RESEARCHREPORT

ANIMAL SCIENCE

Title: Development of Strategies to Improve Sow Productive Lifetime – **NPB #11-111**

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revised

Industry Summary:

Numerous factors, including herd-life (length in days), removal parity, total piglets born and the number of piglets weaned, impact sow productive lifetime. Like many complex traits that are controlled through numerous loci and that are subject to environmental influence, sow productive lifetime can be lowly heritable. This project was conducted to facilitate the identification of physical and blood markers that could be utilized by the swine industry through incorporating this information into the replacement gilt population. The objectives of the project were to determine if specific, easily identifiable factors could be reliable in identifying gilts for the replacement pool that have a high probability of achieving their first estrus prior to 180 days of age. The data in this project demonstrate that by approximately 95 days of age, gilts begin to demonstrate a high degree of variation in the amount of follicular activity, absent at 75 days of age. The variation in follicular activity appears to impact the growth and development of the reproduction tract which can be observed by variation in vulva size. On day 95 of age, only 31% of gilts whose vulva size was more than one standard deviation below the mean achieved their first estrus by 180 days of age, compared to 66% of all other gilts. However, the variability in vulva size at day 75 of age was not useful in identifying gilts that are likely to achieve their first estrus by 180 days of age. Additionally, kisspeptin, a molecule associated with activation of the hypothalamic-pituitary-gonadal axis, was greater on days 75 to 105 of age in gilts that achieved their first estrus by 200 days compared to those that did not. Collectively, we have identified a time point in gilt development when decisions regarding the inclusion or exclusion of gilts in the replacement gilt pool could be made that may reduce the number of non-productive days in the sow herd as age of first estrus is one of the best indicators of sow lifetime production.

Key Findings

- During postnatal ages of 75 to 115 days of age, gilts undergo ovarian follicular reorganization as assessed by having the presence of tertiary follicles.
- Of gilts that achieved their first estrus by 200 days of age, a weak but statistically significant correlation between body weight at 75 days of age of first estrus was observed.
- Changes in vulva development during days 95 to 115 of age, presumably driven by estrogen production from tertiary follicles, may be a useful tool in identification of gilts to include or exclude in a gilt replacement pool.

Keywords: Puberty, Follicle Development, Gilt development

Scientific Abstract:

A valuable indicator of female lifetime productivity is the age at which gilts achieve their first estrus. We have determined that follicular activity, as determined by tertiary follicle development, in pre-pubertal gilts begins during postnatal days (PND) 85-115. The central hypothesis of this study is that gilts demonstrating tertiary follicle development earlier in life are more likely to achieve puberty earlier compared to counterparts of a similar age and weight that lack tertiary follicle development. The objective of this project was to identify markers and specific time-points during pre-pubertal development that could be utilized as valid indices to predict age of first puberty. To accomplish this, 155 gilts of similar age (± 2 days) were weighed and blood drawn for plasma isolation on PND 75, 85, 95, 105 and 115. Circulating plasma kisspeptin levels were measured. Additionally, vulva width, length and area were recorded as proxies for estrogen activity. At each time point, 10 gilts were sacrificed and ovarian follicular activity was recorded. Estrus detection was conducted daily on PND days 126 to 200 for the remaining 105 gilts. Mean vulva area (VA) on PND 75, 85, 95, 105 and 115 was 596 ± 206, 683 ± 190, 864 ± 212, 1014 ± 228 and 1265 ± 252 mm^2, respectively. Of the gilts demonstrating behavioral estrus, 28 were within PND 140-160, 36 between PND 161-180, 15 between PND 181-200, while 26 did not demonstrate estrus within 200 days of age. All gilts euthanized at PND 75 lacked follicular activity as defined by having a minimum of two antral follicles per ovary, while 60%, 80%, 90% and 100% demonstrated follicular activity on PND 85, 95, 105, and 115, respectively, validating PND 75 to 115 as the approximate window for first follicular activity. Body weight at PND75 and vulva width at PND 115 were both correlated to age at first estrus ($P < 0.05$). Of those gilts whose VA was less than one standard deviation from the mean on PND 95 (i.e. < 652 mm^2), 31% and 50% demonstrated their first behavioral estrus by PND 180 and 200, respectively. However, of those gilts whose VA was within or greater than one standard deviation from the mean (i.e. ≥ 652 mm^2), 66% and 79%

exhibited estrus prior to PND 180 and 200, respectively. These data suggest that utilization of VA changes between 95 and 115 days of age could be used as a tool to identify replacement gilts likely to achieve estrus at an early age.

Introduction:

Numerous factors, including herd-life (length in days), removal parity, total piglets born and the number of piglets weaned, impact sow productive lifetime. Like many complex traits that are controlled through numerous loci and that are subject to environmental influence, sow productive lifetime can be lowly heritable (Serenius and Stalder 2004), likely due to the postnatal environment having a profound impact on subsequent production capabilities. This has recently been demonstrated in replacement gilts reared in high (≥ 10 piglets) or low (≤ 7 piglets) lactation litter size (Flowers 2011). Briefly, gilts born of similar litter size (10-12 piglets) were strategically cross-fostered to small (≤ 7; n = 1100) or large (≥ 10; n = 1157) litters during lactation, and monitored over the next six parities. Gilts from small litters had a greater herd-life, with 38% being still in production after six parities compared to 16% of those gilts reared in large litters. In addition to a greater ability to stay in the herd, gilts from smaller litters had better farrowing rates (88.7± 1.8% vs. 83.3± 2.1%) and increased number of piglets born alive (11.0± 0.2 vs. 10.5± 0.2) compared to gilts raised in larger litters during lactation (Flowers 2011). Based on these data, it appears that genetic selection alone is difficult to identify with significant accuracy; the productive lifetime of a replacement gilt as environmental factors can contribute significantly to this outcome. Unfortunately, the feasibility of identifying potential replacement gilts and reducing the litter size in which they are reared is difficult in that it presents a suppressed lactation environment on piglets cross-fostered to support some small litters in addition to the requirement to identify putative replacement gilts within a few hours at birth. Additionally, some gilts reared in larger litters (10-12 piglets) do have the capability of performing in the top 25% of the herd with regard to lifetime productivity.

One indicator that has become utilized as a potential indicator of lifetime productivity is age at first estrus, or puberty onset. As an example, gilts exhibiting their first standing estrus early (< 153 days of age) compared to later (154 to 180 days of age) demonstrate significantly fewer non-productive days (Patterson et al. 2010). Of additional importance is that those gilts that did not exhibit estrus by 180 days of age have a lower service rate than gilts exhibiting estrus prior to day 180 days of age (Patterson et al. 2010). Additional experiments evaluating the utility of restricting feed intake on replacement gilts demonstrated that the younger a gilt is at first estrus the greater likelihood she will produce a first parity litter (Johnson and Miller 2011). Of potentially equal value to the producer is the ability to identify those gilts that are less likely to contribute positively to the sow herd prior to investment by inclusion into the gilt development unit. Of gilts that did not demonstrate estrus by 180 days of age, only 73% were eventually bred compared to approximately 94% of gilts exhibiting estrus between days 140-180 days of age (Patterson et al. 2010).

The ability to identify gilts at a young age that should be included or excluded from the replacement pool based on their likelihood of exhibiting their first estrus prior to day 180 of age is needed. Follicular growth in pigs typically begins around days 60 to 100 days of age (Schwarz et al. 2008). We have identified a time during gilt growth and development when, despite being of similar age and weight, gilts demonstrate significant variation in ovarian development. We sacrificed 48 gilts (98 ± 4d of age; 35 ± 2 kg BW) and collected ovaries from each gilt (Figure 1, below). Of the 48 gilts, 21 contained undeveloped ovaries while 27 contained ovaries that contained numerous tertiary follicles. This project was subsequently designed to test the hypothesis that specific physical signs of advancing ovarian activity, such as vulva development and circulating estrogen, when assessed at a specific stage of pre-pubertal development, could be used identify gilts with an improved or reduced probability of achieving their first estrus by 180 days of age.

VI. Objectives:

Our overall aim in this application was to demonstrate the relationship between pre-pubertal ovarian development and age at first estrus.

Materials & Methods: This section should include experimental design, methods and procedures used, number of animals, etc.

Animals

This study was conducted at Iowa State University with animal procedures having been approved by the Iowa State University Animal Care and Use Committee. Gilts (n = 155) of a similar age (65 ± 2 days) were selected and housed in pens of four and provided *ad libitum* access to feed and water for the project duration.

Data and Blood Collection

On postnatal days (PND) of age 75, 85, 95, 105, and 115, each gilt was weighed, sampled for blood (serum and plasma), and vulva measurements were collected. Additionally, transabdominal ultrasound was conducted and scores were assigned to each gilt as a prediction of the presence of tertiary follicle development. A score of 0 indicated no tertiary follicles were predicted to be observed and a score of 4 indicated that a substantial number of tertiary follicles were present. Vulva measurements of vulva length (VL) and vulva width (VW) were taken with Ultra Tech calipers and recorded in millimeters. Vulva area (VA) was later calculated by multiplying the vulva length and the vulva width.

Tissue collection

At each of the PND time points, 10 gilts were randomly selected and sacrificed. The uterus and ovaries were harvested from each gilt following euthanasia. The uterine tract was weighed and recorded and the ovaries collected and evaluated to get a quantitative assessment of the number and size of tertiary follicles.

Puberty Detection

Of the remaining 105 gilts, boar exposure was conducted from 126 – 200 days of age to detect the onset of each gilts first estrus (puberty). Two mature age boars (> 12 months) were exposed twice daily to all individual gilts. During heat checking, gilts were inspected for signs of puberty onset (swollen and red vulva, interest in boars) as well as response to back pressure applied by a researcher during boar contact. The age of puberty onset for each gilt was determined to be the first day that behavioral estrus (standing heat) was observed. At the conclusion of boar exposure, gilts that demonstrated behavioral estrus were categorized into three puberty date groups: early puberty (140-160 days), intermediate puberty (160-180 days), and late puberty (180-200 days). Gilts who did not display estrus by 200 days were considered non responsive.

Hormone Assays

Initially, our intentions were to utilize an estradiol assay to determine if blood estradiol levels were indicative of the tertiary follicle activity on the ovaries of developing gilts (objective 3). However, the quantity of estradiol in the pre-pubertal gilts from this study was less than the detectable range of our assay (1 pg/mL). Due to these difficulties, anti-müllerian hormone (AMH) and kisspeptin levels were measured in serum and plasma. Kisspeptin is a ligand known to be an established contributor to the activation of the hypothalamic-pituitary-gonadal axis (Lents et al. 2008) and AMH is a marker of the follicular reserve as it is a product of granulosa cells in females (Kevenaar et al. 2006).

Kisspeptin

Plasma kisspeptin levels were determined using a fluorescent enzyme-linked immunosorbent assay (ELISA) labeled for the Kisspeptin-10 (Metastin 45-54) polypeptide (Cat# FEK-048-56, Phoenix Pharmaceuticals, Burlingame, CA, USA). All assays were performed under the instruction of the manufacturers assay protocol. Samples were randomized so that each plate had at least one representative of each puberty date group. The sensitivity of the ELISA kit was a minimum detectable level of 6.6 pg/mL.

Anti-Müllerian Hormone

Serum anti-müllerian hormone levels were measured using a DuoSet ELISA labeled for human MIS/AMH (Cat # DY1737, R&D Systems, Minneapolis, MN, USA). All assays were performed under the instruction of the manufacturers assay protocol. For the AMH assays only samples from PND 95 time point were used. Samples were randomized so that each plate had at least one representative of each puberty date group. The microplate reader was set to read light absorbance at 450nm with a wavelength correction of 540-570nm. The sensitivity of the ELISA kit was a minimum detectable level of 93.8 pg/ml.

Statistical Analysis

All physical measurements where plotted using Excel and then given a correlation to the age of puberty of each gilt using the PROC-CORR program of SAS. Physical measurements were broke into categories of puberty date group as previously described as well as gilts achieving puberty before 200 days and those considered non responsive (after 200 days). Chi Square (X^2) analysis was performed using the PROC-FREQ program of SAS to determine the association of physical measurements and ability to achieve puberty by 180 & 200 days or after 180 & 200 days of age. Hormone concentrations for gilts were correlated to age of puberty using the PROC-CORR program of SAS.

Results:

Variation in Initial Follicular Development

In the group of sacrificed gilts (10 each on days 75, 85, 95, 105 and 115) we observed a tremendous amount of variation in the number of follicles on the ovaries collected (Figures 1 and 2). During the five time points, there was a progression from complete lack of follicular activity on day 75 to all gilts demonstrating follicular activity by 115 (Figure 2). Concomitant with increased follicular development we observed the uterine weights of gilts continued to progress rapidly at each stage of development (Figure 3). The average uterine weights on the five PND time points were 12.8, 25.7, 35.6, 43.9, and 54.7 grams respectively. Unfortunately, of the gilts sacrificed, the utility of transabdominal ultrasound proved to not be useful in predicting the presence or absence of follicular actively with any accuracy.

Puberty Onset

Puberty onset, as determined by the age of first estrus, was determined in the remaining 105 gilts. Following daily boar exposure from PND 126, the earliest behavioral estrus was not detected until PND 140

(Figure 4). Heat detection continued to PND 200 and a total of 79 gilts demonstrated a behavioral estrus with 26 gilts not achieving estrus by 200 days of age (Figure 4). Of the 79 gilts achieving puberty, the average age of first estrus was approximately 165 days of age. Gilts were then categorized based on the age of first estrus into four groups: early puberty (EP; first estrus by PND 140-160) intermediate puberty (IP; first estrus by PND 161-180), late puberty (LP; first estrus by PND 181-200), and non-responsive (NR; first estrus not observed by PND 200).

Relationship between body weight on PND 75, 85, 95, 105 and 115 and age of first estrus.

The body weight (BW) of the remaining 105 gilts increased (Figure 5) across the five PND time points ($P < 0.05$). The average BW in kilograms (kg) of gilts at each PND (75 - 115) was 38.4, 48.9, 57.7, 67.8, and 78.6 kg respectively (Table 1). Chi square statistics showed there to be no significant association between BW and a gilts ability to achieve puberty by either 180 or 200 days of age or after ($\chi^2 > 0.05$; data not shown). However, of the gilts that demonstrated estrus behavior, the correlation between body weight and the age of first estrus was closest to significance at PND 75 ($P = 0.055$; Figure 5) but was not significant at all remaining PND's (Table 1). The correlation suggested that of the gilts cycling by PND 200, the heavier gilts at PND 75 demonstrated an earlier age of first estrus than their lighter counterparts (Figure 5). To further visualize the data, when broken down into age of puberty groups (EP, IP, LP, and NR) the mean BW was greater for gilts in the earlier age of puberty groups (EP & IP) than those of the later groups (LP & NR) (See Supplemental Data Page 1).

Relationship between vulva development on PND 75, 85, 95, 105 and 115 and age of first estrus.

Vulva width (VW), vulva length (VL) and vulva area (VA) all increased over the five PND time points ($P < 0.05$; mean values ± SEM are presented in Tables 2 - 4). The average VW measurement in millimeters (mm) of gilts on the five time points were 22.7, 24.4, 27.4, 30.1, and 33.1 respectively (Table 2). X^2 values for VW on PND 75, 85, 95, 105 and 115 to determine any association between VW and a gilts ability to achieve puberty by 200 days of age were 0.56, 0.74, 0.07, 0.24 and 0.84, respectively, suggesting that VW on PND 95 was marginally predictive of whether or not a gilt would achieve estrus prior to PND 200. However, of the gilts that demonstrated estrus behavior, a correlation between VW and the age of first estrus existed on PND 105 ($P = 0.07$; Figure 6) and 115 ($P = 0.01$; Figure 7) and was not significant at all remaining PND's (Table 2). This data shows that between days 105 and 115 the gilts with larger VWs have a greater chance of achieving puberty at an earlier age than those with smaller VWs at the same age. When the average VWs were broken down into puberty groups, gilts in the earlier puberty groups (EP & IP) averaged larger VW that those in the later puberty groups (LP & NR) (See Supplemental Page 2).

The averages for VL measured in millimeters on the five PND time points are 25.6, 27.9, 31.1, 33.5 and 38.0, respectively (Table 3). VL showed no correlation to age of puberty onset. X^2 values for VL on PND 75, 85, 95, 105 and 115 to determine any association between VL and a gilts ability to achieve puberty by 200 days of age were 0.97, 0.93, 0.10, 0.69 and 0.47, respectively, suggesting that VL on PND 95 was marginally predictive of whether or not a gilt would achieve estrus prior to PND 200. Unlike VW, no correlation on any of the PNDs that data was collected existed between VL and the age of first estrus for the gilts that achieved puberty by 200 days of age (Table 3). The lack of correlation in VL to age of estrus can be further visualized when the average VLs were broken down into puberty groups. Gilts in the earlier puberty groups (EP & IP) had larger VL than those in the later puberty groups (LP & NR) though on some days although this data lacked the consistency observed with VW (See Supplemental Page 3).

The averages for VA (VW x VL) for PND 75, 85, 95, 105 and 115 were 596.7, 683.5, 864.0, 1015.3 and 1265.3, respectively (Table 4). X^2 values for VA on PND 75, 85, 95, 105 and 115 to determine an association between VW and a gilts ability to achieve puberty by 200 days of age were 0.56, 0.90, 0.10, 0.39 and 0.64, respectively, suggesting that VA on PND 95 was marginally predictive of whether or not a gilt would achieve estrus prior to PND 200. Unlike VW, and similar to VL, no significant correlation between VA and the age of first estrus existed for the gilts that demonstrated estrus behavior for any of the PNDs that data was collected (Table 4). The average VAs were plotted and then broken down into puberty groups. Gilts in the earlier puberty groups (EP & IP) had larger VA than those in the later puberty groups (LP & NR) on some days although this data lacked the consistency observed with VW (See Supplemental Page 4) and is likely the influence of VL impacting the VA calculation.

To further characterize the value of assessing the utility to assess changes in vulva development and their value in predicting a gilts ability to achieve their first estrus by PND 180 or 200 we grouped gilts based on VA into two groups for each PND that data was collected. Groups included those gilts with a VA less than 1 standard deviation from the mean and the remaining gilts (VA within or greater than one standard deviation of the mean). For those gilts who had a VA less than one standard deviation of the mean at PND 75 and 85 of age, 54% and 53%, respectively, had achieved puberty by 180 days of age, which was about 8 - 10% less than all other gilts (Figure 8). However, at PND 95. 105 and 115 days of age only 31, 38 and 36% of gilts with a VA less than one standard deviation from the mean achieved estrus by 180 days of age compared to 66, 66, and 64%, respectively, of all other gilts reaching the same benchmark (Figure 8). This result was consistent with determining the ability of gilts grouped by their VA to achieve their first estrus by 200 days of age (Figure 9). On PND 75 and 85, the percentage of gilts achieving their first estrus by 200 days of age was the same between gilts that had small vulvas and normal vulva size. However, 20-30% less gilts achieved estrus by PND 200 if their VA was below one standard deviation from the mean on days 95, 105 or 115 (Figure 9). Collectively, these data suggest that VA could be a useful tool to eliminate gilts from the gilt pool after PND 95, but is not useful prior to PND 85.

Relationship between blood markers and age of first estrus

Kisspeptin

Plasma kisspeptin levels were tested in all samples and mean values per day are indicated in Table 5. Gilts that achieved puberty within 200 days of age had numerically greater kisspeptin on PND 75, 85 and 95 although not statistically significant ($P > 0.05$). Of the gilts that achieved estrus before 200 days of age, other than a weak, and statistically marginal correlation ($P = 0.07$) no significant correlations between plasma kisspeptin and the age of first estrus existed on any of the PNDs that data was collected (Table 5).

Anti-Müllerian Hormone

Anti-müllerian hormone (AMH) was only measured in PND 95 serum samples. We chose this time-point based on the amount of variation observed in tertiary follicle development of the sacrificed gilts at this time point. We compared AMH levels across the four puberty groups (EP, IP, LP, NR) and did not observe a numerical or statistical difference between groups (Figure 11).

Discussion

Age at puberty onset represents a useful phenotype that can be utilized to select or reject gilts entering the breeding herd. As gilts make up a substantial portion (20% - 25%) of the breeding herd, the time when they can be bred can greatly affect the overall performance of the herd as well as the animal's individual lifetime performance (Spörke 2005). However, determining the specific day of age that a gilt experiences her first estrus is labor intensive as it requires early boar exposure and time investment. The objective of this study was to determine the age at which variation in ovarian development occurs and to identify associated markers so that gilts might be selected based on their progress towards achieving first estrus prior to puberty. Blood sampling and body weight collection was conducted on PNDs 75, 85, 95, 105 and 115 in addition to vulva measurements and assessment via transabdominal ultrasound on 155 gilts. We sacrificed 10 gilts at each time-point to verify the follicular activity at each age. Importantly, the sacrificed gilts enabled us to decipher the specific days where a cohort of pre-pubertal gilts begin follicular development and demonstrate that those gilts with increased follicular activity also had greater reproductive tract weight. We were able to determine across 40 days of growth and development, the progression of follicular activity in gilts which transitioned from complete absence of tertiary follicular activity on PND 75 to almost all gilts possessing numerous tertiary follicles on their ovaries by PND 115. Our interest was to determine if we could utilize physical or blood markers to determine which gilts demonstrate folliclular activity earliest, and if those with the earliest tertiary follicle development were also more likely to achieve their first estrus earlier, a potential indicator of sow lifetime productivity.

We then began boar exposure on the remaining 105 gilts on PND 129 until PND 200 to identify the age of first estrus. Age at puberty onset is highly regulated by the individual animal's genetic clock and is variable in the time of developmental onset (Foster et al., 1994). However, there is a relationship in optimal timing of sexual maturation and growth in reaching a threshold to achieve puberty onset (Rozeboom et al., 1995). This was also suggested by Le Cozler et al. (1999) that through restriction of feed and growth the timing at which a female reaches puberty is significantly altered and delayed. In contrast, Foxcroft et al. (1996) suggested that age of puberty onset in a female is reached well after the female has achieved the threshold of growth needed to undergo sexual maturation. Rozeboom et al. (1995) showed an average age of puberty in a cohort of gilts reared *ad libitum* was 172.5 days with a standard deviation of 23.4 days. This study was similar to reports of Young et al. (1990) and Zimmerman et al. (2000) of 167.2 and 168 days, respectively, which are similar to the results of this study.

The utilization of BW on PNDs 75-115 was not useful in predicting whether or not gilts would achieve their first estrus by 200 days of age although BW on day 75 did have a statistical correlation with the age of estrus of those that did cycle. Essentially, heavier gilts on PND 75 could achieve estrus earlier than their lighter counterparts on PND 75. With respect to vulva parameters, the utilization of vulva parameters on PND 95 had the closest association and utility for predicting whether or not a gilt would cycle by 200 days of age while all other PNDs were not useful. This made biological sense, as the most variation in follicular activity occurred on PND 85 and 95 (Figure 2) followed by increased reproductive tract weights (Figure 3). What was not clear before this study was if those gilts that demonstrated earlier follicular activity also achieved estrus earlier. We detected statistically significant correlation between VW and age of first estrus of the gilts that did cycle within 200 days.

In summary, 1) advanced follicular activity begins in gilts around PND 85 and variation persists in a cohort of gilts until about PND 115, 2) advanced follicular activity is associated with increased reproductive tract weights, 3) X^2-square statistics suggested that the most valuable day in using vulva measurement to predict whether or not a gilt would achieve estrus was PND 95 ($X^2 = 0.07$) while all other days were not significant, 3) VW provided the best correlation with age of first estrus for the gilts that did cycle by 200 days of age, 4) blood kisspeptin levels were numerically greater on PND 75-105 in gilts that achieved puberty prior to 200 days compared to those that did not.

To determine how this could impact replacement gilt selection, we categorized gilts based on their vulva area on PND 95 in a way that producers could subjectively sort gilts (i.e. removing the bottom 10-20 percent based on comparative vulva size). For each PND, we identified the percentage of gilts that achieved their first estrus by 180 (Figure 8) and 200 (Figure 9) days of age gilts whose VA was less than 1 standard deviation from the mean of the percentage of gilts achieving estrus whose VA was within or greater than one standard deviation from the mean. What these data suggest is that the exclusion of the bottom approximately 15% of gilts based on vulva size on PND 95, PND 105 or PND 115 can eliminate a set of gilts for which only about a third will achieve puberty by 180 days of age whereas approximately two-thirds of their counterparts with larger vulvas on PND 95 will accomplish the same benchmark. It is difficult to determine if an additive value of using multiple parameters (i.e. blood parameters and physical markers) could provide more accuracy to the ability to predict the age of puberty achievement. This may be possible although the parameters we measured had only weak correlations with age of first estrous and were statistically marginal in the case of kisspeptin. Collectively, these data suggest that utilization of a physical marker, such as changes in vulva parameter which may be reflective of multiple endocrinological and environmental factors, could enable to the classification of the gilts that have manifested the ability to achieve puberty earlier or later compared to their counterparts. Importantly, categorizing gilts this way prior to follicular activity (PND 75 and 95) was not useful in distinguishing groups of gilts that have different capacities to cycle by 180 days of age. Data from this project suggest that evaluation of vulva development on PND 95-115 could be a useful tool in making replacement gilt selection decisions.

Tables

Table 1. Body weight correlations with age of first estrus

PND[1]	N[2]	r-value[3]	p-value[4]	BW ± SEM[5]
75	79	-0.217	0.055	38.6 ± 0.5
85	79	-0.136	0.232	49.1 ± 0.6
95	78	-0.141	0.216	57.8 ± 0.7
105	79	-0.068	0.549	68.1 ± 0.7
115	79	-0.102	0.371	78.8 ± 0.8

[1]Postnatal day of age.
[2]Number of animals included in analysis.
[3]Correlation between body weight and age of first estrus.
[4]*P*-Value.
[5]Body weight mean ± standard error on each day.

Table 2. Vulva width correlations with age of first estrus

PND[1]	N[2]	r-value[3]	p-value[4]	VW ± SEM[5]
75	79	-0.083	0.465	22.8 ± 0.5
85	79	-0.118	0.299	24.7 ± 0.4
95	78	-0.117	0.307	27.8 ± 0.4
105	79	-0.204	0.071	30.1 ± 0.4
115	78	-0.282	0.012	33.0 ± 0.4

[1]Postnatal day of age.
[2]Number of animals included in analysis.
[3]Correlation between vulva width (VW; mm) and age of first estrus.
[4]*P*-Value.
[5]Vulva width mean ± standard error (mm) on each day.

Table 3. Vulva length correlations with age of first estrus

PND[1]	N[2]	r-value[3]	p-value[4]	VL ± SEM[5]
75	79	-0.063	0.579	26.2 ± 0.6
85	79	-0.006	0.958	27.7 ± 0.4
95	78	-0.065	0.570	31.3 ± 0.5
105	79	-0.043	0.703	34.1 ± 0.5
115	78	-0.040	0.727	38.2 ± 0.5

[1]Postnatal day of age.
[2]Number of animals included in analysis.
[3]Correlation between vulva length (VL; mm) and age of first estrus.
[4]*P*-Value.
[5]Vulva length mean ± standard error (mm) on each day.

Table 4. Vulva area correlations with age of first estrus

PND[1]	N[2]	r-value[3]	p-value[4]	VA ± SEM[5]
75	79	-0.075	0.510	612.6 ± 24.6
85	79	-0.065	0.569	694.0 ± 21.2
95	78	-0.119	0.301	879.9 ± 22.7
105	79	-0.135	0.236	1036.8 ± 26.4
115	78	-0.167	0.143	1269.5 ± 28.0

[1]Postnatal day of age.
[2]Number of animals included in analysis.
[3]Correlation between vulva length (VL; mm) and age of first estrus.
[4]*P*-value.
[5]Vulva length mean ± standard error (mm) on each day.

Table 4: Plasma kisspeptin correlations with age of first estrus

PND[1]	N[2]	r-value[3]	p-value[4]	pg/mL ± SEM[5]
75	71	0.017	0.886	50.0 ± 4.0
85	70	-0.216	0.072	54.1 ± 3.9
95	66	-0.130	0.299	59.1 ± 5.6
105	69	-0.086	0.481	55.3 ± 4.6
115	70	-0.011	0.931	57.6 ± 5.2

[1]Postnatal day of age.
[2]Number of animals included in analysis.
[3]Correlation between kisspeptin (pg/mL) and age of first estrus.
[4]*P*-Value.
[5]Vulva length mean ± standard error (mm) on each day.

Figures

Figure 1.

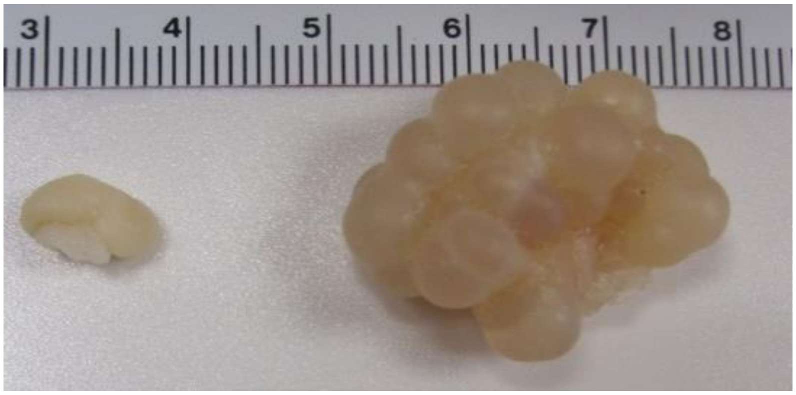


Figure 1. A representative image showing ovaries collected from gilts of the same age but having distinct differences in the number on tertiary follicles present on their ovaries.

Figure 2.

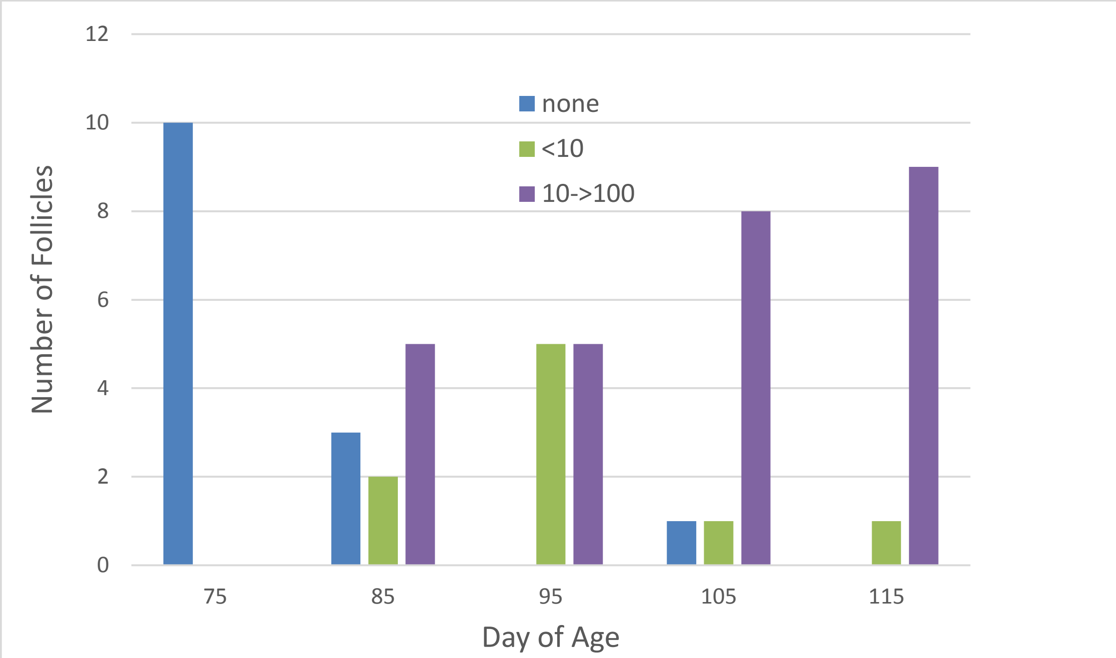


Figure 2. Follicular development increased as gilts progressed in age. On Day 75 all sacrificed gilts possessed ovaries with no tertiary follicles. By PND 85 and 95, tertiary follicle distribution was greatest among sacrificed gilts. By PND 115, the majority of gilts had a significant number of tertiary follicles. Blue bars represent the number of gilts sacrificed at each day having no tertiary follicle development. Green bars represent the number of the 10 gilts sacrificed on each day having a total of 1 to 10 tertiary follicles present. Purple bars represent the number of gilts have a total of 10 to greater than 100 tertiary follicles present.

Figure 3.

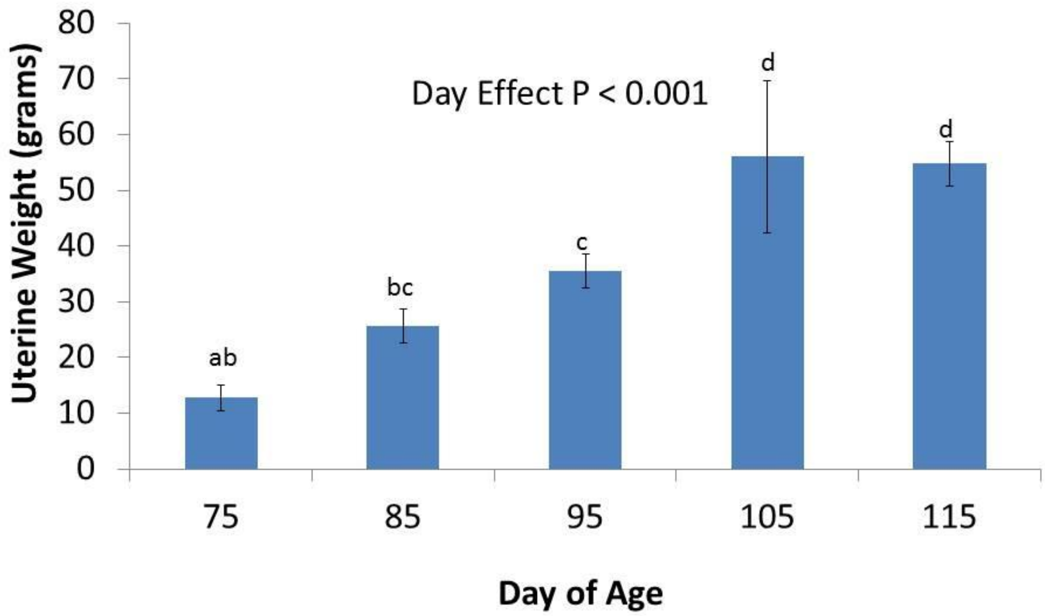


Figure 3. Uterine weight increased as gilts progressed in age from 75-115 days. The weight of the reproductive tract increased concomitant with the increased ovarian activity. Different superscripts represent statistical significance ($P < 0.05$)

Figure 4.

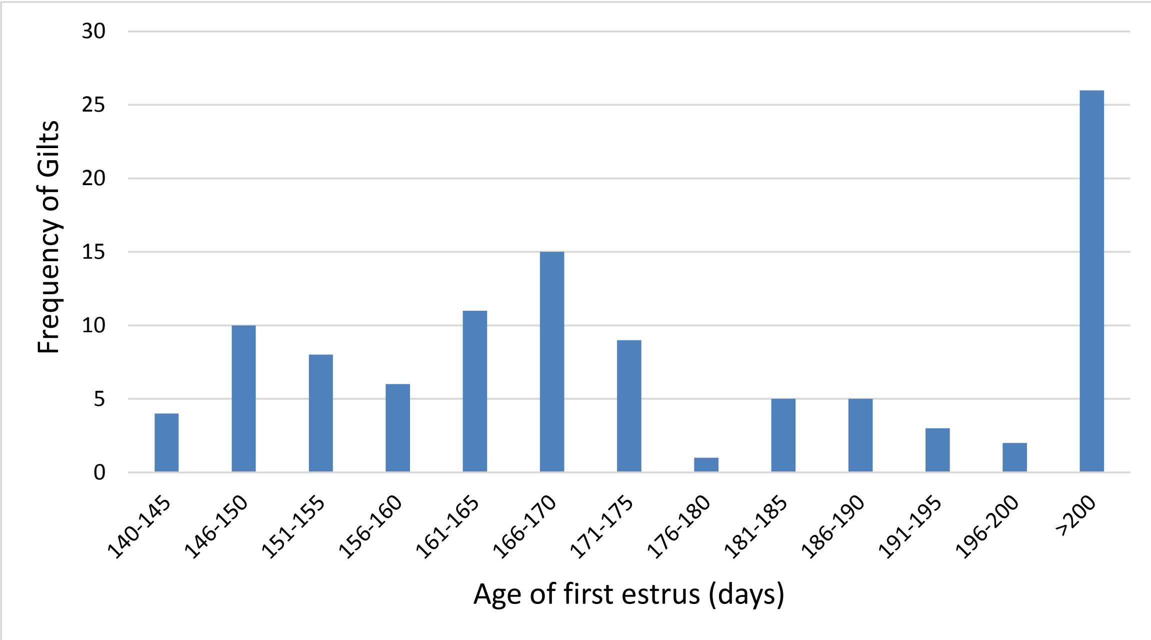


Figure 4. Distribution of the number of gilts achieving their first estrus from PND 140 through PND 200. Boar exposure and daily heat detection was initiated on PND 129.

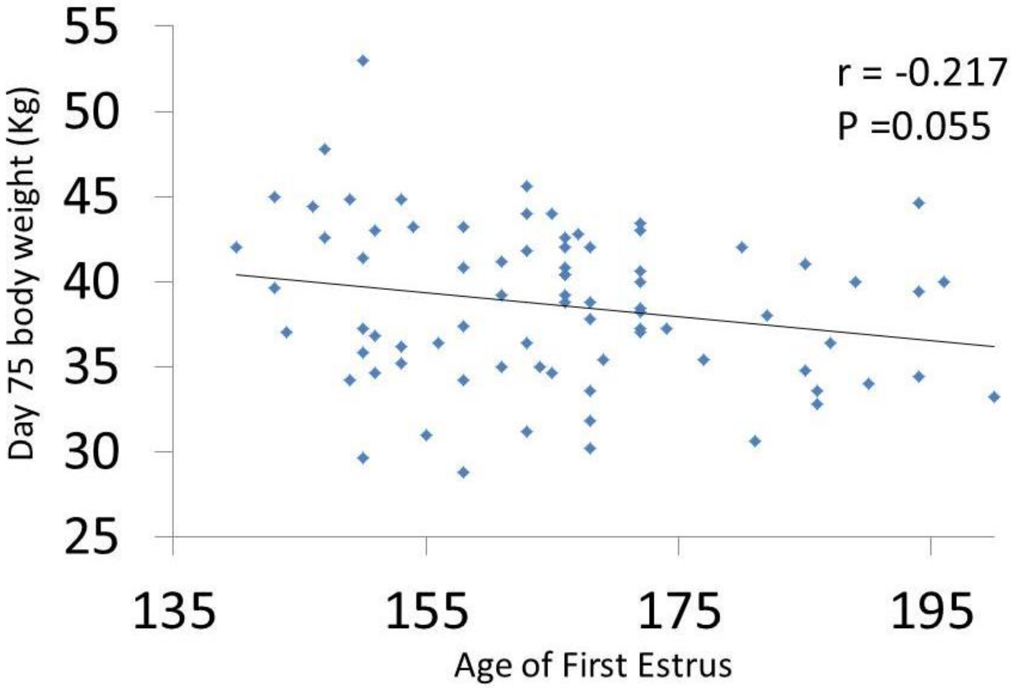


Figure 5.

Figure 5. Correlation between body weight on PND 75 of gilts achieving their first estrus by 200 days of age and their age of first estrus. r=-0.217, *P*=0.055.

Figure 6.

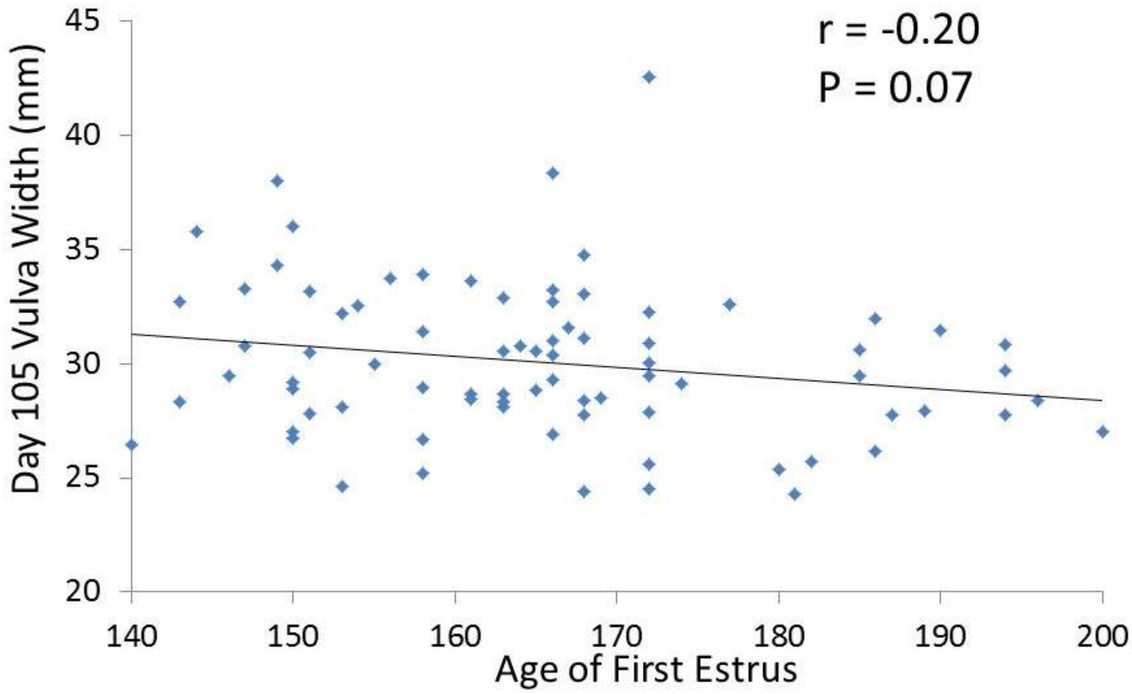


Figure 6. Correlation between vulva width on PND 105 of gilts achieving their first estrus by 200 days of age and their age of first estrus. (r=-0.20, *P*=0.07)

Figure 7.

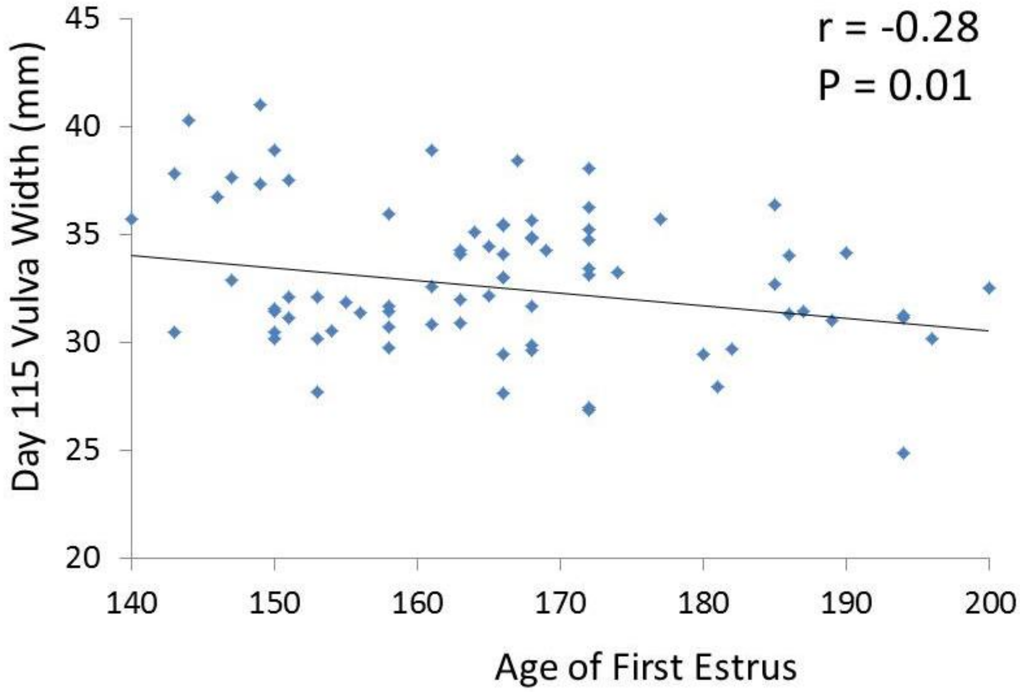


Figure 7. Correlation between vulva width on PND 115 of gilts achieving their first estrus by 200 days of age and their age of first estrus. (r=-0.28, *P*=0.01)

Figure 8.

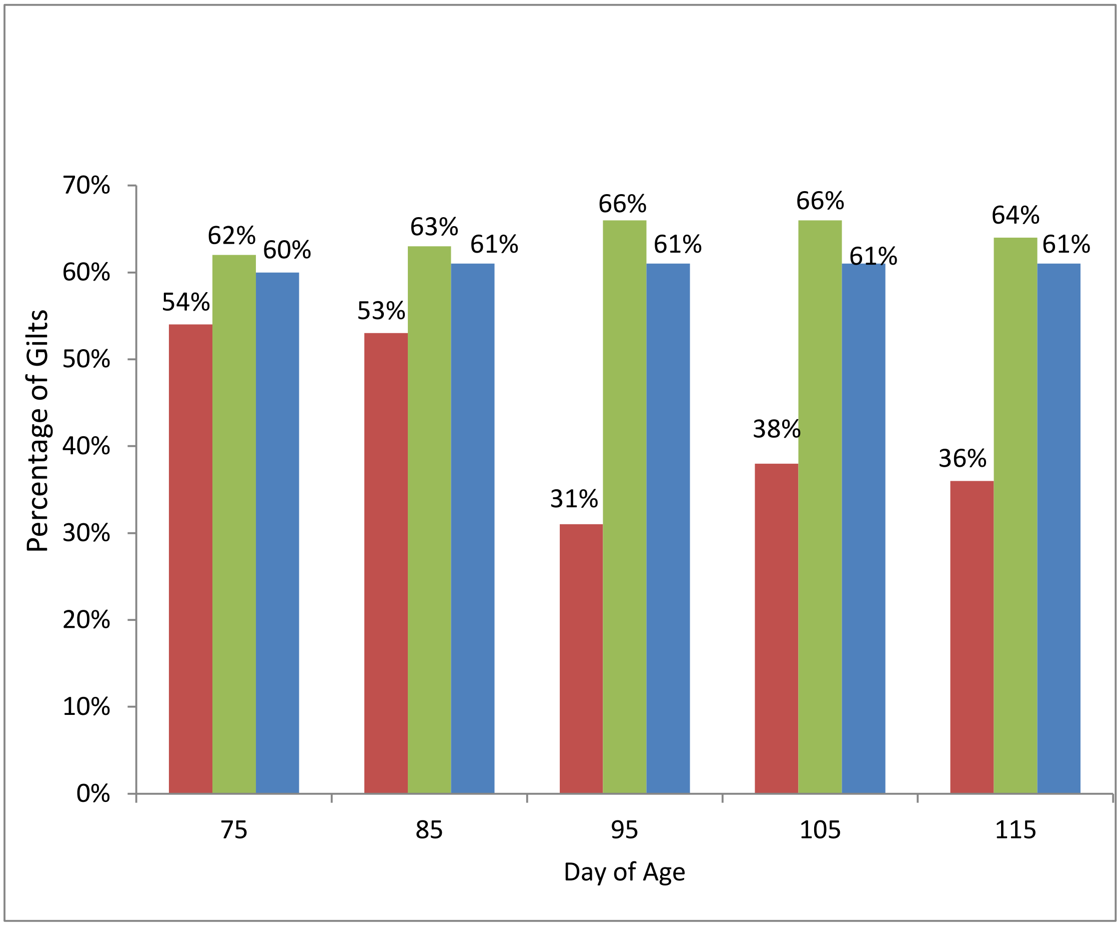


Figure 8. Percentage of gilts achieving estrus by 180 days of age. Gilts were grouped based on the calculated vulva area (VA). Red bars represent the percentage of gilts whose VA was less than 1 standard deviation from the mean and achieved estrus prior to 180 days of age. The green bars represent the percentage of gilts achieving estrus by PND 180 whose VA was within or greater than one standard deviation from the mean. Blue bars repsent the percentage of all gilts achieve estrus by 180 days of age. The utility of screening gilts based on vulva size on PND 95, 105 and 115, and not on days 75 and 85, appears to be using in identifying those gilts that may have a reduced liklihood of achieving estrus by 180 days of age.

Figure 9.

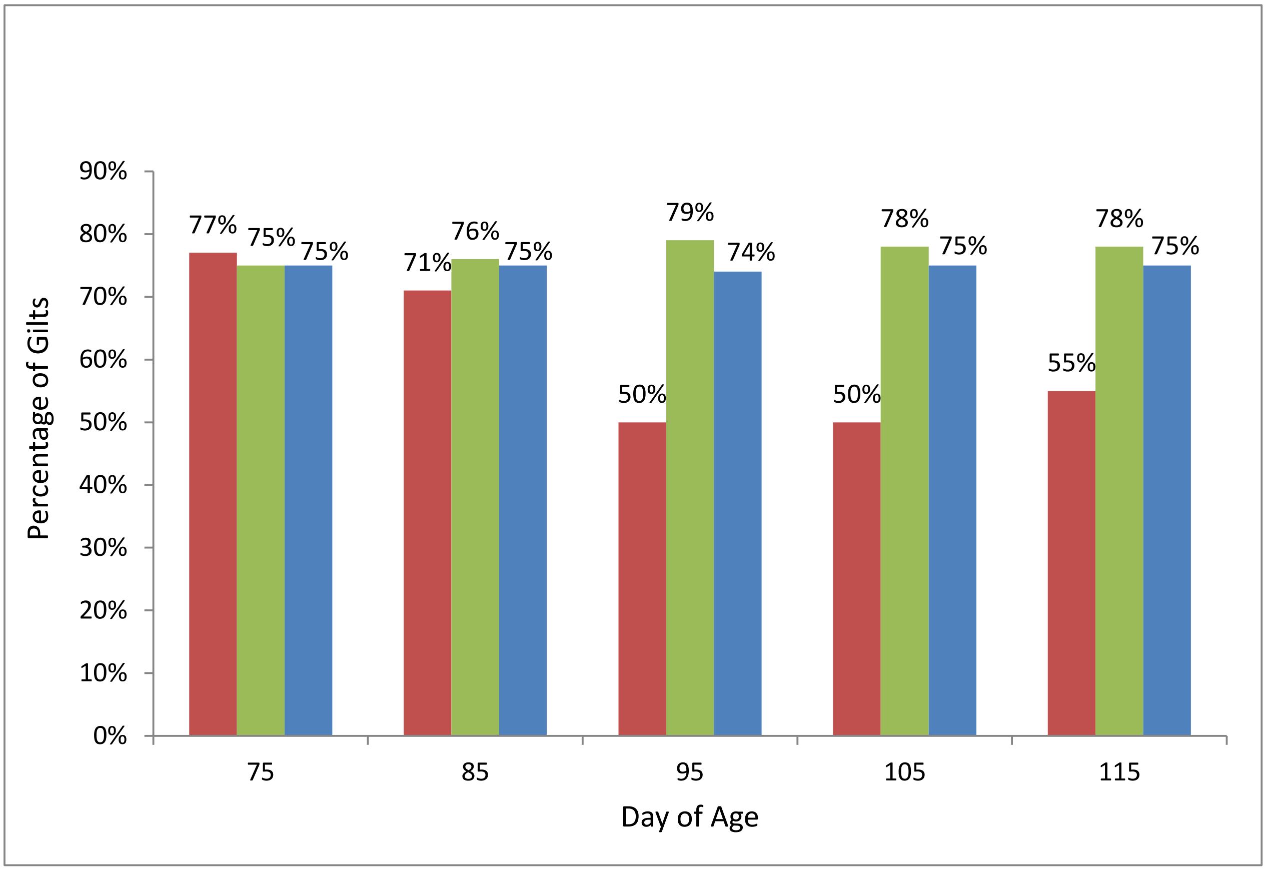
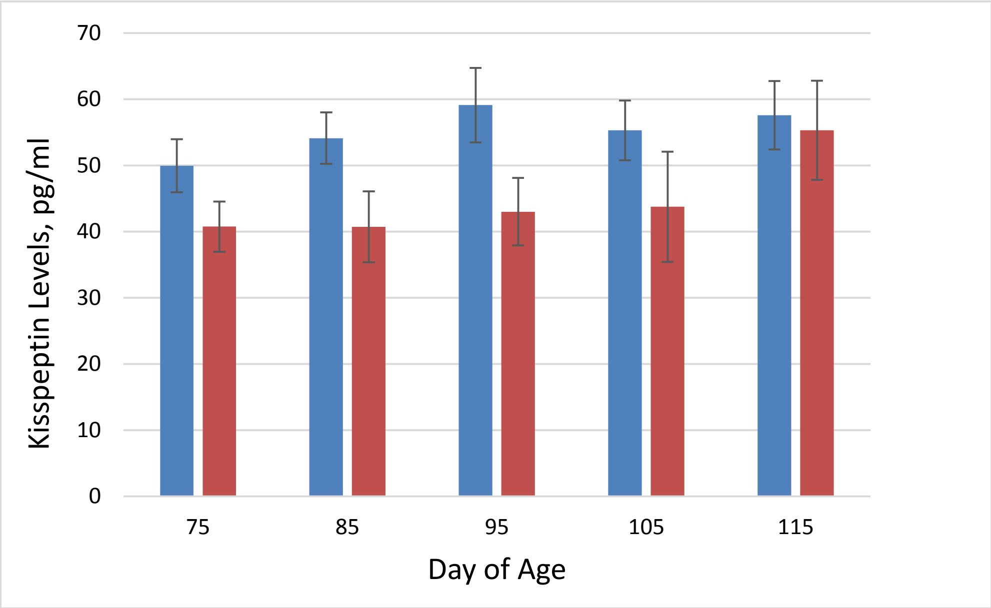


Figure 9. Percentage of gilts achieving estrus by 200 days of age. Gilts were grouped based on the calculated vulva area (VA). Red bars represent the percentage of gilts whose VA was less than 1 standard deviation from the mean and achieved estrus prior to 200 days of age. The green bars represent the percentage of gilts achieving estrus by PND 200 whose VA was within or greater than one standard deviation from the mean. Blue bars repsent the percentage of all gilts achieve estrus by 200 days of age. The utility of screening gilts based on vulva size on PND 95, 105 and 115, and not on days 75 and 85, appears to be using in identifying those gilts that may have a reduced likelihood of achieving estrus by 200 days of age.

Figure 10.



Plasma kisspeptin levels on PND 75, 85, 95, 105 and 115 in gilts that achieved their first estrus within 200 days of age (blue bars) and gilts that did not achieve estrus within 200 days of age (red bars).

Figure 11.

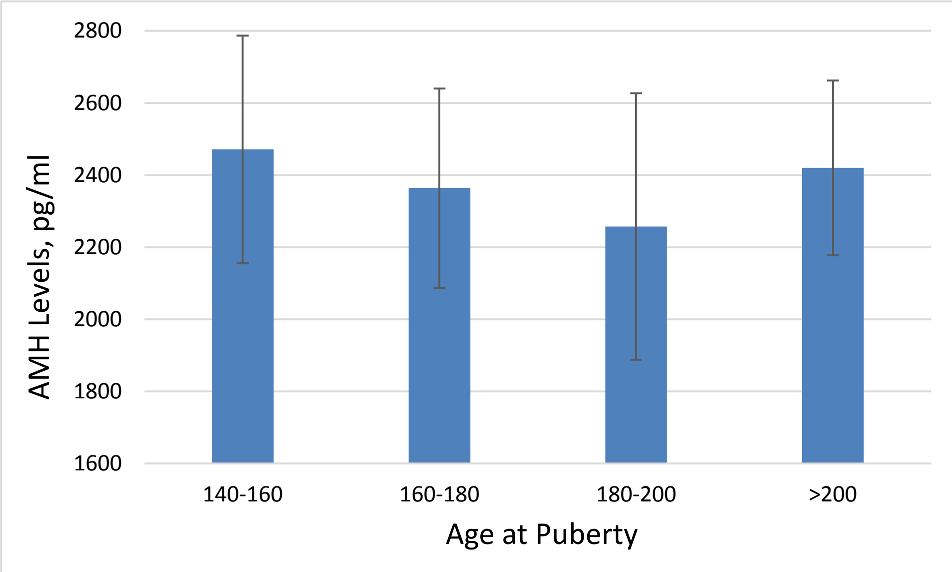


Figure 11. Anti- müllerian hormone (AMH) in serum collected from gilts on PND 95. No differences were observed in AMH concentrations between groups gilts based on when they achieved their first estrus.

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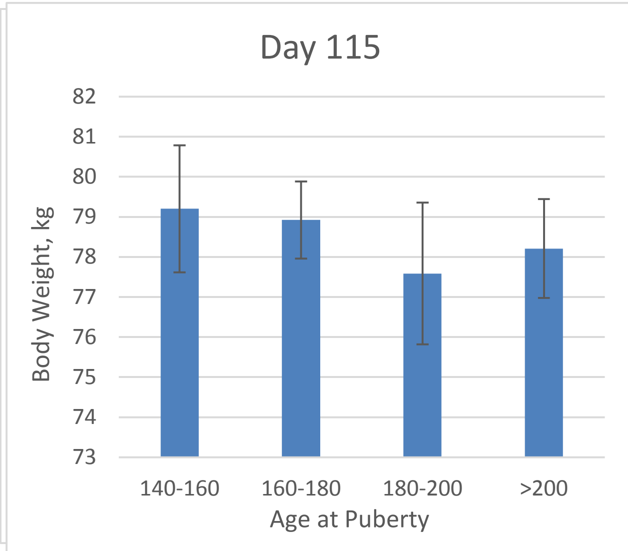
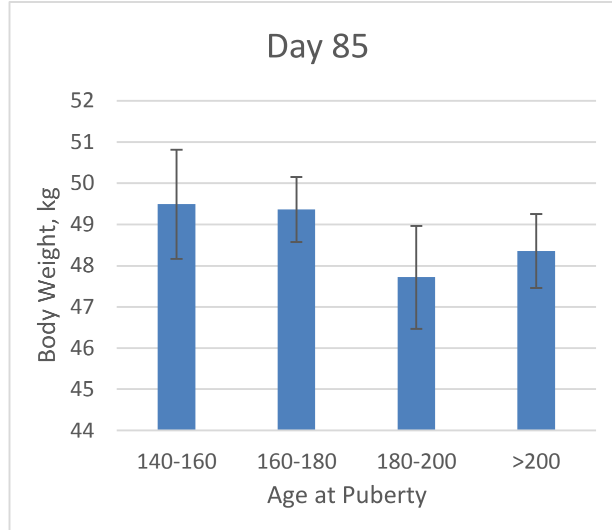
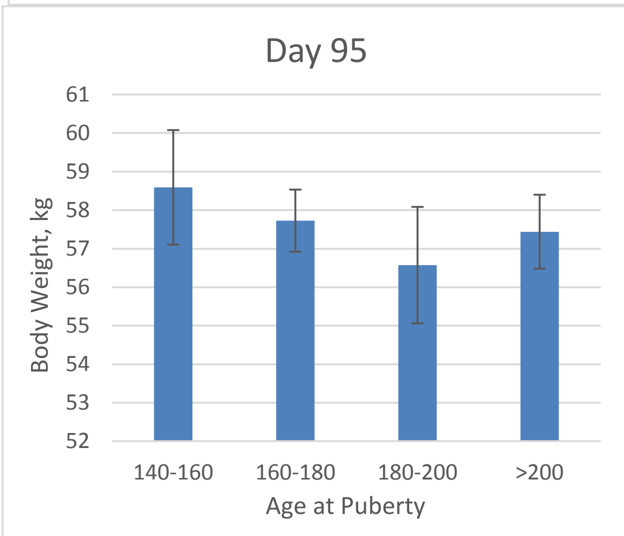
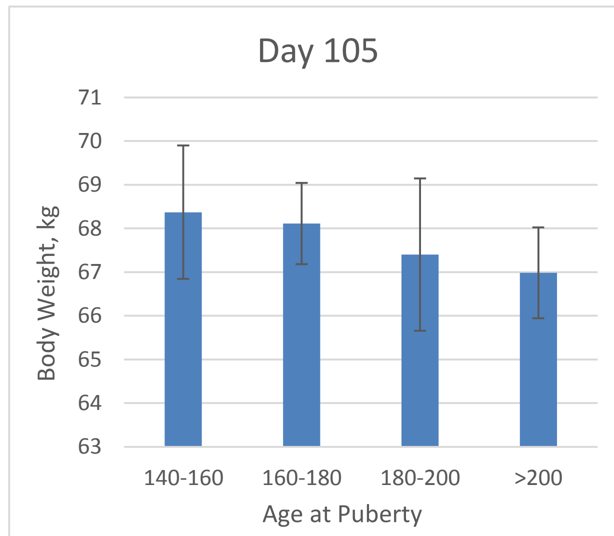
Supplemental Data Sheet 1 – Body Weight



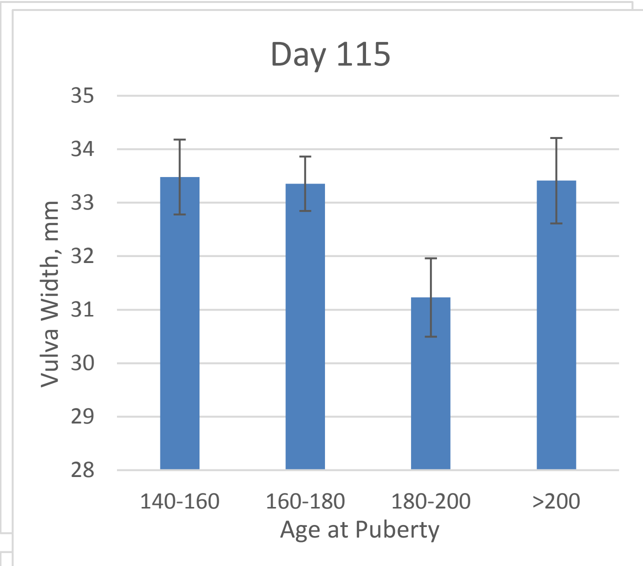


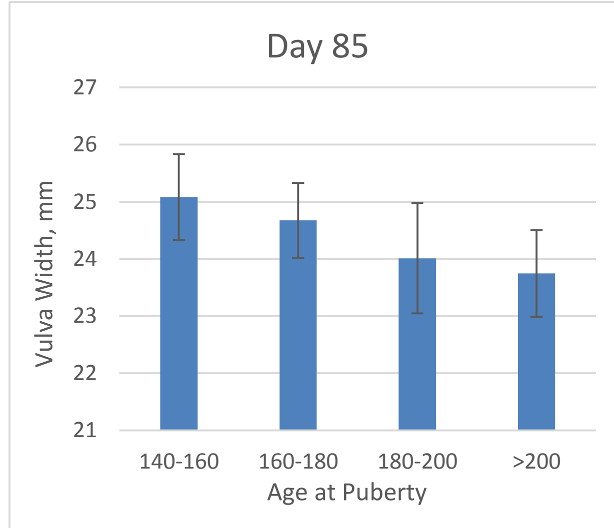


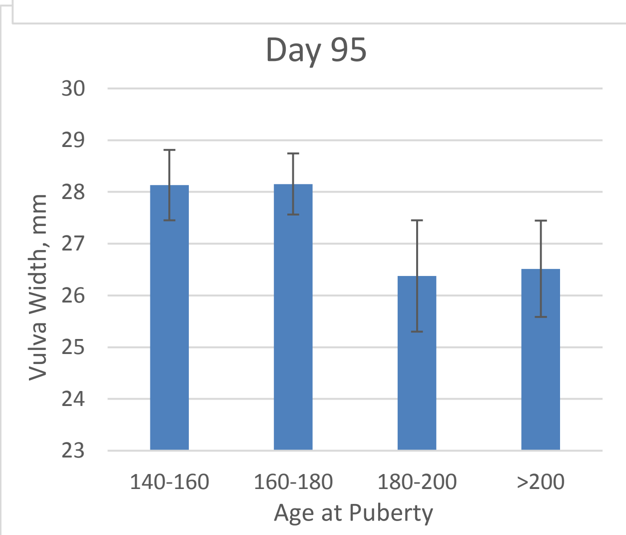


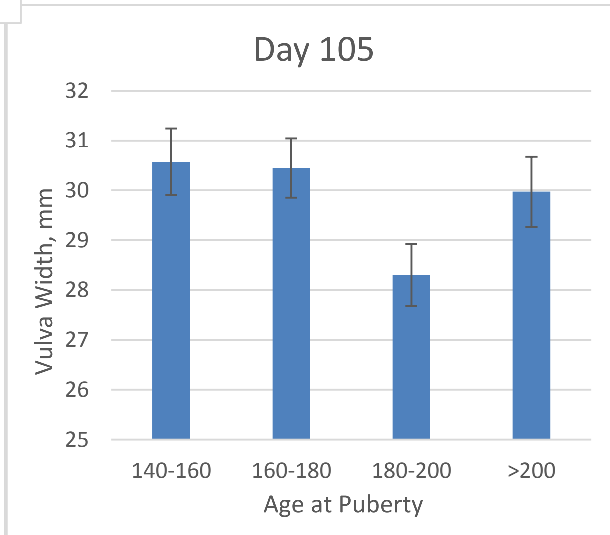
Graphs represent average body weight associated with age at puberty on all five PND time points. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days).

Supplemental Data Sheet 2 – Vulva Width



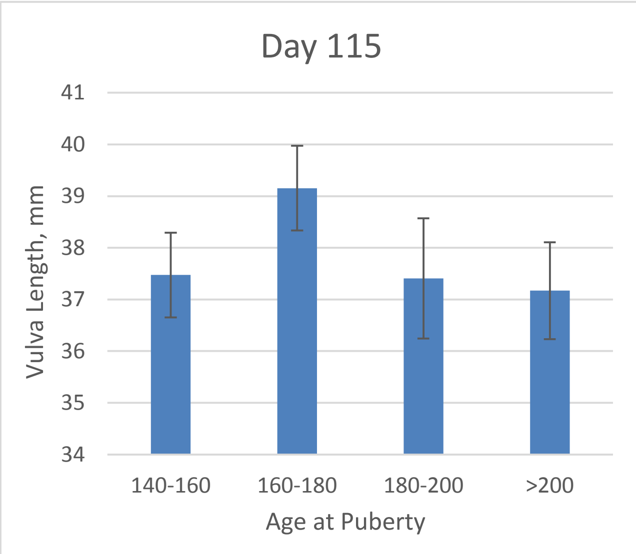


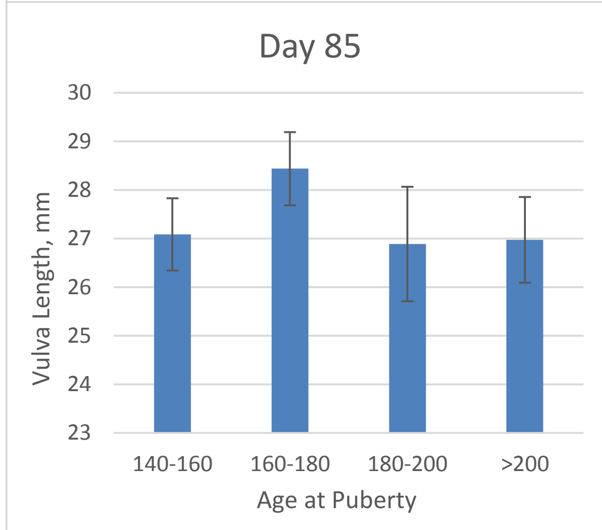


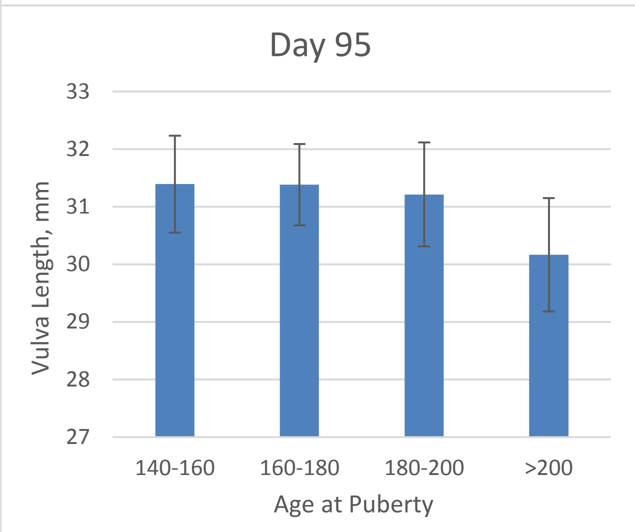


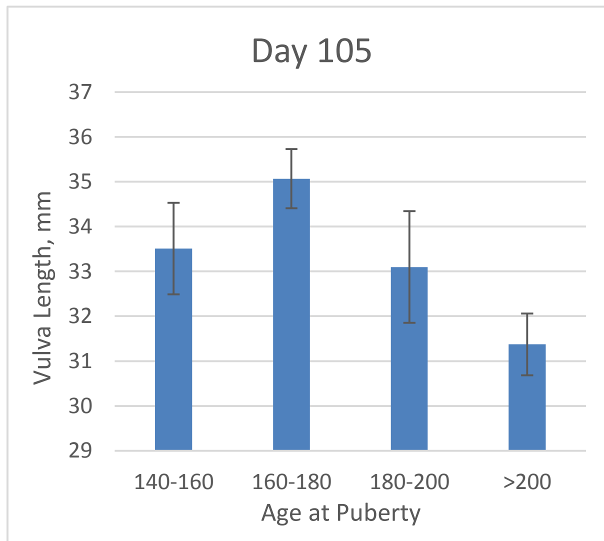
Graphs represent average vulva width associated with age at puberty on all five PND time points. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days).

Supplemental Data Sheet 3 – Vulva Length



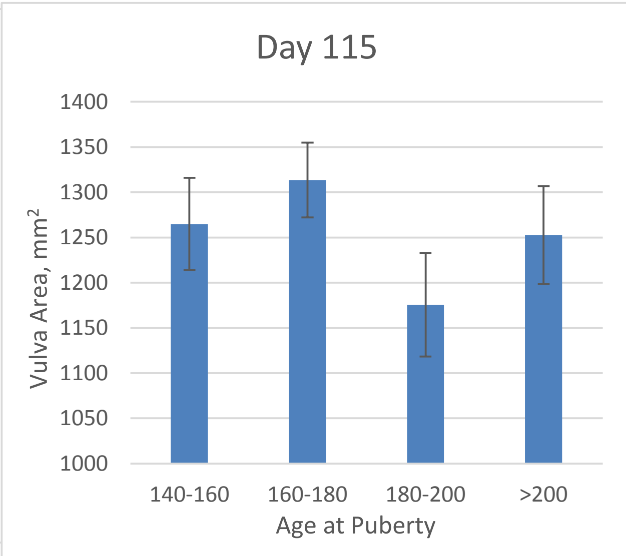


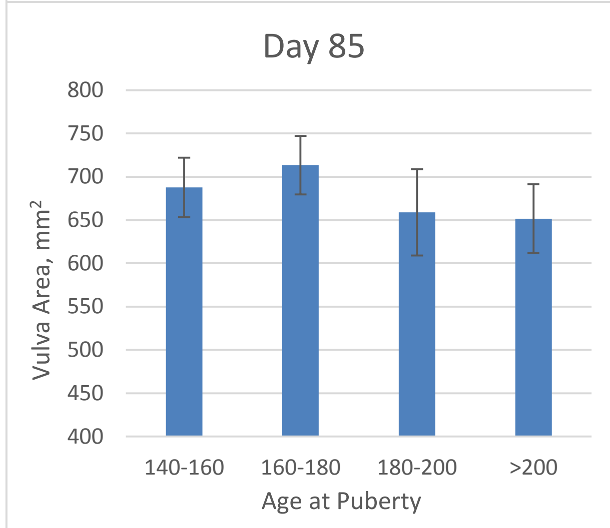




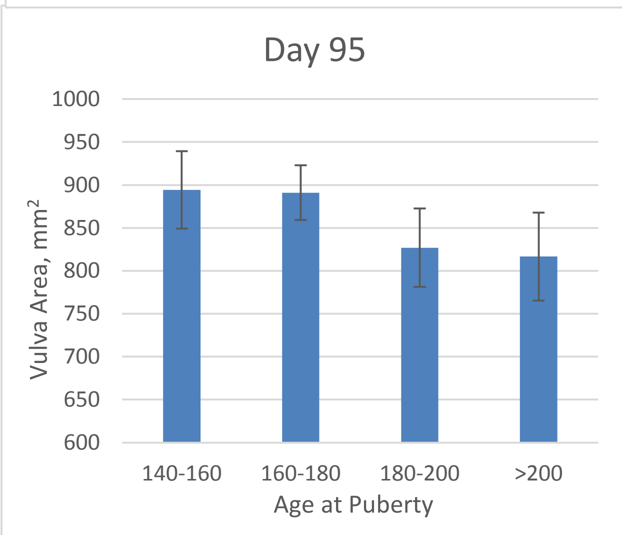
Graphs represent average vulva length associated with age at puberty on all five PND time points. Gilts are broken into four groups based on when they achieved puberty: early puberty (140-160 days), intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days).

Supplemental Data Sheet 4 – Vulva Area

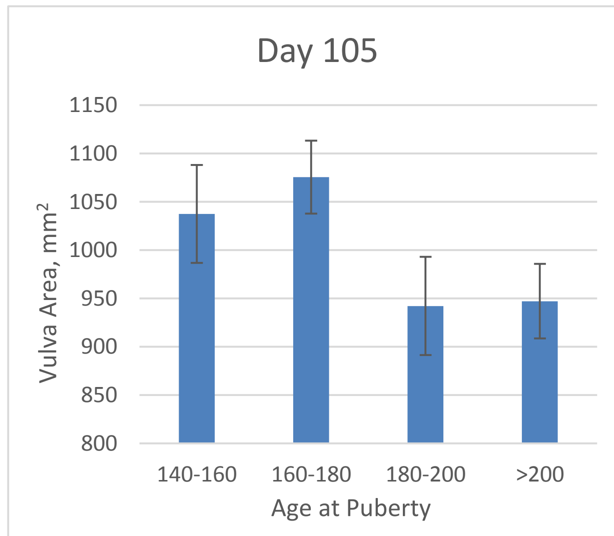




Graphs represent average vulva area associated with age at puberty on all five PND time points. Gilts are broken into four groups based on



when they achieved puberty: early puberty (140-160 days),



intermediate puberty (160-180 days), late puberty (180-200 days), and non-responsive (>200 days).