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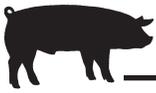
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# Regulation of Pituitary Gene Expression in Lines of Swine with Different Ovulation Rates

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## Summary and Implications

Litter size plays a major role in the economics of pork production. Even modest increases in average litter size can have considerable effects on overall profitability. Two major components of litter size – ovulation rate and embryonic survival – have been used in a selection index project ongoing for several generations at the University of Nebraska–Lincoln (UNL). Additionally, the Chinese Meishan breed is one of the most prolific breeds, producing four to five more pigs per litter than white crossbred females. We investigated the role of the gonadotropin-releasing hormone (GnRH) receptor and gonadotropin subunit genes in determination of ovulation rate between lines of swine. Ten UNL Index and Control line white crossbred gilts and 12 Meishan gilts were ovariectomized following three (Index and Control) or 6 (Meishan) successive estrous cycles. After a 21-day recovery period, gilts from each line were treated with either a specific GnRH antagonist (SB-75; 10 µg/kg of body weight) or 0.9% saline at 60, 36 and 12 hours prior to slaughter. Blood samples were collected prior to the first treatment and at slaughter before anterior pituitary collection. Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were determined by radioimmunoassay and RNA was extracted from anterior pituitary tissue. In all lines, LH was reduced to basal levels by SB-75 treatment, confirming the efficacy of SB-75. In contrast, levels of FSH decreased only in Control gilts following treatment with SB-75. Pituitary levels

of GnRH receptor and gonadotropin subunit gene expression were measured by quantitative PCR. Levels of gene expression for the GnRH receptor and gonadotropin subunits decreased following treatment with the GnRH antagonist in pituitaries of gilts from the Index and Control lines; however, these values remained unchanged in pituitaries from Meishan gilts. Identification of unique genetic changes in swine strains with increased ovulation rates, such as the Chinese Meishan and the UNL Index selection line, may allow for a better understanding of prolificacy. This critical information may also be used to enhance litter size in other lines of pigs and improve efficiency of pig production.

## Background and Introduction

Prolificacy is an important economic measure of productivity in the pork industry. However, many generations of selection are required to increase the number of live born piglets per litter within an applied breeding program. Thus, it is important to identify the genes and underlying biological mechanisms contributing to increased litter size. While many factors can influence prolificacy, a primary component of litter size is ovulation rate, or the number of oocytes (eggs) available to be fertilized after insemination. From a research perspective, ovulation rate can be measured via visualization of ovaries by surgical procedures (i.e., laparotomy or laparoscopy) or ultrasound. In addition, this trait can be improved in a number of different ways, including nutrition (flushing), hormonal treatment (superovulation), and genetic selection. Despite its importance as a primary component of litter

size, however, there is very little known about the genes influencing increased ovulation rate.

Ovulation rate is influenced by circulating levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH), also known as the gonadotropins. The production of these hormones is controlled by the reproductive axis (Figure 1), consisting of the hypothalamus, anterior pituitary gland, and gonads (ovaries or testes). Specifically, gonadotropin-releasing hormone (GnRH), released from the hypothalamus, binds to its receptor on gonadotrope cells of the anterior pituitary gland. Upon binding to its receptor, GnRH stimulates the expression of the GnRH receptor gene itself, as well as the subunit genes that lead to the production of FSH (common alpha- and FSHbeta-subunits) and LH (common alpha- and LHbeta-subunits). The secreted gonadotropins then act on the ovaries to recruit follicles (FSH) or induce ovulation (LH). Steroids, produced by the ovaries, such as estrogen and progesterone provide feedback at the level of both the hypothalamus and anterior pituitary gland to regulate subsequent production of GnRH and gonadotropins, respectively. Therefore, reproductive function is highly dependent on the interaction of GnRH and its receptor.

Sensitivity, or number of GnRH receptors, present on gonadotrope cells of the anterior pituitary gland, may stimulate higher levels of gonadotropin production in lines of swine with increased ovulation rates. In the pig, the sequences for the genes encoding the subunits comprising the gonadotropins, FSH and LH

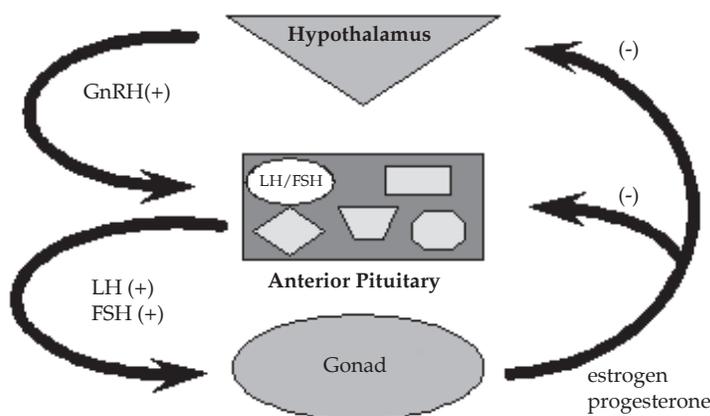
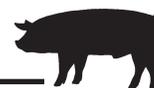


Figure 1. The reproductive axis.

(alpha-, FSHbeta- and LHbeta-subunits), have recently been reported. In addition, the sequence for the porcine GnRH receptor gene was identified by researchers at the University of Guelph. Upon isolation of the gene, investigators at the USDA Meat Animal Research Center have determined that it is located in a similar region as a chromosomal marker for ovulation rate. Therefore, the GnRH receptor gene represents both a physiological and positional candidate for genes influencing ovulation rate in swine. Isolation of these sequences allows for quantification of GnRH receptor and gonadotropin subunit gene expression levels, so comparisons can be made between lines of pigs with ovulation rate differences.

To examine the role of these genes in determining ovulation rate between swine lines, we used swine lines with divergent ovulation rates. Females of the Chinese Meishan breed have a higher ovulation rate than occidental breeds, resulting in four to five more piglets per litter. Thus, Meishan pigs may harbor genetic differences with the potential to enhance reproductive performance of white crossbred pigs. Consistent with the Meishan model, researchers at UNL have developed a line of white crossbred pigs that were selected 11 generations for an index of ovulation rate and

embryonic survival, followed by nine generations of selection for increased litter size. At generation 10, females from the UNL Index line ovulated 7.4 more eggs and at generation 19, produced 2.53 more live born piglets per litter than unselected, control animals.

## Materials and Methods

### Animals and Treatments

All animal procedures conducted in this experimentation were approved by the UNL Institutional Animal Care and Use Committee. White crossbred Index and Control line gilts (Generation 23) were obtained from the University of Nebraska Swine Unit. Gilts of the Meishan breed were obtained from the United States Department of Agriculture, Roman L. Hruska U.S. Meat Animal Research Center in Clay Center, Neb. Gilts were housed in pens with a minimum of 8 square feet of floor space and received 4 pounds of feed per day with water available *ad libitum*. Estrous detection was initiated at 155 days of age for Index and Control gilts and at 95 days of age for Meishan gilts. Upon completion of the third (Index and Control) or sixth (Meishan) estrus, 10 gilts from the Index and Control lines and 12 Meishan gilts were ovariectomized during the luteal phase (day 5 to

15 of the estrous cycle) to remove any confounding effects of steroid hormones (i.e., estrogen) on expression patterns of the genes of interest. During the third estrous cycle, Meishan gilts have similar ovulation rates to that of gilts from occidental breeds. Thus, Meishan females were ovariectomized during the sixth estrous cycle to assure that they would have increased ovulation rates than gilts from the Control line. Eighteen days after ovariectomy, gilts from each line were randomly assigned to treatment groups and received an injection of either the specific GnRH antagonist, SB-75 (10 µg biologically active compound per kg of body weight; UNL Protein Core Facility), or vehicle (0.9% saline) at 60, 36 and 12 hours prior to slaughter. The GnRH antagonist was used to block the effects of GnRH, which is dramatically increased in gilts following ovariectomy. Therefore, GnRH levels were expected to be elevated in ovariectomized gilts treated with vehicle and significantly reduced in ovariectomized gilts treated with the GnRH antagonist, SB-75.

### Data Collection

Blood samples were collected prior to ovariectomy and the first treatment, as well as at slaughter. Following slaughter, the anterior pituitary gland was removed. Serum concentrations of FSH and LH were determined using a radioimmunoassay validated in our laboratory. Levels of FSH and LH were determined using a known standard curve of the respective hormone, run simultaneously with the unknown samples. Total RNA, which was extracted from the anterior pituitary tissue, was converted to cDNA and used in quantitative real-time polymerase chain reaction assays to measure expression of the GnRH receptor, glycoprotein alpha-subunit, FSH-beta-subunit and LHbeta-subunit

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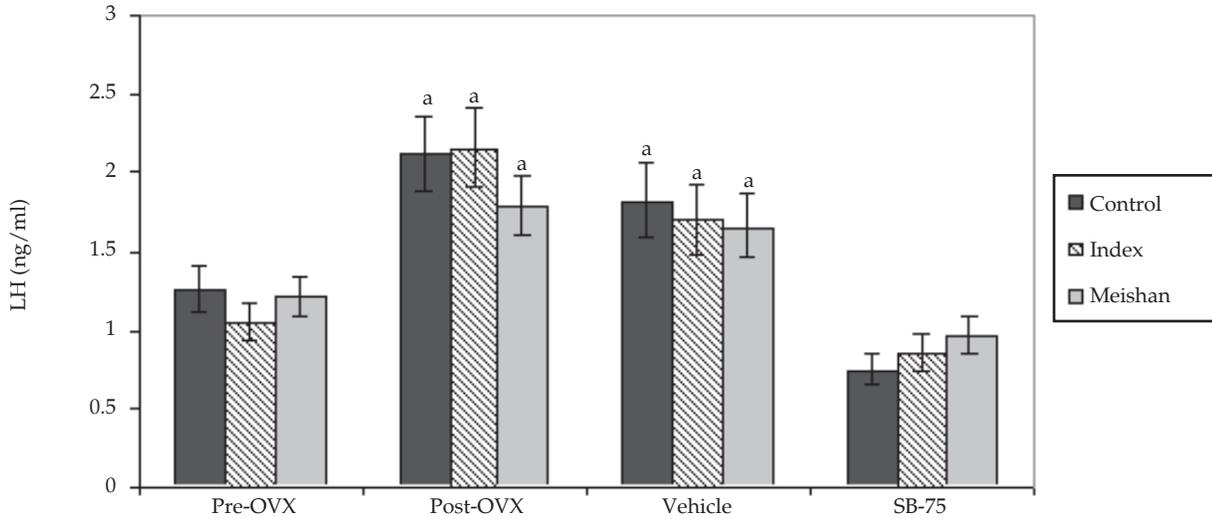
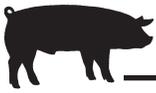


Figure 2. Serum LH levels prior to (Pre-OVX) and after (Post-OVX) ovariectomy and following treatment with the GnRH antagonist, SB-75, or vehicle in Control, Index and Meishan gilts. Each bar represents the least-squares mean  $\pm$  SEM of 5-6 gilts. Bars with superscripts are different than Pre-OVX groups ( $P < 0.05$ ) and different superscripts indicate differences between lines ( $P < 0.05$ ).

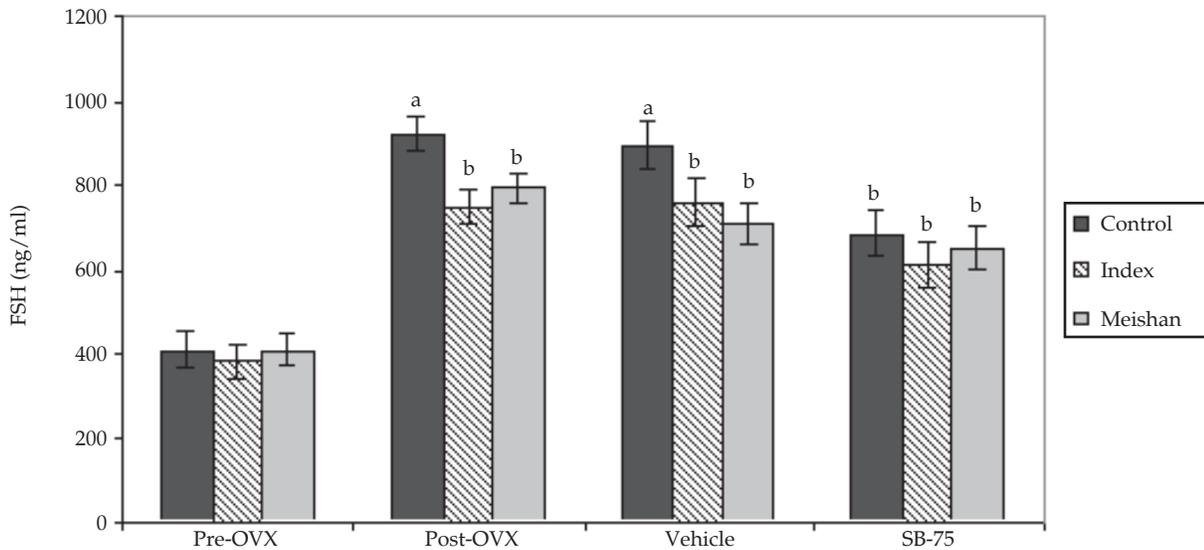


Figure 3. Serum FSH levels prior to (Pre-OVX) and after (Post-OVX) ovariectomy and following treatment with the GnRH antagonist, SB-75, or vehicle in Control, Index, and Meishan gilts. Each bar represents the least-squares mean  $\pm$  SEM of 5-6 gilts. Bars with superscripts are different than Pre-OVX groups ( $P < 0.05$ ) and different superscripts indicate differences between lines ( $P < 0.05$ ).

genes. The 18s ribosomal RNA kit (Applied Biosystems) was used as an endogenous control gene to normalize each assay.

#### Statistical Analysis

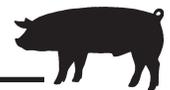
Statistical evaluation was conducted using the General Linear Models procedure of the SAS. The LH means were logarithmically transformed due to non-normality, analyzed for significance and

back-transformed to the original scale. Least-squares means for LH and FSH were compared using least significant differences. Least squares means for expression levels of GnRH and glycoprotein alpha-subunit, FSHbeta-subunit and LHbeta-subunit genes were logarithmically transformed due to non-normality, analyzed for significance, back-transformed to the original scale and compared

using least significant differences. Means for normalized gene expression data are expressed as a ratio of the gene of interest relative to 18s rRNA.

#### Results and Discussion

Levels of LH (Figure 2) and FSH (Figure 3) were similar in females from all three lines prior to ovariectomy. Following



**Table 1. Change in GnRH receptor and gonadotropin subunit gene expression levels following treatment with a GnRH antagonist in lines of swine with differing ovulation rates.**

Gene <sup>b</sup>	Genetic Line <sup>a</sup>		
	Control	Index	Meishan
GnRH receptor	Decrease	Decrease	No Change
Common alpha-subunit	Decrease	Decrease	No Change
FSHbeta-subunit	Decrease	Decrease	No Change
LHbeta-subunit	Decrease	Decrease	No Change

<sup>a</sup>Gilts were ovariectomized after the 3rd (Control and Index) or 6th (Meishan) estrus. Following a two-week recovery period, females were treated with the GnRH antagonist, SB-75, or vehicle (0.9% saline) at 60, 36 and 12 hours prior to slaughter.

<sup>b</sup>Changes in gene expression are significant ( $P < 0.05$ ).

ovariectomy, levels of LH were similar among swine lines; however, FSH levels were higher in Control compared to Index and Meishan gilts ( $P < 0.05$ ). Treatment of gilts with the GnRH antagonist, SB-75, decreased LH levels in all lines but levels of FSH decreased significantly only in the Control line compared to animals receiving the vehicle treatment ( $P < 0.05$ ).

Expression of the GnRH receptor gene was greatest in pituitaries of Meishan, intermediate in Index and lowest in Control line gilts treated with vehicle ( $P < 0.05$ ). Treatment with SB-75 reduced expression of the GnRH receptor gene in the Index and Control lines ( $P < 0.05$ ), but not in Meishan gilts (Table 1). Following vehicle treatment, expression of the common glycoprotein alpha-subunit gene was less in pituitaries from Meishan gilts than in Control and Index gilts ( $P < 0.05$ ). Treatment with SB-75 reduced expression of the alpha-subunit gene in pituitaries from females of the Control and Index lines ( $P < 0.0001$ ) but, similar to the GnRH receptor gene, levels of alpha-subunit mRNA in anterior pituitaries of Meishan gilts were unchanged (Table 1). As was observed with the alpha-subunit, expression of the FSHbeta-subunit gene after vehicle treatment was lower in pituitaries of females

from the Meishan compared to Control and Index lines. There was a decrease in expression of the FSHbeta-subunit gene in anterior pituitaries of both Control and Index gilts receiving treatment with SB-75 ( $P < 0.0001$ ); however, no change occurred in Meishan gilts (Table 1). After receiving the vehicle treatment, anterior pituitary expression of the LHbeta-subunit gene was lower in Meishan than Control gilts, with Index gilts being intermediate. A decrease in expression of the LHbeta-subunit gene was observed in pituitaries from Control and Index gilts treated with SB-75 ( $P < 0.0001$ ). In contrast, expression of the LHbeta-subunit gene in pituitaries of Meishan females did not decline (Table 1).

Expression of the GnRH receptor, common glycoprotein alpha-subunit, and specific FSHbeta- and LHbeta-subunit genes was reduced in pituitaries of gilts from the Index and Control lines, but not females from the Meishan line following SB-75 treatment. This suggests that differential mechanisms may be involved in gene regulation and production of gonadotropins between Meishan and white crossbred lines of swine. Also, post-treatment expression of the GnRH receptor and LHbeta-subunit genes was significantly

greater in anterior pituitaries from females of the Meishan line compared to both Control and Index lines, suggesting that basal expression of these genes is elevated in Meishan gilts. Identification of a trait(s) that could be easily screened and correlated with ovulation rate in young females would be of great interest for selection purposes. Further research needs to be conducted to reveal the mechanisms controlling the observed differences in expression of the GnRH receptor and gonadotropin subunit genes between Meishan and white crossbred lines of swine.

## Conclusion

Ovulation rate is very important to swine production, as it is a primary determinant of litter size. Even a modest increase in average litter size of 0.2 pigs per litter on a 10,000 sow operation could net a producer nearly \$99,000 in additional profit, depending on pork prices. If differences in pituitary gene expression between Meishan, Index, and Control lines are determined, a region of a particular gene may be isolated to provide a genetic test for ovulation rate. Ultimately, the unique gene sequences from individuals with increased ovulation rates could be incorporated into transgenic swine. This would allow the opportunity to increase ovulation rate in any breed or line of pigs, while maintaining the beneficial characteristics of that breed or line. These animals would be very valuable to pork production worldwide.

<sup>1</sup>Benjamin E. Bass is a graduate student, Rebecca A. Cederberg a research technologist, Ginger A. Mills an agricultural research technician, and Brett R. White an assistant professor in the Animal Science Department at the University of Nebraska-Lincoln.