Sex bias of the birth litter affects surge but not tonic LH secretion in gilts¹

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ABSTRACT: The physiology and behavior of gilts that develop in a male-biased litter can differ from gilts that develop in a female-biased litter. We hypothesized that gilts from male-biased litters will have a delayed and attenuated luteinizing hormone (LH) surge, and reduced LH pulse frequency and amplitude compared to gilts from female-biased litters. Gilts were selected at birth from male-biased (>60% males $n = 10$) or female-biased (>60% females $n = 9$) litters. From 18 wk of age, detection of puberty using daily boar contact began and their subsequent estrous periods were synchronized with oral progestogen (altrenogest). On day 3 after altrenogest withdrawal, blood samples were obtained from 6 gilts per sex bias group at 10 min intervals from 0900 to 2100 h to determine LH pulse amplitude and

frequency. From 0900 on day 4, all 19 gilts were sampled every 4 h until the end of estrus to characterize LH surge dynamics. There were no differences between groups in LH pulse characteristics. Compared to gilts from female-biased litters, the LH surge in gilts from male-biased litters was delayed [56.00 ± 3.32 h vs. 43.11 ± 3.76 h (mean $\pm$ standard error of the mean (SEM)), $P < 0.05$], the duration was decreased [29.78 ± 2.12 h vs. 37.71 ± 1.19 h (mean $\pm$ SEM), $P < 0.05$] and the total secretion as measured by area under the curve was decreased (91.42 ± 9.52 ng/mL vs. 120.28 ± 9.48 ng/mL, $P < 0.05$). Our results indicate that a male-biased uterine environment has different effects on the tonic secretion of LH than the LH surge, with only some elements of the LH surge being affected.

Key words: gilts, litter sex bias, luteinizing hormone

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INTRODUCTION

The physiology and behavior of gilts that develop in a male-biased litter can differ from gilts that develop in a female-biased litter. For example, as the number of males in a litter increased the age of first estrus for gilts in the litter declined (Lamberson et al., 1988).

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Gilts that failed to conceive on their first mating were more likely to be from a male-biased litter and gilts from a male-biased litter were more sensitive to gonadotropins, more aggressive and more males in a litter resulted in less teats (Drickamer et al., 1997; Drickamer et al., 1999; Seyfang et al., 2017, 2018). The physiology and behavior of a gilt can also be affected by its intrauterine position. Gilts that developed in utero between 2 males reached puberty earlier than gilts that developed between 2 females while gilts that developed between 2 females showed enhanced receptivity (Parfet et al., 1990a, 1990b). These differences resemble the effects of exposure to excessive concentrations of testosterone in utero. Exogenous treatment of sows with testosterone increased

testosterone in amniotic fluid and in maternal and fetal circulation. Responsiveness to the positive feedback effects of estrogen on luteinizing hormone (LH) secretion was reduced when gilts born to sows treated with testosterone were 160 d of age (Elsaesser and Parvizi, 1979; Petric et al., 2004). This indicates that gilts exposed to excessive concentrations of testosterone in utero had deficits in the secretion of LH. Gilts that developed in utero between 2 males, however, did not have greater testosterone concentrations than females that developed between 2 females (Framstad et al., 1990; Wise and Christenson, 1992). We aimed to determine if there were differences in the secretion of LH between gilts that developed in a male-biased litter and gilts that developed in a female-biased litter and tested the hypothesis that gilts from a male-biased litter would have a delayed LH surge onset, an attenuated LH surge amplitude, reduced LH pulse frequency, and reduced LH pulse amplitude.

MATERIALS AND METHODS

This experiment was approved by the University of Adelaide Animal Ethics Committee (approval number S-2015-060C). It was conducted at the Roseworthy Piggery, The University of Adelaide (South Australia) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013) and the Animal Welfare Act, 1985.

An indicative timeline of the methods is illustrated in Fig. 1. Nineteen terminal line gilts from Large White $\times$ Landrace sows were selected from 14 litters consisting of $> 60\%$ male (male biased; $n = 10$) or $> 60\%$ female (female biased; $n = 9$) piglets, including stillborn piglets. Gilts were selected at birth and only 1 or 2 females from each litter were selected to minimize the effect of litter. Pigs were weaned at 28 d into a straw-based open sided group shed and raised under standard Australian commercial conditions. At 16 wk of age, the gilts

were moved from the ecoshelter to a controlled environment barn where they were kept in pens (1.3 m $\times$ 3.0 m) in groups of 4. They had access to water ad libitum and access to 4 kg/d each of a gilt developer diet formulated to provide 13 MJ digestible energy (DE)/kg and 0.87% total lysine.

Detection of puberty began at 18 wk of age, with the gilts having fenceline contact for 60 min/d with mature boars in a detection-mating area. Detection of puberty was confirmed by the standing response to back pressure in the presence of a boar.

From 12 d after detection of puberty, gilts were orally administered 20 mg/d of a progestogen, altrenogest, to synchronize estrus. Gilts remained on altrenogest daily until all gilts had exhibited puberty and had been on altrenogest for a minimum of 16 d. Altrenogest was withdrawn from all gilts on the same day.

All gilts were moved into individual stalls (225 cm $\times$ 62 cm) 1 mo prior to altrenogest removal. During this time all gilts were acclimatized to the stalls, feeding schedule and human interactions. This minimized the effects of housing on any measurements. On day 2 after altrenogest withdrawal gilts were fitted with indwelling jugular catheters accessed via their ear veins. On day 3 after altrenogest withdrawal, a subset of 12 gilts (6 gilts from male-biased litters and 6 gilts from female-biased litters) were sampled to assess tonic LH secretion. Blood samples were collected into heparinized tubes at 10 min intervals from 0900 to 2100 h from 6 gilts from male-biased litters and 6 gilts from female-biased litters to characterize the dynamics of tonic LH secretion indicated by the frequency and amplitude of LH pulses.

To characterize the dynamics of the LH surge, starting at 0900 on day 4 after altrenogest withdrawal, all 19 gilts were sampled every 4 h until the end of estrus as determined by a failure to stand to back pressure in the presence of a boar. All samples were centrifuged at 1,360 g for 10 min and the plasma harvested and stored at -20°C until

Figure 1. Timeline of methods from when the gilts were selected at birth, then weighed at 24 h, boar exposed from 18 w of age until puberty, administered altrenogest, altrenogest withdrawn, and then blood sampled for luteinizing hormone (LH) concentrations before and during ovulation, as well as progesterone (P4) concentrations after ovulation. The LH pulse samples were collected every 10 min and the LH surge samples collected every 4 h. Each gilt had a minimum of 16 d on altrenogest.

required for LH assay. A double antibody radioimmunoassay was used to measure LH, using an AFP-15103194 antibody with porcine LH preparation AFP-11043B as standard, as described previously (Niswender et al., 1969). The lowest detectable concentration was 0.4 ng/mL. The intra- and interassay coefficients of variation were 11.9% and 20.3%, respectively.

Statistical Methods

Pulses of LH were defined as increases in concentration of LH that exceeded the preceding value by at least 3 times the mean standard deviation of the LH concentrations and was followed by a decline at a rate consistent with the half-life of LH in pigs of 28 min (Turner et al., 1999). The number of pulses (frequency) per hour, the pulse amplitude, and overall mean LH concentrations were analyzed using a 1-way analysis of variance (ANOVA). The LH surge was defined as described by previous literature (Barb et al., 1982), with the onset beginning when the concentration was 50% greater than the baseline and ended when the concentration decreased to 50% below the baseline. The baseline was calculated by averaging the data prior to an increase in concentration and removing outliers until the values were all within 2 standard deviations of the mean. The data were first analyzed with a repeated measures ANOVA to determine that there was a time $\times$ treatment interaction in the LH surge between gilts from male-biased litters and gilts from female-biased litters. The time of onset of the LH surge, the duration of the LH surge, the total secretion as determined by the area under curve, and the maximum concentration of the LH surge (peak amplitude) were analyzed using a 1-way ANOVA. The normality of the data was tested using the homogeneity of variance test, and Kolmogorov-Smirnov and Shapiro-Wilk tests of normality. The duration of the LH surge and the time to the peak of the LH surge were not normally distributed, however, this was not improved by log 10 or square root transformation so the raw data were used.

RESULTS

Descriptive Data

Gilts from male-biased litters came from litters of 11.80 ± 0.74 total born piglets with 3.60 ± 0.37 females and 8.20 ± 0.49 males. Gilts from female-biased litters came from litters of 12.89 ± 0.66

total born piglets with 8.56 ± 0.53 females and 4.33 ± 0.23 males. There was no difference in litter size between gilts from male-biased litters and gilts from female-biased litters ($P = 0.291$). The percentage of females in male-biased litters averaged $29.48 \pm 1.90\%$ and females from female-biased litters averaged $66.543 \pm 1.43\%$. Gilts from male-biased litters reached puberty at 183.20 ± 3.74 d and gilts from female-biased litters at 175.89 ± 3.77 d [$P = 0.188$, mean $\pm$ standard error of the mean (SEM)]. At the time of LH sampling, there was no difference in weight between gilts from male-biased litters and gilts from female-biased litters with 157.90 ± 3.73 kg vs. 156.94 ± 2.58 kg for gilts from male- and female-biased litters, respectively ($P = 0.839$, mean $\pm$ SEM). There was no difference in duration of altrenogest feeding between gilts from male-biased litters and gilts from female-biased litters with 30.50 ± 3.34 d vs. 38.56 ± 3.36 d, respectively ($P = 0.108$, mean $\pm$ SEM). There was no difference in the interval from progesterone withdrawal to estrus with 6.20 ± 0.36 d vs. 5.78 ± 0.40 d for gilts from male- and female-biased litters, respectively ($P = 0.442$, mean $\pm$ SEM). There was no difference in duration of estrus at the time of LH sampling. Gilts from male-biased litters stood for 3.10 ± 0.41 d and gilts from female-biased litters stood for 2.78 ± 0.43 d ($P = 0.595$, mean $\pm$ SEM). All gilts ovulated during the LH sampling period as determined by presence of fresh corpora lutea on the ovaries at slaughter within 12 d of estrus and the concentration of progesterone in plasma. Both biases had a range of 13 to 19 corpora lutea with an average of 15.3.

Tonic Secretion of LH

There were no significant differences (mean $\pm$ SEM) between gilts from male-biased litters and gilts from female-biased litters for overall LH concentrations during the 12 h sampling period (0.60 ± 0.03 ng/mL for both). Similarly, there were no differences in pulse frequency [0.81 ± 0.11 vs. 0.78 ± 0.05 (pulses/h); $P = 0.77$] or pulse amplitude (0.52 ± 0.03 vs. 0.53 ± 0.06 ng/mL; $P = 0.893$) for gilts from male-biased and female-biased litters, respectively. These results can be seen in Fig. 2. The individual profiles of LH during tonic secretion for gilts from female-biased litters and gilts from male-biased litters are shown in Fig. 3 and Fig. 4.

Surge Secretion of LH

As shown in Fig. 5, gilts from male-biased litters had a delayed onset of the LH surge ($P < 0.05$),

Figure 2. The overall concentration of luteinizing hormone (LH), number of LH pulses per hour and amplitude of LH pulse for pigs that developed in a male-biased or female-biased litter. Panel A depicts the overall concentration of LH for the 12 h sampling period, panel B depicts the number of pulses per h, and panel C depicts mean amplitude of LH for the 12 h sampling period. There were no significant differences between the groups.

Figure 3. The tonic secretion of luteinizing hormone (LH) for pigs that developed in a male-biased litter. On day 3 after altrenogest withdrawal, blood samples were collected at 10 min intervals from 0900 to 2100 h and analyzed for the concentration of LH in ng/mL. Asterisk denotes pulse.

a reduced duration of the LH surge ($P < 0.05$), and a reduced area under the LH curve ($P < 0.05$) compared to gilts from female-biased litters. The amplitude of the LH surge was not significantly different ($P = 0.093$) between gilts from male-biased litters and gilts from female-biased litters. The individual profiles for LH during the preovulatory LH surge for gilts from male-biased litters are shown in Fig. 6 and the

gilts from male-biased litters are shown in Fig. 7. The change in mean LH for gilts from male-biased and gilts from female-biased litters is shown in Fig. 8.

DISCUSSION

Our data indicate that the LH surge of gilts that developed in a male-biased uterine environment was

Figure 4. The tonic secretion of luteinizing hormone (LH) for pigs that developed in a female-biased litter. On day 3 after altrenogest withdrawal, blood samples were collected at 10 min intervals from 0900 to 2100 h and analyzed for the concentration of LH in ng/mL. Asterisk denotes a pulse.

Figure 5. The characteristics of the preovulatory luteinizing hormone (LH) surge for gilts from male-biased and female-biased litters. The characteristics are the time of onset of the LH surge (panel A), the duration of the LH surge (panel B), the peak amplitude of the LH surge (panel C), and the area under curve of the LH surge (panel D). Asterisk denotes significant difference $P < 0.05$.

Figure 6. The profile of luteinizing hormone (LH) secretion during the LH surge for gilts that developed in a male-biased litter. To characterize the dynamics of the LH surge, starting at 0900 on day 4 after altrenogest withdrawal, blood was collected and analyzed for the concentration LH in ng/mL every 4 h until the end of estrus as determined by a failure to stand to back pressure in the presence of a boar. Hours on the x-axis denotes hours after commencement of sampling.

inhibited whereas the tonic secretion of LH was not affected. In gilts that developed in a male-biased uterine environment, the onset of the LH surge was delayed, the duration of the LH surge was reduced, and the plasma concentration of LH released during the surge was decreased. In contrast, none of the parameters of pulsatile secretion of LH differed between gilts that developed in a male-biased or female-biased uterine environment. A key point of difference between a male- and female-biased uterine environment in sheep and rodents is the predominance of sex steroids that are synthesized, with the

male-biased uterine environment being dominated by the synthesis of androgens. While this is a tempting explanation for our results in the pig, there is good evidence to suggest that steroids produced by male pigs in utero do not influence the concentration of testosterone in their female littermates (Framstad et al., 1990; Wise and Christenson, 1992). The mechanisms that cause the LH surge are complex and multifaceted and they are initiated by the positive feedback of estrogens on the hypothalamus and anterior pituitary gland (Goodman, 2014). Disruption of the LH surge will impact the onset of estrus, the timing of

Figure 7. The profile of luteinizing hormone (LH) secretion during the LH surge for gilts that developed in a female-biased litter. To characterize the dynamics of the LH surge, starting at 0900 on day 4 after altrenogest withdrawal, blood was collected and analyzed for the concentration LH in ng/mL every 4 h until the end of estrus as determined by a failure to stand to back pressure in the presence of a boar. Hours on the x-axis denotes hours after commencement of sampling.

Figure 8. The mean luteinizing hormone (LH) during the LH surge for gilts that developed in a female-biased litter and gilts that developed in a male-biased litter. To characterize the dynamics of the LH surge, starting at 0900 on day 4 after altrenogest withdrawal, blood was collected and analyzed for the concentration LH in ng/mL every 4 h until the end of estrus as determined by a failure to stand to back pressure in the presence of a boar. Hours on the x-axis denotes hours after commencement of sampling. The within subjects standard error of the mean was 0.85.

ovulation, and the synchrony between these events which will, in turn, affect the chances of conception whether from natural or artificial insemination. Irrespective of the mechanisms involved, our data indicate that the sex bias of the uterine environment in

which a gilt develops has the potential to affect her reproductive success and this is consistent with much of the published literature in pigs (Lamberson et al., 1988; Parfet et al., 1990a, 1990b; Drickamer et al., 1997; Drickamer et al., 1999).

The mechanisms by which a male-biased uterine environment affects the LH surge in gilts cannot be determined from this study. Research in sheep that has unraveled the effects of the steroidal environment of the uterus in which females develop on the secretion of LH when the females have attained puberty (Robinson, 2006; Cheng et al., 2010; Abi Salloum et al., 2012) may provide some insight. When the circulating concentration of testosterone was increased in sows by injecting testosterone propionate, the stimulatory estrogen feedback on LH secretion was impaired in gilts at 160 d of age (Elsaesser and Parvizi, 1979; Petric et al., 2004). The concentration of testosterone in amniotic fluid and maternal and fetal circulation was increased in female fetuses when sows were injected with testosterone propionate (Ford and Christenson, 1987). This indicates that elevation of testosterone in maternal circulation via exogenous treatment can increase testosterone in the developing fetus. Female pigs that developed between 2 males, however, were not exposed to greater concentrations of testosterone than females that developed between 2 females (Framstad et al., 1990; Wise and Christenson, 1992). The deficits we have detected in the dynamics of the LH surge do resemble the effects of exposure to excessive concentrations of testosterone in utero and are consistent with previous research in the pig. This previous research indicated gilts from a male-biased litter were less likely to conceive at their first mating, were more sensitive to gonadotropins, were more aggressive and more males in a litter resulted in less teats (Drickamer et al., 1997; Drickamer et al., 1999, Seyfang et al., 2017, 2018). Gilts that developed in utero between 2 males reached puberty earlier than gilts that developed between 2 females while gilts that developed between 2 females showed enhanced receptivity (Parfet et al., 1990a, 1990b). Given the evidence in pigs that the gonadal steroids synthesized by males in utero did not influence the concentration of their female littermates and the epitheliochorial placenta of the pig (Framstad et al., 1990; Wise and Christenson, 1992) the pig may present a unique model. Exogenous testosterone treatment can affect the LH surge via the action of the androgens, however, the relevance of this in a natural situation is questionable and not yet clear. Further research is needed to better understand how a male-biased uterine environment affects a number of aspects of gilt reproduction including the effects on surge secretion of LH described in the current experiment.

We detected no difference in the frequency or amplitude of LH pulses between gilts that

developed in a male-biased litter and gilts that developed in a female-biased litter. This may be because the regions of the brain that control tonic and surge secretion of LH in pigs can be influenced at different periods of development in utero. Unlike sheep and rodents, little information on the precise mechanisms or brain regions that control surge and tonic secretion of LH exists in pigs, nor does information regarding the timing of development of these brain regions. If there are differences in the timing of development of the mechanisms that regulate tonic and surge secretion of LH in pigs, this would help explain our findings. We acknowledge that this is a hypothesis and requires testing, however, indirect evidence directly related to the gonadal steroids suggest that specific aspects of the reproductive axis can be affected at different times during gestation in gilts (Elsaesser and Parvizi, 1979; Petric et al., 2004). When pigs were treated with testosterone at day 30 of gestation the external genitalia of the female piglets were masculinized but when pigs were treated with testosterone at day 40 of gestation the external genitalia were unaffected (Petric et al., 2004). In addition, Petric et al. (2004) showed that the critical window for masculinizing the LH surge of female pigs was between days 35 and 39 of gestation and that the tonic release of LH was not affected by treatment with testosterone between days 30 and 36 of gestation or days 40 and 46 of gestation (Petric et al., 2004). Combined, these data suggest that the critical period for the organizational effects of the uterine environment on the mechanisms that regulate LH are unlikely to be one specific period during gestation but are likely to occur prior to day 90 of gestation. Our data support this view and support the likelihood that the mechanism which controls the LH surge in pigs may be influenced by the prenatal environment at a different time during gestation than the mechanism that controls tonic release of LH. Further research is needed to determine the mechanism and regions of the brain that regulate LH release in the pig and this may help explain the current data and advance our understanding of the critical period during gestation that the mechanism that regulates LH production in the pig can be affected.

The sex ratio of the birth litter affected surge secretion of LH but did not have an effect on tonic secretion of LH in female pigs. This partially supports our hypothesis. The current study has highlighted that there may be different periods during gestation where the prenatal environment can affect the control of the secretion of LH in female pigs

and reaffirms the need to better understand the neuroendocrine control of reproduction in the pig. This includes the mechanisms that regulate the effects of a male-biased litter on gilt reproduction. While the sex bias of a gilts birth litter can affect many aspects of the reproductive axis and this includes the surge secretion of LH, the mechanism remains elusive.

Conflict of interest statement. None declared.

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