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D. V. Mariscal
University of Nebraska-Lincoln

P. L. Wolfe
University of Nebraska-Lincoln

E. G. M. Bergfeld
University of Nebraska-Lincoln, ebergfeld@sciencesocieties.org

A. S. Cupp
University of Nebraska-Lincoln, acupp2@unl.edu

F. N. Kojima
University of Nebraska-Lincoln

See next page for additional authors

Comparison of Circulating Concentrations of Reproductive Hormones in Boars of Lines Selected for Size of Testes or Number of Ovulations and Embryonal Survival to Concentrations in Respective Control Lines[1,2]

D. V. Mariscal*[,3], P. L. Wolfe*, E. G. Bergfeld*, A. S. Cupp*[,4], F. N. Kojima*, K. E. Fike*, T. Sánchez*[,5], M. E. Wehrman*, R. K. Johnson*, R. J. Kittok*, J. J. Ford†, and J. E. Kinder*[,6]

*Department of Animal Science, University of Nebraska, Lincoln 68533-0908 and †Roman L. Hruska U.S. Meat Animal Research Center, USDA, ARS, Clay Center, NE 68933-0166

ABSTRACT: The objectives of this study were to determine whether circulating concentrations of gonadotropins and gonadal hormones of boars were altered as a result of selection of pigs for size of testes or for embryonal survival and(or) number of ovulations. Included in Exp. 1 and 2 were boars with the greatest estimated paired weight of testes (TS) and boars from a control (C) line. Concentrations of FSH were similar ($P > .10$) in boars from the TS and C lines. In Exp. 3, 4, and 5, circulating concentrations of FSH and 17β-estradiol (E_2) were evaluated in neonates, during pubertal development, and in mature boars of lines selected for an index of number of ovulations and embryonal survival (I), and data were compared to those for boars from a respective C line. Concentrations of E_2 were not different in boars from the I line and those from the C line during the early neonatal period but were greater ($P < .05$) in boars of the C line than in those from the I line during pubertal development. Concentrations of FSH were greater ($P < .05$) in mature boars from the I line than in those from the C line. In summary, selection for size of testes did not influence circulating concentrations of FSH in mature boars. The secretory pattern of E_2 in boars before puberty changed as a result of selection for embryonal survival and number of ovulations in females of the I line, and the different patterns of circulating E_2 early in life may result in enhanced circulating concentrations of FSH in adult boars of the I line compared with boars of the C line.

Key Words: Follicle-Stimulating Hormone, Luteinizing Hormone, Testosterone, Estrogen, Ovulation, Boars

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Introduction

Testes were larger in boars of a line selected for size of testes at 150 d of age (**TS**) than in boars of a randomly selected control (**C**) line (Johnson et al., 1994). Boars used in Exp. 1 and 2 of the present study were from these lines. In another study, an average of three more ovulations for each gilt occurred in a line selected for number of ovulations (**RL**) than in a randomly selected C line (Cunningham et al., 1979). Boars in Exp. 6 of the present study were from the RL and respective C line (Cunningham et al., 1979). Selection for number of ovulations and embryonal survival in another study resulted in significant increases in litter size of sows in the selection line (I line) compared with the respective C line (Neal et al., 1989; Casey et al., 1994). Boars used in Exp. 3, 4, and 5 of the present study were from the I and respective C line. The objective of the present experiments was to

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[3]Present address: Departamento de Zootecnia, Universidad Autónoma Chapinga, Mexico.

[4]Present address: Univ. of California, Reproductive Endocrinology Ctr., San Francisco, CA.

[5]Present address: Colegio de Posgraduados, Montecillos, Edo. Mexico.

[6]To whom correspondence should be addressed: 224j Animal Science.

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compare concentrations of FSH, LH, testosterone (**T**), or 17β-estradiol (**E_2**) in boars from the various selected lines to those of boars from respective C lines.

Materials and Methods

Genetic Composition and Management of Boars

Descriptions of lines and selection criteria for each line in the six experiments are given in Table 1. Boars were fed appropriately balanced diets, weaned from their dams at 28 d of age, and placed in a nursery unit. Boars were exposed to continuous lighting and were maintained in environmentally controlled rooms while in the farrowing and nursery units. At approximately 2 mo of age, boars were moved to a finishing unit, where they were exposed to natural lighting. Animals in Exp. 1, 2, and 5 were housed in the Animal Science Building on the University of Nebraska campus during the periods when experiments were performed. During experimental periods, boars used in these experiments were tethered in a single room that did not contain other pigs. Animals used in Exp. 3, 4, and 6 were maintained at the Swine Research Facility located near Mead, NE during the period when experiments were performed. Boars used in Exp. 3, 4, and 6 were housed in pens with other boars during the experimental periods. Ten boars were housed/pen with .89 m^2 allowed for each boar.

Experiment 1

Boars (14 to 15 mo of age; Large White × Landrace composite) from the second generation of a line selected for larger size of testes (TS; n = 6) and boars from a C line (n = 7) that were randomly selected were used. Boars from the TS line that were used in the experiment had the greatest predicted weight of testes at 150 d of age of all boars of this line, and these boars had been previously used to produce the third generation of pigs for this line. Boars of the C line used in this experiment were selected at random to produce the third generation of pigs. Body weights were similar for boars from the TS and C lines (182.5 ± 5.2 and 182.1 ± 6.9 kg, respectively), but excised testes weights at 15 to 16 mo of age were greater ($P < .05$) in boars from the TS than in those from the C line (867.83 ± 27 and 689.22 ± 29 g, respectively). Data for size of testes and for LH, T, and E_2 from boars used in Exp. 1 were previously reported (Wolfe et al., 1989b).

Indwelling ear vein catheters were inserted as described by Kelly (1986). Blood samples (4 mL) were collected during three periods over a 4-wk interval to determine concentrations of FSH. At each period, blood samples were obtained hourly for 12 h. Then an exogenous dose of LHRH was administered i.v. (188 ng/kg of BW), and blood samples were collected at 12-min intervals for an additional hour. Boars were intact during the first period of blood collection and then castrated. The second period of blood collection occurred 14 d after castration. Immediately after the second blood collection, boars were administered Compudose implants (Elanco Products, Indianapolis, IN) containing E_2 (.528 mg of E_2/kg BW) in the ventral midline to achieve physiological concentrations of E_2 (Wolfe et al., 1989b). The third period of blood collection occurred 7 d after implantation of E_2. Blood samples were mixed with 15 mg of EDTA. Samples were stored at 4°C until centrifugation at 3,500 × *g* for 30 min. Plasma was stored at –20°C until it was assayed for FSH.

Experiment 2

Boars (12 to 13 mo of age; Large White × Landrace) from the third generation of the TS line (n = 5) and boars from the respective C line (n = 6) were used. Boars from the TS line that were used in the study had the greatest predicted weight for testes at 150 d of age of all boars of the third generation in the

Table 1. Description of lines and selection criteria for lines used in each experiment

Selection line	Selection criteria[a]	Generation	Exp.
	Large White-Landrace composite population		
TS	Increased predicted weight of testes at 150 d of age	2	1
TS	Increased predicted weight of testes at 150 d of age	3	2
I	Increased index of OR and ES in females at 50 d of gestation	10	3, 4, and 5
C	Random		1 to 5
	Gene pool population (14-breed composite)[b]		
R	Increased OR	1 to 9	—
L	Random	10 to 22	—
RL	Cross of R × L	1	6
C	Random	1 to 22	6

[a]OR = ovulation rate; ES = embryo survival; LS = litter size.
[b]See Zimmerman et al. (1975).

TS line. Boars from the third generation of the C line were selected at random from all boars of this line. Boars used in this experiment were the boars of the TS and C lines that had been previously used to produce the fourth generation of pigs. Boars from the TS and C lines had similar BW (160.1 ± 5.8 and 151.9 ± 4.5 kg, respectively), whereas excised weights of testes differed ($P < .05$; 768.9 ± 59 and 544.06 ± 19 g, respectively) at castration at 12 to 13 mo of age. Data for size of testes and circulating LH were previously published (Wolfe et al., 1989a; Exp. 3). Ear veins were catheterized as described in Exp. 1.

Blood samples (4 mL) were collected at three periods and assayed for concentration of FSH. During the first period, blood samples were collected hourly for 12 h from intact boars then LHRH was administered i.v. (188 ng/kg BW) to determine the FSH response. Blood sampling continued at 12-min intervals for an additional hour. Boars were castrated immediately after the first period of blood collection. The second period was 14 d after castration, and blood samples were collected using the same protocol as that for the first period. In the third period, sodium pentobarbitol (20 mg i.v./kg BW) was used to anesthetize the castrated males and block the endogenous release of LHRH. While the castrated males were anesthetized, LHRH was injected to determine FSH release. This model was used to determine the amount of FSH released in response to a known amount of LHRH in the absence of testosterone feedback at the hypothalamic-pituitary axis. Anesthesia was maintained with sustaining doses of sodium pentobarbitol throughout a 5-h period of blood collection. Starting 1 h after induction of anesthesia, blood samples were collected at 6-min intervals for 1 h. After the first hour of sample collection, anesthetized boars were given a series of four i.v. injections of LHRH (94 ng/kg BW) 1 h apart. Blood samples were collected at 6-min intervals throughout the 4-h period during which the four injections of LHRH were administered and for 1 h after the last injection of LHRH. Blood samples were treated as indicated for Exp. 1.

Experiment 3

Boars from the selection line (**I**; n = 20) were from the 10th generation of a line in which females were selected on an index for number of ovulations and embryonal survival (Neal et al., 1989). Boars from the I line were offspring of sows with the greatest index for number of ovulations and embryonal survival to 50 d of gestation. Boars from a C line (n = 17) in which gilts were selected at random regardless of number of ovulations and rate of embryonal survival were used for comparison to boars of the I line. Collection of blood samples started when boars were approximately 7 d of age. Blood samples were collected at weekly intervals until boars were 28 d of age. Concentrations of FSH, LH, T, and E_2 in all samples were determined. Each blood sample (2 mL) was collected into a tube containing 7.5 mg of EDTA. Samples were stored on ice for approximately 2 h until they were centrifuged (20 min, 3,500 × *g*) for removal of plasma. Plasma was stored at –20°C until samples were used for quantification of hormones.

Experiment 4

Boars (n = 12) from the 10th generation of the I line were used. Boars were from sows that had high (**HI**; n = 6) or low (**LI**; n = 6) estimated breeding values for an index of number of ovulations and embryonal survival to 50 d of gestation. Boars (n = 12) of the same age from the C line also were used. They were from sows that had a high (**HC**; n = 6) or low (**LC**; n = 6) estimated breeding value for the index. Breeding values were predicted using pedigree and phenotypic index data back to the base generation of selection. Sows that were dams of the boars in the high and low groups were those with the greatest within-line difference in expected genetic value for the index. Predicted numbers of ovulations for gilts sired by boars used in this experiment based on the pedigree data were > 25 (HI), 14 (LI), 14 (HC), and 8 (LC). All boars used in this experiment were progeny of different sires.

Blood samples (4 mL) were collected via jugular venipuncture at 28-d intervals for 6 mo starting at the time pigs were weaned from their dams (28 d of age). Blood samples were collected into a tube containing 7.5 mg of EDTA and plasma was separated within 3 h after collection by centrifugation at 3,000 × *g* for 20 min. Plasma was stored at –20°C. Concentrations of FSH, LH, T, and E_2 were quantified in individual samples.

Experiment 5

Boars (15 mo old) from the I (n = 8) and C lines (n = 6) were used. Boars from the I line were offspring of sows with the greatest index, and boars from the C line were randomly selected. Boars were fitted with indwelling jugular catheters via ear vein cannulation techniques as described by Kelly (1986).

Blood samples (4 mL) were collected at 15-min intervals for 24 h and were mixed with 15 mg of EDTA. Plasma samples were separated by centrifugation at 3,000 × *g* for 20 min. Plasma was stored at –20°C. Concentrations of FSH, LH, and T were quantified for individual samples. A composite sample was made from samples collected every 15 min over 24 h for each boar. Composite samples for each day were used for quantification of circulating E_2. Characteristics of LH pulses were analyzed for each boar with procedures described in Statistical Analyses.

Experiment 6

Boars used in this experiment were from the first generation of a cross of a relaxed selection line and a

litter size line (RL =13) or from a respective C line (n = 14). All lines were from the University of Nebraska Gene Pool Population (Zimmerman and Cunningham, 1975). All lines were derived from the same base generation. The relaxed selection line had been selected for nine generations for increased number of ovulations (Cunningham et al., 1979) followed by 13 generations of random selection. The litter size line was derived from a line selected for number of ovulations for nine generations followed by two generations of random selection. The litter size line was then selected for eight generations for increased number of pigs per litter (Lamberson et al., 1991) followed by two generations of random selection. For 22 generations, pigs from the C line that were chosen to produce pigs in subsequent generations were selected at random.

Two single blood samples (4 mL) were collected 2 wk apart from mature boars (15 mo of age). Blood samples were collected via jugular venipuncture into tubes containing 15 mg of EDTA and placed immediately on ice. Samples were stored on ice for 2 h before centrifugation at 3,000 × *g* for 20 min. Plasma was aspirated, divided into aliquots, and stored at –20°C until hormone assays were performed. Concentrations of FSH, LH, T, and E_2 were determined in all samples.

Hormone Assays

Plasma FSH was quantified in duplicate 300-μL aliquots with a homologous double-antibody RIA described by Bolt (1981). Antiserum to FSH (USDA-398-04P, pFSHβ antiserum) was kindly provided by D. J. Bolt (USDA Animal Hormone Program, Beltsville, MD) and was used as first antibody (Guthrie and Bolt, 1983). Highly purified porcine FSH (USDA-pFSH-I 1), also provided by D. J. Bolt, was used as standard and for iodination. The FSH was labeled with ^{125}I using the iodogen (1,3,4,6-tetrachloro-3α, 6α-diphenylglycoril) coated vial method (Fraker and Speck, 1978), and the free ^{125}I was separated on a Bio-Gel P-100 column. Donkey anti-rabbit gamma globulin was used as second antibody. The assay was validated as follows. Serial dilutions of three different pooled samples were assayed at volumes of 200, 300, 400, and 500 μL. Assay determinations from these dilutions from each of the three samples were correlated (r = .97). Recovery of added mass (.2, .4, .8, and 1.6 ng of FSH) from 300 μL of sample averaged 90%. Intra- and interassay CV were 2.7 and 14.9%, respectively.

Concentrations of LH in plasma were analyzed with a RIA (Niswender et al., 1970; Wolfe et al., 1989b). Intra- and interassay CV were 2.5 and 13.6%, respectively. Plasma concentrations of T and E_2 were determined with RIA (Grotjan and Steinberger, 1978a,b; Redmer et al., 1984, respectively). Intra- and interassay CV for T assays were 2.4 and 10.9%, respectively. Intra- and interassay CV for E_2 assays were 4.0 and 15.0%, respectively.

Statistical Analyses

In Exp. 1 and 2, the fitted model included line as a fixed effect and boar within line as a random effect. In Exp. 3, fixed effects fitted in the model were line and period of blood collection, and boar was a random effect. In Exp. 4, the fitted model included line, period of blood collection, and ovulation rate as fixed effects, and boar was a random effect. In Exp. 5, the fitted model included line as a fixed effect and boar within line as a random effect. In Exp. 6, line and period of blood collection were included as fixed effects and boar within line was included as a random effect.

All data were analyzed with PROC MIXED of SAS (1992) using mixed-model procedures. To account for the covariance between observations from the same animal at different periods of blood collection in Exp. 3, 4, and 6, the different options of covariance structure for residuals available in the Repeated Statement of PROC MIXED were used, and the model with the best fit was chosen as the final model. Models were compared using likelihood ratio tests (Dobson, 1990). Means were compared with the *t*-test of PROC MIXED of SAS (1992). Because of unequal variances between treatment groups, mean concentrations of FSH in boars from Exp. 4 and 5 were transformed to logarithms (SAS, 1985).

The pattern of secretion of LH for boars in Exp. 5 was characterized by determination of mean concentration, frequency, and amplitude of pulses of FSH and LH. Characteristics of FSH and LH were determined with the Pulsar algorithms (Pulsar software modified for IBM-PC by J. F. Gitzen and V. D. Ramírez, Urbana, IL). Values of G for FSH were G(1) 3.2, G(2) 2.8, G(3) 2.5, G(4) 10.0, and G(5) 10.0. Values of G for LH were G(1) 100.0, G(2) 2.4, G(3) 2.0, G(4) 1.8, and G(5) 100.0.

Results

Experiment 1

Data for mean concentrations of FSH in boars from TS and C lines at the different periods of blood collection are in Table 2. Concentrations of FSH before administration of LHRH were similar ($P > .10$) in boars of both lines while they were intact, castrated, and after implantation of E_2. Concentrations of FSH were similar ($P > .10$) in boars from TS and C lines after administration of LHRH at all periods of blood collection.

Experiment 2

Mean concentrations of FSH were similar ($P > .10$) during all three periods in boars from both lines (Table 3). Release of FSH in response to administration of LHRH was similar ($P > .10$) in boars of both

Table 2. Mean concentrations of follicle-stimulating hormone (FSH) before and after administration of luteinizing hormone-releasing hormone (LHRH) in boars selected for size of testes (TS) and in a randomly selected control line (C; Exp. 1)

Selection line	FSH, ng/mL[a]	
	Before LHRH[b]	After LHRH[c]
	Intact[d]	
TS	.25 ± .13	.32 ± .14
C	.27 ± .12	.37 ± .13
	Castrated[e]	
TS	.77 ± .14	.97 ± .17
C	.79 ± .16	.96 ± .20
	Implant of E_2[f]	
TS	.72 ± .16	.77 ± .14
C	.75 ± .18	.76 ± .17

[a]Least squares means ± SE.
[b]Mean concentration of FSH from samples taken hourly for 12 h before administration of LHRH (188 ng/kg BW).
[c]Mean concentration of FSH from samples taken every 12 min for 1 h after administration of LHRH.
[d]Blood collection in intact boars.
[e]Blood collection at 14 d after castration.
[f]Blood collection at 7 d after implantation of estradiol (E_2; 21 d after castration).

lines when intact and after castration. Concentrations of FSH were similar ($P > .10$) in boars from TS and C lines during anesthesia and after administration of four injections of LHRH at 1-h intervals.

Experiment 3

There were no differences in concentrations of FSH, LH, and T in pigs from the I and C lines (Table 4). Concentrations of FSH, LH, and T in circulation changed during the 1st mo after birth (Table 4). Mean concentrations of FSH and LH were greater the first 2 wk after birth than in the next 2 wk. Concentrations of T were greater ($P < .05$) at 2 and 3 than at 1 wk of age. By 4 wk after birth, concentrations of T were similar to those detected during the 1st wk after birth. For concentrations of E_2, there was an age × line interaction ($P < .05$). Mean concentrations of E_2 were greater in boars from the I line than in those from the C line during the 1st wk after birth. Concentrations of E_2 were greater ($P < .05$) at 2 and 3 than at 1 and 4 wk of age.

Experiment 4

Ovulation rate classification within line of boars (high or low) did not affect concentrations of any of the hormones analyzed ($P > .10$). This effect was deleted from the mathematical model, which then had line and age as main effects. Data for mean concentrations of FSH and LH from boars of the different lines are given in Table 5. Transformed means were not different for boars from I and C lines (–1.52 ± .19 and –1.31 ± .19, respectively). The analysis of log-transformed data indicated that concentrations of FSH were affected ($P < .05$) by period of collection. Concentrations of FSH were lower ($P < .05$) at 2 mo than at 4 or 5 mo of age, and concentrations varied little from 3 to 6 mo of age.

There was an interaction ($P < .05$) between line and age for concentrations of LH. Concentrations of LH were greater in boars from the I than C line at 2 mo of age, but they were similar thereafter up to 6 mo of age.

Data for concentrations of T are given in Table 5. There was an interaction ($P < .05$) between line and period for concentrations of T in circulation during the first 6 mo after birth. Concentrations of T were greater ($P < .05$) at 2, 3, and 4 than at 1 mo of age and further increased ($P < .05$) at 5 and 6 mo of age in pigs of both lines. The overall mean concentrations of E_2 differed ($P < .05$) between lines; pigs from the C line had greater concentrations of E_2 than pigs from the I line. Concentrations of E_2 were greater ($P < .05$) at 4, 5, and 6 than at 1, 2, and 3 mo of age in boars of both lines (Table 5).

Table 3. Mean concentrations of follicle-stimulating hormone (FSH) before and after administration of luteinizing hormone-releasing hormone (LHRH) in boars selected for size of testes (TS) and a randomly selected control line (C; Exp. 2)

Selection line	FSH, ng/mL[a]	
	Before LHRH[b]	After LHRH[c]
	Intact[d]	
TS	.05 ± .06	.09 ± .04
C	.14 ± .05	.08 ± .03
	Castrated[e]	
TS	1.33 ± .21	1.52 ± .19
C	1.14 ± .19	1.42 ± .17
	Anesthesia[f]	
TS	1.07 ± .38	
1st		1.37 ± .41
2nd		1.42 ± .46
3rd		1.59 ± .49
4th		1.58 ± .42
C	1.31 ± .32	
1st		1.78 ± .36
2nd		1.93 ± .40
3rd		2.05 ± .42
4th		1.82 ± .36

[a]Least squares means ± SE.
[b]Mean concentration of FSH before administration of LHRH.
[c]Mean concentration of FSH after administration of LHRH.
[d]Blood collection in intact boars.
[e]Blood collection 14 d after castration.
[f]Blood collection during anesthesia before and after the first to fourth injections of LHRH (188 ng/kg BW) was at intervals of 1 h.

Table 4. Mean concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), and 17β-estradiol (E_2) during the first month of age in male pigs from a line selected on an index for number of ovulations and embryonal survival (I) and in a randomly selected control line (C; Exp. 3)

	Weeks after birth				
Item	1	2	3	4	$\bar{x}$ ± SE
	FSH, ng/mL[a]				
I	.34	.32	.20	.19	.3 ± .02
C	.37	.42	.26	.17	.3 ± .02
Mean[b]	.36^{A}	.37^{A}	.23^{B}	.18^{B}	—
	LH, ng/mL[a]				
I	1.86	1.98	1.61	1.67	1.8 ± .1
C	1.93	2.29	1.47	1.55	1.8 ± .1
Mean[b]	1.88^{AB}	2.21^{A}	1.54^{B}	1.61^{B}	—
	T, ng/mL[a]				
I	.64	1.10	1.60	.70	.9 ± .06
C	.74	1.30	1.33	.68	1.0 ± .06
Mean[b]	.69^{B}	1.19^{A}	1.24^{A}	.70^{B}	—
	E_2, pg/mL[a]				
I	7.91	11.16	9.45	4.55	8.3 ± .7
C	2.73	8.11	8.82	7.28	6.3 ± .7
Mean[b]	5.32^{B}	9.64^{A}	9.13^{A}	5.93^{B}	—

[a]Least squares means.
[b]Mean of endocrine data pooled across lines during each period of blood sampling.
^{A,B}Means with differing superscripts within rows differ ($P < .05$).

Table 5. Mean concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), and 17β-estradiol (E_2) in weaned male pigs from generation 10 in a line selected on an index for number of ovulations and embryonal survival (I) and in a randomly selected control line (C; Exp. 4)

	Months after birth						
Item	1	2	3	4	5	6	$\bar{x}$ ± SE
	FSH, ng/mL[a]						
I	.23	.13	.24	.27	.23	.23	.22 ± .05
C	.18	.19	.28	.34	.39	.33	.27 ± .05
Mean[b]	.20^{AB}	.16^{B}	.27^{AB}	.30^{A}	.30^{A}	.27^{AB}	—
	LH, ng/mL[a]						
I	.44	.45^{c}	.29	.20	.21	.23	.31 ± .02
C	.48	.24^{d}	.30	.25	.28	.28	.31 ± .02
Mean[b]	.46	.34	.30	.26	.24	.25	—
	T, ng/mL[a]						
I	.49	.71	.97	.82^{d}	1.63	3.40	1.34 ± .18
C	.50	1.01	.75	1.44	1.60	2.28	1.26 ± .18
Mean[b]	.49^{D}	.86^{C}	.86^{C}	1.12^{C}	1.62^{B}	2.84^{A}	—
	E_2, pg/mL[a]						
I	12.9	10.3	11.3	15.5	27.5	24.1	16.7 ± .1^{d}
C	18.1	16.0	18.2	28.7	36.4	35.0	24.4 ± .1^{c}
Mean[b]	15.5^{A}	13.1^{A}	15.3^{A}	22.1^{B}	31.9^{C}	29.1^{CB}	—

[a]Least squares means.
[b]Mean of endocrine data pooled across lines during each period of blood sampling.
^{c,d}Means with differing superscripts within columns differ ($P < .05$).
^{A,B,C}Means with differing superscripts within rows differ ($P < .05$).

Table 6. Mean concentrations of follicle-stimulating hormone (FSH), testosterone (T), and 17β-estradiol (E_2) in mature boars of a line selected on an index for number of ovulations and embryonal survival (I) and a randomly selected control line (C; Exp. 5)

Hormone	Mean concentration[a]	
	I (n = 8)	C (n = 6)
FSH, ng/mL[b]	.22 ± .05[d]	.08 ± .06[e]
T, ng/mL[b]	4.7 ± 2.07	7.3 ± 2.23
E_2, pg/mL[c]	128.7 ± 40.00	116.0 ± 46.00

[a]Least squares means ± SE.
[b]Mean concentrations of samples taken every 15 min for 24 h.
[c]Mean concentrations of pooled samples of all samples taken in 24 h.
[d]Means with differing superscripts differ ($P < .05$).

Experiment 5

Data for mean concentrations of FSH are given in Table 6. No pulses of FSH were detected in boars from the I or C lines. Because the standard deviation was greater in the I than in the C line (.19 and .04 for I and C lines, respectively) statistical analyses were performed on the logarithm of concentrations of FSH. Results from this transformation yielded means of –1.79 ± .7 for boars from I and –2.7 ± .6 for the C line, and concentrations of FSH were greater ($P < .05$) in boars from the I than from the C line. Mean and basal concentrations and pulse amplitude of LH were not different ($P > .05$) among boars of the two lines. Boars from the I line, however, had greater ($P < .05$) numbers of LH pulses than boars from the C line (Table 7). Concentrations of T and E_2 were similar ($P > .05$) in boars of both lines (Table 6).

Experiment 6

Concentrations of FSH (.21 ng/mL of plasma) and LH (1.03 ng/mL of plasma) in boars from RL and C lines were not different ($P > .05$). Boars from the C line had greater ($P < .05$; 1.18 vs 2.51 ng/mL of plasma; SEM = .43) concentrations of T than boars from the RL line. Concentrations of E_2 in boars from RL and C lines were not different ($P > .05$; 66.24 ng/mL of plasma).

Discussion

Results from Exp. 1 and 2 indicated no important differences in circulating concentrations of FSH among boars from TS and C lines. This supports previous work (Schinckel et al. 1984) in which boars with larger testes had concentrations of FSH similar to those of boars with smaller testes during sexual maturation. Based on data from the present experiments, there are no differences in feedback regulation of 17β-estradiol on the hypothalamic-pituitary axis in modulation of FSH secretion between boars in the TS and C lines. There were also no differences between males of the two lines in response of pituitaries to LHRH in releasing FSH. Circulating concentrations of FSH and regulation of FSH secretion are similar, therefore, among boars of the TS and C lines. Interestingly, selection for size of testes in pigs had no effect on ovulation rate in genetically related females (Johnson et al., 1994).

Function of the gonads has been postulated to be controlled by the same genes in both sexes, even though males and females have very different reproductive functions (Land, 1973). The LH and FSH are involved in regulation of reproductive processes in males and females (Cole, 1969), and physiological and endocrine functions are regulated in both sexes by gonadal tissues that specifically bind FSH and LH. Enlargement of human testes during puberty was mainly due to the action of LH and(or) T on FSH-primed testes (August et al., 1972). Increased secretion of FSH in rams, however, may be partially related to testicular enlargement (Courot et al., 1975). The FSH is thought to act synergistically with LH to increase growth of testes. The present research indicates that differences in circulating FSH do not exist among adult boars from lines with large differences in size of testes.

Wolfe et al. (1989a,b), working with the same animals that were used in the present study, found that mean concentrations of LH were similar among boars of both lines. Response to administration of exogenous LHRH, however, was consistently less in boars from the TS than in those from the C line. Thus, they concluded that boars with larger testes had

Table 7. Mean concentration, basal concentration, amplitude, and frequency of luteinizing hormone (LH) pulses over a 24-hour period in mature boars of a line selected on an index for number of ovulations and embryonal survival (I) and a randomly selected control line (C; Exp. 5)

Selection line	Concentration of LH, ng/mL[a]	Basal LH, ng/mL[a]	LH amplitude, ng/mL[a]	LH pulses/24 h[a]
I (n=8)	.93	.80	.58	7.2[b]
C (n=6)	.94	.92	.46	2.5[c]

[a]Least squares means.
[b,c]Means with differing superscript within a column differ ($P < .05$).

altered hypothalamic control of hypophyseal synthesis and(or) release of LH. Selection for size of testes might have affected factors involved in release of LH but not FSH. Treatment with E_2 did not decrease the secretion of FSH in boars of either line; thus, regulation of FSH secretion may require the synergistic actions of T in addition to E_2 (Koppelman and Loriaux, 1983) and other factors produced by the testis such as inhibin (Hasegawa et al., 1988).

Growth of the testes likely involves several sets of genes; thus, changes in several factors may increase the size of testes. By examining previous and present results, variations in size of testes among boars cannot be explained by variation in concentrations of FSH in sexually mature boars.

Changes in secretion of FSH during neonatal and pubertal periods in boars from the I and C lines in the present study were consistent with those reported by Colenbrander et al. (1982) and Kosco et al. (1987). Peaks in concentrations of FSH in plasma were detected during the first 2 wk after birth, they decreased thereafter, and then they remained at fairly steady concentrations until pubertal development. In the present study, concentrations of FSH in mature boars from the I line were greater than in those from the C line. Selection for number of ovulations and embryonal survival in pigs of the I line increased secretion of FSH in mature boars. These results are consistent with those reported by Borg et al. (1993) in which boars of highly prolific Chinese breeds had greater concentrations of FSH in circulation than boars of occidental breeding.

Greater concentrations of LH in plasma were detected in the first weeks after birth and greater concentrations of T may have resulted from the increased LH; this confirms that during the early neonatal period negative feedback of T in modulation of release of LH is absent (Ford and Schanbacher, 1977). Increased T during the experimental period is related to increased numbers and degree of differentiation of interstitial cells in testes (Colenbrander et al., 1977). At 3 wk after birth, concentrations of LH were lower than earlier in the neonatal period, and this pattern of change paralleled changes in concentration of FSH during the 3 wk after birth. Results were similar in pigs from both lines (I and C) in the present study. The decrease in gonadotropins during the first 3 wk after birth is associated with a decrease in tubular growth and Leydig cells returning to the undifferentiated state (Van Straaten and Wensing, 1978).

During pubertal development in pigs used in the present study, pattern of LH in circulation was similar to that reported previously (Romanowicz et al., 1976; Allrich et al., 1982; Kosco et al., 1987). In mature boars, mean concentrations of LH were greater than at earlier ages but were not different between lines. Number of LH pulses, however, was greater in mature boars of the I than C lines, but basal concentrations of LH and amplitude of LH pulses were similar among boars of the two lines. Pattern of LH secretion differed as a result of increased frequency of LH pulses in boars from the I compared with those in the C line, but further research will be necessary to explain the reason for differences in number of LH pulses among boars of the two lines.

Cyclic patterns of T secretion occurred during the postnatal and prepubertal periods in the present study. These developmental changes are reflected by increases in concentrations of T from the 1st to 3rd wk after birth, decreases between the 3rd and 4th wk after birth, and progressive increases in concentrations of T over the next 5 mo. These results are in general agreement with results from previous research (Romanowicz et al., 1976; Colenbrander et al., 1978; Ford, 1983; Martin et al., 1984). Changes in concentration of T during maturation are related to changes in Leydig cell steroidogenic capacity, which results from morphological differentiation of testes (Colenbrander et al., 1978; Van Straaten and Wensing, 1978). Concentrations of T from pubertal development to maturity were not associated with changing concentrations of LH (Romanowicz et al., 1976); therefore, changes in LH during these periods may only be partially responsible for fluctuations in concentrations of T (Allrich et al., 1982; Lunstra et al., 1986).

Concentrations of E_2 in circulation during the first 3 wk after birth of male pigs decreases and stabilizes between 1 and 3 mo of age, and then it progressively increases until maturity (Ford, 1983). Similar changes in concentrations of E_2 in circulation were detected in pigs of the I line in the present study. In pigs from the C line, however, concentrations of E_2 were lower during the 1st wk after birth and increased thereafter, with greater concentrations of E_2 in pigs from the C than I line during the 4th wk after birth and from 4 to 6 mo after birth. The actions of E_2 during sexual maturation of boars may modulate T synthesis by testicles (Dorrington et al., 1978). Small changes in circulating concentrations of E_2 inhibit secretion of LH and testosterone in prepubertal boars (Kittok et al., 1984). Data from the present study indicate that selection for number of ovulations and embryonal survival altered patterns of secretion of E_2 during neonatal and pubertal development of boars from the I line.

In boars from the RL and respective C line, concentrations of FSH were not different; concentrations of FSH in boars of the RL line were similar to those of boars from the I line ($.23 \pm .07$ and $.22 \pm .05$). Of particular interest is the lack of difference in concentrations of FSH among boars of the RL line and respective controls. This is in contrast to previous reports (Borg et al., 1993) and data from the present study when concentrations of FSH of boars from the I

and C lines were compared. In both studies, boars from lines with a greater number of ovulations had greater concentrations of FSH; however, the RL line was also selected for a greater number of ovulations, but there was no difference in concentrations of FSH compared with those in boars of the respective C line. It is intriguing that gilts from the RL line have greater circulating concentrations of FSH during their estrous cycle than those of the C line (Kelly et al., 1988), but females of the I and C lines have similar circulating concentrations of FSH throughout the estrous cycle (Mariscal-Aguayo, 1994). Neither were there differences in concentrations of FSH in females of the high-ovulating Chinese pigs compared with concentrations of FSH in occidental pigs (Hunter et al., 1993). There were, therefore, no differences in circulating concentrations of FSH during the estrous cycles of females selected for a greater number of ovulations, whereas boars of these lines had greater FSH than boars of respective C lines. In contrast, females from the selection line (RL) had greater FSH than respective controls, but there was no difference in FSH among boars of the two lines (RL vs C lines). It will be interesting to determine whether the relationship of greater FSH in either females or males, but not both sexes, of lines selected for ovulation rate will hold true with evaluation of more lines of pigs. A greater number of ovulations in lines selected for this trait likely results from expression of different genes in the various studies. This would explain why differences in circulating concentrations of FSH exist between the sexes.

In summary, selection for size of testes did not influence secretion of FSH in adult boars. Selection for an increased number of ovulations and embryonal survival altered the pattern of secretion of E_2 before puberty, and this might have resulted in differences in secretion of FSH in mature boars. In Exp. 6, however, boars from a line selected for number of ovulations and litter size did not have circulating concentrations of FSH different from those of boars of the respective C line.

Implications

Selection for reproductive traits is usually based on records of females. Inclusion of a male trait or development of a practical criterion for the presence of genes that have a positive effect on female performance and can be evaluated in males would potentially increase the rate of response to genetic selection. Including concentrations of follicle-stimulating hormone in boars as a part of a selection index may enhance efficiency of selection for prolificacy in swine.

Literature Cited

Allrich, R. D., R. K. Christenson, J. J. Ford, and D. R. Zimmerman. 1982. Pubertal development of the boar: Testosterone, estradiol-17β, cortisol and LH concentrations before and after castration at various ages. J. Anim. Sci. 55:1139.

August, G. P., M. M. Grumbach, and S. L. Kaplan. 1972. Hormonal changes in puberty: III. Correlation of plasma testosterone, LH, FSH, testicular size, and bone age with male pubertal development. J. Clin. Endocrinol. 34:319.

Bolt, D. J. 1981. Development of a homologous radioimmunoassay for ovine follicle stimulating hormone: Studies after estrus, ovariectomy, estradiol and releasing hormone. J. Anim. Sci. 53: 730.

Borg, K. E., D. D. Lunstra, and R. K. Christenson. 1993. Semen characteristics, testicular size, and reproductive hormone concentrations in mature Duroc, Meishan, Fengjing, and Minzhu boars. Biol. Reprod. 49:515.

Casey, D., T. A. Rathje, and R. K. Johnson. 1994. Response to ten generations of index selection for components of litter size in swine. Proc. 5th World Congr. Genet. Appl. Livest. Prod. 17: 315.

Cole H. H. 1969. In: H. H. Cole and P. T. Cupps (Ed.) Reproduction in Domestic Animals. Academic Press, New York.

Colenbrander, B., F. H. de Jong, and C.J.G. Wensing. 1978. Changes in serum testosterone concentrations in the male pig during development. J. Reprod. Fertil. 53:377.

Colenbrander B., M. T. Frankenjuis, and C.J.G. Wensing. 1982. Male sexual development. In: D.J.A. Cole and G. R. Foxcroft (Ed.) Control of Pig Reproduction. Butterworths Scientific, London.

Colenbrander, B., T.A.M. Kruip, S. J. Dieleman, and C.J.G. Wensing. 1977. Changes in serum LH concentrations during normal and abnormal sexual development in the pig. Biol. Reprod. 17: 506.

Courot, M., M. de Reviers, and J. Pelletier. 1975. Variation in pituitary and blood LH during puberty in the male lamb. Relation to time of birth. Ann. Biol. Anim. Biochem. Biophys. 15:509.

Cunningham, P. J., M. E. England, L. D. Young, and D. R. Zimmerman. 1979. Selection for ovulation rate in swine: Correlated response in litter size and weight. J. Anim. Sci. 48:509.

Dobson, A. 1990. An Introduction to Generalized Linear Models. Chapman and Hall, New York.

Dorrington, J. H., I. B. Fritz, and D. T. Armstrong. 1978. Control of testicular estrogen synthesis. Biol. Reprod. 18:55.

Ford, J. J. 1983. Serum estrogen concentrations during postnatal development in male pigs. Proc. Soc. Exp. Biol. Med. 174:160.

Ford, J. J., and B. D. Schanbacher. 1977. Luteinizing hormone secretion and female lordosis behavior in male pigs. Endocrinology 100:1033.

Fraker, P. J., and D. C. Speck, Jr. 1978. Protein and cell membrane iodination with a sparingly soluble chloroamide, 1, 3, 4, 6-tetrachloro-3α, 6α-diphenylglycoluril. Biochem. Biophys. Res. Commun. 80:840.

Grotjan, H. E., Jr., and E. Steinberger. 1978a. A microscale method for liquid scintillation counting, applicable to radioimmunoassay of steroids and substances of comparable relative molecular mass. Clin. Chem. 24:1193.

Grotjan, H. E., Jr., and E. Steinberger. 1978b. On the relative biological and immunoreactive potencies of luteinizing hormone on the oestradiol benzoate-treated male rat. Acta Endocrinol. 89:538.

Guthrie, H. D., and D. J. Bolt. 1983. Changes in plasma estrogen, luteinizing hormone, follicle-stimulating hormone and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ during blockade of luteolysis in pigs after human chorionic gonadotropin treatment. J. Anim. Sci. 57:993.

Hasegawa, Y., K. Miyamoto, S. Iwamura, and M. Igarashi. 1988. Changes in serum concentrations of inhibin in cyclic pigs. J. Endocrinol. 118:211.

Hunter, M. G., C. Biggs, G. R. Foxcroft, A. S. McNeilly, and J. E. Tilton. 1993. Comparisons of endocrinology and behavioural events during the periovulatory period in Meishan and Large-White hybrid gilts. J. Reprod. Fertil. 97:4745.

Johnson, R. K., G. R. Eckardt, T. A. Rathje, and D. K. Drudik. 1994. Ten generations of selection for predicted weight of testes in swine: direct response and correlated response in body weight, backfat, age at puberty, and ovulation rate. J. Anim. Sci. 72: 1978.

Kelly, C. R. 1986. Characterization of follicle populations and gonadotropins during the estrous cycle in swine. M.S. Thesis. University of Nebraska-Lincoln.

Kelly, C. R., T. E. Socha, and D. R. Zimmerman. 1988. Characterization of gonadotropic and ovarian steroid hormones during the periovulatory period in high ovulating select and control line gilts. J. Anim. Sci. 66:1462.

Kittok, R. J., J. E. Kinder, and R. K. Johnson. 1984. Relationship between serum luteinizing hormone and estradiol in prepubertal boars. Theriogenology 21:303.

Koppelman, M.C.S., and D. L. Loriaux. 1983. Regulation of gonadotropin secretion in men. In: A. S. Bhatnagar (Ed.) The Anterior Pituitary Gland. Raven Press, New York.

Kosco, M. S., D. J. Bolt, J. E. Wheaton, K. J. Loseth, and B. G. Crabo. 1987. Endocrine responses in relation to compensatory testicular growth after neonatal hemicastration in boars. Biol. Reprod. 36:1177.

Lamberson, W. R., R. K. Johnson, D. R. Zimmerman, and T. E. Long. 1991. Direct responses to selection for increased litter size, decreased age at puberty, or random selection following selection for ovulation rate in swine. J. Anim. Sci. 69:3129.

Land, R. B. 1973. The expression of female sex-limited characters in the male. Nature (Lond.) 214:208.

Lunstra, D. D., J. J. Ford, R. K. Christenson, and R. D. Allrich. 1986. Changes in Leydig cell ultrastructure and function during pubertal development in the boar. Biol. Reprod. 34:145.

Mariscal-Aguayo, D. V. 1994. Reproductive Hormones in Pigs Selected for Reproductive Traits. Ph.D. Dissertation. University of Nebraska.

Martin, T. E., Jr., B. Baker, H. W. Miller, and T. F. Kellogg. 1984. Circulating androgen levels in the developing boar. Theriogenology 21:357.

Neal, S. M., R. K. Johnson, and R. J. Kittok. 1989. Index selection for components of litter size in swine: Response to five generations of selection. J. Anim. Sci. 67:1933.

Niswender, G. D., L. E. Reichert, Jr., and D. R. Zimmerman. 1970. Radioimmunoassay of serum levels of LH throughout the estrous cycle in pigs. Endocrinology 87:576.

Redmer D. A., R. K. Christenson, J. J. Ford, and B. N. Day. 1984. Effect of unilateral ovariectomy on compensatory ovarian hypertrophy, peripheral concentrations of follicle-stimulating hormone and luteinizing hormone, and ovarian venous concentrations of estradiol-17β in prepuberal gilts. Biol. Reprod. 31: 59.

Romanowicz, K., R. Stupnicki, B. Barcikowski, A. Madej, and M. Walach. 1976. Plasma testosterone and LH in growing boars. In: Tischner and J. Pilch. Proc. VIIIth Int. Cong. Anim. Reprod. A.I. Krakow. 3:89.

SAS. 1985. SAS User's Guide: Statistics (Version 5 Ed.). SAS Inst. Inc., Cary, NC.

SAS. 1992. Changes and Enhancements. Technical Report P-229. SAS/STAT Software. Cary, NC.

Schinckel, A. P., R. K. Johnson, and R. J. Kittok. 1984. Relationships among measures of testicular development and endocrine function of boars. J. Anim. Sci. 58:1255.

Van Straaten, H.W.M. and C.J.G. Wensing. 1978. Leydig cell development in the testis of the pig. Biol. Reprod. 18:86.

Wolfe, P. L., M. W. Wolfe, T. T. Stumpf, R. J. Kittok, R. K. Johnson, and J. E. Kinder. 1989b. Response to luteinizing hormone releasing hormone in postpubertal boars with large testes. Domest. Anim. Endocrinol. 6:211.

Wolfe, P. L., M. W. Wolfe, T. T. Stumpf, J. A. Stotts, M. L. Day, R. J. Kittok, R. K. Johnson, and J. E. Kinder. 1989a. Control of luteinizing hormone in postpubertal boars with large testes. J. Anim. Sci. 67:1334.

Zimmerman, D. R., and P. J. Cunningham. 1975. Selection for ovulation rate in swine: Population, procedures and ovulation response. J. Anim. Sci. 40:61.