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Follicular development and maturation in gilts selected for an index of high ovulation rate and high prenatal survival

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ABSTRACT: Seventy-one 10th-generation gilts from White Line-1 (WL-1 = randomly selected control line) and White Line-2 (WL-2 = selected for an index of ovulation rate and prenatal survival rate) were used to compare the pattern of follicular development and atresia during the follicular phase of the estrous cycle. Gilts were treated with PGF\textsubscript{2\alpha} on d 13 of the estrous cycle (d 0 of induced follicular development) to induce luteolysis and assigned randomly within line and sire for ovary recovery on d 0, 2, 3, 4, 5, and the day after estrus. Ovaries were evaluated for numbers of corpora albicantia and small (2 to 2.9 mm), medium (M1 = 3 to 4.9 mm; M2 = 5 to 6.9 mm), and large (≥7 mm) follicles. The concentration of estradiol-17\beta in follicular fluid was used to classify individual M2 and large follicles as estrogen-active (≥100 ng of estradiol-17\beta/mL) or inactive (<100 ng of estradiol-17\beta/mL). The WL-2 gilts had a greater ovulation rate than WL-1 gilts at their pre-treatment estrus (20.4 vs. 13.8 corpora albicantia; \(P < 0.001\)). The small and M1 follicle populations decreased rapidly in both lines over time (\(P < 0.001\)). The M2 follicle population increased in both lines between d 0 to 4 and then decreased. Mean estradiol concentration of M2 follicles increased in both genetic lines over time (\(P < 0.02\)). All large follicles were estrogen-active in both lines; the number of large follicles increased with day (\(P < 0.001\)) and was similar in both lines. The number of estrogen-active M2 follicles was similar in both lines, increasing to d 3 and 4 and then decreasing (\(P < 0.01\)) thereafter. However, the total number of estrogen-active follicles (sum of estrogen-active M2 and large follicles) was greater in WL-2 than in WL-1 gilts (\(P < 0.04\)), increasing to the ovulatory potential by d 3 in WL-1 gilts, but continuing to increase through d 4 in WL-2 gilts. Selection of an additional six ovulatory follicles from the estrogen-active M2 follicle pool after d 5 was required in both lines to achieve the projected ovulation rate, and after estrus, the number of large follicles remained insufficient to attain the ovulatory potential of each line.

Key Words: Corpora Lutea, Estrogen, Follicles, Ovary, Selection

Introduction

Nine generations of selection for increased number of corpora lutea in the University of Nebraska Gene Pool population increased ovulation rate by about 3.2 ova (Zimmerman and Cunningham, 1975). However, litter size increased by approximately 0.85 pigs (Cunningham et al., 1979) due to greater prenatal loss in the select line (Geisert et al., 1977). Johnson et al. (1984) proposed and initiated a second selection experiment to improve litter size by selection based on an index of ovulation rate and prenatal survival at 50 d of gestation in a composite population of Large White and Landrace, White Line pigs (WL; Neal et al., 1989). The index-selected line (WL-2) averaged 6.7 more corpora lutea and 3.3 more fetuses after 10 generations of selection than the randomly selected control line (WL-1) (Casey et al., 1994).

Gilts selected for high ovulation rate from the University of Nebraska Gene Pool maintained a larger pool of medium (3 to 6.9 mm) follicles during the mid- to late-follicular phase and continued to select ovulatory follicles from this pool 1 d later than randomly selected control gilts (Zimmerman and Kopf, 1988; Zimmerman et al., 1990; Vatzias et al., 1991). Gilts selected for high ovulation rate also maintained more estrogen-active follicles 5 to 6.9 mm in diameter during the mid- to late-follicular phase (Vatzias et al., 1993), and they con-
Materials and Methods

Animals

The gilts came from the 10th generation of the University of Nebraska population, a Large White-Landrace composite population that included a control line randomly selected within sire group each generation (WL-1), and a line selected for increased ovulation rate and prenatal survival (WL-2). Gilts from WL-1 (n = 37 gilts from 15 sires) and WL-2 (n = 34 gilts from 14 sires) lines were assigned and stratified within sire to day of ovary collection. Gilts were grouped by estrous date and were maintained in groups of three or four in an environmentally regulated facility on concrete-slotted floors under constant light and controlled temperature (24°C). Gilts were allowed daily access to 1.6 kg of a 14% CP (as-fed basis) corn-soybean diet. These gilts were 8 to 11 mo old, weighed 95 to 150 kg, and had experienced two or more estrous periods before assignment to an ovary recovery group. Estrous activity was evaluated twice daily throughout the study by exposing gilts to a sexually mature boar. Luteolysis was induced with PGF₂α (10 mg of Lutalyse, i.m.; Pharmacia Upjohn, Kalamazoo, MI) in the morning of d 13 of the estrous cycle (d 0 of induced follicular development), and ovaries were recovered during follicular maturation on d 0, 2, 3, 4, 5 after PGF₂α injection, and the day after PGF₂α-induced estrus (d 6 or 7). None of the gilts used for collection of follicles on the mornings of d 0 to 5 was in estrus the evening before slaughter; estrous gilts were slaughtered the day after they were first detected in estrus. Procedures for handling animals complied with those specified in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Ovarian Evaluation

Ovaries were recovered at a local abattoir and immediately placed in 0.15 M saline on ice for transport back to the laboratory for dissection. The number of corpora albicantia (CA) was recorded as a measure of ovulation rate at the previous estrus. Surface follicles ≥2 mm in diameter were categorized and recorded as follows: small (2 to 2.9 mm), medium-1 (M1 = 3 to 4.9 mm), medium-2 (M2 = 5 to 6.9 mm), and large (≥7 mm).

Follicular maturation in M1, M2, and large follicles was assessed on fluid concentrations of estradiol-17β (estradiol). Follicles were classified as estrogen-active (≥100 ng of estradiol/mL) or inactive (<100 ng of estradiol/mL) as described by Foxcroft et al. (1987). Follicular fluid was aspirated from each medium and large follicle with a 1-mL tuberculin syringe and a 25-gauge, 1.6-cm hypodermic needle. The fluid was then placed into a microfuge tube and centrifuged at 15,600 × g for 10 min. The supernatant fluid was then collected, frozen, and stored at −20°C until assayed for estradiol.

Radioimmunoassay

Concentrations of estradiol in follicular fluid samples were determined by RIA validated in our laboratory (Vatzias, 1992). Follicular fluid from M1, M2, and large follicles was diluted to 1:2,000, 1:8,000, and 1:32,000, respectively, in PBS −0.1% gelatin to obtain optimal binding ranges (20 to 80%) in the estradiol assays. The intra- and interassay CV were 8.7 and 10.3%, respectively.

Data Analyses

Count data, including the total number of follicles, number of follicles of each size, and number of CA, were fitted to a GLM assuming data for each trait had a Poisson distribution. The SAS (SAS Inst., Inc., Cary, NC) procedures described by Littell et al. (1996) for count data were used. The model was:

\[
\ln(y_{ijk}) = m + L_i + D_j + (LD)_{ij} + e_{ijk}
\]

where \(y_{ijk}\) is the observed count for each trait for the \(k\)th gilt of line \(i\) evaluated on day \(j\); \(L_i\) is the effect of the \(i\)th line (control or select); \(D_j\) is the effect of the \(j\)th day of measurement (0, 2, 3, 4, and 5 d after treatment or estrus for gilts detected in estrus the day before slaughter); \((LD)_{ij}\) is the line × day interaction; and \(e_{ijk}\) is the residual. The natural logarithm was used as the link function, and tests of significance of effects in the model and least squares means were produced on the logarithmic scale. Means on the observed scale were calculated with the inverse link function as \(\exp[\ln(\hat{y}_{ij})]\), where \(\hat{y}_{ij}\) is the least squares mean in the logarithmic scale. Count data for estrogen-active follicles was restricted to d 2 to 5, as there were no estrogen-active follicles on d 0, and some estrous gilts had mean estradiol concentrations in M2 and large follicles below 100 ng/mL, which is indicative of follicular maturation beyond the ovulatory release of LH.

Mean estradiol concentrations in M2 follicles of each gilt were used to evaluate the influence of day after PGF₂α treatment on follicular maturation (GLM procedure of SAS). For the M2 follicles, the statistical analysis was restricted to the mean estradiol concentration within gilts that had M2 follicles.
Results

Ovulation Rate

Overall, WL-2 gilts ovulated an average of 6.6 more follicles than WL-1 gilts (20.4 ± 3.4 vs. 13.8 ± 4.5 CA; P < 0.001). Ovulation rate was not significantly influenced by day of ovary recovery or by the interaction between line and ovarian location (right or left side; P > 0.10). Number of CA (pooled across days) averaged 6.3 and 9.9 for the right ovary and 7.5 and 10.5 for the left ovary for WL-1 and WL-2 gilts, respectively.

Follicular Pattern

The mean numbers of small, M1, M2, and large follicles are summarized by genetic line and day in Figure 1. Total number of follicles was similar in WL-1 and WL-2 gilts (P = 0.18) and decreased with day after PGF2α treatment (P < 0.001); there was no line × day interaction (P = 0.40). No differences were observed between WL-1 and WL-2 gilts for number of follicles within each size classification (P = 0.10 for small; P = 0.35 for other sizes), and there was no line × day interaction for any of the size classifications (P = 0.18). The number of small and M1 follicles decreased (P < 0.001) and the number of large follicles increased (P < 0.001) with day after PGF2α. Number of M2 follicles changed with day (P < 0.01), with few present on d 0 and estrus; the highest numbers were present on d 3 and 4, and intermediate numbers were observed on d 2 and 5.

Concentration of Estradiol and Number of Estrogen-Active Follicles

Medium-1 follicles during the follicular phase in both genetic lines had low estradiol concentrations (<60 ng/mL), as did M2 follicles on d 0 (<100 ng/mL). Concentrations of estradiol in M2 follicles increased over time (P < 0.02; Table 1) but were similar in the two lines (P > 0.10). Mean concentrations of estradiol in M2 follicles more than doubled between d 2 and 5 in WL-1 gilts (P < 0.05). Concentration of estradiol in M2 follicles of WL-2 gilts increased rapidly between d 3 and 4 (P < 0.05). All large follicles were estrogen-active (≥100 ng estradiol/mL) in both genetic lines. Only three of 12 gilts had large follicles on d 3, with 296 ± 63 ng of estradiol/mL. Concentration of estradiol in large follicles was not affected by day or line on d 4 and 5 (P > 0.30), with an overall mean of 440 ± 51 ng/mL (n =

Table 1. Concentrations of estradiol-17β (mean ± SE, ng/mL) in follicular fluid of M2 follicles following PGF2α on d 13 of the estrous cycle (d 0 of follicular development)

<table>
<thead>
<tr>
<th>Daya</th>
<th>WL-1a</th>
<th>Ratiob</th>
<th>WL-2a</th>
<th>Ratioa</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>108.7 ± 24.2bc</td>
<td>3/7</td>
<td>125.2 ± 47.6b</td>
<td>5/6</td>
</tr>
<tr>
<td>3</td>
<td>223.1 ± 55.2bc</td>
<td>5/6</td>
<td>150.9 ± 49.8b</td>
<td>5/6</td>
</tr>
<tr>
<td>4</td>
<td>224.3 ± 59.1bc</td>
<td>5/6</td>
<td>275.9 ± 51.5bc</td>
<td>6/6</td>
</tr>
<tr>
<td>5</td>
<td>240.3 ± 56.9bc</td>
<td>6/7</td>
<td>353.6 ± 35.4bc</td>
<td>3/4</td>
</tr>
</tbody>
</table>

aDay, P < 0.05; Line, P > 0.10; WL-1 = control line; WL-2 = line selected for ovulation rate and prenatal survival.

bNumbers with different superscripts within a column differ, P < 0.05.

cNumber of gilts with M2 (5.0 to 6.9 mm) follicles relative to number of gilts slaughtered.
Table 2. Mean number of estrogen-active medium and large follicles on d 2, 3, 4, and 5 following prostaglandin F2$_{α}$ on d 13 of the estrous cycle (d 0 of follicular development)

<table>
<thead>
<tr>
<th>Follicle size</th>
<th>WL-1</th>
<th>WL-2</th>
<th>WL-1</th>
<th>WL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2 follicles$^b$</td>
<td>3.1 ± 1.64</td>
<td>5.7 ± 1.49</td>
<td>0.14 ± 7.6</td>
<td>0</td>
</tr>
<tr>
<td>Large follicles$^c$</td>
<td>12.0 ± 1.31</td>
<td>11.5 ± 1.32</td>
<td>1.7 ± 1.90</td>
<td>1.3 ± 2.05</td>
</tr>
</tbody>
</table>

$^a$WL-1 = control line; WL-2 = line selected for ovulation rate and prenatal survival.

$^b$Follicles from 5.0 to 6.9 mm; Line, $P > 0.075$; Day, $P < 0.04$; line × day, $P > 0.30$.

$^c$Follicles ≥7.0 mm; Line, $P > 0.90$; Day, $P < 0.003$; line × day, $P > 0.60$.

19 gilts). All gilts slaughtered on d 4 and 5 had estrogen-active follicles.

Mean numbers of estrogen-active M2 follicles did not differ ($P = 0.075$) between lines (Table 2), increased to d 3 and 4, and then decreased. The greatest numerical difference between WL-1 and WL-2 gilts in number of estrogen-active M2 follicles was 12.5, which was observed on d 4. Mean numbers of estrogen-active large follicles increased with day ($P < 0.003$) and were similar in both lines ($P = 0.90$; Table 2). Total numbers of estrogen-active follicles (sum of estrogen-active M2 and large) increased with day after PGF2$_{α}$ injection ($P < 0.001$; Figure 2) and were greater in WL-2 than in WL-1 gilts ($P < 0.04$). There was no interaction of line with day ($P = 0.30$), but WL-1 gilts reached their ovulatory potential by d 3, whereas WL-2 acquired estrogen-active follicles longer and achieved their ovulatory potential on d 4 (Figure 2). In the estrous gilts, two of four WL-1 and two of seven WL-2 gilts had estrogen-active follicles. Of these four estrous gilts, the number of estrogen-active M2 follicles was zero and 12 for the WL-1 gilts, and two and 15 for the WL-2 gilts.

**Discussion**

The ovulation rate difference between WL-2 gilts and WL-1 gilts (6.6 CA; $P < 0.01$) observed in the present study was similar to the difference of 6.7 corpora lutea reported at the end of 10 generations of index selection for increased ovulation rate and prenatal survival (Casey et al., 1994). Ovulation rate was similar for the right and left ovaries within each line, indicating that increased activity of both ovaries contributed to the higher ovulation rate in WL-2 gilts.

The decrease in the number of small follicles in the current study between d 0 and estrus in both lines reflects atresia and disappearance of follicles from the surface of the ovary. Consistent with this, the number of M1 follicles, the next largest category, also decreased; however, the decrease in M1 follicles reflects, in part, movement of follicles into the next larger category, M2 follicles, with the progression of follicular development. Atresia is the means by which follicles that contain oocytes in an inappropriate stage of development are eliminated from advancing to ovulatory status (Guthrie and Garrett, 2001). The decrease in M2 follicles between d 4 and estrus is associated with a rapid increase in large follicles during the same period. These changes in the number of follicles within each general size classification during the follicular phase of the porcine estrous cycle are similar to those reported by others (Hunter and Wiesak, 1990).

Increased concentrations of estradiol were observed in follicular fluid of M2 follicles during the follicular phase in both genetic lines. The total number of estrogen-active M2 and large follicles indicates that WL-2 gilts were able to maintain selection of estrogen-active follicles for a longer period during the mid- to late-follicular phase than WL-1 gilts. Vatzias et al. (1992, 1993) proposed a similar mechanism for greater ovulation rate in the Nebraska Gene Pool line. The length of estrous cycle in WL-1 and WL-2 gilts was similar.
after synchronization with a synthetic progestin (Mariscal et al., 1998), and there were no apparent differences between these two lines in the profiles of decreasing progesterone and increasing estradiol during the follicular phase. Also, in the current study, all gilts in both lines that were slaughtered on d 5 had estrogen-active follicles, indicating that they had not yet experienced the full magnitude of an ovulatory release of LH. In the estrous gilts, similar proportions within each line had experienced an apparent ovulatory release of LH based on the absence of estrogen-active follicles. Thus, greater numbers of estrogen-active follicles on d 4 and 5 in WL-2 relative to WL-1 gilts was not associated with obvious differences in duration of the follicular phase of the estrous cycle.

The number of large follicles observed on d 5 and after estrus (Figure 1) did not reflect the expected ovulation rate of either genetic line based on ovulation rate (CA count) from the previous estrus. Therefore, continued selection of four to six ovulatory follicles from the pool of estrogen-active M2 follicles was necessary in both lines in order for the lines to achieve their final ovulation rate. This lack of homogeneity in rate of development is characteristic of porcine ovarian follicles (Hunter and Wiesak, 1990). However, the lack of adequate numbers of large follicles at estrus raises the possibility that some follicles may ovulate before achieving the size of large follicles.

The pathway to expressing high ovulation rate may reflect differences in the numbers of follicles recruited and maintained during the luteal phase and, in turn, the size and health status of the pool of medium follicles available for selection and maturation into large ovulatory follicles during the follicular phase. Foxcroft and Hunter (1985) suggested that the origin of the ovulatory follicles can be traced back to follicles 2 to 4 mm in diameter at the beginning of the follicular phase. Because total numbers of follicles ≥2 mm that were recruited by the beginning of the follicular phase (d 13) were similar (WL-1 = 81.8 vs. WL-2 = 84.8) for each line, the ovulation rate advantage of WL-2 gilts does not seem to relate to ovaries containing more antral follicles. The superiority in ovulation rate of the high ovulation line (RS line) from the University of Nebraska Gene Pool population (Vatazias, 1992) and Hyperprolific sows from France (Driancourt and Terqui, 1996) was also unrelated to greater numbers of follicles at the beginning of the follicular phase. Similar to RS gilts and Hyperprolific sows cited above, WL-2 gilts gained their ovulation rate superiority by selecting more ovulatory follicles during the mid-follicular phase and were able to maintain a larger pool of healthy, estrogen-active follicles during the mid- to late- follicular phase. The WL-2 gilts achieved their ovulation rate advantage, as reflected by total number of estrogen-active follicles, by d 4.

Gilts selected for high ovulation rate from the University of Nebraska Gene Pool had greater concentrations of FSH during the late luteal to early follicular phase than control gilts (Knox et al., 2003). Likewise, greater FSH secretion was associated with greater ovulation rate in other studies (Shaw and Foxcroft, 1985; Kelly et al., 1988). It was suggested that the elevated concentrations of FSH are involved with the maintenance of a larger pool of healthy M2 follicles, from which ovulatory follicles are selected and develop. However, no clear differences in FSH concentrations were detected between higher ovulating Meishans and females from maternal white lines (Hunter et al., 1996; Wise et al., 2001). Mariscal et al. (1998) reported that FSH concentrations did not differ between WL-1 and WL-2 sows during the estrous cycle, and concentrations of LH, estradiol, and progesterone were similar in these two lines. Thus, actions other than changes in circulating reproductive hormones regulate ovulation rate in WL-2 gilts selected for high ovulation rate and prenatal survival.

In summary, follicular dynamics have changed in response to genetic selection for high ovulation rate and high prenatal survival. Selected WL-2 gilts achieved a numerically larger pool of estrogen-active M2 follicles by d 4 that contributed to a significantly greater total number of estrogen-active follicles than that observed in WL-1 gilts. Further investigation is needed to determine what factor(s) during the late luteal and early follicular phase are responsible for development of a larger pool of estrogen-active follicles in WL-2 gilts. The signal(s) that triggers the rapid maturation of M2 follicles between d 3 and 4 in WL-2 gilts is of great interest.

**Literature Cited**


Follicle numbers in index selected gilts


