

Allelic variation in the secreted folate binding protein gene is associated with uterine capacity in swine¹

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ABSTRACT: Previous comparisons between the cDNA and gene sequences for secreted folate binding protein (sFBP) indicated a 12-bp insertion/deletion (ins/del) polymorphism in exon 1 and a SNP that altered (Ser-Arg) the protein AA sequence. The effect of the Ser-Arg SNP on reproductive traits was examined in three groups of Meishan-White European breed crossbred gilts. The gilts for all three groups were unilaterally hysterectomized-ovariectomized (UHO) at 100 d of age. Group 1 gilts (n = 77) were mated at estrus, slaughtered at d 105 of pregnancy, and a blood sample was collected from each fetus to determine fetal hematocrit. The number of corpora lutea and fetuses and the fetal and placental weights were recorded. Group 2 gilts (n = 46) were mated, the remaining uterine horn was flushed with 20 mL of saline on d 11 of pregnancy, conceptuses were counted, and flushings were measured for total sFBP. Gilts were allowed an estrous cycle to recover, mated again at estrus, slaughtered at 105 d of gestation, and the data as described for Group 1 were collected. Groups 1 and 2 gilts were genotyped for the Ser-Arg SNP. In Group 3, gilts (n = 70) and boars (n = 30) were genotyped for the Ser-Arg SNP

before mating, and like genotypes were mated. Gilts were then treated as described for Group 2. The effect of the 12-bp ins/del on reproductive traits was examined in 407 white crossbred UHO gilts from a randomly selected control line and from lines selected for ovulation rate (OR) and uterine capacity (UC). Gilts were mated and slaughtered at 105 d of age, and the numbers of corpora lutea and live fetuses, and fetal and placental weights and fetal hematocrits were recorded. The 12-bp ins/del also was evaluated in 131 intact gilts from the OR selected line. These gilts were mated at approximately 250 d of age and farrowed. The numbers of fully formed and live piglets were recorded. A significant effect ($P < 0.05$) of the Ser-Arg SNP was detected on the number of embryos present on d 11 of pregnancy and on UC. The sFBP 12-bp ins/del was associated with UC ($P < 0.01$) and the number of CL ($P < 0.05$) in UHO gilts, but not with litter size in intact gilts from the OR line. Results suggest that the 12-bp ins/del polymorphism could be exploited to increase litter size in swine, provided that the negative effect of the polymorphism on OR is overcome.

Key Words: Embryo, Erythropoiesis, Fetus, Folate, Pregnancy, Swine

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Introduction

With current technology, uterine capacity (UC), or the number of fetuses that can be maintained by the uterus until the end of gestation, represents the major limit to litter size in swine (Vallet, 2000). The Meishan is a Chinese breed of pigs known to have greater UC than European breeds (Haley and Lee, 1993). In addition,

using the unilaterally hysterectomized-ovariectomized (UHO) surgical model (Christenson et al., 1987) to measure UC, selection for UC increased UC by approximately one live fetus per uterine horn (Christenson and Leymaster, 2002). Thus, gene alleles associated with increased UC should be present in greater frequency in both Meishans and gilts selected for UC, although the same alleles may not occur in both populations. Previous reports from our laboratory have suggested that fetal erythropoiesis may influence UC (Pearson et al., 1998; Vallet et al., 2001, 2003), and folate is required for rapidly dividing tissues (Babior, 1990; Blount et al., 1997) such as the conceptus and fetal erythron. Folate transport to the conceptus involves secreted folate binding protein (Vallet et al., 1998, 1999a,b). Secreted folate binding protein seems to be a product of the endometrial glands (Kim and Vallet, 2004), but the contribution of the conceptus to

¹Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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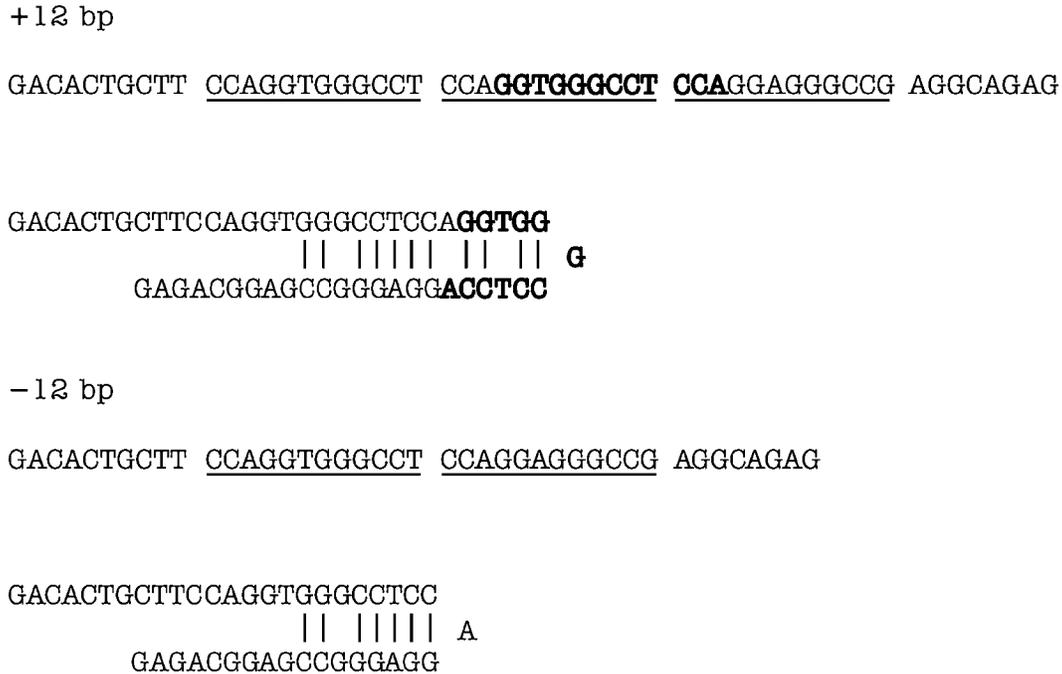


Figure 1. Sequence of exon 1 of secreted folate binding protein (Vallet et al., 2001) showing the presence or absence of the 12-bp (bold type) insertion deletion along with potential folding patterns for each structure. The underlined region indicates the repeated region of this exon.

intrauterine secreted folate binding protein (**sFBP**) is not known. Because of the role of sFBP in folate transport, polymorphisms in the sFBP gene are candidates for association with UC. A previous report (Vallet et al., 2001) suggested several sequence polymorphisms in the sFBP gene. Among these, one polymorphism was a SNP that coded for a difference (Ser-Arg) in the AA sequence of the sFBP protein. Another was a 12-bp insertion/deletion (**ins/del**) polymorphism in exon 1 that is part of a repeat region in the 5' untranslated region. The presence or absence of this polymorphism determines whether two or three repeats are present, and it may change the secondary structure of the sFBP mRNA (Figure 1) and thereby affect mRNA stability or translation efficiency. The objective of the current study was to determine whether either of these polymorphisms in the sFBP gene is associated with reproductive traits in swine.

Materials and Methods

The Secreted Folate Binding Protein Ser-Arg Single-Nucleotide Polymorphism

A genotyping assay using primer extension and mass spectrometry (Sequenom, San Diego, CA) was developed for the Ser-Arg sFBP SNP using the primers in Table 1. Using this assay, a preliminary survey of Meishan-White crossbred gilts, and crossbred gilts of Yorkshire, Landrace and Duroc mixed breeding indicated that this polymorphism was only present in gilts of Meishan descent. Thus, the effects of this genotype on

reproductive traits could only be evaluated in groups of Meishan-White crossbred gilts.

The collection of phenotypic data from Meishan-White crossbred Groups 1 and 2 was described in Vallet et al. (2002). The Meishan-White crossbred gilts used in this experiment are the result of several generations of inter se matings between Meishan-White crossbred gilts and boars. Briefly, for Group 1, UC, number of corpora lutea (**CL**), fetal hematocrits, fetal weights, and placental weights were recorded for 77 UHO gilts on d 105 of gestation. The UHO surgery involves the removal of one ovary and one uterine horn. Because of compensatory ovarian hypertrophy, ovulation rate (**OR**) is unaffected. After fertilization, this results in embryo numbers that exceed the capacity of the remaining uterine horn (Christenson et al., 1987). Thus, litter size in UHO gilts equals half the UC for that gilt. For Group 2, the number of embryos, CL, average embryo diameter, and total intrauterine sFBP on d 11 of pregnancy were recorded for 46 UHO gilts, the gilts were rebred, and the same information as that collected for Group 1 was collected on d 105 of gestation. Genomic DNA for each gilt in Groups 1 and 2 was isolated from tail tissue using the salt extraction method (Kappes et al., 2000), and was then used to determine the Ser-Arg SNP genotype for each gilt. For Group 3, an approximately equal number of gilts and boars of each genotype were generated by making specific matings between Meishan-White crossbred gilts and boars possessing specific Ser-Arg genotypes. Genomic DNA was isolated from tail tissue from select male (n = 30) and all female (n = 70) progeny of these matings and used to determine Ser-

Table 1. Primers used to genotype regions of the secreted folate binding protein (sFBP) gene

Polymorphism	Primer sequence ^a
Ser-Arg SNP	
Forward genotyping	agcggataacaatttcacacaggGCCCAGGAATTCTGATCTGAGG
Reverse genotyping	TCACCATCTCCGTCAAGTTGG
Universal biotinylated primer	AGCGGATAACAATTTACACACAGG
Probe primer	GAAGCGGTGGCAGGCGGC
12-bp insertion/deletion	
Forward genotyping	GGCCTGAAACTGAAAGACAAG
Reverse genotyping	CTTTCCTCCCGTCTCTG

^aLowercase letters indicate sequence of the biotinylated universal primer used for Sequenom genotyping assays at Roman L. Hruska U.S. Meat Animal Research Center. Uppercase letters are sFBP gene specific.

Arg SNP genotypes. Each gilt from these matings was UHO at 100 d of age, and then mated to a boar of like genotype at approximately 200 d of age. Surgery was performed on d 11 of pregnancy, each gilt was remated, again to a boar of like genotype, and slaughtered at d 105 of gestation. At surgery and at slaughter, data were collected as described for Group 2 gilts. In addition, fetal brain, liver, heart, and spleen weights were collected at 105 d of gestation. Gilts were mated to boars of like genotype to simplify the experiment, such that for homozygous gilts, litter genotypes also would be homozygous.

For Group 3 gilts, intrauterine sFBP was measured using a specific RIA (Vallet et al., 1999a). To ensure that the assay was valid for both the Ser and Arg forms of sFBP, parallelism and ability of the assay to measure added exogenous sFBP were compared for the two homozygous genotypes. Dilutions of uterine flushings from a homozygous Ser gilt and a homozygous Arg gilt were both parallel to the standard curve. Measurement of the same amount of added exogenous sFBP to both samples did not differ. Group 3 uterine flush samples were measured in a single assay with an intraassay CV of 11.6%.

The Secreted Folate Binding Protein 12-Base Pair Insertion/Deletion

To genotype this polymorphism, primers were developed flanking the region of the sFBP gene containing the 12-bp ins/del (Table 1). Genomic DNA samples were isolated from tail tissue using the salt extraction method, and then the genotyping primers were used to amplify genomic DNA from the UHO and intact gilts described below. The resulting products were predicted to be 169 and 181 bp, and were resolved and visualized by electrophoresis using a 10% polyacrylamide gel in Tris-borate-EDTA buffer followed by staining with ethidium bromide (Figure 2). A preliminary survey indicated that this polymorphism segregates in European breed pigs; thus, we chose to evaluate it in white crossbred gilts.

Collection of the phenotypic data for evaluation of the effect of this polymorphism was described by Chris-

tenson and Leymaster (2002). Briefly, white crossbred gilts from a randomly selected control line, a line selected for OR, and a line selected for UC were UHO at 160 d of age, and then mated at approximately 250 d of age at standing estrus. A total of 407 gilts were slaughtered at 105 d of gestation, the remaining uterine horn was recovered, opened, and a blood sample was obtained from each living fetus. Each fetus and placenta were then removed and weighed. Finally, the ovary was dissected to count the number of CL. Fetal blood samples were used to determine hematocrit.

Because we were interested primarily in genes affecting UC, we felt that litter size in intact gilts from the OR line, which has greater OR and therefore more potential embryos, also would provide a useful measure of UC. Thus, phenotypic information was used from intact gilts from the OR line, collected as described in Christenson and Leymaster (2002). Briefly, OR gilts were bred at approximately 250 d of age and farrowed. At farrowing, the number of fully formed piglets born and the number of piglets born alive were recorded.

Statistical Analyses

Embryo diameters, fetal hematocrits, fetal and placental weights, and fetal brain, liver, heart, and spleen

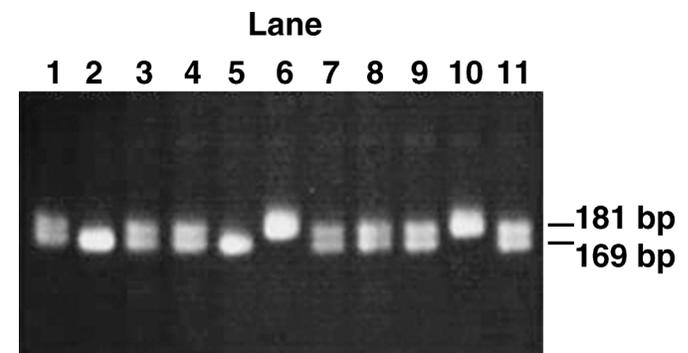


Figure 2. An image of a gel showing the bands resulting from PCR of 11 genomic DNA using the primers specific for the 12-bp insertion/deletion is illustrated. Lanes 2 and 5 are homozygous for the deletion. Lanes 6 and 10 are homozygous for the insertion. Lanes 1, 3, 4, 7, 8, 9, and 11 are heterozygous.

Table 2. Least squares means of secreted folate binding protein (sFBP) single-nucleotide polymorphism genotypes for reproductive traits measured on d 11 or 105 of pregnancy in Meishan-White crossbred gilts from Groups 1 and 2 combined

Variable	Genotype		
	Ser-Ser	Ser-Arg	Arg-Arg
d 11			
No. of observations	33	9	4
Corpora lutea	13.9 ± 0.5	14.7 ± 1.0	13.3 ± 1.5
No. of embryos	11.2 ± 0.7	11.0 ± 1.3	9.3 ± 1.9
Blastocyst average diameter, mm	2.8 ± 0.2	3.4 ± 0.4	3.1 ± 0.5
Total intrauterine sFBP, mg	5.9 ± 1.6	7.1 ± 3.2	11.5 ± 4.8
d 105			
No. of observations	104	14	5
CL	14.8 ± 0.3	15.1 ± 0.8	14.0 ± 1.2
Uterine capacity per uterine horn	6.5 ± 0.2	5.8 ± 0.7	6.0 ± 1.0
Fetal hematocrit, %	33.8 ± 0.4	33.6 ± 1.1	33.6 ± 1.7
Fetal weight (wet tissue), g	728 ± 18	782 ± 50	853 ± 80
Placental weight (wet tissue), g	153 ± 5	154 ± 14	216 ± 22 ^a

^aAdditive contrast (Ser-Ser vs. Arg-Arg) was significant, $P < 0.01$.

weights were averaged within each litter, and litter averages were used for further analyses. Data from Groups 1 and 2 of the Meishan-White crossbred gilts were combined and analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC), with a model that included effect of the Ser-Arg sFBP genotype. For Group 3, the effect of boar was nested within genotype, so the MIXED procedure of SAS was used to analyze the data using a model that included the effect of the Ser-Arg sFBP genotype as a fixed effect and the effect of the sire of the litter nested within Ser-Arg sFBP genotype as a random effect.

For the sFBP 12-bp ins/del in UHO gilts from the three selected lines, genotype frequency data between selected lines were analyzed by χ^2 analysis, and other data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The model included effects of sFBP genotype, selected line, season, and the line \times season interaction as fixed effects. The effect of the sire of the litter nested within the line \times season interaction, and the interaction between sFBP genotype and the sire of the litter within the line \times season interaction were included in the model as random effects.

The numbers of fully formed and live piglets born to intact OR gilts were analyzed using the MIXED procedure with a model that included the effects of season and the sFBP 12-bp ins/del genotype as fixed effects; the effect of the sire of the litter nested within season and the interaction between sFBP genotype and the sire of the litter within season were included as random effects.

For both polymorphisms, the following orthogonal contrasts were used to determine the additive and dominance effects for each locus: 1) additive: gilts that were homozygous for allele 1 were compared with gilts that were homozygous for allele 2; and 2) dominance: the average of gilts that were homozygous for allele 1 and

gilts that were homozygous for allele 2 was compared with gilts that were heterozygous.

Results

Results from Meishan-White crossbred gilts from Groups 1 and 2 combined and Group 3 are presented in Tables 2 and 3, respectively. For Groups 1 and 2, in which the genotype of the sire of the litter for the Ser-Arg sFBP SNP was unknown, the only significant effect of maternal genotype detected was on placental weight ($P < 0.05$). Gilts that were homozygous for the Arg allele had greater placental weight. However, only five gilts with this genotype were available in Groups 1 and 2 combined; thus, Group 3 was produced to give similar numbers of observations for each genotype, and to simplify the relationships between maternal and fetal genotypes. In Group 3, an additive effect of sFBP genotype on the number of embryos present on d 11 was observed ($P = 0.05$), indicating that the presence of the Arg allele was associated with a decrease in the number of embryos recovered on d 11 of pregnancy. This occurred with no effect on the number of CL on d 11. In addition, a significant additive effect of genotype ($P < 0.05$) on total intrauterine sFBP on d 11 of pregnancy was obtained. On d 105 of pregnancy, the significant additive effect of genotype on placental weight obtained for Groups 1 and 2 was not confirmed (Table 3). Finally, on d 105 of pregnancy, a significant dominance effect ($P < 0.05$) was observed for UC, indicating that UC was greater for heterozygous gilts than the average of the two groups of homozygous gilts, which did not differ. No other effects of the Ser-Arg sFBP genotype were detected in these gilts.

Results of the associations between the 12-bp ins/del and reproductive traits in UHO gilts from the three selected lines are summarized in Table 4. The frequency

Table 3. Least squares means of secreted folate binding protein (sFBP) single-nucleotide polymorphism genotypes for reproductive traits measured on d 11 or 105 of pregnancy in Group 3 Meishan-White crossbred gilts

Variable	Genotype		
	Ser-Ser	Ser-Arg	Arg-Arg
d 11			
No. of observations	24	20	26
Corpora lutea	13.8 ± 0.5	13.4 ± 0.6	13.1 ± 0.5
No. of embryos	11.5 ± 0.8	10.1 ± 0.9	9.1 ± 0.8 ^a
Blastocyst average diameter, mm	5.6 ± 0.6	4.9 ± 0.6	5.7 ± 0.6
Total intrauterine sFBP, mg	17.7 ± 4.6	20.4 ± 5.0	32.9 ± 4.2 ^a
d 105			
Corpora lutea	13.8 ± 0.6	12.9 ± 0.6	14.0 ± 0.6
Uterine capacity per uterine horn	5.6 ± 0.4	6.7 ± 0.4	5.1 ± 0.4 ^b
Fetal hematocrit, %	37.8 ± 1.0	36.5 ± 0.9	37.5 ± 0.9
Fetal weight (wet tissue), g	863 ± 39	882 ± 39	836 ± 37
Placental weight (wet tissue), g	229 ± 18	219 ± 17	205 ± 16
Fetal brain weight (wet tissue), g	26.9 ± 0.5	27.4 ± 0.5	26.7 ± 0.4
Fetal heart weight (wet tissue), g	8.0 ± 0.5	8.2 ± 0.4	7.7 ± 0.4
Fetal liver weight (wet tissue), g	24.4 ± 1.3	26.3 ± 1.3	24.7 ± 1.3
Fetal spleen weight (wet tissue), g	1.4 ± 0.1	1.7 ± 0.1	1.5 ± 0.1

^aAdditive contrast (Ser-Ser vs. Arg-Arg) was significant, $P \leq 0.05$.

^bDominance contrast (average of Ser-Ser and Arg-Arg vs. Ser-Arg) was significant, $P < 0.05$.

of the deletion allele was 17.6, 38.3, and 22.1% for the control, OR, and UC lines, respectively. Chi-square analysis indicated that the frequency of the deletion allele did not differ between the control and UC lines, but it was different ($P < 0.01$) between the control and OR lines. Gilts homozygous for the deletion of 12 bp in exon 1 had increased (additive contrast; $P = 0.01$) UC and decreased (additive contrast; $P < 0.05$) CL compared with gilts that were homozygous for insertion of the 12 bases. Dominance contrasts for these traits were not significant. No differences between sFBP 12-bp ins/del genotypes in the other traits were detected.

In contrast to the results from UHO gilts, no significant effects of the sFBP 12-bp ins/del on either the total

piglets born or the number born alive (Table 5) were detected for intact gilts from the OR line.

Discussion

This is the first report of polymorphisms in the sFBP gene associated with reproductive traits. Both polymorphisms were significantly associated with UC, although for the Ser-Arg SNP, a dominance effect was detected, whereas the effect of the 12-bp ins/del was additive. Although an effect of this polymorphism on UC was evident, there was no significant effect of the 12-bp ins/del on litter size in intact gilts from the OR selected line, although a trend similar to the effect of the poly-

Table 4. Least squares means of secreted folate binding protein (sFBP) 12-bp insertion/deletion genotypes (+ indicates presence of a 12-bp insertion) for traits measured on d 105 of pregnancy in unilaterally hysterectomized-ovariectomized gilts from the control, ovulation rate (OR), and uterine capacity (UC) selected lines

Variable	Genotype		
	++	+-	--
No. of gilts	215	169	23
No. of control gilts	95	49	1
No. of OR gilts	54	76	19
No. of UC gilts	66	44	3
Uterine capacity per uterine horn	6.8 ± 0.1	7.2 ± 0.2	8.0 ± 0.5 ^a
No. of corpora lutea	15.2 ± 0.2	15.2 ± 0.2	13.7 ± 0.6 ^b
Fetal hematocrit, %	37.2 ± 0.2	37.1 ± 0.2	36.5 ± 0.6
Fetal weight (wet tissue), g	783 ± 10	786 ± 12	797 ± 33
Placental weight (wet tissue), g	186 ± 4	186 ± 4	190 ± 11

^aAdditive contrast (++ vs. --) was significant, $P < 0.01$.

^bAdditive contrast (++ vs. --) was significant, $P < 0.05$.

Table 5. Least squares means of secreted folate binding protein 12-bp insertion/deletion genotypes (+ indicates the presence of the 12-bp insertion) for the number of fully-formed and the number of live piglets born to gilts from the ovulation rate (OR) selected line

Variable	Genotype		
	++	+-	--
No. of gilts	48	60	23
No. of fully formed piglets born	10.5 ± 0.4	11.1 ± 0.4	11.3 ± 0.6
No. of live piglets born	10.0 ± 0.4	10.4 ± 0.4	10.2 ± 0.6

morphism on UC was observed. The Ser-Arg SNP was also associated with the number of embryos recovered on d 11 of pregnancy, but the differences in this trait between genotypes are unlikely to explain the effect of this locus on UC. Nevertheless, the effects of sFBP polymorphisms on OR, the number of embryos recovered on d 11 of pregnancy, and litter size in UHO gilts on d 105 of pregnancy suggest that sFBP may play roles in follicle development, fertilization rate, and/or early embryo survival and UC.

Numerous chromosomal regions contain variations that have been associated with litter size or UC in swine, including SSC 6 (Wilkie et al., 1999), 8 (Rohrer et al., 1999; King et al., 2003) and 11 (Cassady et al., 2001). In addition, polymorphisms in several genes have been associated with either litter size or UC. These include the estrogen receptor (Rothschild et al., 1996), prolactin receptor (Rothschild et al., 1997; Van Rens et al., 2002), retinol binding protein (Rothschild et al., 2000), and the erythropoietin receptor (Vallet et al., 2005) genes. The sFBP gene maps to SSC 9 (J. L. Vallet, G. A. Rohrer, and B. A. Freking, unpublished observations), and it therefore does not correspond to any of the chromosomal regions previously identified. This is not surprising, considering that phenotypic measurements of litter size alone would be unlikely to detect associations with this locus because of opposing effects of the locus on the components of litter size (Bennett and Leymaster, 1989). Only Rohrer et al. (1999) performed association analysis with the litter size component trait of UC; however, because the Ser-Arg polymorphism is not fixed in Meishans (J. L. Vallet and B. A. Freking, unpublished observations), and because of the dominance effect of the Ser-Arg locus on UC, associations would be unlikely to be detected using the methods of Rohrer et al. (1999).

The sFBP Ser-Arg and 12-bp ins/del polymorphisms are likely to cause different changes in sFBP gene function, potentially explaining the various associations of each with component reproductive traits. The Ser-Arg SNP changes both the mRNA sequence and the AA sequence of the sFBP protein, and thus could have a variety of effects on gene function. A significant effect of the Ser-Arg SNP on total intrauterine sFBP content was detected in Group 3 gilts, and a similar trend was present in gilts from Groups 1 and 2, indicating that along with a change in AA sequence, the polymorphism

also increased the amount of sFBP protein. The validation of the sFBP assay for the two different forms indicated that the assay measured each form of sFBP equally, so differences in assay reactivity do not seem to explain the differences between genotypes. It is possible that the polymorphism could affect translation efficiency by changes in codon usage. The Ser codon found in the sFBP Ser allele represents only 7% of Ser codons in a sample of porcine genes, whereas the Arg codon of the Arg allele represents 65% of the Arg codons from the same sample of genes (from <http://www.kazusa.or.jp/codon/>, Nakamura et al., 2000). Codon usage affects protein translation, but the effect of a specific change on translation is complex (Kim et al., 1997; Duan et al., 2003). It is possible that the sFBP mRNA containing the Arg allele is translated more efficiently than the Ser allele. Alternatively, by changing the protein sequence, the SNP could alter the susceptibility of the sFBP protein to degradation by proteases, thereby changing the static amount of sFBP present in the intrauterine lumen at the time the uterus was sampled. Finally, in addition to the effect of the SNP on overall protein amount, the change in AA sequence could affect protein function, such as folate binding affinity. Further biochemical characterization of the mRNA and proteins produced from sFBP genes containing the Ser and Arg polymorphisms are necessary to fully understand how this SNP affects folate transport.

In contrast to the Ser-Arg polymorphism, the 12-bp ins/del occurs in the 5' untranslated region of the sFBP mRNA. Thus, this polymorphism could influence either transcription of the gene or translation or stability of the mRNA. Interestingly, previous results indicated that the amount of sFBP protein within the uterine lumen during early pregnancy was not significantly correlated with endometrial sFBP mRNA levels (Vallet et al., 1999a,b), suggesting that sFBP intrauterine protein concentrations may primarily be controlled by translation of the mRNA or by secretion of the protein. Whether mRNA transcription, translation, or susceptibility to degradation is increased or decreased by each allele remains to be investigated.

Alternatively, the effects on reproductive traits observed for both the Ser-Arg SNP and the 12-bp ins/del in this experiment could be accounted for by linkage disequilibrium to other functional polymorphism(s). The sFBP gene is one of a family of related folate recep-

tor genes (including a placental membrane folate receptor gene; Vallet et al., 1999b, 2001; Kim and Vallet, 2004) that are within a short distance from each other (Vallet et al., 2001). Because of linkage disequilibrium, polymorphisms in these or other nearby genes also could be responsible for the significant effects reported here.

An effect of the Ser-Arg locus on the number of embryos on d 11 of pregnancy was detected in Group 3 gilts, although a similar but nonsignificant trend was present in gilts from Groups 1 and 2. This occurred despite no significant effects on OR, leaving differences in fertilization rate or embryonic survival as the only remaining explanations. In Group 3, the boars were the same genotype as the gilt; thus, the genotype could have influenced sperm function in the male. Nonetheless, given the presence of a similar trend in Groups 1 and 2 gilts, where the boar genotype was unknown, this explanation seems unlikely. A more likely explanation is that some aspect of oocyte development (subsequently affecting fertilization rate) or embryo survival was affected by the Ser-Arg locus. Although there is currently no evidence for a role for sFBP in follicle development, expressed sequence tags corresponding to this gene have been isolated from ovarian RNA libraries (www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=pig).

The difference in UC between heterozygous and homozygous gilts for the Ser-Arg SNP was observed in Group 3 gilts, but not in Groups 1 and 2 combined. The difference in results may be due to differences in the numbers of observations for each genotype in the different groups. Because of the low frequency of the Arg allele in the population of Meishan-White crossbred gilts from which the Groups 1 and 2 gilts were sampled, the numbers of observations for heterozygous and homozygous Arg gilts may have been insufficient to detect differences in UC in Groups 1 and 2.

The dominance effect of the Ser-Arg locus on UC is difficult to explain. Although the Ser-Arg locus also was associated with the number of embryos present on d 11 of pregnancy, this cannot be used to explain the UC results because the average number of embryos on d 11 for all three genotypes still exceeded average UC by 3 to 4 embryos. Because it seems clear that sFBP genotype affects more than one trait, the results could be due to a combination of negative and positive influences of the genotype on various mechanisms that together determine UC. To fully understand how the Ser-Arg SNP affects UC, a great deal more information on the role of sFBP in conceptus development, and the differences between the Ser and Arg forms of sFBP with regard to susceptibility to degradation and folate affinity are needed.

In contrast to the Ser-Arg locus, the 12-bp ins/del locus had an additive effect on UC and is thus of potential value for marker-assisted selection for litter size. Unfortunately, it also had an opposing additive effect on OR; thus, the net effect on litter size may be limited except in high ovulating lines of gilts. The effect on OR

is counterintuitive, considering that the frequency of the deletion allele, which is associated with decreased OR, is significantly greater in the OR line compared with the control, suggesting that selection for OR increased the frequency of the allele. The control and OR lines began with gilts sampled from a common population of white crossbred gilts, however, and the frequency of the deletion allele in the founder gilts and boars of each line are not known. It is possible that by chance the allele frequencies were already significantly different in the founder animals. It also is possible that the deletion allele frequencies in the control and OR lines may have diverged due to genetic drift.

The opposing effects of the ins/del locus on UC and OR may have decreased the association between the genotype at the ins/del locus and litter size in intact OR gilts. Despite the opposing effects of the ins/del locus on OR and UC, the depression in OR by the locus could potentially be overcome either by selection or by hormonal treatment, which would result in increased litter size. The effect of the ins/del on UC was approximately 1.2 extra piglets per uterine horn in UHO gilts, which translates into potentially 2.4 piglets per litter in intact gilts if OR is sufficient. Further experiments are needed to confirm the potential utility of this locus for altering litter size of swine.

Implications

Because both secreted folate binding protein polymorphisms were significantly associated with uterine capacity, these results suggest that the secreted folate binding protein gene plays a role in the uterine capacity of gilts. More information is needed on the role of secreted folate binding protein in reproduction in the pig and on the consequences of the genetic variation in the secreted folate binding protein gene on gene transcription, messenger RNA, and protein function. Nevertheless, these results confirm that sequence variation in the secreted folate binding protein gene is associated with differences in various factors affecting litter size, including ovulation rate, fertilization rate or embryonic survival, and uterine capacity. Because of the dominance effect of the serine-arginine single nucleotide polymorphism and the opposing effects of both secreted folate binding protein polymorphisms on the component traits of litter size, these genetic markers are unlikely to be immediately useful for marker-assisted selection for litter size without further research.

Literature Cited

- Babior, B. M. 1990. Erythrocyte disorders: Anemias related to disturbances of DNA synthesis (megaloblastic anemias). Pages 453–481 in *Hematology*. 4th ed. W. J. Williams, E. Beutler, A. J. Erslev, and M. A. Lichtman, ed. McGraw-Hill, New York, NY.
- Bennett, G. L., and K. A. Leymaster. 1989. Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. *J. Anim. Sci.* 67:1230–1241.
- Blount, B. C., M. M. Mack, C. M. Wehr, J. T. MacGregor, R. A. Hiatt, G. Wang, S. N. Wickramasinghe, R. B. Everson, and B. N. Ames.

1997. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: Implications for cancer and neuronal damage. *Proc. Natl. Acad. Sci. USA* 94:3290–3295.
- Cassady, J. P., R. K. Johnson, D. Pomp, G. A. Rohrer, L. D. Van Vleck, E. K. Spiegel, and K. M. Gilson. 2001. Identification of quantitative trait loci affecting reproduction in pigs. *J. Anim. Sci.* 79:623–633.
- Christenson, R. K., and K. A. Leymaster. 2002. Correlated responses in gravid uterine, farrowing and weaning traits to selection of pigs for ovulation rate or uterine capacity. *Comm. No. 08–25 in Proc. 7th World Cong. Genet. Appl. Livest. Prod., Montpellier, France.*
- Christenson, R. K., K. A. Leymaster, and L. D. Young. 1987. Justification of unilateral hysterectomy-ovariectomy as a model to evaluate uterine capacity in swine. *J. Anim. Sci.* 65:738–744.
- Duan, J., M. S. Wainwright, J. M. Comeron, N. Saitou, A. R. Sanders, J. Gelernter, and P. V. Gejman. 2003. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum. Mol. Genet.* 12:205–216.
- Haley, C. S., and G. J. Lee. 1993. Genetic basis of prolificacy in Meishan pigs. *J. Reprod. Fertil. Suppl.* 48:247–259.
- Kappes, S. M., G. L. Bennett, J. W. Keele, S. E. Echternkamp, K. E. Gregory, and R. M. Thallman. 2000. Initial results of genomic scans for ovulation rate in a cattle population selected for increased twinning rate. *J. Anim. Sci.* 78:3053–3059.
- Kim, C. H., Y. Oh, and T. H. Lee. 1997. Codon optimization for high-level expression of human erythropoietin (EPO) in mammalian cells. *Gene* 199:293–301.
- Kim, J. G., and J. L. Vallet. 2004. Secreted and membrane forms of folate binding protein occur sequentially during pregnancy in swine. *Biol. Reprod.* 71:1214–1219.
- King, A. H., Z. Jiang, J. P. Gibson, C. S. Haley, and A. L. Archibald. 2003. Mapping quantitative trait loci affecting female reproductive traits on porcine chromosome 8. *Biol. Reprod.* 68:2172–2179.
- Nakamura, Y., T. Gojobori, and T. Ikemura. 2000. Codon usage tabulated from international DNA sequence databases: Status for the year 2000. *Nucleic Acids Res.* 28:292.
- Pearson, P. L., H. G. Klemcke, R. K. Christenson, and J. L. Vallet. 1998. Uterine environment and breed effects on erythropoiesis and liver protein secretion in late embryonic and early fetal swine. *Biol. Reprod.* 58:911–918.
- Rohrer, G. A., J. J. Ford, T. H. Wise, J. L. Vallet, and R. K. Christenson. 1999. Identification of quantitative trait loci affecting female reproductive traits in a multigeneration Meishan-White composite swine population. *J. Anim. Sci.* 77:1385–1391.
- Rothschild, M., C. Jacobson, D. Vaske, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen, A. Mileham, and G. Plastow. 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. *Proc. Natl. Acad. Sci. USA* 93:201–205.
- Rothschild, M. F., L. A. Messer, A. Day, R. Wales, T. Short, O. Southwood, and G. Plastow. 2000. Investigation of the retinol-binding protein 4 (RBP4) gene as a candidate gene for increased litter size in pigs. *Mamm. Genome* 11:75–77.
- Rothschild, M. F., L. A. Messer, and A. Vincent. 1997. Molecular approaches to improved pig fertility. *J. Reprod. Fertil. Suppl.* 52:227–236.
- Vallet, J. L. 2000. Fetal erythropoiesis and other factors which influence uterine capacity in swine. *J. Appl. Anim. Res.* 17:1–26.
- Vallet, J. L., R. K. Christenson, and H. G. Klemcke. 1998. Purification and characterization of intrauterine folate-binding proteins from swine. *Biol. Reprod.* 59:176–181.
- Vallet, J. L., R. K. Christenson, and H. G. Klemcke. 1999a. Technical note: A radioimmunoassay for porcine intrauterine folate binding protein. *J. Anim. Sci.* 77:1236–1240.
- Vallet, J. L., B. A. Freking, K. A. Leymaster, and R. K. Christenson. 2005. Allelic variation in the erythropoietin receptor gene is associated with uterine capacity and litter size in swine. *Anim. Genet.* 36:97–103.
- Vallet, J. L., H. G. Klemcke, and R. K. Christenson. 2002. Interrelationships among conceptus size, uterine protein secretion, fetal erythropoiesis, and uterine capacity. *J. Anim. Sci.* 80:729–737.
- Vallet, J. L., H. G. Klemcke, R. K. Christenson, and P. L. Pearson. 2003. The effect of breed and intrauterine crowding on fetal erythropoiesis on day 35 of gestation in swine. *J. Anim. Sci.* 81:2352–2356.
- Vallet, J. L., T. P. L. Smith, T. S. Sonstegard, M. Heaton, and S. C. Fahrenkrug. 2001. Structure of the genes for porcine endometrial secreted and membrane folate binding proteins. *Domest. Anim. Endocrinol.* 21:55–72.
- Vallet, J. L., T. P. L. Smith, T. Sonstegard, P. L. Pearson, R. K. Christenson, and H. G. Klemcke. 1999b. Isolation of complementary deoxyribonucleic acids encoding putative secreted and membrane-bound folate binding proteins from endometrium of swine. *Biol. Reprod.* 61:372–379.
- van Rens, B. T. T. M., and T. van der Lende. 2002. Litter size and piglet traits of gilts with different prolactin receptor genotypes. *Theriogenology* 57:883–893.
- Wilkie, P. J., A. A. Paszek, C. W. Beattie, L. J. Alexander, M. B. Wheeler, and L. B. Schook. 1999. A genomic scan of porcine reproductive traits reveals possible quantitative trait loci (QTLs) for number of corpora lutea. *Mamm. Genome* 10:573–578.