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Elevation of Plasma FSH with a Low Level of FSH-P During the Early to Mid Follicular Phase Blocks the Loss of Greater Numbers of Medium Follicles in Control Line Gilts Compared to Gilts Selected for High Ovulation Rate

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The number of follicles ovulated (ovulation rate) at estrus is an important determinant of litter size in the pig because it sets the upper limit for litter size.

Ovulation rate is a moderately heritable trait in pigs (h^2 = 40%). Gilts selected for high ovulation rate (Relax Select, RS line) in the University of Nebraska Gene Pool population ovulated about 3.5 more follicles than randomly selected Control (C) line gilts after nine generations of selection. This difference continues to be maintained after many generations of random selection following the end of deliberate selection.

Evaluation of the pattern of follicular development showed that RS gilts maintain a larger pool of 3 to 6.9 mm follicles than C gilts during the mid to late follicular phase of the estrous cycle. Also, a greater proportion of the 5 to 6.9 mm follicles were healthier in RS than C gilts during this period.

Large preovulatory follicles were slower to develop in RS gilts and it was not until late in the follicular phase that RS gilts developed the number of large preovulatory follicles needed to achieve their advantage in ovulation rate. Other studies showed that RS gilts maintain elevated concentrations of follicle-stimulating hormone (FSH), but not

luteinizing hormone (LH), during the late luteal and early follicular phases of the estrous cycle (days 12 to 14). The elevated concentrations of FSH in RS gilts during the late luteal and early follicular phases may be involved with the maintenance of a larger pool of healthy 5 to 6.9 mm follicles from which preovulatory follicles are selected.

Therefore, the present study was conducted to: 1) further characterize relationships between development and maturation of follicles and concentrations of gonadotropic and gonadal hormones in RS and C line gilts during the early to mid follicular phase of the estrous cycle; and 2) determine whether FSH therapy (treatment with FSH-P, a commercially available porcine FSH preparation) during the early to mid follicular phase of the estrous cycle will prevent the loss of medium follicles and reverse the pattern of development and/or maintenance of fewer medium follicles in C than RS gilts during the mid follicular phase.

Materials and Methods

Thirty-nine RS and 40 C line gilts from the University of Nebraska Gene Pool herd were assigned randomly within sire to a replicated experiment that evaluated follicle development and maturation at two intervals (24 or 48 hour) after initiation of FSH-P treatment. Gilts from the RS and C lines represented the progeny of 13 and 14 sires, respectively. The gilts were 9 to 11 months of age and weighed between 100 and 150 kg when evaluated. They had experienced two or more estrous cycles before assignment to experiment.

Two injections of $PGF_{2\alpha}$ (10 mg Lutalyse) were given at 12 h intervals to all gilts on day 13 of the estrous cycle to induce luteolysis and initiate the start of the follicular phase on the same day. FSH-P treatment started 36 hours after first $PGF_{2\alpha}$. Gilts received 1.5 Armour Units (AU) of FSH-P at 12 hour intervals over 1 or 2 days until ovariectomy (OVX). Blood samples were collected at 12 hour intervals from day 13 to OVX and assayed for concentrations of FSH, LH, estradiol and progesterone.

At ovariectomy, the numbers of corpora albicantia were recorded as a measure of ovulation rate at the previous estrus. Numbers of follicles (F) equal or greater than 3 mm in diameter were categorized and recorded as follows: medium-1 (M1F, 3 to 4.9 mm), medium-2 (M2F, 5 to 6.9 mm) and large (LF, equal or above 7 mm). Follicle numbers for the different size categories were not normally distributed, so the data concerning follicle numbers were converted to relative percentage for each gilt (dividing numbers of follicles in a given size category by the total number of follicles in all three size categories) before the data were analyzed statistically.

The follicular fluid was assayed for estradiol (E) and the concentration of E was used to assess the health status of individual follicles. Follicles with E greater than 100 ng/mL were classified as healthy (estrogen-active) and follicles with E less than 100 ng/mL as atretic or degenerate (estrogen inactive).

Results and Discussion

Overall, RS gilts ovulated about two more ova than C gilts at the pretreatment estrus (14.3 vs 12.3, $p<.001$). The ovulation rate difference between lines was less than observed in earlier studies. Gilts in the present study were given only 3.5 lb. of diet per day whereas gilts in most of the earlier studies were "flushed" (fed 7 lb of diet per day) for 10 to 14 days before estrus to stimulate maximal expression of ovulation rate.

Gonadotropic and Gonadal Hormone Concentrations in Plasma during the Pretreatment Period

Relax Select gilts maintained higher ($p<.02$) concentrations of FSH during the pretreatment period but the advantage decreased between 12 and 36 hour after PGF_{2a} treatment (Figure 1). This confirmed previous findings that RS gilts maintain greater concentrations of FSH between day 12 and day 14 of the estrous cycle. Concentrations of LH did not differ between genetic lines, also confirming previous observations.

Estradiol concentrations increased over time in both lines but the increase tended to be greater in RS than C gilts between 12 and 24 hours (Line x Hour, $p<.07$). This resulted in higher concentrations of E in RS gilts at both 24 and

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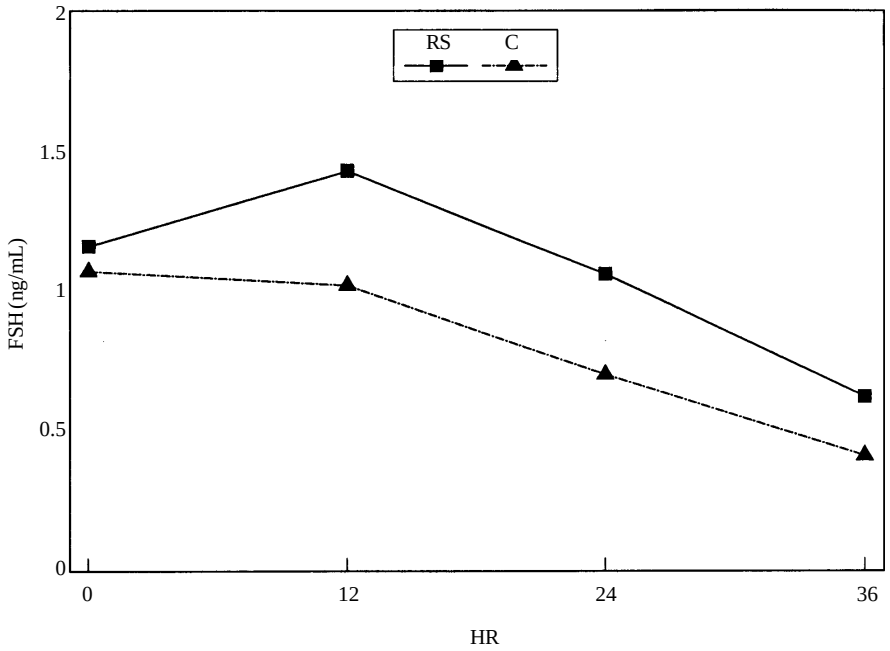

Figure 1. Mean concentrations of plasma FSH during the pretreatment period (0 to 36 h after $PGF_{2\alpha}$) as affected by genetic line and hour.

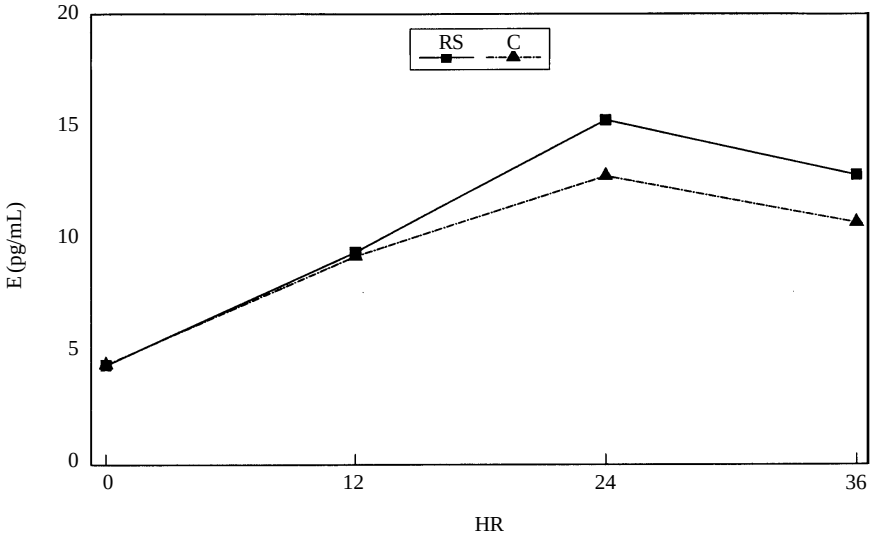

Figure 2. Mean concentrations of plasma estradiol (E) during the pretreatment period (0 to 36 h after $PGF_{2\alpha}$ injection) as affected by genetic line and hour.

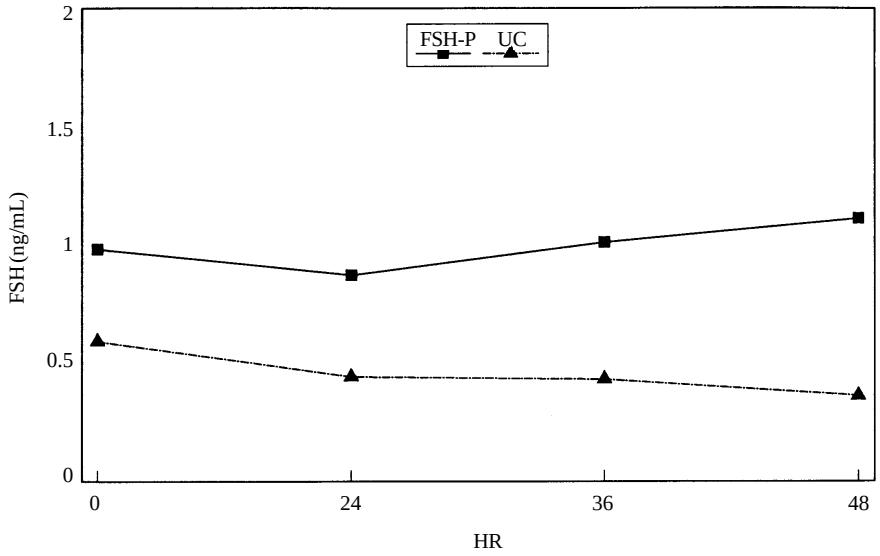

Figure 3. Mean concentrations of plasma FSH from12 to 48 h after first FSH-P injection as affected by treatment and hour.

36 hours after $PGF_{2\alpha}$ (Figure 2). The higher concentrations of plasma estradiol in RS gilts may have resulted from the higher concentrations of FSH observed during the early follicular phase in RS gilts.

Gonadotropic and Gonadal Hormone Concentrations in Plasma during the Treatment Period

Plasma FSH concentrations were elevated in FSH-P treated gilts over the entire treatment period but the difference in FSH levels increased with interval after first FSH-P treatment (Figure 3). This occurred because FSH levels tended downward over time in UC gilts while increasing after 24 hours in FSH-P treated gilts; FSH concentrations were more than 2-fold higher in FSH-P treated gilts than in UC gilts 48 hours after the first FSH-P treatment (1.1 vs .36 ng/mL at 48 hour, Figure 3).

Plasma LH concentrations declined over time in UC gilts of both genetic lines but tended to increase over time in FSH-P treated gilts. The different patterns of LH in UC and FSH-P treated gilts resulted in a .2 to .3 ng/mL elevation in LH at 36 and 48 hours in gilts treated with FSH-P. The increase in LH concentration probably reflects accumulation over time of the LH contamination in the FSH-P preparation.

Treatment of gilts with FSH-P stimulated elevated concentrations of estradiol during the treatment period. Estradiol levels increased 1.5-fold (16 to 40 pg/mL) in UC gilts compared to 2-fold (25 to 78 pg/mL) in FSH-P gilts between 12 and 48 hours after the first FSH-P treatment; concentration of estradiol was nearly twice as high in FSH-P treated gilts at 48 hour, when the maximum difference in estradiol concentration was observed (Figure 4). The higher concentrations of plasma estradiol in FSH-P treated gilts may be due to the development of greater numbers of estrogen-active (healthy) follicles in FSH-P treated gilts.

Number and Relative Percentage of Medium and Large Follicles

The data concerning numbers and relative percentage of medium and large follicles as affected by line, treatment and interval from first FSH-P treatment to OVX (24 vs 48 hour) are presented in Table 1 and Table 2, respectively.

The relative percentage of M1F (3 to 4.9 mm) was affected by treatment with FSH-P ($p<.01$) and interval to OVX (HR) after first FSH-P injection ($p<.01$) but not by genetic line. However, treatment with FSH-P had a differential effect on numbers of M1F at 24 and 48 hours after first FSH-P treatment.

Number of M1F increased in response to FSH-P at 24 hour but declined in response to FSH-P at 48 hour (Table 1). Thus, the loss of M1F between 24 and 48 hours was accelerated in FSH-P treated gilts as compared to UC gilts (Table 2).

The decrease in percentage of M1F between 24 and 48 hour after first FSH-P injection (60 and 84 hours after $PGF_{2\alpha}$ on day 13) may have resulted in part from growth of M1F into M2F. The greater loss of M1F may be due as well

to the elevated concentrations of LH described earlier. USDA researchers recently reported that administration of a highly purified porcine LH preparation increased the number of LF but decreased the number of small follicles by 70% by 72 hours after the first injection.

There was also a trend ($p<.08$) for an interaction between FSH-P treatment and genetic line. Mean numbers of M1F decreased in C while increasing in RS gilts in response to FSH-P. This suggests that RS gilts were more responsive to FSH-P. But it may have occurred in part because UC gilts from the RS line had lower numbers of M1F than UC gilts from C line at both 24 and 48 hours (60 and 84 hours post $PGF_{2\alpha}$); the numbers of M1F in FSH-P treated gilts were similar for the C and RS lines. The greater decline in number of M1F in C than RS gilts during this period verifies the results of a previous UNL study reported in the 1993 Swine Report.

The relative percentage of M2F declined as expected in UC gilts but increased in FSH-P treated gilts of both genetic lines between 24 and 48 hours (Table 2). Gilts treated with FSH-P had developed a greater number of M2F than UC gilts in both genetic lines at both 24 and 48 hours (Table 1). Purified porcine FSH (pFSH-B-1) has been reported by USDA researchers to increase numbers of small and/or medium follicles but not large follicles in pigs.

Untreated control gilts from the RS and C lines had similar percentages of M2F at 60 (C, 35.0 vs RS, 39.9%) and at 84 hours (C, 32.2% vs RS, 33.2%) post $PGF_{2\alpha}$. These results failed to confirm the findings of a previous study (1993 Swine Report) which reported a greater loss of M2F in C than RS gilts between day 3 and day 4 after $PGF_{2\alpha}$ treatment on day 13. However, follicle evaluation in the present study was done earlier in the follicular phase (day 2.5 and day 3.5 post $PGF_{2\alpha}$ on day 13). It is possible that the difference in follicle loss between day 3 and day 4 is not yet expressed at day 3.5 (84 hour post $PGF_{2\alpha}$) but still may be reflected in the percentage of M2F that are estrogen

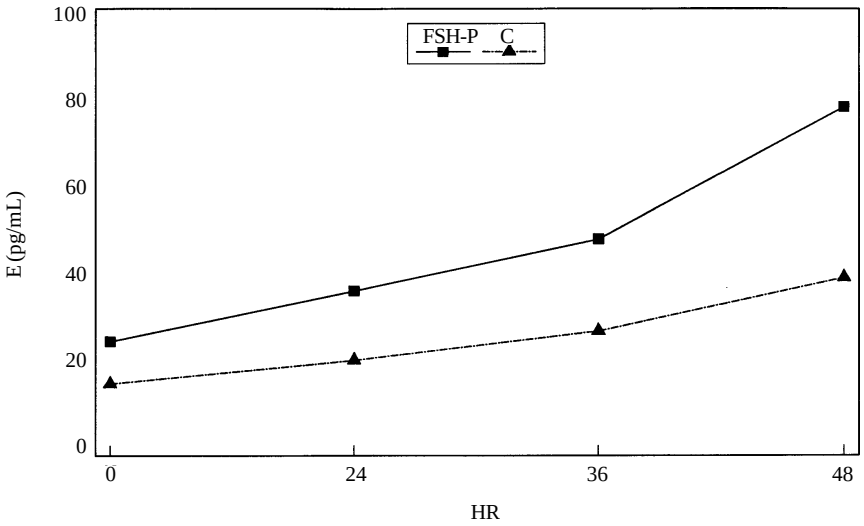

Figure 4. Mean concentrations of plasma estradiol (E) from 12 to 48 h after first FSH-P injection as affected by treatment and hour

Table 1. Mean number of medium and large follicles as affected by genetic line, treatment and hour after first FSH-P treatment

		M1F[a]		M2F[a]		LF[a]	
TRT	HR[c]	RS[e]	C[e]	RS	C	RS	C
UC[b]	24	16.0	21.5	11.0	11.8	1.1	1.0
FSH-P	24	25.9	23.1	17.5	16.4	1.8	1.8
UC	48	9.3	13.2	8.6	9.9	7.7	6.8
FSH-P	48	6.8[d]	5.0[d]	14.5	14.1	13.0	

[a]M1F = 3 to 4.9 mm, M2F = 5 to 6.9 mm, LF above 7 mm
[b]UC = untreated controls
[c]Hours to OVX after first FSH-P injection
[d]Zero values are included in mean
[e]RS, Relax Select line; C, Control line

Table 2. Relative percentage of medium and large follicles as affected by genetic line, treatment and hour after first FSH-P treatment.

		M1F[a]		M2F[a]		LF[a]	
TRT	HR[c]	RS[d]	C[d]	RS	C	RS	C
UC[b]	24	55.7	62.1	39.9	35.0	4.4	2.9
FSH-P	24	56.6	56.9	39.5	38.0	3.9	5.1
UC	48	34.0	43.6	33.2	32.2	32.9	24.2
FSH-P	48	17.6	14.3	41.7	42.6	40.7	43.2

[a]M1F = 3 to 4.9 mm, M2F = 5 to 6.9 mm, LF above 7 mm
[b]UC = untreated controls
[c]Hours to OVX after first FSH-P injection
[d]RS, Relax Select line; C, Control line

inactive (unhealthy). RS and C gilts showed similar patterns of development of M2F in response to FSH-P treatment (Tables 1 and 2).

The large follicle population was also influenced by treatment with FSH-P but, as with M1F and M2F, the effect differed at 24 and 48 hours after first FSH-P treatment. Large follicles accounted for about 4% of all follicles at 24 hours, regardless of treatment. However, LF developed more rapidly in FSH-P treated gilts of both genetic lines between 24 and 48 hours (Table 2). Mean numbers of LF were similar in UC and FSH-P treated gilts at 24 hour but were nearly 1-fold higher ($p<.01$) in

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Table 3. Mean estradiol concentrations (ng/mL) in follicular fluid from medium and large follicles.

		M1F[a]		M2F[a]		LF[a]	
TRT	HR[c]	RS[d]	C[d]	RS	C	RS	C
UC[b]	24	28.4	20.4	170.3	129.5	358.7	203.4
FSH-P	24	51.5	67.7	129.7	167.6	159.8	222.4
UC	48	7.2	6.8	135.0	117.1	279.7	261.4
FSH-P	48	93.0	19.0	226.5	210.2	335.5	287.6

[a]M1F = 3 to 4.9 mm, M2F = 5 to 6.9 mm, LF above 7 mm
[b]UC = untreated controls
[c]Hours to OVX after first FSH-P injection
[d]RS, Relax Select line; C, Control line

FSH-P treated gilts at 48 hour (Table 1). Numbers of LF developed to a similar level in response to FSH-P in C and RS gilts and numbers and relative percentages of LF were similar in both genetic lines on day 2.5 and day 3.5 post $PGF_{2\alpha}$ (Tables 1 and 2). The more rapid development of LF in C than RS gilts between day 3 and day 4 reported in a previous study (1993 Swine Report) was not evident between day 2.5 and day 3.5 in the present experiment. This difference may develop later in the follicular phase.

Estradiol (E) Concentrations in Follicular Fluid (FF)

Mean concentrations of E in FF are presented in Table 3. M1 follicles from gilts treated with FSH-P showed elevated ($p<.001$) concentrations of E, but the response differed between genetic lines and with interval to OVX after first FSH-P ($p<.01$). The E response to FSH-P was higher in C than RS gilts at 24 hour but the reverse was true at 48 hour. Concentrations of E declined in UC gilts in both lines between 24 and 48 hours but M1F from RS gilts continued to respond to FSH-P at 48 hour and achieved higher concentrations of E than occurred at 24 hour. In contrast, M1F from FSH-P treated C gilts at 48 hours showed less than a two-fold elevation in E above UC gilts and did not achieve the levels of E expressed by FSH-P treated C gilts at 24 hours (Table 3).

Treatment with FSH-P also increased E concentrations in M2F but the E responses differed at 24 and 48 hours after first treatment with FSH-P ($p<.003$). Estradiol concentrations in FF were comparable in UC and FSH-P treated gilts at 24 hour. But E concentrations in M2F had decreased slightly in UC gilts while increasing about 70% in FSH-P treated gilts at 48 hour (Table 3).

Treatment with FSH-P tended to exert a differential effect on E concentrations in FF from LF at 24 and 48 hours after first FSH-P treatment ($p<.07$). Estradiol concentrations were similar in FF from UC gilts at 24 and 48 hours but were suppressed at 24 hours and elevated at 48 hours in FSH-P treated gilts compared to UC gilts. The reasons for this tendency remain obscure.

The low concentration of E in FF of M1F from UC gilts at 24 and 48 hours does not reflect their inability to respond to gonadotropin. FSH-P treatment, with one exception, stimulated major elevations in E concentrations in FF at 24 and 48 hours after the first FSH-P injection. The exception was the M1F obtained from C gilts at 48 hour (84 hours post $PGF_{2\alpha}$). These follicles failed to increase their production of E in response to FSH-P. They may have lost their gonadotropin receptors and they may soon disappear from the ovaries. Histological evaluation may be required to assess atresia rate in M1F since all M1F are producing relatively low concentrations of E and are estrogen inactive.

Mean E concentrations in FF of M2F were similar to the levels reported during the same period in an earlier study conducted at UNL (1993 Swine Report). They found that concentrations of E in FF of M2F reached comparable levels (220 ng/mL) in C and RS gilts on day 3 and then either showed a small increase (RS line) or substantial decrease in E concentration (50% reduction, C line) to day 4. The decline in E concentration in FF from M2F in C gilts corresponded to the same time frame (day 3 to day 4) when major losses of M2F were occurring in C line gilts. The low E concentrations served as the basis for classifying the majority of these follicles atretic.

Health Status of the 5 to 6.9 mm Medium Follicles

Lower relative percentages and numbers of M2F were not observed in C line gilts on day 3.5 in the present study. However, these follicles may be undergoing biochemical changes leading to atresia and later loss from the surface of the ovaries. Therefore, the health status of M2F was assessed by classifying individual M2 follicles as healthy (estrogen-active, > 100 ng E/mL of FF) or atretic (estrogen inactive, < 100 ng E/mL of FF).

Untreated control gilts from the RS line had higher percentages of healthy M2F than C line gilts at both 60 (RS, 80% vs C, 69% healthy) and 84 hours (RS, 74% vs C, 52% healthy) post $PGF_{2\alpha}$ ($p<.01$). These differences are similar to those observed in an earlier study at 72 (RS, 86% vs C, 78% healthy) and 96 hours post $PGF_{2\alpha}$ (RS, 72% vs C, 50% healthy). Treatment with FSH-P lowered the percentage of healthy M2F in both genetic lines (RS, 68% vs C, 62%) at 24 hour (60 hours post $PGF_{2\alpha}$) but improved the percentage of healthy M2F (RS, 91% vs C, 92% healthy) at 48 hours after first FSH-P treatment (84 hours post $PGF_{2\alpha}$). Thus, enhanced gonadotropin concentration beginning at 48 hours (12 hours after first FSH-P injection) post $PGF_{2\alpha}$ was able to reverse the pattern of greater atresia of M2 follicles (5 to 6.9 mm) in C line gilts during the mid follicular phase of the estrous cycle. Since FSH-P treated RS and C line gilts have similar numbers of LF and healthy M2F at this stage (84 hours post $PGF_{2\alpha}$) of the follicular phase, it is probable that C line gilts will be able to continue to select follicles from the M2F pool. This should allow them to develop and ovulate comparable numbers of large preovulatory follicles as RS gilts. This will be evaluated in future studies.

[1]Hui-Wen Yen is a graduate student, Steve Christian is a Research Technologist and Dwane Zimmerman is a Professor in the Animal Science Department, University of Nebraska, Lincoln.