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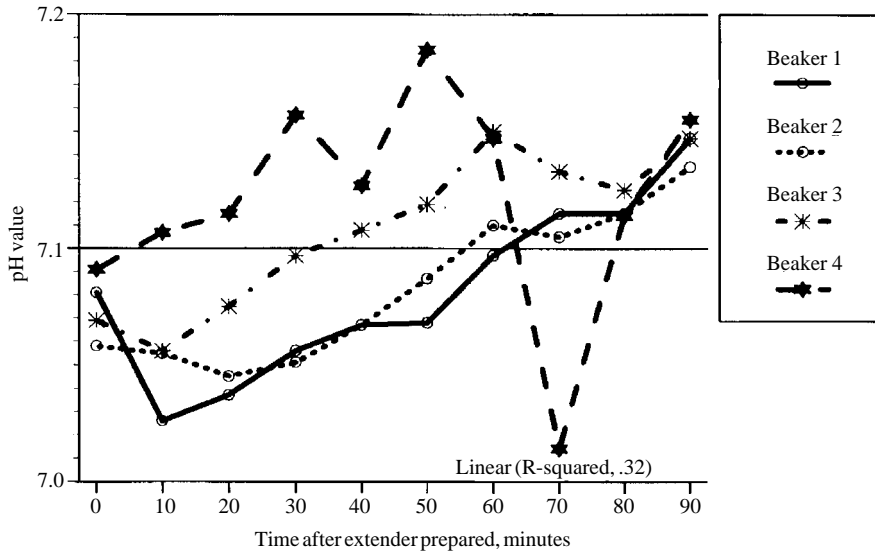


Figure 8. Pattern of change in pH over time for BTS semen extender.

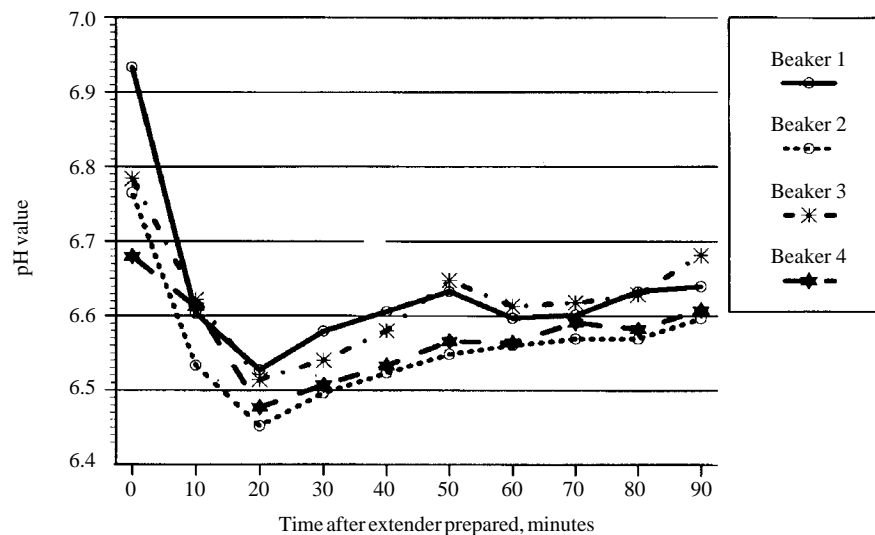


Figure 9. Pattern of change in pH over time for SpermAid semen extender.

# Follicular Selection and Atresia in Gilts Selected for an Index of High Ovulation Rate and High Prenatal Survival

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## Summary and Implications

Previously, we reported (See Yen et al., Nebraska Swine Report 1998) White Line gilts selected for an index of high ovulation rate and high prenatal survival (White Line-2, WL-2) maintained a larger pool of medium follicles (3 to 6.9 mm) during the early- to mid-follicular phase than randomly selected controls (White Line-1, WL-1). The present study evaluated the health status of the medium follicles to determine whether WL-2 gilts maintain a healthier pool of medium follicles and are able to continue selection of ovulatory follicles later in the follicular phase to achieve their ovulation rate advantage (6.6 ova). Ovaries were recovered on days zero, two three, four and five after induced luteolysis with PGF2 $\alpha$  on day 13 (day zero) of the estrous cycle. Numbers of follicles (F) equal or greater than 3 mm in diameter were categorized by size and recorded as follows: medium-1 (M1F, 3 to 4.9 mm), medium-2 (M2F, 5 to 6.9 mm) and



large ( $LF \geq 7$  mm). Estradiol (E) concentration in follicular fluid was used to classify individual M2F and LF as healthy ( $\geq 100$  ngE/mL) or atretic ( $< 100$  ngE/mL). M1F were not estrogen-active ( $< 60$  ngE/mL) and could not be evaluated for atresia with this method. Mean E concentrations in M2F increased linearly from day two to day five in WL-2 gilts while E concentrations increased rapidly between day two and day three and then plateaued in WL-1 gilts. All LF were estrogen active ( $\geq 100$  ngE/mL) and classified as healthy in both genetic lines. The percentage of healthy M2F increased rapidly in WL-2 gilts between day three and day five whereas percent of healthy M2F remained unchanged in WL-1 gilts during this period. Mean numbers of healthy M2F increased rapidly in WL-2 gilts between day two and day four and then declined to day five. Numbers of healthy M2F in WL-1 gilts increased between day two and day three and then declined to day four and day five. The greatest difference occurred on day four. WL-2 gilts maintain a larger pool of healthy M2F to day four of the follicular phase and rapidly select and mature these follicles into ovulatory follicles to achieve their ovulation rate advantage. Both genetic lines need to select about six additional ovulatory follicles from the M2F pool after day five to achieve final ovulation rates. Greater understanding of the biological basis of the improvement in follicular dynamics in the WL-2 population may prove useful in developing more efficient methods for improving ovulation rate and enhancing litter size in swine.

## Introduction

Increasing litter size can improve efficiency of swine production. Variation in litter size is determined by the number of follicles that ovulate and release viable ova, the percentage of ova fertilized by sperm and the percentage of beginning embryos and fetuses that survive in utero during gestation and are born alive.

Selection for high ovulation rate in the University of Nebraska gene

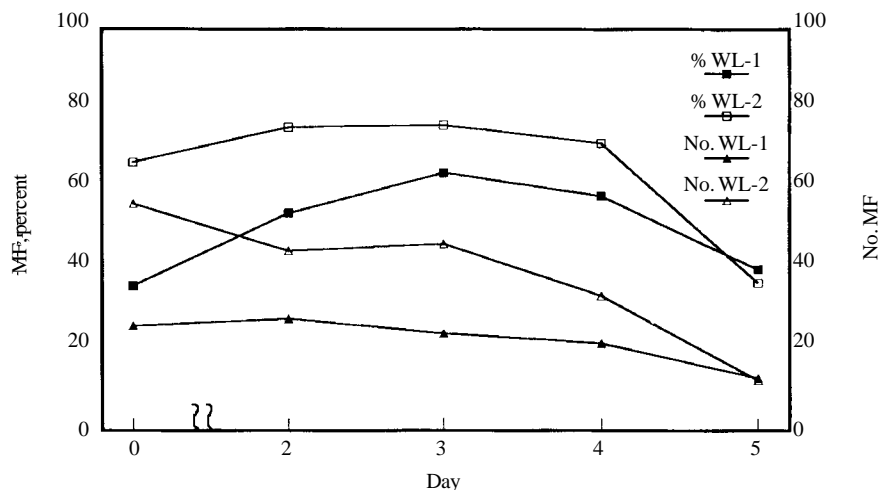


Figure 1. Mean numbers and relative percentage of medium follicles (3 to 6.9 mm) following PGF2-alpha on day 13 (day 0) of the estrous cycle.

pool population increased ovulation rate by about 3.2 ova over randomly selected control line gilts, but the number of pigs born alive increased less than one pig per litter after nine generations of selection. The limited litter size response led to the design of a second selection experiment where selection was based on both an index of the ability of females to maintain large litters to day 50 of gestation (percent prenatal survival) and ovulation rate (see Johnson, Nebraska Swine Report 1990). This experiment utilized a Large White x Landrace composite population and has proved more effective at increasing both ovulation rate and litter size. The ovulation rate and litter size advantages for the index selected line over the randomly selected control line averaged 6.7 ova and 1.5 pigs after 10 generations of selection (see Johnson, Nebraska Swine Report 1998).

The pathway to expressing high or low 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.

Our laboratory reported previously that WL-2 gilts maintain a larger pool of medium follicles (3 to 6.9 mm) than WL-1 gilts during the early- to mid-follicular phase of the estrous cycle

(Figure 1) and rapidly mature the larger M2 (5 to 6.9 mm) follicles into large ovulatory follicles ( $\geq 7$  mm) between day four and day five of the follicular phase (Figures 2 and 3). We hypothesized the pool of medium follicles maintained by WL-2 gilts during the follicular phase is not only larger but also healthier and that the ovulation rate advantage is achieved by continued selection of ovulatory follicles from the medium follicle pool during the mid- to late-follicular phase. The objective of the present study was to determine differences in the health status of medium follicles during the follicular phase in gilts selected for an index of high ovulation rate and prenatal survival (WL-2) versus randomly selected control line gilts (WL-1).

## Materials and Methods

Fifty-nine tenth generation WL-1 and WL-2 gilts were assigned randomly within sire for ovary recovery on days zero, two, three, four and five after induced luteolysis (regression of corpora lutea) with PGF2 $\alpha$  (10 mg Lutalyse) on day 13 (day zero) of the estrous cycle. Gilts from WL-1 and WL-2 represented the progeny of 11 and nine sires, respectively. These gilts were 8 to 11 months of age and weighed between 209 and 330 pounds when evaluated. They had experienced two

(Continued on next page)



or more estrous periods before assignment. Distribution of gilts by line and day of evaluation were: day zero (n=7 WL-1 and 5 WL-2), day two (n=7 WL-1 and 6 WL-2), day three (n=5 WL-1 and 6 WL-2), day four (n=5 WL-1 and 6 WL-2) and day five (n=7 WL-1 and 5 WL-2).

Ovaries were recovered at slaughter and placed in .9 percent saline on ice. The numbers of corpora ablicantia (CA) 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 by size and recorded as follows: medium-1 (M1F, 3 to 4.9 mm), medium-2 (M2F, 5 to 6.9 mm) and large (LF,  $\geq 7$  mm). Estradiol (E) concentration in follicular fluid was used to classify individual M2F and LF as healthy ( $\geq 100$  ngE/mL) or atretic ( $< 100$  ngE/mL). M1F were not yet estrogen-active ( $< 60$  ngE/mL) and could not be evaluated for atresia with this method. Mean concentrations of E in follicular fluid, percentage of healthy M2F and mean number of healthy M2F were analyzed statistically by the GLM procedure of SAS with line and day as main effects.

### Result and Discussion

Overall, WL-2 gilts ovulated 6.6 more follicles than WL-1 gilts at the pretreatment estrus (20.4 versus 13.8,  $P<.01$ ). This difference is similar to the line difference reported earlier (See Johnson, 1998 Nebraska Swine Report).

Concentrations of estradiol (E) in follicular fluid of M1F were low ( $< 60$  ng/mL) throughout the follicular phase in both genetic lines. Mean E concentrations in M2F more than doubled between days two and three (108.7 versus 223.1 ng/mL) and then plateaued to day five in WL-1 gilts (Figure 4). In contrast, E increased rapidly in WL-2 gilts between day three and day four (150.9 versus 275.9 ng/mL) and had increased to a substantially higher level on day five (352.6 ng/mL,  $P<.01$ ) than in WL-1 gilts (Figure 4). These data suggest the M2F population of WL-1 and WL-2 gilts

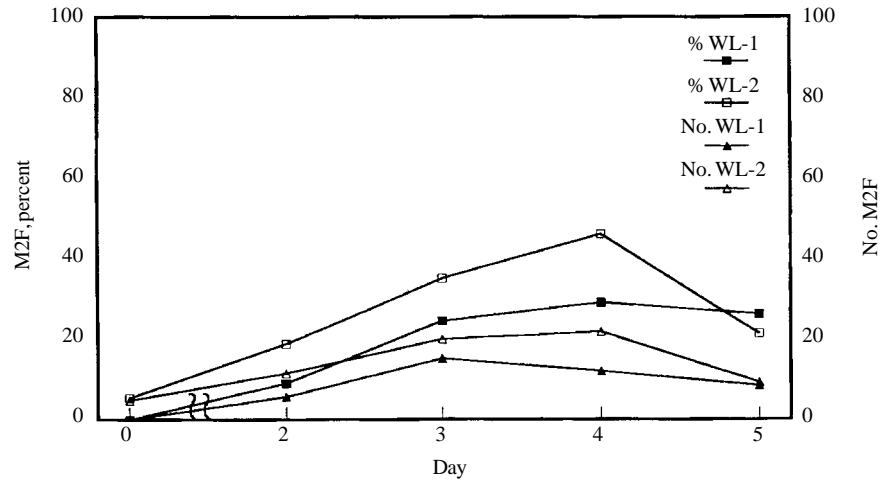


Figure 2. Mean numbers and relative percentage of medium-2 follicles (5 to 6.9 mm) following PGF2-alpha on day 13 (day 0) of the estrous cycle.

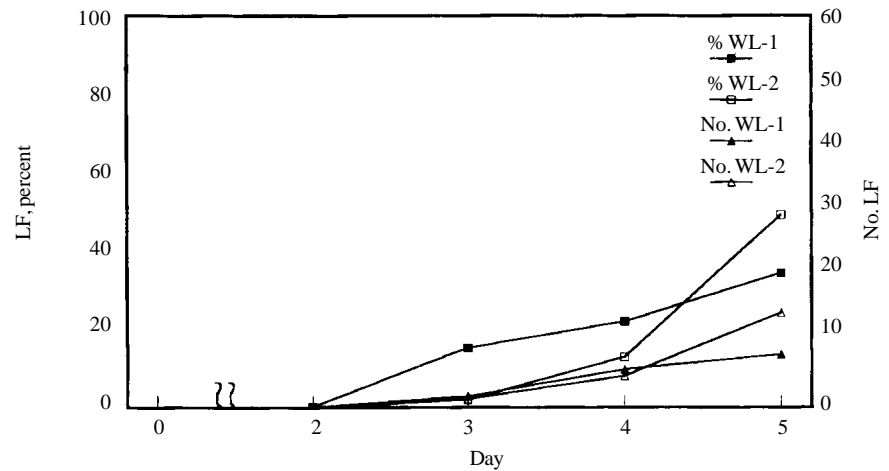


Figure 3. Mean numbers and relative percentage of large follicles ( $\geq 7$  mm) following PGF2-alpha on day 13 (day 0) of the estrous cycle.

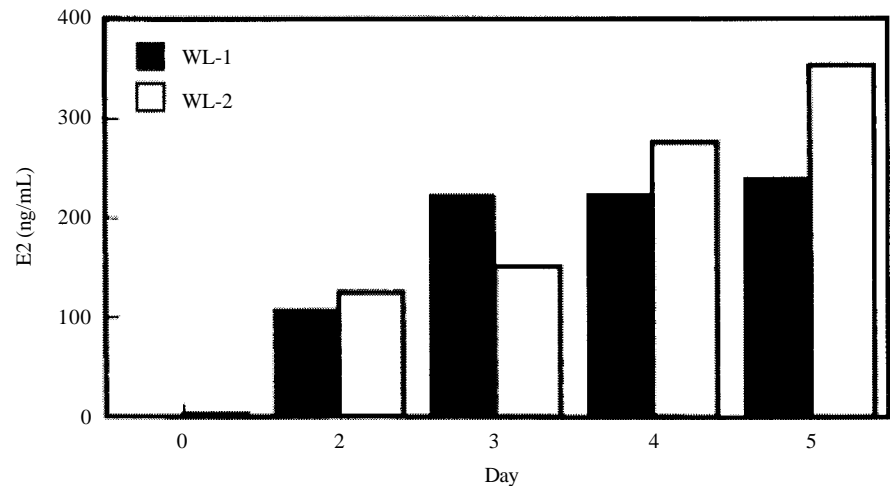
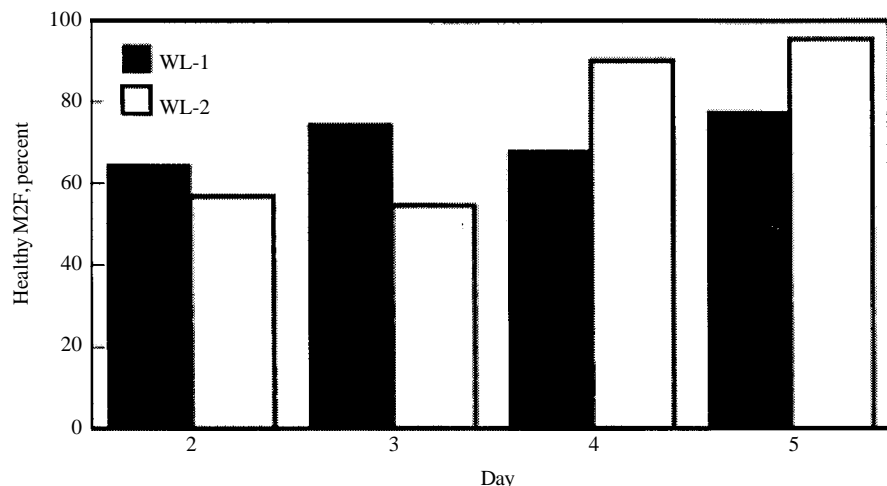
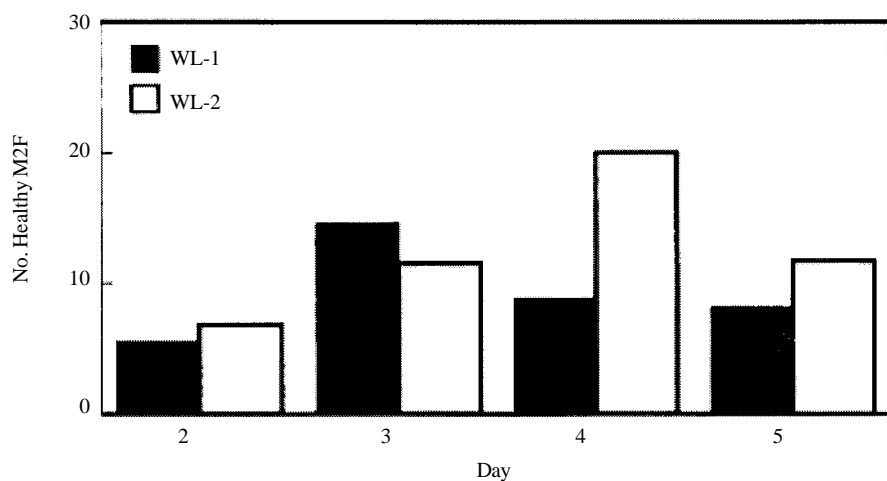


Figure 4. Mean concentrations of estradiol (E2) in follicular fluid from individual M2 (5 to 6.9 mm) follicles following PGF2-alpha on day 13 (day 0) of the estrous cycle.



**Figure 5.** Percentage of healthy M2 (5 to 6.9 mm) follicles following PGF2-alpha on day 13 (day 0) of the estrous cycle.



**Figure 6.** Mean numbers of healthy M2F (5 to 6.9 mm) following PGF2-alpha on day 13 (day 0) of the estrous cycle.

are in different functional states during the follicular phase. M2 follicles in WL-1 show a major increase in functional activity between days two and three but do not continue to increase after day three, whereas WL-2 gilts sustain increased functional activity after day three of the follicular phase. The M2F population of WL-2 gilts is probably more mature, and perhaps healthier, later in the follicular phase than the M2F pool of WL-1 gilts.

Medium follicles would have to be healthy to be selected and develop into large ovulatory follicles. Therefore, the health status of individual M2F was evaluated and compared between lines. Percentages of healthy M2F were greater

numerically, but not statistically, in WL-1 gilts on days two and three (WL-1, 64.6 and 74.6 percent versus WL-2, 56.8 and 54.6 percent,  $P > .1$ ; Figure 5). Percentages of healthy M2F increased rapidly in WL-2 from days three to five (54.6 versus 95.5 percent,  $P < .07$ ) whereas percentage of healthy M2F remained unchanged in WL-1 gilts from days three to five (74.5 versus 77.8 percent, Figure 5). Mean numbers of healthy M2F increased about 2.6 fold (5.5 versus 14.6,  $P < .06$ ) in WL-1 gilts between day two and day three and then declined to day five (Figure 6). In contrast, mean numbers of healthy M2F in WL-2 gilts increased linearly from 6.8 to 20 between day

**Table 1.** Line differences in number of corpora albicantia (CA), healthy medium and large follicles on day five after PGF2-alpha on day 13 (day 0).

Line	Follicle Size <sup>a</sup>		
	M2	L	No. CA <sup>b</sup>
WL-1	8.1	8.1 <sup>c</sup>	13.8 <sup>c</sup>
WL-2	11.6	14.5	20.4

<sup>a</sup>M2, 5 to 6.9 mm; L, 7 mm and above.

<sup>b</sup>Ovulation rate at pretreatment estrus.

<sup>c</sup> $P < .01$ .

two and day four and then declined to day five (Figure 6). These results suggest that WL-2 gilts were able to maintain a larger pool of healthy M2F during the mid- to late-follicular phase and mature these healthy M2F rapidly into LF after day four to achieve their ovulation rate advantage.

All LF were estrogen active ( $\geq 100$  ngE/mL) and classified as healthy in both genetic lines. The number of LF observed on day five differed between the two genetic lines (WL-1, 8.1 versus WL-2, 14.5,  $P < .01$ ) but did not reflect the expected ovulation rate of either line (Table 1). Both lines must continue selecting ovulatory follicles from the healthy pool of M2F in order to achieve their final ovulation rate. To achieve ovulation rates comparable to that expressed at the previous estrus, each line would have to mature about six M2F into large ovulatory follicles before time of ovulation.

## Conclusion

Follicular dynamics have been changed in response to genetic selection for high ovulation rate and high prenatal survival. Selected WL-2 gilts develop M2F earlier in the follicular phase and achieve a larger pool of healthy M2F from which they select LF later into the follicular phase. After day four, WL-2 gilts rapidly select and mature their healthy M2F into LF to achieve their ovulation rate advantage.

<sup>1</sup>Hui-Wen Yen is a graduate student and Rodger K. Johnson and Dwane R. Zimmerman are professors in the Department of Animal Science.