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Negative Relationship Between Blood Concentrations of Follicle-Stimulating Hormone and Testicular Size in Mature Boars^{1,2,3}

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ABSTRACT: Relationships between blood concentration of FSH and testicular size and daily sperm production were evaluated with data obtained from five studies originally designed to investigate regulation of FSH secretion in Meishan (MS), White composite (WC), and crossbreds of these. A minimum of three blood samples/boar were obtained at greater than 4-d intervals for determination of FSH, and testes were obtained at castration or slaughter. In a random sample of boars, FSH was fivefold greater ($P < .01$) in MS than in WC boars ($n = 22/\text{group}$). Daily sperm production (DSP)/gram of testis (estimated by counting elongated spermatid nuclei in testicular homogenates) was similar in the groups, but testicular weight (TWT), adjusted for body weight, was less ($P < .01$) in MS than in WC, yielding lower total daily sperm production (TDSP; $P < .05$) in MS boars. In

four populations (one with MS, one with WC, and two with crossbreds; $n = 34$ males), boars were selected for extremes in FSH concentrations from larger groups. Across all populations, a threefold greater plasma FSH concentration was associated with a 32% smaller TWT ($P < .01$). Coincident with increased FSH, TDSP was 33% less ($P < .05$). In 48 MS \times WC boars that were selected for divergence in plasma FSH during pubertal development (4 to 6 mo of age), this divergence was retained at 1 yr ($P < .01$). Retrospectively, the divergence in FSH was also apparent at 2 and 8 wk of age ($P < .05$), and the boars with elevated FSH had smaller testicles, lower DSP, and lower TDSP ($P < .01$). These studies document a negative relationship in mature boars between FSH secretion and testicular size accompanied with decreased TDSP.

Key Words: Swine, Testes, FSH

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Introduction

Artificial insemination within U.S. swine production units and sales of semen are currently experiencing increased growth and demand. Because total daily sperm production correlates positively with testicular size in boars (Swierstra, 1968; Wilson et al., 1977;

Hemsworth et al., 1983; Rathje et al., 1995), testicular size becomes of greater economic importance. In boars from a line selected for testicular size, Huang and Johnson (1996) reported a 29% increase in sperm per ejaculate (semen collected daily for 3 wk at 1 yr of age), relative to control boars, in association with a 25% increase in testicular size. Sexually mature Fengjing and Meishan (MS) boars had testicles that were one-half the size of those in Duroc boars and produced one-half the number of sperm per ejaculate (Borg et al., 1993). In these two Chinese breeds, plasma concentrations of FSH were seven times greater than in the Durocs. Our objective was to evaluate the relationship between circulating FSH concentrations and testicular size in mature boars.

Materials and Methods

Animals

In the first study, the relationship between plasma FSH concentrations and testicular size was examined

¹A preliminary summary of a portion of these data was presented at the 1995 International Symposium on Swine in Biomedical Research, College Park, MD.

²Mention of names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the same by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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in sexually mature MS and White composite (**WC**; a line composed equally of Chester White, Landrace, Large White, and Yorkshire) boars ($n = 22$ /genetic group) selected at random from larger populations. Sperm production was evaluated in 17 of the MS and 13 of the WC boars. Jugular blood samples ($n = 3$) were collected at 4- to 10-d intervals before testicles were obtained at castration or slaughter. Mean age was 16.8 mo for MS and 14.5 mo for WC boars ($SEM = 1.4$ mo).

For the second study, boars were selected within genetic groups for divergence in plasma FSH. Initially, jugular blood samples were obtained when boars were 7 to 9 mo of age. Selected boars were retained for subsequent blood sampling and collection of testicles. The genetic groups included 14 MS from a pool of 27 boars, 8 WC boars from 110 individuals stratified within sire families, 6 $\frac{3}{4}$ MS \times $\frac{1}{4}$ WC from the 22 that were available, and 6 $\frac{1}{4}$ MS \times $\frac{3}{4}$ WC from a group of 20. Three jugular blood samples were obtained from the selected boars during the 2-mo period before collection of testicles. At castration, mean age was 10.9, 16.5, 27.0, and 22.8 mo for MS, WC, $\frac{3}{4}$ MS \times $\frac{1}{4}$ WC, and $\frac{1}{4}$ MS \times $\frac{3}{4}$ WC boars, respectively, and was not influenced by FSH classification (19.4 vs 19.2 ± 1.0 mo). Sperm production was evaluated in 10 of the 14 selected MS boars and in all selected boars from the other three genetic groups.

In the third study, $\frac{3}{4}$ MS \times $\frac{1}{4}$ WC sires were mated to $\frac{1}{4}$ MS \times $\frac{3}{4}$ WC females, or the reciprocal combination, to produce $\frac{1}{2}$ MS \times $\frac{1}{2}$ WC boars. Jugular blood samples were obtained from each boar at 1, 2, 8, 16, 20, and 24 wk of age. Based on divergence in FSH at 16 to 24 wk, 48 boars from 10 sire families, 23 litters, were selected and reared to 1 yr of age. Each sire was represented by at least one boar classified as having high plasma FSH and one having low plasma FSH. An additional three blood samples were obtained at 55 to 58 wk of age, before collection of testicles, and the mean of these was designated as 55 wk for presentation.

Blood and Testicular Evaluations

All plasma samples were assayed for FSH concentration with previously established procedures (Trout et al., 1992). The antiserum was anti-ovine FSH #JAD17-679 in the first study and anti-ovine FSH #AFP-C5288113 in the second and third studies. The reference preparation for all studies was USDA-B1. For the first study, interassay CV were 17 and 7% for pools of porcine sera that assayed 262 and 1,100 ng FSH/mL. The interassay CV in the second and third studies were 16 and 13% for pools of porcine sera that assayed 272 and 1,370 ng FSH/mL. Testicles were obtained at slaughter or by castration of anesthetized boars. Anesthetic was either Telazol (A. H. Robins, Richmond, VA) or pentothal (Abbott Laboratories,

North Chicago, IL) followed by closed-circuit halothane (Halocarbon Laboratories, North Augusta, SC) and oxygen. Testicles were trimmed and weighed, and one was frozen for subsequent determination of sperm production by counting elongated spermatid nuclei in testicular homogenates (Amann and Almquist, 1961; Rathje et al., 1995).

Statistical Evaluations

For all studies, data were evaluated with GLM procedures of SAS (1990). In the first study, body weight was a covariate in the model that evaluated breed differences in testicular weight and total daily sperm production (**TDSP**). The model for the second study included genetic group, FSH classification (high or low), and the interaction between these two. Additionally, the distribution of mean FSH in the 110 WC boars was tested for normality with the univariate procedure. For the third study, the model included FSH classification blocked within sire family or FSH classification blocked within litter. When an *F*-test indicated statistical significance ($P < .05$), comparisons were conducted with the Bonferroni procedure. Data are presented as least squares means \pm SEM.

Results

Plasma concentrations of FSH were five times greater in MS than in WC, but body weight, testicular weight, and TDSP were significantly less (Table 1). At a constant body weight, testicular size remained smaller in MS than in WC (2.92 vs. 3.85 g/kg; $P < .01$).

For the second study, boars were identified within four populations on the basis of divergence in plasma FSH concentrations. The group of WC boars was large enough to determine that plasma FSH was not ($P < .01$) a normally distributed, phenotypic trait for this population (Figure 1). Across all four populations, testicular weight differed by 1.6 times, in association with the selected difference in plasma FSH (201 vs 611 ± 34 ng/mL; $P < .01$; Figure 2). This relationship held for all four populations of boars, and body weight within genetic line was not influenced by FSH classification ($P > .10$). The relative difference in FSH between high and low boars was 2.4 for MS, 3.0 for WC, 4.0 for $\frac{3}{4}$ MS \times $\frac{1}{4}$ WC, and 3.3 for $\frac{1}{4}$ MS \times $\frac{3}{4}$ WC. In the boars with high plasma FSH, TDSP was less ($P < .01$) than in those with low FSH (13.8 vs $20.5 \pm 1.7 \times 10^9$ /boar), but daily sperm production/gram of tissue (**DSP/g**) was not influenced ($P > .10$) by FSH classification (35.1 vs $34.1 \pm 2.6 \times 10^6$ /g).

Crossbred MS \times WC boars that had 4.6-fold greater FSH during pubertal development maintained a 2.9-fold advantage at 55 wk ($P < .01$; Figure 3). These boars with high FSH at 4 to 6 mo of age also had greater ($P < .05$) plasma FSH at 2 and 8 wk of age.

Table 1. The interrelationships of plasma FSH concentration, body weight, testicular weight, and daily sperm production in sexually-mature Meishan and White Composite boars

Genetic group	Body wt, kg	Plasma FSH, ng/mL	Testicular wt, g	DSP/g ^a	TDSP ^b
MS	115	704 ^c	324.8 ^c	21.6	7.3 ^d
WC	147	136	547.4	20.4	10.7
SEM	6	48	24.7	1.3	.9

^aDSP = daily sperm production, 10⁶ per gram of testicle.

^bTDSP = total daily sperm production, 10⁹ per boar.

^cMS differs from WC; *P* < .01.

^dMS differs from WC; *P* < .05.

Testicular weight, independent of body weight, decreased (*P* < .01) in association with this increase in plasma FSH (Figure 4), as did DSP/g of testis and TDSP (Figure 5). The reduction in testicular size (39%) and DSP/g (27%) produced a dramatic reduction in TDSP (56%). When data were restricted to the 16 litters that included at least one boar with high plasma FSH concentration and at least one other with low FSH (36 total boars), the conclusions did not differ from those summarized above.

Discussion

From the current observations, we conclude that testicular size and total daily sperm production are negatively related to plasma FSH when boars were selected on the basis of divergence in plasma FSH concentrations. This confirms breed differences

reported by Borg et al. (1993) and extends this relationship to boars within five different genetic combinations. The mating of $\frac{3}{4}$ MS \times $\frac{1}{4}$ WC boars to $\frac{1}{4}$ MS \times $\frac{3}{4}$ WC females or the reciprocal combination maximized the opportunity to establish extreme phenotypic differences. Such extremes in phenotypes likely account for the FSH-associated decrease in DSP/g that was observed in the $\frac{1}{2}$ MS \times $\frac{1}{2}$ WC boars but not in the other groups of boars.

The number of Sertoli cells determines subsequent testicular size (Steinberger and Steinberger, 1971; Orth, 1982). Proliferation of Sertoli cells in boars occurs during the 1st mo of life when FSH is elevated, and maturation of Sertoli cells continues through early puberty with formation of the blood-testis barrier by 4 mo of age (Tran et al., 1981; Putra and Blackshaw, 1985; Kosco et al., 1987, 1989). In MS \times WC crossbred boars, the timing of these events should occur earlier due to earlier age of puberty in MS boars

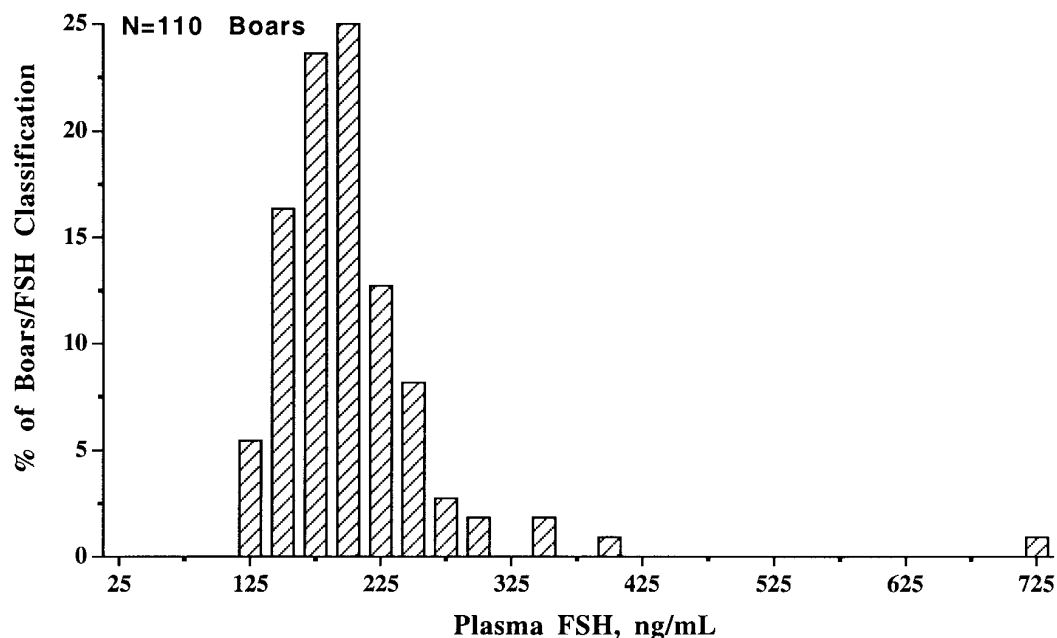


Figure 1. Distribution of boars based on mean of three plasma FSH determinations/boar conducted at 7 to 9 mo of age. Boars were classified into groups at increments of 25 ng/mL.

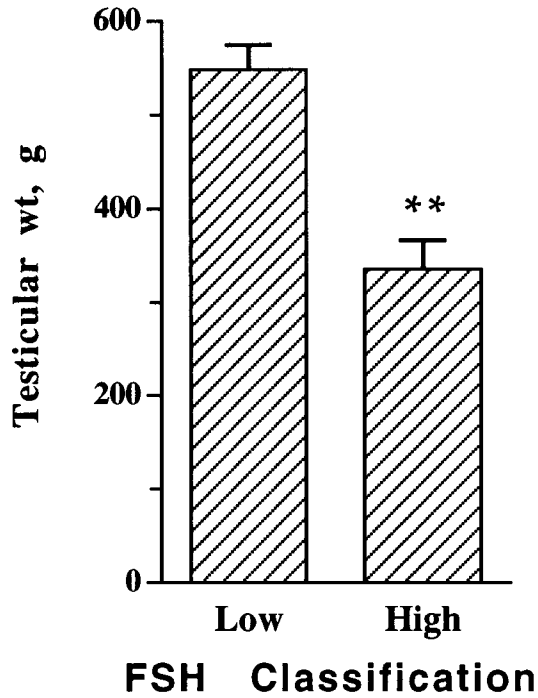


Figure 2. Testicular weights in sexually mature boars that were selected for divergence in plasma FSH (n = 17 boars/FSH classification).

(Harayama et al., 1991; Lunstra et al., 1993). Treatment of boars with exogenous FSH from 8 to 40 d of age increased the length of seminiferous tubules at 100 d of age and, theoretically, would produce larger

testicles (Swanlund et al., 1995). Furthermore, rats treated with FSH during the period of Sertoli cell proliferation had larger testicles as adults (Meachem et al., 1996). Thus, failure of 2- to 8-wk-old MS × WC boars with high FSH to develop large testicles is contrary to expectations.

In contrast to identification of extremes in FSH secretion, boars can be selected for increased testicular size. This procedure produced an increase in testicular size (Rathje et al., 1995; Huang and Johnson, 1996), but FSH secretion was not affected during the early generations of selection (Schinckel et al., 1984; Mariscal et al., 1996). Selection of males differed between these studies and the current studies, and this likely accounts for the lack of association of testicular size with FSH in the earlier study. Because plasma FSH in mature boars is not distributed normally, boars with high FSH would have a lower probability of being selected at random than in our studies, in which selection was based on maximal divergence.

Although elevated FSH in mature boars may result from diminished sensitivity to negative feedback mechanisms, experimental evidence does not support this hypothesis (Wise et al., 1996). Boars with high FSH also had greater plasma LH and testosterone concentrations than those with low FSH. Accepting the limitations of the RIA for the alpha-subunit of inhibin, plasma inhibin concentrations did not differ between MS boars with high or low FSH (Wise et al., 1996). Furthermore, pubertal boars from a line in which ovulation rate of females increased through

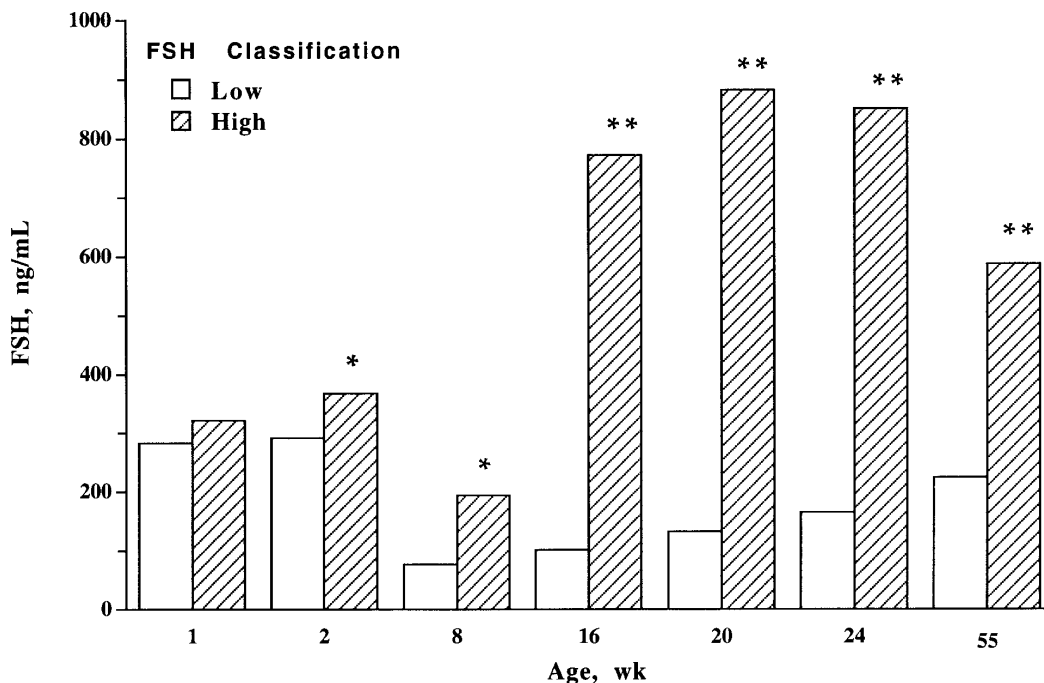


Figure 3. Plasma FSH concentrations in Meishan × White Composite boars that were selected within sire families for divergence in plasma FSH at 16 to 24 wk of age (n = 24 boars/FSH classification).

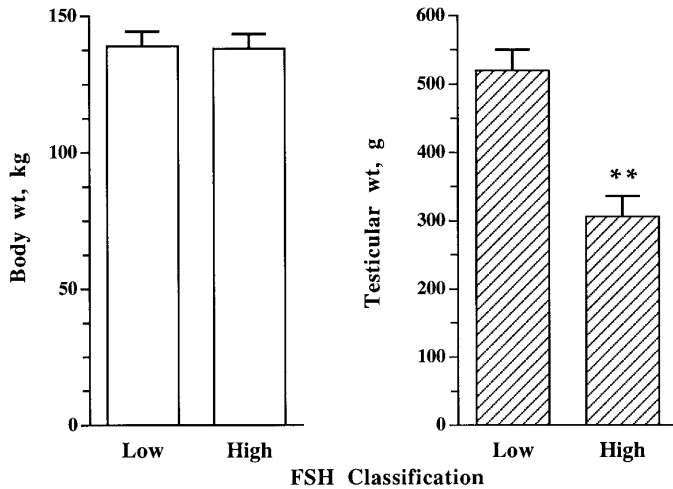


Figure 4. Body and testicular weights at 1 yr of age in Meishan \times White Composite boars that were selected within sire families for divergence in plasma FSH at 16 to 24 wk of age ($n = 24$ boars/FSH classification).

direct selection had higher plasma FSH and higher inhibin concentrations than boars in the respective control line (Cassady et al., 1995). Confirmation of these situations in which plasma FSH and inhibin lack the classical negative correlation must await more refined inhibin assays.

As an alternative, divergence in plasma FSH may result from differences in FSH synthesis and secretion in the face of similar feedback regulation. Historically, lack of absolute correlation between FSH and LH secretion fostered the search for an FSH-releasing hormone, but none has yet been identified within the hypothalamus. More recently, isolation of activin, a

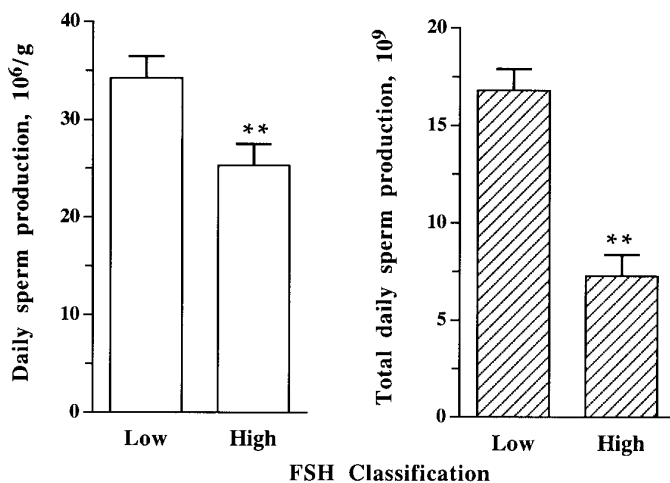


Figure 5. Sperm production/gram of testicle and total daily sperm production at 1 yr of age in Meishan \times White Composite boars that were selected for divergence in plasma FSH at 16 to 24 wk of age ($n = 24$ boars/FSH classification).

dimeric protein of the beta-subunits of inhibin, provides a means for intrapituitary regulation of FSH independent of LH (Corrigan et al., 1991; Bilezikjian and Vale, 1992; Weiss et al., 1995). Boars with high pituitary FSH concentrations have higher expression of the inhibin beta-B gene than boars with low FSH concentrations, and expression of the inhibin alpha subunit gene is nondetectable (Li et al., 1996). Thus, if inhibin beta-B mRNA is translated into protein, activin should exist within the anterior pituitary gland, not inhibin, and support greater FSH concentrations.

Returning to testicular development, why do not prepubertal boars with high FSH secretion develop large testicles? Studies with cultured rat Sertoli cells document that FSH and activin act synergistically to promote proliferation of Sertoli cells (Boitani et al., 1995). Thus, factors other than FSH may be limiting during neonatal development in boars destined to have small testicles, and this regulatory factor could be coupled, in some unknown manner, to FSH secretion. Furthermore, regulation of spermatogenesis by FSH is not fully understood (Zirkin et al., 1994). In contrast to our prediction, observations to date indicate that selection of boars with exceedingly high plasma FSH will lead to smaller testicles and reduced capacity to produce sperm. Whether this negative relationship between plasma FSH and sperm production in boars relates to conditions in infertile men who have elevated plasma FSH (Martin-du-Pan and Bischof, 1995) remains to be established.

Implications

Although follicle-stimulating hormone (FSH) is a positive regulator of Sertoli cell development and sperm production in males, FSH and sperm production were negatively related in mature boars due to smaller testicles in boars with high plasma FSH. Selection solely for greater plasma FSH concentrations in boars is not recommended at this time.

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