litter size over the lifetime of the sow (Freking et al., 2016). This provides potential explanation as to why an improvement in litter size was observed in the current study, where it is possible through distinguishing gilts with large vulvas indirect selection for larger reproductive tract also occurred.

The dam's responsiveness to estrogen is also critical in many instances for successful reproduction to take place in pigs (Bazer and Thatcher, 1977; Geisert et al., 1982). The variation in reproductive tract development at 100 d of age is presumed to be the result of increased ovarian estrogen production caused by an activated hypothalamic-pituitary gonadal axis (HPGx). By characterizing gilts based on variation in prepubertal VS, preference was given to gilts average or above average VS. In addition to early HPGx activation, increased sensitivity to estrogens could also be manifested in gilts with early reproductive tract development, of which would be beneficial in reducing early embryonic loss around the time of pregnancy establishment (Morgan et al., 1987). Taken together, these potential explanations provide further incentive in the continual investigation to improve SLP.

## **Conclusion**

Gilts below average for reproductive tract development at approximately 100 d of age, as assessed by VS, achieve their first two parities later in life and produce fewer piglets compared to their contemporaries. The current body of work suggests that

assessing and scoring VS in gilts prior to puberty achievement can serve as a potential strategy for selecting replacements or identifying culls. Further implementation using a subjective assessment of VS is needed to confirm the efficacy of being a primary selection criteria across different genetic lines. Additionally, the fundamental biology causing the improvement in litter size and age at farrowing presents additional opportunities for research.

### **Acknowledgements**

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### **Declaration of Interest**

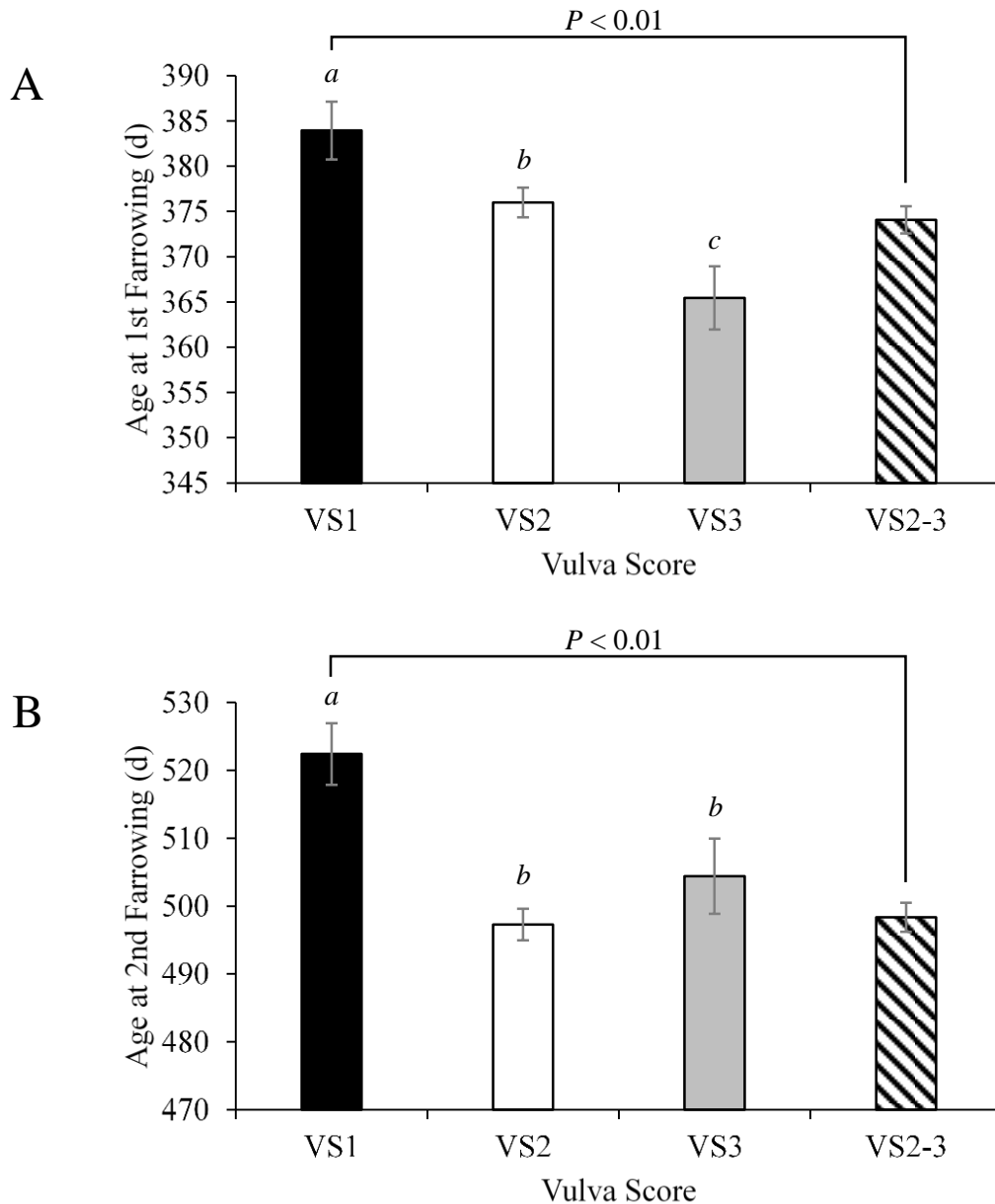
This study was supported by the Iowa Pork Producers Association and TriOak Foods Inc. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of TriOak Foods Inc. or Iowa Pork Producers Association. No conflicts of interest, financial or otherwise are declared by the authors.

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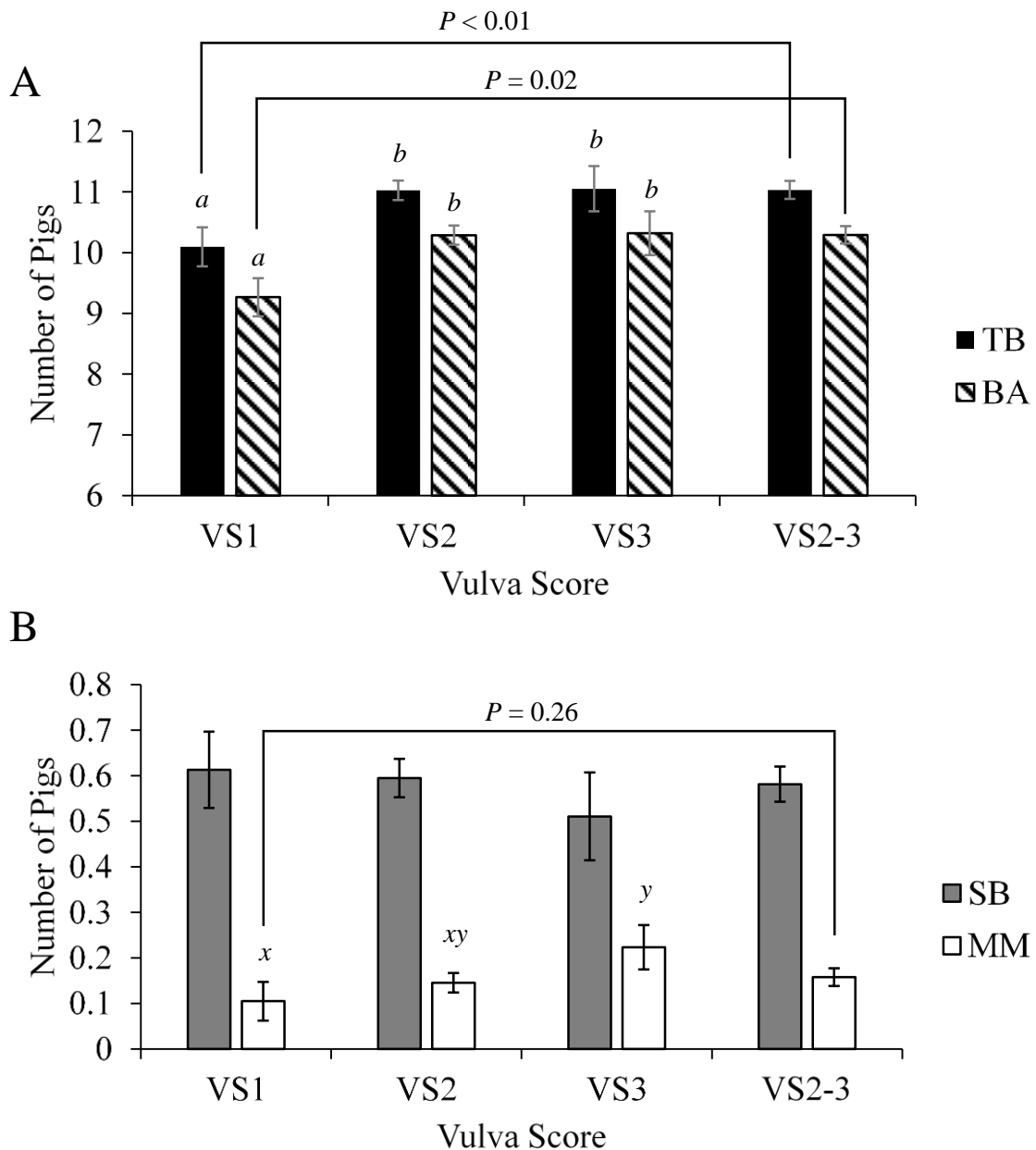
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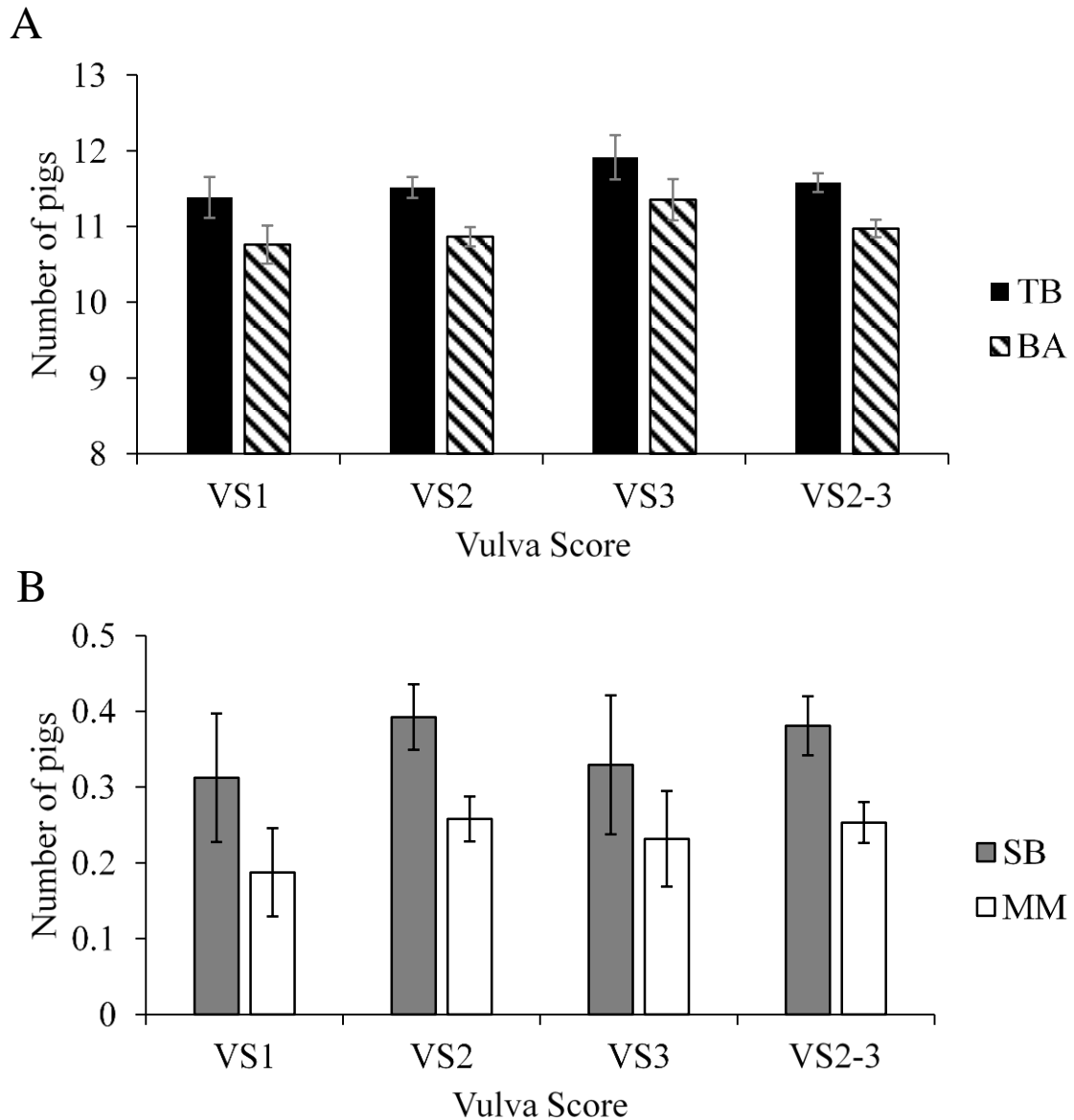


**Figure 4.1 Age at Parity 1 (P1) and 2 (P2) is affected by Prepubertal Vulva Score.**

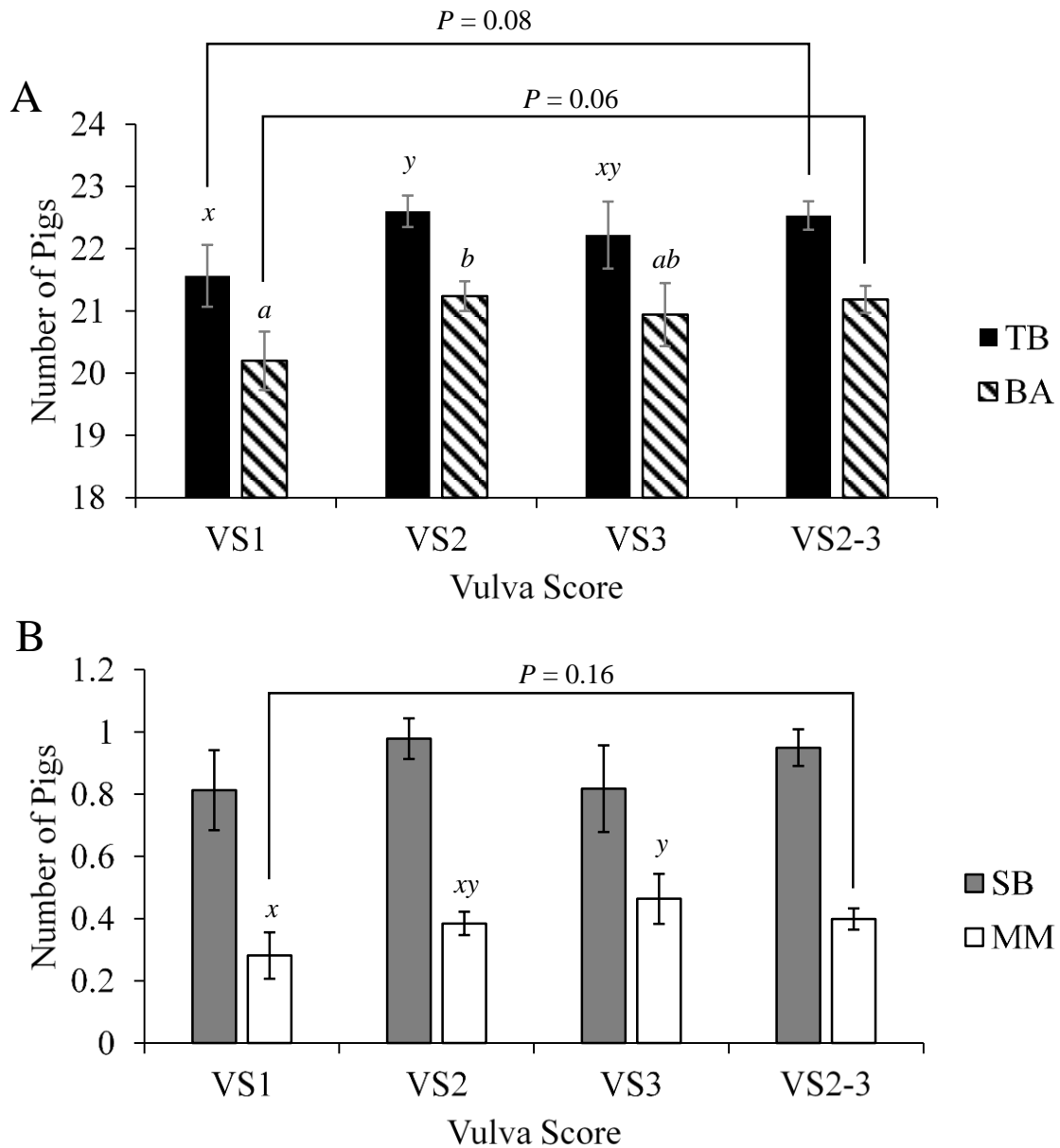
Gilts were classified by visual assessment at approximately 100 d of age. Gilts received a vulva score 1 (VS1) if subjectively estimated to be below average for vulva size within a contemporary group. Gilts receiving a vulva score 2 (VS2) were considered average for their contemporary group. Likewise, gilts receiving vulva score 3 (VS3) were estimated to be above average for vulva size within a contemporary group. For P1 (**A**) and P2 (**B**) the number of d from birth to farrowing was affected ( $P < 0.01$ ) by vulva score. Bars depict least squares means  $\pm$  SEM; different superscripts differ significantly ( $P \leq 0.05$ ). Gilts scored VS1 had greater ( $P < 0.01$ ) d to P1 (**A**) and P2 (**B**) when compared to all gilts scoring average or above (VS2-3) within the contemporary group.



**Figure 4.2 Parity 1 Litter Size as a result of Prepubertal Vulva Score.** Gilts were classified by visual assessment at approximately 100 d of age. Gilts received a vulva score 1 (VS1) if subjectively estimated to be below average for vulva size within a contemporary group. Gilts receiving a vulva score 2 (VS2) were considered average for their contemporary group. Likewise, gilts receiving vulva score 3 (VS3) were estimated to be above average for vulva size within a contemporary group. **A.** Parity 1 total born (TB) and born alive (BA) were affected by VS ( $P < 0.03$ ). **B.** Parity 1 stillborn (SB) pigs and mummified fetuses (MM) were not affected by VS ( $P > 0.18$ ). Bars of the same color with different superscripts denote significant differences ( $^{ab}$ ,  $P \leq 0.05$ ;  $^{xy}$ ,  $P \leq 0.1$ ). Gilts scored VS1 had fewer TB ( $P < 0.01$ ) and BA ( $P = 0.02$ ) when compared to all gilts scoring average or above (VS2-3) within the contemporary group (**A**).



**Figure 4.3 Parity 2 Litter Size as a result of Prepubertal Vulva Score.** Gilts were classified by visual assessment at approximately 100 d of age. Gilts received a vulva score 1 (VS1) if subjectively estimated to be below average for vulva size within a contemporary group. Gilts receiving a vulva score 2 (VS2) were considered average for their contemporary group. Likewise, gilts receiving vulva score 3 (VS3) were estimated to be above average for vulva size within a contemporary group. **A.** Parity 2 total born (TB) and born alive (BA) were not affected by VS ( $P > 0.21$ ) **B.** Parity 2 stillborn (SB) pigs and mummified fetuses (MM) were not affected by VS ( $P > 0.55$ ).



**Figure 4.4 Combined Litter Performance of Parity 1 and 2 as a result of Prepubertal Vulva Score.** Gilts were classified by visual assessment at approximately 100 d of age. Gilts received a vulva score 1 (VS1) if subjectively estimated to be below average for vulva size within a contemporary group. Gilts receiving a vulva score 2 (VS2) were considered average for their contemporary group. Likewise, gilts receiving vulva score 3 (VS3) were estimated to be above average for vulva size within a contemporary group. **A.** Combined Parity 1 and 2 total born (TB) and born alive (BA) pigs **B.** Parity 1+2 stillborn (SB) pigs and mummified fetuses (MM) were not significant across VS ( $P > 0.24$ ). Bars depict least squares means for litter parameter  $\pm$  SEM. Differences in letter denote significance ( $^{ab}$ ,  $P \leq 0.05$ ;  $^{xy}$ ,  $P \leq 0.1$ ). Gilts scored VS1 had tended to have fewer TB ( $P = 0.08$ ) and BA ( $P = 0.06$ ) when compared to all gilts scoring average or above (VS2-3) within the contemporary group (**A**).

**CHAPTER 5:  
EFFECTS OF INCREASED AMBIENT TEMPERATURES AND  
SUPPLEMENTAL ALTRENOGEST PRIOR TO PREGNANCY  
ESTABLISHMENT IN GILTS**

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Contribution Statement

MRR conducted statistical analysis and wrote the manuscript. MRR and KLB were responsible for the live animal portion of the project and conducted sample analysis. LHB, AFK, and JWR designed the experiment. JWR advised statistical analysis, edited the manuscript and served as corresponding author.

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### Abstract

Heat stress (HS) mitigation strategies are critically needed to combat the substantial economic effects on livestock production systems. The manifestations of seasonal infertility include delayed puberty onset, reduced conception rates, decreased litter size, and increased wean to estrus interval. To assess the effects of HS during early gestation and evaluate the potential benefit of supplementing altrenogest (ALT) as a mitigation strategy, thirty crossbred post-pubertal gilts ( $157 \pm 11$  kg) were subjected to a 14-d synchronization protocol via oral ALT administration. After synchronization, estrus detection was conducted followed by artificial insemination during estrus. After insemination, gilts were assigned to one of four treatments; heat stress (HS;  $35 \pm 1^\circ\text{C}$  for 12h/d and  $31.60 \pm 1^\circ\text{C}$  for 12h/d) with (HSALT,  $n = 7$ ) or without (HSCON,  $n = 7$ ) 15 mg/d ALT supplementation or thermal neutral (TN;  $20 \pm 1^\circ\text{C}$  for 24 h/d) conditions with (TNALT,  $n = 8$ ) or without (TNCON,  $n = 8$ ) 15 mg/d altrenogest supplementation until 12 d following the onset of estrus. ALT administration occurred at 0600 h from 3-12 days post estrus (DPE) and rectal temperatures ( $T_R$ ) and respiration rates (RR) were recorded to assess effects of diurnal heat load. Blood collection was performed via jugular venipuncture on 0, 4, 8 and 12 DPE. Animals were euthanized humanely at 12 DPE followed by collection of ovarian tissues, uterine flushing and present conceptuses for further analysis. RR and  $T_R$  were increased ( $P < 0.01$ ) in HS compared to TN and neither variable was affected by ALT supplementation. Feed Intake (FI) was reduced ( $P < 0.01$ ) in HS animals although it was not affected by ALT treatments. CL weight was reduced ( $P < 0.01$ ) in HSCON gilts when compared to TNCON and HSALT gilts despite progesterone (P4) concentrations in serum or luteal tissue not being affected by treatment

( $P > 0.05$ ). CL diameter was reduced for HSALT gilts compared to other treatment groups ( $P < 0.05$ ). IL1B concentration in uterine flushings, an indicator of conceptus growth, was not affected ( $P > 0.05$ ) by environment or ALT supplementation although moderate ( $P = 0.06$ ) interaction between environment and altrenogest existed as IL1B concentration for TNALT was greater ( $P = 0.03$ ) compared to TNCON. While environment did not affect conceptus stage of development ( $P > 0.05$ ) ALT supplementation advanced conceptus elongation ( $P < 0.01$ ). Collectively, these data demonstrate that HS appears to affect luteal development prior to pregnancy establishment, and ALT improves the rate at which a conceptus develops *in utero*.

**Keywords:** heat stress, altrenogest, conceptus, pregnancy

## Introduction

The seasonal reduction in gilt and sow productivity observed in the swine industry imposes significant economic strain on pork production (St-Pierre et al., 2003, Pollmann, 2010). Occurring in conjunction with summer months, increased temperatures and thermal load imposed on livestock is thought to be a significant contributor to seasonal swine fertility (Love, 1978; Auvigne et al., 2010). Characteristic of season infertility is delayed puberty onset (Paterson et al., 1991) and reduction of sows returning to estrus post weaning (Omtvedt et al., 1971; Love, 1978). Sows experiencing irregular inter-estrus intervals (25-35 d) after mating coupled with reduced farrowing rates and litter sizes is also manifested during seasonal infertility (Omtvedt et al., 1971; Love, 1981; Xue et al., 1994).

Effective communication between the developing conceptus and dam is a prerequisite to pregnancy establishment (Bazer et al., 1982), and if disrupted can result in failure to carry a pregnancy to term. Exposure to stressors prior to implantation can be detrimental to conceptus survival and pregnancy maintenance. Increased ambient temperatures during the first 15 d of gestation has been shown to negatively impact conceptus viability (Edwards et al., 1968; Wettemann et al., 1988). Additionally, progesterone (P4), while essential for pregnancy maintenance, is also vital for the induction of endometrial secretions important for conceptus development and implantation (Ka et al., 2007). Furthermore, P4 production has been shown to be negatively impacted with seasonal changes in pregnant sows (Wrathall et al., 1986). Separate from environmental and nutritional effects, supplemental P4 from the time of corpus luteum (CL) formation to d 30 of pregnancy has shown to have beneficial effects on conceptus survivability (Ashworth, 1991). Collectively, we hypothesized that increased ambient temperatures during early gestation negatively impacts CL function and conceptus development during the pre-implantation window; and supplementation with a P4 analogue would alter endometrial function during HS.

### **Materials and Methods**

All animal procedures in this study were conducted with approval by the Iowa State University Institutional Animal Care and Use Committee.



## ***Animals***

This experiment was conducted utilizing thirty crossbred gilts with average body weights of  $157 \pm 11$  kg. Gilts were housed in individual stalls (57 x 221 cm) at the Iowa State University Swine Nutrition Farm research facility (Ames, IA), each stall equipped with a stainless-steel feeder and nipple drinker. Gilts were provided *ad libitum* water throughout the duration of the experiment, and fed 2.7 kg of a standard diet once daily.

## ***Estrus Synchronization, Acclimation and Artificial Insemination***

Prior to synchronization, all gilts were intramuscularly injected with 5 mL of commercially available gonadotropins (P.G. 600®, Merck Animal Health, Summit, NJ 07901) to induce estrus. Behavioral estrus was monitored using an intact boar.

Additionally, at approximately eighteen d following P.G. 600 injection, blood samples were taken to assess P4 levels on a subset of gilts to validate CL function and cyclicity. After cyclicity was confirmed, estrus was synchronized by orally administering 6.8 mL of 15 mg altrenogest (ALT, Matrix®, Merck Animal Health, Summit, NJ 07901) at the time of feeding (0700) for fourteen d. An ALT dosing gun was used to ensure a complete dose was effectively administered. Animals were moved into stalls during synchronization process, to allow for acclimation prior to starting the experiment. See experimental timeline (Figure 5.1).

Following the last d of ALT administration, gilts were subjected to boar exposure for estrus detection and breeding. Boar exposure occurred twice daily (0600 and 1800 h) for seven d during the ALT withdrawal period. Beginning on d two of the ALT withdrawal period,  $T_R$ ,  $RR$ , and  $FI$  was recorded to establish a baseline prior to onset of

treatment conditions. During boar exposure period, gilts were declared in estrus once exhibiting standing behavior in response to back pressure. Gilts were artificially inseminated twelve hours following onset of estrus behavior using pooled terminal Duroc semen (Swine Genetics International, Cambridge, IA 50046) and received additional inseminations every twelve hours, up to thirty-six hours after onset of estrus or until estrus behavior ceased. All inseminations were performed by the same technician to minimize variation.

### ***Treatment Period***

Following estrus, gilts were randomly assigned to one of four treatment groups; diurnal heat stress (HS;  $35 \pm 1^\circ\text{C}$  for 12h/d and  $31.6 \pm 1^\circ\text{C}$  for 12h/d) supplemented with (HSALT, n = 7) or without (HSCON, n = 7) 15 mg/d ALT or thermal neutral (TN;  $21 \pm 1^\circ\text{C/d}$ ) also supplemented with (TNALT, n = 8) or without (TNCON, n = 8) ALT.

All gilts were introduced to their assigned environmental conditions at 1900 h, to normalize the initial thermal load experienced for those assigned to HS conditions. Gilts were given a minimum of 48 hours from the beginning of estrus onset to the time of introduction to thermal conditions, to assure ovulation was not confounded by ambient temperature. In the case of gilts detected in standing heat in the AM, introduction to their respective thermal environment occurred 60 h after estrus was detected. Gilts experienced estrus onset in the PM, were introduced to their thermal environment 48 hours after estrus was detected. Once placed into environmental treatment, gestational ALT supplementation began, with ALT gilts being orally administered 6.8 mL Matrix® (15 mg

altrenogest) once daily at feeding (0600 h) from d 3-12 and CON gilts receiving no supplementation.

### ***Response to Thermal Conditions***

$T_R$  was measured using a digital thermometer (Welch Allyn Sure Temp<sup>®</sup> Plus 690, Skaneateles Falls, NY, USA) three times in the AM (0700, 0800 and 0900 h) and PM (1700, 1800 and 1900 h). The three AM and three PM readings were subsequently averaged for statistical analysis. RR was measured at the same times as  $T_R$ , and performed by counting flank movements in a 15 second interval, and multiplying by four to obtain breaths per minute (bpm). FI was determined by administering a known amount in the AM and recording feeder weights during both PM and AM time points. Both ambient temperature and humidity were recorded every 15 min by four data loggers (Lascar EL-USB-2-LCD, Erie, PA) placed equidistantly apart in the room and later condensed into averages for each time point.

### ***Blood Sampling***

Blood was obtained via jugular venipuncture (10 mL; BD<sup>®</sup> vacutainers; Franklin Lakes, NJ; K3EDTA; EDTA) on 0, 4, 8 and 12 DPE. Serum samples were collected by centrifugation at 1500 x g for 10 min, aliquoted and stored at -80° C until further analysis.

### ***Harvesting and Tissue Collection***

At approximately 288 h following detection in standing estrus, gilts were humanely euthanized via captive bolt followed by exsanguination. Timing of euthanasia from the onset of estrus was performed to target the narrow window of conceptus elongation where differences in conceptus growth could be discernable by classifying conceptuses as spherical or filamentous. Following confirmation of insensibility, reproductive tracts were removed and ovaries collected to assess CL number, diameter, and weight. Following ovary removal, each uterine horn was flushed for retrieval of luminal contents. Briefly, uterine flushing was performed by positioning hemostats at the proximal end of each horn next to the uterine bifurcation, and injecting of 20 mL sterile saline at the distal end of the uterine horn through the oviduct. Saline injection was manipulated through the uterine horn, followed by flush contents being collected. Following classification of conceptus stage of development, uterine flushings were placed on ice before being centrifuged at 600 x g for 10 min for removal of cell debris. Uterine flush samples were then stored at -80°C until further analysis. Conceptuses recovered were classified by stage of stage of development, counted and measured if the conceptuses could be individually distinguished after flushing. Following initial measurements, conceptuses were flash frozen in liquid nitrogen and later stored at -80°C.

### ***Hormone and Protein Assays***

Lysed CL and serum P4 levels were determined using a solid phase enzyme-linked immunosorbent assay (ELISA) (Cat # EIA-1561, DRG Instruments GmbH, Germany) that utilized competitive binding principle to detect P4. All assays were performed per manufacturer's guidelines. For CL P4 analysis, fluid was first extracted

from flash frozen tissue in 5% trichloroacetic acid and homogenized. Extractions were diluted 1:100 for detectability by the assay. Serum collected from d 12 post estrus was diluted 1:3 for detectability. Samples were run in triplicate, with samples from estrous cycle d being balanced across assigned plates, and treatments being randomized for representation within plates.

Uterine flush interleukin-1 $\beta$  (IL1B) levels were assessed using a solid phase ELISA that employed a quantitative sandwich enzyme immunoassay technique (Cat # DLB50, R&D Systems, Minneapolis, MN, USA). All assays were conducted in accordance with manufacturer's guidelines. Uterine flush samples from each horn were first pooled for representation of whole uterine IL1B and to account for inter-horn variation in conceptus spacing at the time of flushing. Samples were run in duplicate, and randomized within the plate.

### ***Statistical Analysis***

Statistical Analysis SAS University Edition (Cary, NC 27513) was used for all statistical analysis. PROC MIXED was used for analysis of  $T_R$ ,  $RR$ , and  $FI$  with timepoint of the experiment as the repeated measure. The model tested the effect of treatment, environment, and the treatment x environment interaction. Hormone and protein assays were analyzed using PROC MIXED as well, again examining the effect of treatment, environment, and the treatment x environment interaction.

## **Results**

***HS reduced feed intake and increased  $T_R$  and  $RR$ , which were unaffected by ALT supplementation***

FI was reduced ( $P < 0.01$ ) by 18% in HS gilts compared to gilts in TN conditions, but did not differ between ALT treatments ( $P = 0.98$ ; Figure 5.2). Thermal conditions also effected RR, with HS gilts having increased ( $P < 0.01$ ) breaths per minute compared to TN counterparts (102 bpm vs. 27 bpm; Figure 5.3). No difference was observed for RR between ALT and CON groups ( $P = 0.99$ ; Figure 5.3). HS  $T_R$  was increased compared to TN  $T_R$  (39.1 vs. 38.6°C;  $P < 0.01$ ; Figure 5.4).  $T_R$  did not differ between gilt between ALT and CON treatments ( $P = 0.98$ ; Figure 5.4).

***HS conditions did not affect serum or luteal progesterone production***

Serum P4 concentrations did not differ between treatment groups at 0, 8, or 12 DPE (Figure 5.5). Serum P4 concentration in TNALT gilts was increased ( $P = 0.04$ ) compared to HSCON on 4 DPE. TNALT P4 concentrations also tended to be greater ( $P = 0.06$ ) than P4 concentrations for TNCON gilts at 4 DPE (Figure 5.5A). Luteal P4 was not affected by thermal conditions ( $P = 0.90$ ) or ALT treatment ( $P = 0.52$ ; Figure 5.5B).

***Corpus luteum weight and diameter was altered by treatments.***

Average CL weight was reduced ( $P < 0.01$ ) in HSCON (0.45 g) compared to TNCON (0.51 g) and HSALT (0.52 g; Figure 5.6A). Average CL weight for TNALT gilts (0.48 g) did not differ from contemporaries. Average CL diameter was decreased ( $P \leq 0.05$ ) in HSALT group compared to TNCON, HSCON and TNALT (10.20 vs. 10.56, 10.57, 10.62  $\pm$  0.11 mm, respectively; Figure 5.6B).

***Effect of increased ambient temperature and altrenogest supplementation on conceptus interleukin-1 $\beta$  production***

Conceptus IL1B concentration was increased ( $P = 0.03$ ) in TNALT compared to TNCON (785.38 vs. 205.28 pg/mL). No difference was detected in HSALT or HSCON when compared to the contemporary groups ( $P > 0.05$ ; Figure 5.7A). When evaluating only environmental effect IL1B production no difference was observed ( $P = 0.77$ ; Figure 5.7B). Similarly, when the effect of ALT supplementation on IL1B was analyzed, no difference was detected ( $P = 0.20$ ; Figure 5.7C).

***Conceptus development was advanced by altrenogest supplementation although unaffected by HS***

Environment did not affect stage of conceptus elongation rate (71.4%, HS vs 69.2%, TN;  $P = 0.66$ ; Figure 5.8A). For gilts administered ALT, 12 of 13 (92.5%) yielded conceptuses that had begun rapid elongation and were filamentous in morphology, increased ( $P < 0.01$ ) from the gilts not supplemented ALT, where only 6 of 14 (42.9%) yielded filamentous conceptuses (Figure 5.8B).

## **Discussion**

The predictable, seasonal reduction in gilt and sow reproductive performance associated with seasonal increases in ambient temperature results in significant economic losses annually to the pork industry (St-Pierre et al., 2003; Pollmann, 2010; Ross et al., 2017). This continual challenge to swine production is characterized by numerous factors, including delay in puberty achievement (Paterson et al., 1991), prolonged or

irregular returns to estrus (Sterning et al., 1990; Peltoniemi et al., 1999), and the reduction in farrowing rate and litter size (Omtvedt et al., 1971; Xue et al., 1994). Though the decrease in performance is likely the result of several contributing factors, including photoperiod (Love et al., 1993), the effects of increased temperature also impedes reproductive performance in pigs (Auvigne et al., 2010).

Establishment of pregnancy is a highly coordinated process in which sufficient communication between conceptus and dam must occur to be successful (Bazer et al., 1982; Geisert et al., 1982a; Geisert et al., 1982b). This peri-implantation window is thought to be sensitive to the negative effects of HS, specifically on conceptus viability (Edwards et al., 1968; Wettemann et al., 1988). Additionally, early pregnancy disruption has been shown to have a greater incidence during the summer/autumn months compared to winter/spring months (Tast et al., 2002). It is unclear whether the increased thermal load itself causes conceptus death, or if conceptus death is an indirect effect as a result of compromised CL function and subsequent P4 production (Wrathall et al., 1986). This prompted the current investigation, in which the effects of HS conditions from the time of luteinization to pregnancy recognition were assessed in gilts. Simultaneously, half the gilts were supplemented with a P4 analogue in effort to rescue any potential negative effects increased ambient temperatures exerted on luteal P4 production.

Inherently, pigs struggle to cope with HS largely due to their lack of functional sweat glands coupled with selection for increased lean tissue accretion, resulting in greater metabolic activity (Brown-Brandl et al., 2001; Baumgard and Rhoads, 2013). In the current study, HS animal exhibited notable behavioral signs of stress.  $T_R$  was increased in HS gilts at the onset of the thermal treatment along with a significant



increase in RR. FI was reduced in the HS gilts when compared to the TN counterparts. This reduction in FI is one of the consistent initial responses observed when pigs are experiencing HS. By reducing feed consumption, it is thought that pigs can better regulate core body temperature by subsequently reducing metabolic heat production (Baumgard and Rhoads, 2013).

CL weight was reduced in HSCON gilts compared to TNCON counterparts. Conversely, HSALT was increased compared to HSCON, but was not different from TN counterparts. Interestingly, despite being heavier in weight, HSALT CL were decreased in diameter in comparison to other treatment groups. However, this variation in weight and diameter was not reflected in luteal or circulating P4 concentration. The relationship between CL size and P4 production has shown to be dissimilar in cattle as well (Sartori et al., 2002). It is worth noting, however, that at timepoints when differences would have been expected to be greatest for serum P4 between treatments (d 8 and 12), HS gilts had become partially acclimated to the environment, as demonstrated by the gilt's ability to resume homeostatic temperatures at night. Despite the lack of difference observed in this study for serum P4, further work is needed to test the effect of initial thermal load response (observed d 3-6) on circulating P4 when production is near or at its highest levels (d 8-12).

The rate at which the CL develops in the pig is very rapid, with the luteal mass initially growing by cellular hypertrophy then dramatically increasing the mitotic activity and cell proliferation to approximately d 15 (Ricke et al., 1999). During luteinization, angiogenic factors act to increase the vascularity of the rapidly growing structure (Murphy et al., 2001). After ovulation, the early luteal structure experiences hypoxic

conditions due to reduced blood flow around ovulation (Niswender et al., 1976). Hypoxia is thought to be the initial stimulus of angiogenic factors to promote new vascular development (Reynolds et al., 2000). Additionally, it was reported that hypoxic conditions increased vascular endothelial growth factor (VEGF) in pig granulosa cells (Basini et al., 2004). Based on the current knowledge on early luteal formation, hypoxic conditions appear to be beneficial in prompting the development of the necessary vasculature. In pigs, one mechanism of dissipating heat is to increase circulation to the periphery, creating a hypoxic environment for the internal organs (Lambert et al., 2002). In the current study where gilts were subjected to intense heat for majority of the luteal phase, it is possible that the initial internal hypoxic environment did not influence luteal formation. Further investigation is needed to determine the duration of hypoxic conditions on CL function, and if prolonged duration of intense heat challenge effects the viability of the luteal tissue due to deprived blood flow. Interestingly, HS did not affect CL weight or P4 concentrations. Unexpectedly, when HS gilts were supplemented with ALT an increase in CL weight was observed compared to HSCON gilts. This is interesting as supplemental P4 has previously been shown to have negative effects on CL size and function (Spies et al., 1960), although it is worth mentioning that this study administered P4 from d 10 to 25, not all of which were pregnant, and at a greater dose than what we administered. The effect of ALT on CL size and P4 production capability is limited in the literature, justifying further work to determine any effects of ALT on CL function.

Around d 12 of pregnancy, pig conceptus undergo morphological changes, transitioning from spherical confirmation to an filamentous conceptus (Heuser and

Streeter, 1929). The elongation process occurs very rapidly, transitioning to the filamentous shape in a matter of hours (Geisert et al., 1982a). This process of elongation is critical for conceptus survival to ensure adequate surface area at time of attachment. Concomitant with elongation, the conceptus increases the synthesis and release of IL1B. IL1B activates numerous cell signaling pathways facilitating implantation (Ross et al., 2010; Jeong et al., 2016). The window of expression for IL1B is very narrow being only during the short time it takes the conceptus to complete the morphological changes from spherical to filamentous. In the current study, animals were sacrificed for tissue collection at a precise time relative to estrus onset, in effort to target this narrow window of conceptus elongation. In the uterine flush, differences in IL1B production were not observed across environmental or ALT treatments. An interacting effect of environment and ALT treatment was observed wherein TNALT gilts had increased IL1B concentration in uterine flush compared to TNCON. We suspect this difference is a product of the increased elongation percentage in the ALT treated gilts compared to CON.

It is worth noting, however, that since IL1B is produced exclusively by the conceptus, concentration is not only dependent on that stage of development but also the number of conceptuses present. For gilts that yielded filamentous conceptuses, accurate distinction and count of individual conceptus is not possible due to entanglement during the flushing process. Although IL1B in the uterine flush was not different across treatments, further work is warranted to examine the direct effects of HS on conceptus viability at the time of elongation. Earlier reports have concluded that at d 16, conceptus viability was compromised by HS, evident by the increase in fragmentation of the

trophectoderm (Wettemann et al., 1988). This illustrates the sensitivity of the conceptuses to the direct effects of environment during the peri-implantation window, in which we believe IL1B could be a valuable marker for conceptus health during this critical time of development.

ALT treated gilts show an increased percentage of filamentous conceptuses compared to the CON treated counterparts. The regulation of this trophoblastic elongation is not well defined, as it is unclear whether it is conceptus mediated or initiated through maternal signaling. These findings suggest that P4, or in this case a P4 analogue, plays a critical role in facilitating events leading up to maternal recognition and implantation. The ability of P4 to mediate uterine secretion was validated when exogenous P4 administered on d 2 and 3 of gestation increased endometrial secretions and conceptus production of estrogen on d 11 (Vallet et al., 1998). The evidence of supplemental P4 benefits during early pregnancy are somewhat inconclusive in the literature. Ashworth et al., (1991) showed an improvement in fetus viability when the dam was supplemented P4 from d 4 to 30 of pregnancy. Conversely, Mao and Foxcroft (1998) showed that conceptus survival rate decreased following exogenous P4 treatment which began 36 h after estrus onset. The time of P4 supplementation appears to be critical, as an improvement in pregnancy rate when ALT was provided on d 2-4 (83%) compared to d 1-4 where pregnancy rate was reduced to 38% (Soede et al., 2012). Considering the increased rate of conceptus elongation observed in ALT treated gilts in this study, coupled with the lack of reduction in CL weight, suggests the need for further investigation on possible luteotropic factors produced by the elongating conceptus prior to maternal recognition.

For pregnancy to be established, P4 first suppresses its receptor in the uterine endometrium. This down regulation of P4 receptor allows for conceptus derived estrogens to interact with its receptor, ERS1, subsequently promoting the upregulation of growth factors important for trophoblastic elongation (Ka et al., 2007). Considering the current knowledge of the role P4 has during the peri-implantation window, the production of various growth factors in response to ALT acting on the uterine endometrium is a possible explanation as to why we observed an increased number of conceptus reaching the filamentous stage. Further work is needed to fully comprehend the effect that ALT has on the gilt endometrium at d 12 of pregnancy.

### **Conclusion**

Developing and implementing HS mitigation strategies is needed to combat the economic burden associated with seasonal infertility. HS impacts CL weight but its ability to produce P4 during early pregnancy appeared unscathed. Supplementation of a P4 analogue accelerated conceptus elongation and during HS improved CL weight compared to HSCON counterparts. These findings provide useful implications for improving conceptus viability during HS as well as possibly mitigating the negative effects of HS on CL development, both of which require further investigation to confirm application efficacy.

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### **Declaration of Interest**

This project was supported by the Iowa Pork Producers association. Any opinion, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the Iowa Pork Producers Association. No Conflicts of interest, financial or otherwise are declared by the authors.

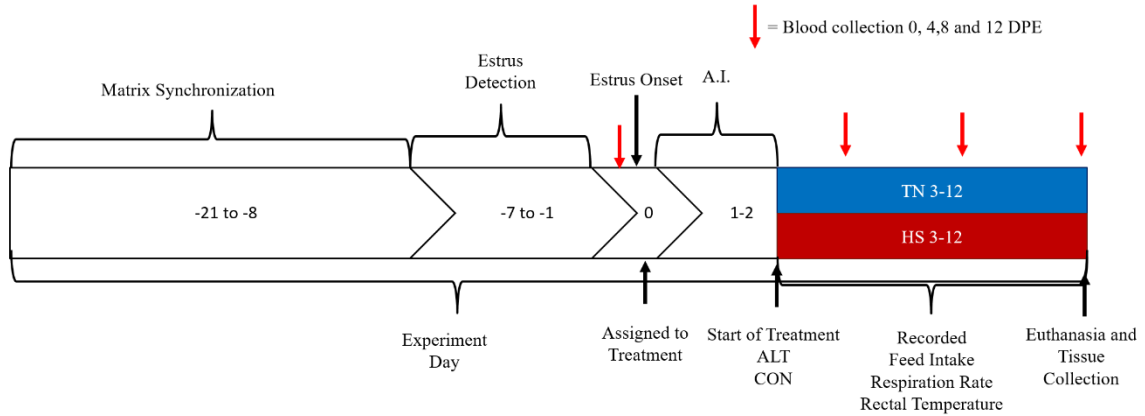
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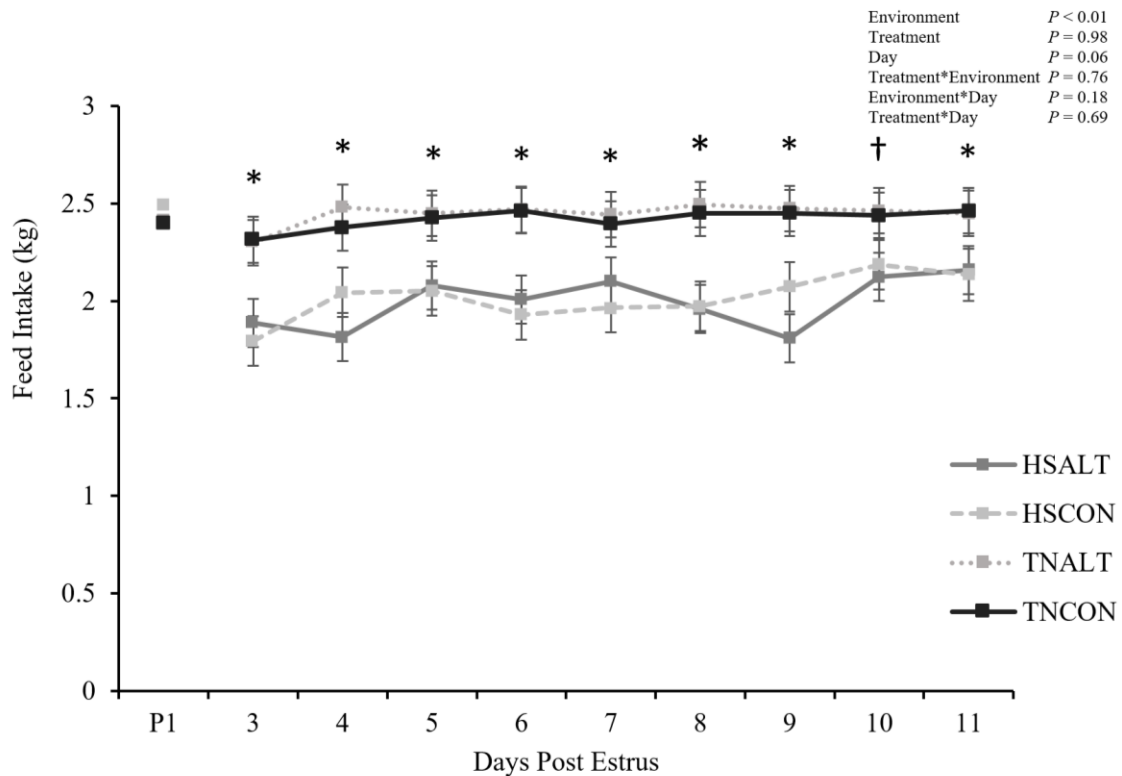
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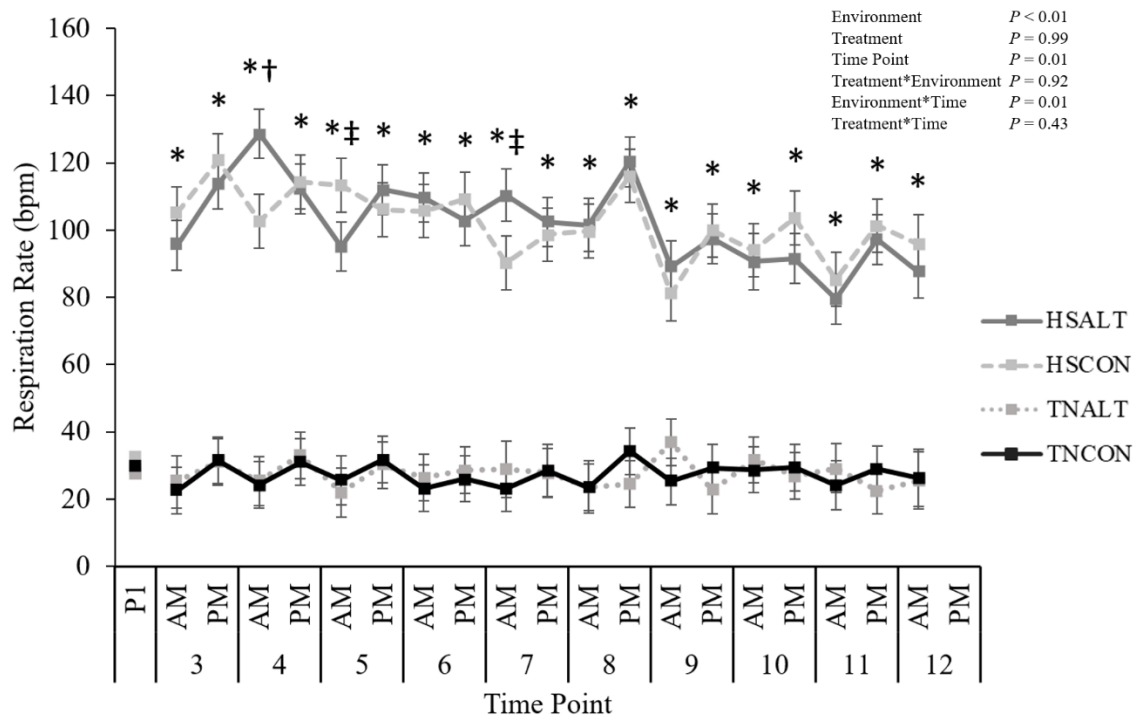




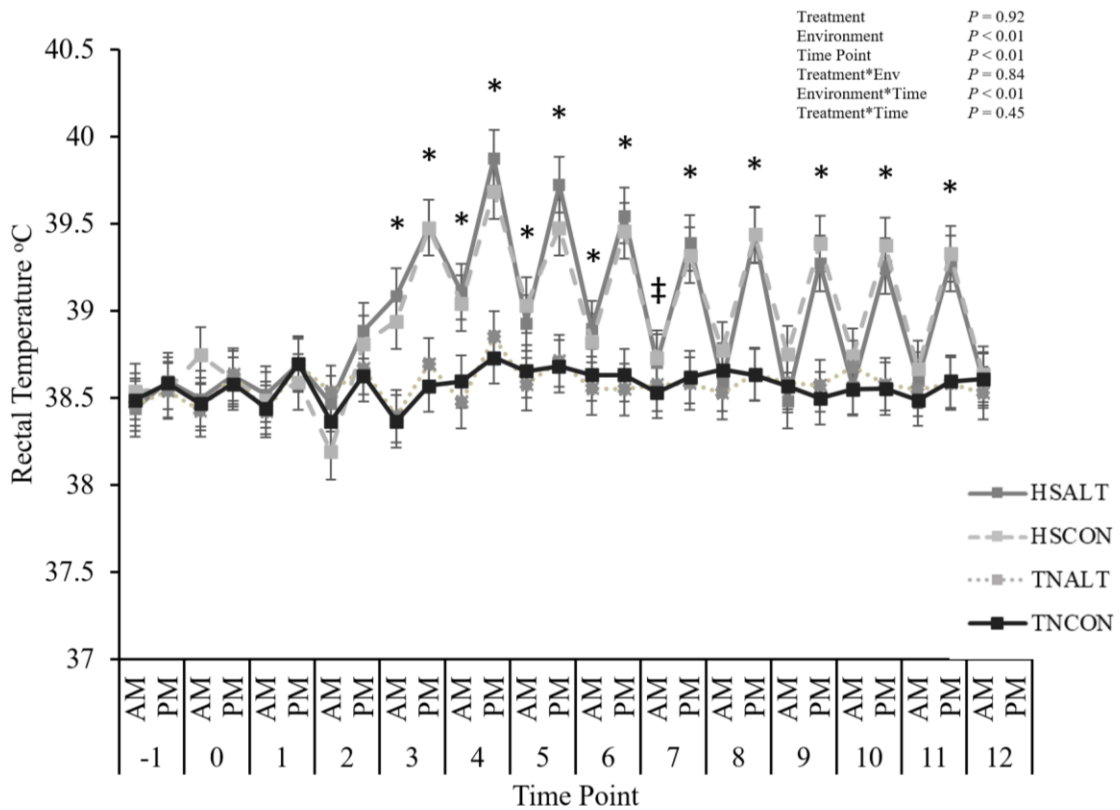
**Figure 5.1 Experimental Timeline.** Estrus onset was designated as experimental day 0. Gilts were inseminated 12 and 24 hours after estrus onset. Gilts were introduced to thermal treatment 48-60 hours after declared in standing heat. Blood was collected on experimental days 0, 4, 8 and 12 for hormone evaluation. Feed intake, respiration rate, and rectal temperatures were recorded from d 3-12 post estrus. At 12 DPE, gilts were euthanized for tissue collection.



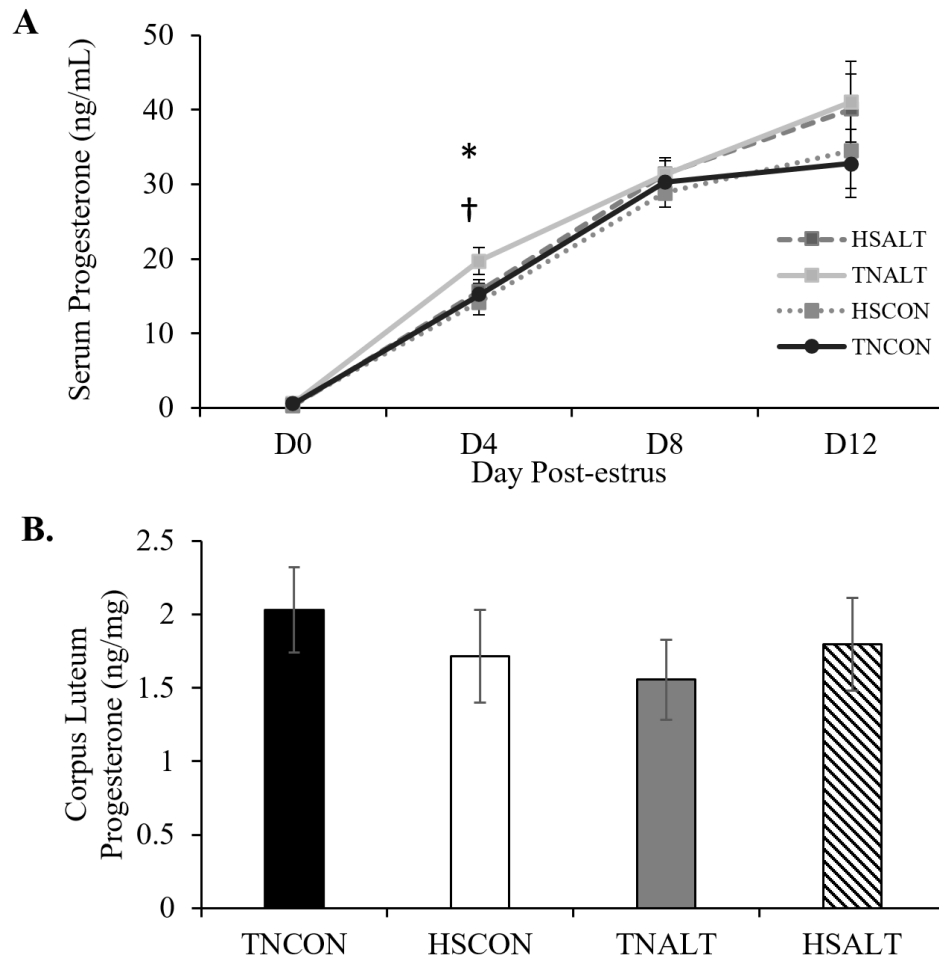
**Figure 5.2 Impact of Cyclical Heat Stress on Feed Intake.** Post-pubertal gilts were assigned to one of four different treatments, heat stress (HS) conditions ( $35 \pm 1^\circ\text{C}$  for 12h,  $31 \pm 6^\circ\text{C}$  for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions ( $20 \pm 1^\circ\text{C}$ , 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Environment assignment and ALT administration lasted from d 3-12 post estrus. Animals were limit fed 2.7 kg at 0600h. Feed intake (FI) was tracked daily, both A.M. and P.M. Line graphs denote FI each d post estrus for each group  $\pm$  SEM. \* indicates difference between environment at specified time points ( $P < 0.01$ ); † indicated difference between environment ( $P < 0.05$ ). No Differences were detected across ALT treatments.



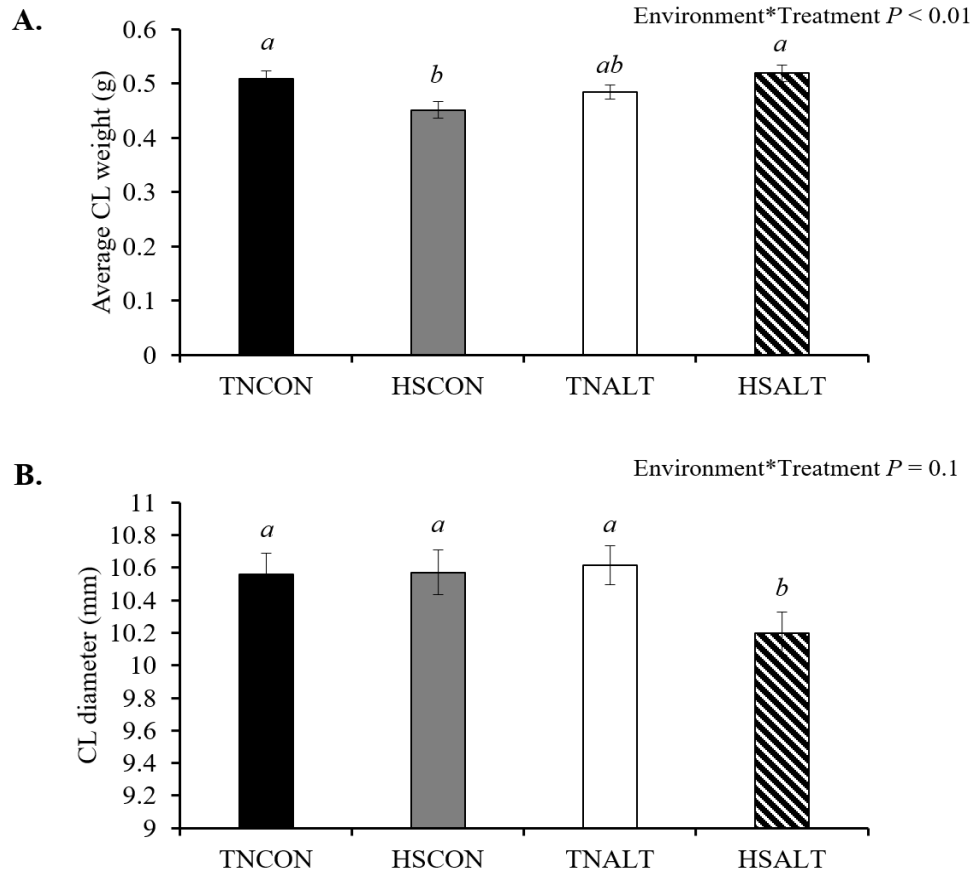
**Figure 5.3 Impact of Cyclical Heat stress on Respiration Rate.** Post-pubertal gilts were assigned to one of four different treatments, heat stress (HS) conditions ( $35 \pm 1^\circ\text{C}$  for 12h,  $31 \pm 6^\circ\text{C}$  for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions ( $20 \pm 1^\circ\text{C}$ , 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Respiration rate (RR) was recorded by observing flank movements for 15 seconds and multiplied to determine beats per minute (bpm). RR was assessed three times in morning (AM) and evening (PM) and averaged together for each time point per treatment and environment  $\pm$  SEM. Line graphs denote bpm for each time point post estrus for each group  $\pm$  SEM. \* indicates difference between environment at specified time points ( $P < 0.01$ ); † denotes difference between ALT treatments in HS group ( $P < 0.05$ ); ‡ denotes a tendency for difference between ALT treatments in HS group ( $P < 0.10$ ).



**Figure 5.4 Impact of Cyclical Heat Stress on Rectal Temperature.** Post-pubertal gilts were assigned to one of four different treatments, heat stress (HS) conditions ( $35 \pm 1^\circ\text{C}$  for 12h,  $31 \pm 6^\circ\text{C}$  for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions ( $20 \pm 1^\circ\text{C}$ , 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Environment assignment and ALT administration lasted from d 3-12 post estrus. Rectal temperatures ( $T_R$ ) was recorded three times each morning (AM) and evening (PM). Line graph represents average morning and evening  $T_R$  per treatment  $\pm$  SEM. There was an increase ( $P < 0.01$ ) in average  $T_R$  in the HS environment compared to TN. \* and ‡ indicate a difference of ( $P < 0.01$ ) and ( $P < 0.1$ ) respectively.

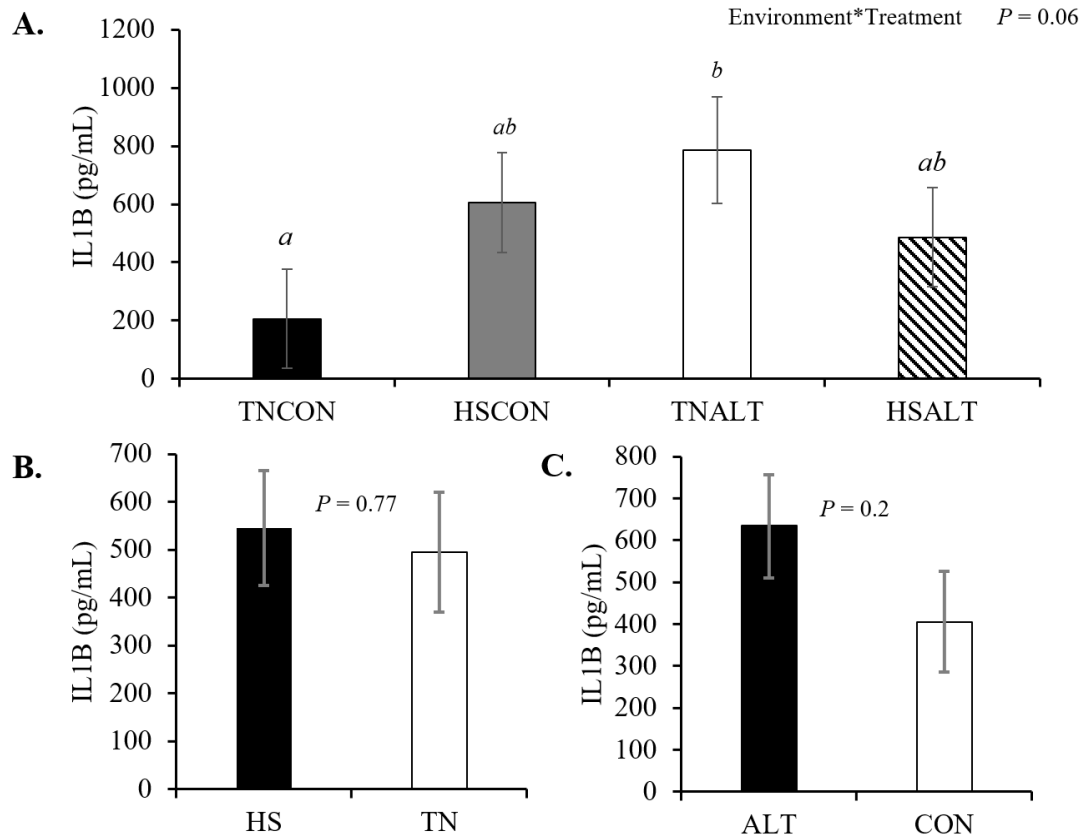


**Figure 5.5 Impact of Increased Ambient Temperature and Altrenogest Supplementation on Serum and Luteal Progesterone Concentrations.** Bred gilts were subject to either heat stress (HS) conditions ( $35 \pm 1^\circ\text{C}$  for 12h,  $31 \pm 6^\circ\text{C}$  for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions ( $20 \pm 1^\circ\text{C}$ , 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Graphs represent LS means for serum and luteal P4 concentrations in each group  $\pm$  SEM. **A.** Blood was collected to assess progesterone (P4) concentrations on 0, 4, 8, 12 d post estrus (DPE) and analyzed using colorimetric ELISA. Serum P4 concentrations did not differ at 0, 8 and 12 DPE. Serum P4 concentrations at 4 DPE were increased ( $P < 0.05$ ; \*) for TNALT compared to HSCON (19.73 vs. 14.18 ng/ml). A tendency for increased serum P4 concentrations exists at 4 DPE between TNALT and TNCON ( $P < 0.1$ ; †). **B.** Corpora Lutea (CL) were collected from each animal, powdered and homogenized in 5% trichloroacetic acid. Homogenates were diluted and analyzed to quantify P4 concentrations. No differences were detected across environment ( $P = 0.90$ ) or ALT treatment ( $P = 0.52$ ).

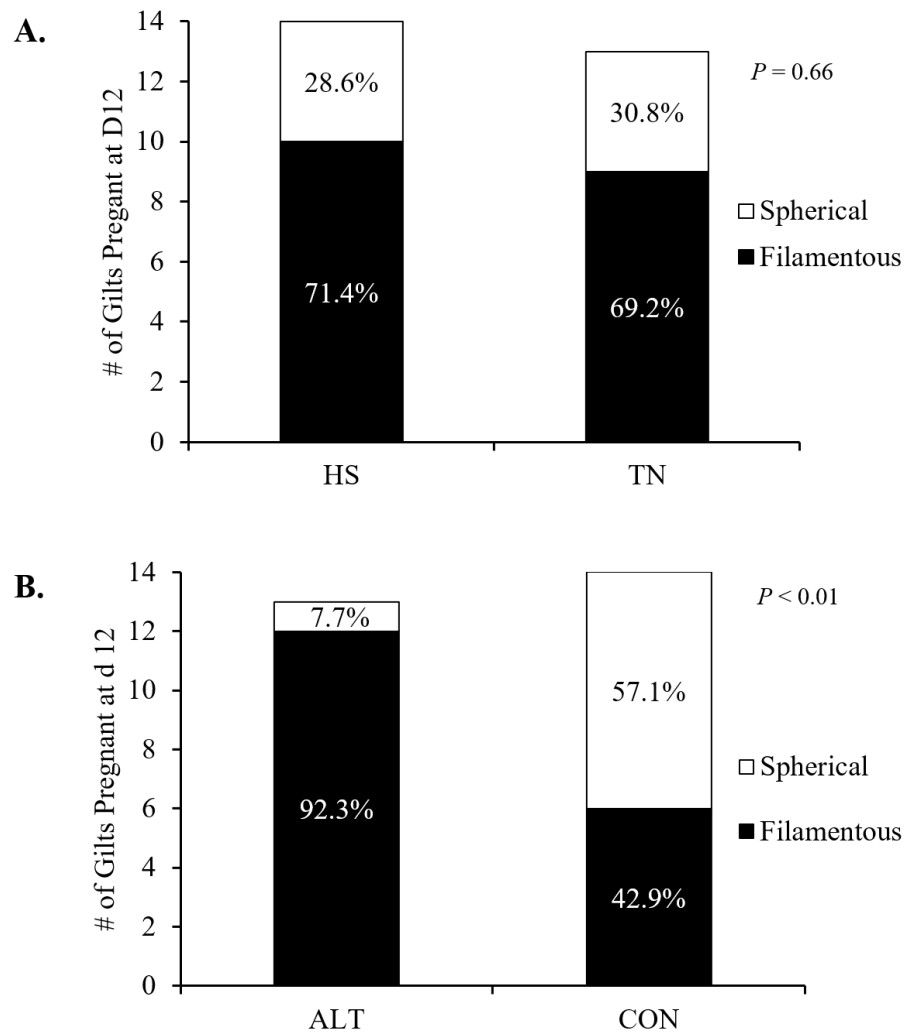


**Figure 5.6 Impact of Increased Ambient Temperatures and Altrenogest**

**Supplementation on Corpus Luteum Size.** Bred gilts were subject to either heat stress (HS) conditions ( $35 \pm 1^\circ\text{C}$  for 12h,  $31 \pm 6^\circ\text{C}$  for 12h, 21-31% relative humidity) with (HSALT) or without (HSCON) 15 mg/d of orally administered altrenogest (ALT) or thermal neutral (TN) conditions ( $20 \pm 1^\circ\text{C}$ , 36-57% relative humidity) with (TNALT) and without (TNCON) ALT supplementation during d 3-12 post estrus. Bar charts depict LS means for CL weight (g) and diameter (mm) for each group  $\pm$  SEM. **A.** Average corpora lutea (CL) weight for each group were recorded. An environment by altrenogest treatment interaction was observed ( $P < 0.01$ ). CL weight was decreased ( $P < 0.01$ ) in HSCON compared to TNALT and HSALT (0.45 vs. 0.51 and 0.52 g respectively). CL of TNALT gilts tended to be heavier ( $P < 0.1$ ) compared to CL of HSCON gilts. Differenced in letters denote statistical difference ( $P < 0.01$ ). CL diameter was measured and averaged within treatments. **B.** A tendency for environment by altrenogest treatment interaction was observed ( $P = 0.1$ ) CL diameter was decreased ( $P \leq 0.05$ ) in HSALT compared to remaining group. Differenced in letters denote statistical difference ( $P < 0.05$ ). Ovulation rates did not differ across individuals (data not shown).



**Figure 5.7 Effect of Increased Ambient Temperatures and Altrenogest Supplementation on Conceptus Interleukin-1 $\beta$  Production.** Uterine flushes were collected by injection of 20 mL sterile saline into the uterine lumen and collected after manipulating flush volume proximally and distally to the uterine bifurcation. Interleukin-1 $\beta$  (IL1B) was measured in the uterine flush using colorimetric ELISA. Data presented represent IL1B concentration (pg/mL) LSMEANS  $\pm$  SEM. **A.** An increase ( $P = 0.03$ ) was observed between TNCON and TNALT (205.28 vs. 785.38 pg/ml). **B.** No difference was observed between gilts in HS or TN conditions on conceptus IL1B production ( $P = 0.77$ ). **C.** No difference was observed between gilts in ALT or CON treatment groups on conceptus IL1B production ( $P = 0.2$ ).



**Figure 5.8 Effect of Environmental Conditions and Altrenogest Treatment on Conceptus Development.** Conceptuses were recovered from 27 of 30 gilts inseminated. Bars represent number of gilts under specified environment or treatment that yielded conceptuses. Black portion indicates conceptuses filamentous in morphology, white portion represents gilts with spherical conceptuses at the time of flushing. **A.** No effect was observed between environment on conceptus development ( $P = 0.66$ ). **B.** An increase ( $P < 0.01$ ) in the percentage of gilts with filamentous conceptuses was observed for those treated with ALT compared to CON gilts (92.3 vs 42.9%).



## CHAPTER 6: SUMMARY AND CONCLUSIONS

In this thesis, strategies were investigated in attempt to improve gilt reproduction. As described, optimizing gilt development and management is critical in maximizing sow farm productivity. Understanding the application and biology associated with selecting replacement gilts with the greater reproductive potential, and managing them during heat stress (HS) events, provides new insight for producers to improve productivity.

### *Gilt Development*

In the modern swine industry, the average sow does not contribute profit to an operation until after reaching parity 3 (Stalder et al., 2003). Until that point, any revenue accrued by the sow is used to offset her cost of development and maintenance. This poses a problem in the swine industry, where ~50% of sows in U.S. breeding herds are second parity or younger, and ~60% of females are culled prior to reaching parity 3 (Koketsu, 2007). This skew in parity structure results in greater costs for developing replacement gilts and reduced total production from younger parity females (Lucia et al., 1999), ultimately putting a significant financial burden on the remaining sows (i.e. those at or beyond their 3<sup>rd</sup> parity). Age at puberty onset is recognized for its predictive value pertaining to sow longevity. Demonstrated by Patterson et al. (2010), gilts reaching puberty prior to 180 days of age were more likely to remain in production to parity 3. However, using age at puberty for selection has limitations as it is labor intensive to detect and variable across individuals. Additionally, value for late puberty gilts dramatically decreases over time due to increased feed cost as well as the additional weight gain acquired during this time positions these gilts outside the marketing

standards set by the industry. Therefore, the ability to identify gilts as replacement or cull candidates at a younger age offers significant value to sow farms.

In pigs, reproductive maturation begins long before estrus behavior is expressed. Activation of the hypothalamic-pituitary-gonadal axis (HPGx) begins approximately 10 weeks after birth (Dyck and Swierstra, 1983), evident by the developmental changes the reproductive tract undergoes in response to gonadotropins and ovarian-produced estrogen. This stage of maturation is variable as demonstrated by Graves et al. (2015), where tertiary follicle development increased from postnatal day 75 to 115, with the greatest variation in follicular development occurring on postnatal days 95, 105, and 115. This same study also concluded that variation in VW from days 95, 105 and 115 were correlated with the gilts ability to reach puberty by 200 days of age. Establishment of these developmental timepoints warranted investigation into whether prepubertal variation in VW could serve as a marker for reproductive development and distinguish gilts with greater reproductive potential.

The current study (Chapter 3) tested the effectiveness of prepubertal vulva scoring in a commercial system as a means for identifying potential replacement and/or culls, as well as the relationship to sow reproductive performance. This study concluded, when evaluated at fifteen weeks of age, gilts with scores representing greater VW had an increased likelihood of successfully reaching parity 1 (P1). In addition to improved inclusion rate, gilts defined using Vulva Score methods A and B (VSA; VSB) showed an increase in total born (TB) and born alive (BA) over two parities when gilts assigned the lowest score possible were compared to the average of all other gilts scored. The farm score method (FS) in this study did not reveal as robust differences in litter size through

two parities compared to the more defined methods; VSA and VSB. This was unexpected, although plausible, as we believe this is partly due to the increased variation attributed to the subjective nature of visually evaluating vulva size. Additionally, overall representation of gilts scored using FS method was less compared to the other methods, due to inability of farm personnel availability at time of data collection. We do have sufficient reason to believe that visual assessment of the vulva can be performed effectively to yield similar results to using calibrated tools for screening gilts with small vulvas from the rest of the gilt population (Chapter 4).

The underlying biology associated with earlier reproductive maturation and the increased performance as sows is unclear, however, the association between early follicular development and increased fertility has been validated in the Meishan breed. Meishan pigs, a breed known for high fecundity, start reproductive development as early as 45 days of age (Miyano et al., 1990) suggesting earlier HPGx activation occurs, triggering early ovarian follicle and reproductive tract growth.

Follicular development results in the production of estrogen, and presumably, is the causative factor for the variation in reproductive tract size and VW at 75 to 115 days of age. The reproductive tract responsiveness to ovarian produced estrogens potentially provides insight into why the current work showed increased reproductive performance of gilts with greater reproductive tract development. Estrogen plays key roles in porcine reproduction, namely maternal recognition and establishing that initial cross talk between conceptus and dam, and estrogen receptor loci are associated with litter size (Rothschild et al., 1996). Considering the importance of estrogen, one can speculate that gilts with increased sensitivity to estrogen could possibly have an advantage reproductively, and

that an increase in vulva size prior to puberty essentially serves a biomarker for said sensitivity.

Another possible explanation for the improvement in litter production, is that by identifying gilts larger in vulva size, we are indirectly selecting for gilts with increased total reproductive tract size, and subsequently uterine capacity. The uterine environment is very competitive at the time of implantation, and acquiring sufficient surface area at the conceptus-endometrium interface is vital to the survival of the embryo. Increased uterine capacity is associated with decreased embryonic loss (Bolet et al., 1986) and increased number of pigs produced over four parities (Freking et al., 2016). Considering the known benefit of added uterine capacity on fertility in swine, it could be a contributing factor to our findings.

Being able to distinguish replacement and cull candidates at a young age offers significant incentives to the producer. From the work presented in this thesis, selecting gilts based on vulva size can result in easier identification of individuals less likely to perform as sows. This, in turn, results in savings of the time, labor, and other production costs associated with developing gilts. Additionally, gilts with an increased retention rate to parity 1 also generate more pigs over their first two litters, allowing the sow farm to improve total productivity.

### *Heat Stress*

The economic benefit of identifying gilts with greater reproductive potential offers new opportunities for producers to help maximize production. However, the seasonal effects on fertility observed annually potentially negates these production gains.

HS imposes significant economic losses annually to livestock production (St-Pierre et al., 2003). HS is widely considered to be a primary cause for the seasonal reduction in fertility observed annually in swine (Omtvedt et al., 1971; Tast et al., 2002; Auvigne et al., 2010; Ross et al., 2017), most commonly manifested with reduced farrowing rates, irregular estrus returns, decreased litter size, and prolonged wean-to-estrus interval. To obtain a better understanding of how HS affects swine in early pregnancy we conducted a study (Chapter 5) using sexually mature gilts and subjected them to increased ambient temperatures during the preimplantation period (3-12 days post estrus). Specifically, we investigated the effects of HS on the luteal phase prior to pregnancy establishment. We hypothesized that HS would negatively impact luteal function and embryo development during the preimplantation window; and supplementation with a progesterone (P4) analogue would help mitigate these negative effects. To test this, a 2x2 factorial design was conducted, with gilts assigned to HS or thermal neutral (TN) conditions, with individuals in each environmental group assigned to altrenogest (ALT) supplementation or a control (CON) group who received no ALT. When luteal and serum P4 levels were analyzed, no differences were detected across groups. Interestingly, CL weight was reduced in HSCON treated gilts, but was not in those assigned to HSALT. These findings suggest, that while development of luteal tissue is potentially impacted by environment, its steroidogenic capacity did not appear to be impaired when subjected to increased ambient temperatures or when ALT was supplemented. To evaluate the effects of embryo viability, IL1B was measured in the uterine flush content. IL1B is drastically upregulated during the time of embryo elongation (Ross et al., 2003) and is thought to be critical in communication between the dam and conceptus. No differences in IL1B concentrations

were detected when environmental effect or ALT effect were evaluated. However, number of conceptuses and stage of development are two main factors contributing to IL1B concentration in the uterine flushing, neither of which could be controlled in the experiment. Interestingly, stage of development was not impacted by environment, but was advanced in ALT treated gilts compared to CON. Supplementing P4 in the form of ALT to gilts in early pregnancy is met with some incongruity in the literature regarding its benefit or lack thereof on fertility (Ashworth, 1991; Soede et al., 2012; Szymanska and Blitek, 2016). Considering these results, and the current literature, timing of P4 or ALT supplementation relative to ovulation and fertilization as well as duration of treatment appear to be influential on embryo development and potentially survival.

### *Future Implications*

The findings within this thesis offer potential new strategies for pork producers to improve gilt reproductive performance. Prepubertal vulva scoring demonstrated significant benefit to improving litter size, inclusion rate, and age at farrowing for those not classified into the smallest category. Being able to discern gilts based on vulva size requires personnel training. Once properly trained, however, scoring is easy to implement during the replacement gilt selection process. The timing of evaluation is critical in distinguishing more suitable gilt replacements from their contemporaries, and scoring vulvas on prepubertal gilts offers an additional culling criteria that can be considered prior to first expression of estrus.

Additional research is needed to understand the biology associated with reduced fertility and early pregnancy loss in swine due to HS. While effectiveness of ALT

supplementation was not definitive as a HS mitigation strategy, its effect on stage of embryo development offers interesting implications to improve fertility in pigs.

Conceptus elongation and attachment is a highly competitive period during development in which sufficient crosstalk between conceptus and the dam must be established (Bazer and Johnson, 2014) and is a time that accounts for much of the embryonic loss (Pope et al., 1990). Being able to enhance conceptus elongation could potentially result in greater elongation synchrony within the litter, and help reduce embryonic losses due to insufficient communication at the conceptus-endometrium interface. Further research is needed to determine optimal timing and duration for P4 supplementation to test these potential benefits.

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