RESEARCH REPORT

ANIMAL SCIENCE

Title: Genome-wide association analyses of sow reproduction and lifetime productivity – **NPB #11-070** revised

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Industry Summary:
Concurrent with increased prolificacy, high sow death losses and replacement rates are serious economic and welfare issues facing producers. Reproductive failure is the most frequent reason for culling sows. Lifetime productivity is characterized by moderate heritability and high phenotypic variance and, as a result, substantial genetic variation is expected to exist in most populations. Traditional selection for sow reproductive longevity results is ineffective due to low heritability and late expression of the trait. The primary goal of this research project was to identify DNA markers associated with reproductive and sow lifetime productivity that can be used in nucleus herds to select for increased lifetime productivity. Incorporation of DNA markers into selection programs is potentially a more practical approach for improving sow lifetime productivity. Using a resource population of crossbred gilts developed at UNL, we explored genetic factors associated with reproductive traits and lifetime productivity. Our work generated the following major research findings:

- From all the traits recorded before breeding, age at puberty was the only one that significantly affected the chance of females would produce a first parity litter.
- Genome-wide association analyses uncovered several regions that have an effect across the reproductive traits.
- DNA markers located on three different chromosomal regions were associated with early expression of puberty that led to up to 1.36 greater number of lifetime parities.
- Genomic prediction values of reproductive traits explained an important proportion of the phenotypic differences when training and prediction was performed in the same data set but negligible for litter size traits when training and genomic prediction were performed in different data sets.

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Scientific Abstract:

We performed genome wide association studies in a discovery population resource developed at UNL to identify loci that influence reproductive and lifetime productivity traits. Combined SNP effects explained 26% of phenotypic differences in age at puberty. The contribution of SNP variation to the phenotypic variance of the lifetime number of parities was 19% whereas the contribution to litter size traits (TNB and NBA) at first parity was very limited (5%). Of the traits recorded before breeding, only age at puberty significantly affected the probability that females would produce a first parity litter. The genetic variance explained by 1 Mb windows of the sow genome, compared across traits, uncovered regions that influence both age at puberty and lifetime number of parities. Allelic variants of SNPs located on SSC5 (27-28 Mb), SSC 8 (36-37 Mb) and SSC12 (1.2-2 Mb) exhibited additive effects and were associated with both early expression of puberty and a greater than average number of lifetime parities. Combined analysis of these SNPs showed that an increase in the number of favorable alleles had positive impact on reproductive longevity, increasing number of parities with up to 1.36. The region located on SSC5 harbors non-synonymous alleles in arginine vasopressin receptor 1A (AVPR1A) gene, a G-protein coupled receptor associated with social and reproductive behaviors in voles and humans and a candidate for the observed effects. This region is characterized by high levels of linkage disequilibrium in different lines and could be exploited in marker assisted selection programs across populations to increase sow reproductive longevity.

The litter size traits were the main reproductive traits present in both UNL and ISU data sets. Combined SNP effects of the merged UNL and ISU data sets explained a relatively low proportion of the phenotypic variation of the reproductive traits, varying across parities from 1.9 % for NBA at P1 to 21.3 % at P3. The contribution of SNP variation to the phenotypic variance of the lifetime productivity was minor (0.5 and 1 %). Genomic prediction values of reproductive traits explained an important proportion of the phenotypic differences when training and prediction was performed in the UNL data set but negligible for litter size traits when training was performed in the UNL set and genomic prediction was performed in the ISU data set.

Introduction:

The long-term goal of our research is to reduce culling rates, sow death losses, and enhance the productive life of sows. Our central hypotheses are: 1) there are many genes with relatively small to moderate effects that explain differences in sow reproductive and lifetime productivity, 2) age of puberty is moderately heritable and is a good predictor of lifetime productivity, 3) age at puberty and reproductive longevity are dependent on the function of the hypothalamic-pituitary-gonadal axis and are most likely influenced by the same genetic variants, and 4) identification of genetic markers associated with age at puberty and other reproductive traits influence sow lifetime productivity. The primary goal of this research project was to identify SNP markers and combinations of markers associated with reproductive and sow lifetime productivity that can be used in breeding programs to improve lifetime productivity. We achieved this goal by 1) integrating and expanding UNL and ISU data sets that aim to increase power and accuracy in detection and validation of genetic markers associated with sow reproductive longevity and by 2) using high-density marker effects in the UNL training set to estimate genome prediction values in ISU prediction data set for reproductive and lifetime productivity traits that are common in both datasets.

Objectives:

Objective 1. Predict reproductive and lifetime productive performance using UNL training set
Objective 2. Improve the power to estimate SNP effects for reproductive and lifetime productive performance in traits common in UNL and ISU data sets
Objective 3. Identify procedures to apply markers association results in Genomic Selection programs to improve sow reproductive longevity.

Materials & Methods:

Population:

UNL. The dams of the project gilts were commercial Large White x Landrace crossbreds (LW x LR) or Nebraska Index Line (NIL) (Petry and Johnson, 2004) sows (Johnson et al., 2009; Miller et al., 2011). Project gilts were produced in batches of 100 to 120 individuals using single boar semen from of an unrelated industry Landrace line (L1 and L2). The LR1 sire line was used in the first four (1 - 4) and LR2 was used in the rest of the batches (5 - 10). From 275 litters sired by 56 sires, 852 gilts contributed to this study after being randomly selected at approximately 56 d of age and subsequently exhibiting puberty before 240 d. Gilts received the same diet and management from birth to 123 d of age. From 123 d of age until they were moved to the breeding barn at 225 to 240 d of age, gilts were housed in pens averaging approximately 8 gilts/pen being. From batch 1 to 6 the gilts were allowed either ad libitum access to feed (A) or were placed on a restricted feeding regimen (R), receiving a daily allotment of feed that was 75% of the energy consumed by gilts on the A regimen (Miller et al., 2011). The R diet was formulated similarly to the A diet except that it was fortified to maintain similar intakes of amino acids, minerals, and vitamins (Table 1). From batch 7 all gilts were allowed ad libitum access to feed with half of the gilts having access to a formulated feed that resulted in 75% of the energy consumed by the rest of the gilts. The low energy intake diet was achieved using an addition of soybean hulls. During the breeding period and thereafter all experimental animals received the same diet.

Detection of age at puberty was initiated at 140 d of age and continued until approximately 240 d of age. The majority of the gilts (91%) expressed estrus in this interval. Gilts were moved once daily from their pen to an adjacent room and exposed to a mature boar for 15 min. This process continued until gilts were moved to the breeding barn or until all gilts within a pen had been observed in estrus at least twice. Gilts were maintained through four parities unless they were culled or died. Culling was performed only for failure to conceive or farrow a litter, or structural problems. Total number born of fully formed pigs (TNB), number born alive (NBA), number mummified (MUM) and number stillborn (SB) were recorded through the fourth parity.

ISU. Reproductive and lifetime productivity data were recorded between 2005 to 2009 from 835 sows of Large white (LW) and LW x LR crossbred lines from a single commercial farm. The data includes reproductive traits such as litter size and lifetime productivity traits such as total number born, number born alive to a maximum of nine parities. High-density genotypes of 683 individuals were used in a genome-based association analysis using GenSel Software (Fernando and Garrick, 2008.

Table 1. Composition and calculated analysis (as-fed basis) of ad libitum (A) and restricted (R) diets fed to gilts during the development period (d 123 to 1 wk prior to breeding, batch 1 to 4, Miller et al., 2012).

Item	Diet	
	A	B
Ingredient		
Corn	77.37	67.74
Soybean meal, 46.5% CP	16.60	25.00
Tallow	3.00	3.00
Dicalcium phosphate	1.48	2.38
Limestone	0.65	0.85
Salt	0.50	0.50
Vitamin premix[1,2]	0.25	0.33
Mineral premix[3,4]	0.15	0.20
TOTAL	100.00	100.00
Calculated analysis		
ME, kcal/kg	3,442	3,402
CP, %	14.30	17.50
Lysine, %	0.70	0.93
Ca	0.70	1.00
P	0.60	0.80

[1]For diet A supplied per kilogram of diet: vitamin A (as retinyl acetate), 4,400 IU; vitamin D (as cholecalciferol), 440 IU; vitamin E (as α-tocopherol acetate), 24 IU; vitamin K (as menadione dimethylpyrimidinol bisulfite), 3.5 mg; riboflavin, 8.8 mg; d-pantothenic acid, 17.6 mg; niacin 26.4 mg; vitamin B_{12}.

[2]For diet R supplied per kilogram of diet: vitamin A (as retinyl acetate), 5,878 IU; vitamin D (as cholecalciferol), 588 IU; vitamin E (as α-tocopherol acetate), 32 IU; vitamin K (as menadione dimethylpyrimidinol bisulfite), 4.7 mg; riboflavin, 11.8 mg; d-pantothenic acid, 23.5 mg; niacin 35.3 mg; vitamin B_{12}.

[3]For diet A supplied per kilogram of diet: Zn (as $ZnSO_4$), 128 mg; Fe (as $FeSO_4 \cdot H_2O$), 128 mg; Mn (as MnO), 30 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 11 mg; I (as $Ca(IO_3) \cdot H_2O$), 0.26 mg; Se (as Na_2SeO_3), 0.3 mg.

[4]For diet R supplied per kilogram of diet: Zn (as $ZnSO_4$), 171 mg; Fe (as $FeSO_4 \cdot H_2O$), 171 mg; Mn (as MnO), 40 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 14.7 mg; I (as $Ca(IO_3) \cdot H_2O$), 0.35 mg; Se (as Na_2SeO_3), 0.3 mg.

DNA isolation: DNA was isolated from tail and ear tissue of experimental gilts using the DNeasy blood and tissue kit (Qiagen). The isolated DNA was scored for quality based on the level of DNA degradation that was evaluated by electrophoresis.

DNA genotyping: Genotyping was performed using the PorcineSNP60 BeadChip (Illumina) that contains assays for a total of 62,183 SNPs. The genotyping was contracted out to Gene Seek as described in the proposal. The majority of the SNPs (88.2%) were mapped on the build 10.2 reference assembly of the porcine genome. Illumina data analysis software was used to assign quality scores (GenCall) for each genotype. A minimum GenCall genotype quality score of 0.40, a sample and SNP call rate of 0.80 were used as cutoff thresholds for removing low quality genotypes. As a result, 57 761 SNP representing 822 genotyped samples were used in the genome wide association analyses. Selective genotyping of the AVPR1A gene G256D SNPs using a subset of 78 samples of the UNL resource population was performed using KASPar technology (KBioscience).

Genome-wide association analysis (GWAS): The proportion of genetic variance for age at puberty, litter size traits, reproductive longevity and productivity was estimated from high-density SNPs genotypes using Bayes B model (Kizilkaya et al., 2010) implemented via GenSel software package (Fernando and Garrick, 2009). The analyses were performed using a π value set equal to 0.995, assuming prior probability of 0.005 SNPs having a non-zero effect for the targeted traits. Fixed effects included in the model were line, batch, and diet. The Markov Chain Monte Carlo included 41,000 iterations with the first 1,000 samples being discarded. Effects sampled from Bayes B at every 40^{th} iteration were used to compute the posterior distribution for the genetic variance explained by each 1 Mb window of the swine genome. Associations between single markers located in the major QTL areas and age at puberty and lifetime number of parities were tested by a linear mixed model fitted by JMP that included marker genotype and replicate as fixed effects and sire, litter and diet as random effects. A similar model was used to estimate the effect of the number of favorable alleles of three SNPs (ALGA0064320, ALGA0106255 and BGIS0007637) on age at puberty and lifetime number of parities.

Results:

Objective 1.

Variation in age at puberty is defined by multiple loci. The average genotyping call rate of the samples used in the analyses varied from 80.2 to 93.6% with a mean of 93.0%. The average of the SNP call rate was 98.7% and varied among SNPs with 207 SNPs generating genotypes for all samples and 37,878 SNPs generating genotypes for at least 99.0% of the samples. A large proportion of informative SNPs were characterized by minor allelic frequency of at least 0.10 (77.6%). The fraction of monomorphic SNPs was 4%. Combined SNP effects explained 26% of phenotypic differences in age at puberty (Table 2). The contribution of SNP variation to the phenotypic variance of the lifetime number of parities was 19% whereas the contribution to litter size traits (TNB and NBA) at first parity was very limited (5%). Genomic prediction values were obtained for each gilt using estimated SNP effects (Fernando & Garrick, 2009). Substantial differences between sire's average genome-wide predictions of their daughter's age at puberty existed, providing potential opportunities for selection of sire with propensity to generate gilts with superior reproductive longevity (Figure 1). Analysis of the genetic variance explained by each 1Mb window of the swine genome, based on posterior distributions of the SNP effects, uncovered major regions associated with puberty onset, reproductive longevity and litter size traits. The top 1% of the 2,593 windows of 1 Mb combined explained 11 % of additive genetic variation and included multiple regions from SSC1 (31-32, 94.2-94.9, 287-288.0, 94.1-94.9 Mb), SSC3 (16-16.9, 71.1-72 Mb), SSC6 (115.1-116, 144-145 Mb), SSC8 (36-37, 37-38 Mb), SSC9 (21.1-22, 139-140 Mb), SSC12 (1.2-2, 2.1-3, 11-11.9 Mb), SSC13 (117-117.9, 142.1-142.9 Mb), SSC14 (19-20, 28-29, 66-67, 68-69 Mb) and unique regions from SSC2 (87-88 Mb), SSC4 (7-8 Mb), SSC5 (30-30.9 Mb), SSC7 (39.1-40 Mb), and SSC18 (60-60.9 Mb) (Figure 2). Most of these regions are characterized by clusters of SNPs associated with the largest effects on age at puberty onset (top 0.05%). The region located at

the proximal end of SSC4 (7.0-8.0 Mb) explained the largest amount of additive genetic variation (1.22%), having a model frequency of 36%, although the variation explained by this window was not significantly greater than the average explained by 1 Mb windows (P < 0.32). Several of the detected regions for age at puberty overlap previously reported candidate genes and QTL. For example, the windows at the proximal end of SSC12 (1-2, 2.1-3, 11-11.9 Mb) are adjacent to a QTL for age at puberty reported by Holl et al., (2004). Detailed data and results are described in Tart et al. (2013, Animal Genetics).

In the ISU data set, SNPs explained a negligible proportion of the variance in litter size traits as well in lifetime number of pig produced (Table 3). In this commercial data set SNPs from five regions located on four chromosomes were associated with litter size at parity 1 and SNPs from 10 regions located on eight chromosomes were associated with lifetime total number born alive (Onteru et al., 2010).

Figure 1. Sire's genomic breeding values estimated as the average of their daughter' genomic predicted values for age at puberty.

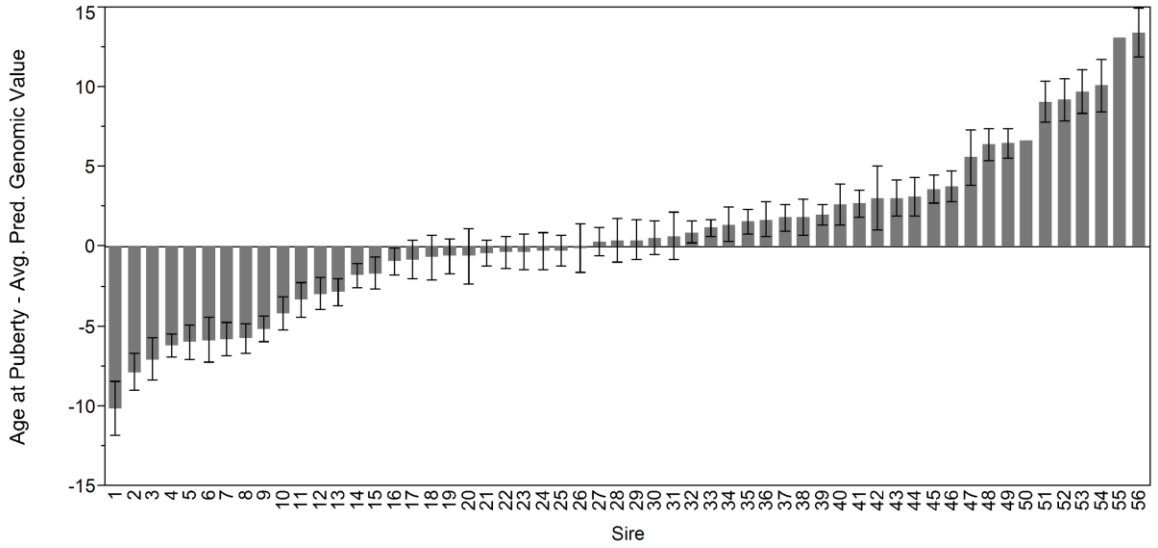

Figure 2: Genome-wide association analysis between 57,761 SNPs and age at puberty. Each dot represents the proportion of genetic variance explained by five consecutive SNPs. The X axis represents the location of the SNPs in the swine genome. The Y axis represents the contribution of that marker to the genetic variance. Alternate colors represent autosomes, from SSC1 to 18, followed by chromosome X.

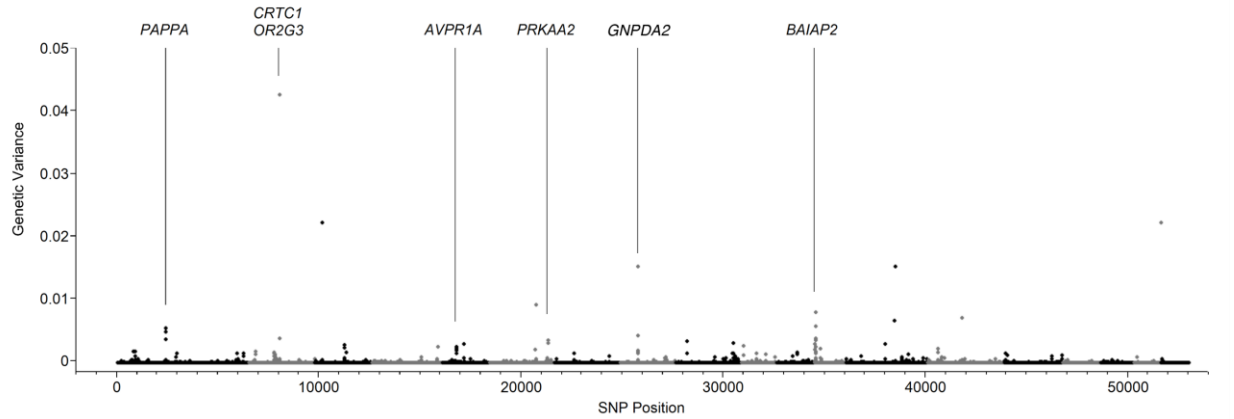

Table 2. Posterior means of variance components of sow reproductive traits based on 57,761 SNP effects estimated by GWAS in the UNL population (Tart et al., 2013).

Trait *	n	Genetic variance	Residual variance	Total variance	Proportion of phenotypic variance explained by SNPs
AP	822	90.37	253.12	343.47	0.26
TNB-LT	550	3.41	301.83	305.24	0.01
NBA-LT	547	5.75	261.70	267.46	0.02
NP	739	0.23	1	1.23	0.19
TNB_P1	622	0.44	8.48	8.92	0.05
NBA_P1	620	0.42	8.60	9.01	0.05

* AP, age at puberty; TNB-LT, lifetime total number born; NBA-LT, lifetime number born alive; NP, number of lifetime parities; TNB, total number born and NBA, number born alive at parity 1 (P1)

Table 3. Posterior mean of variance components explained by whole genome SNP markers for reproductive traits in a study using maternal pig lines as part of ISU study (Onteru et al., 2011).

Trait*	n	Genetic variance	Residual variance	Total variance	Proportion of phenotypic variance explained by SNPs
TNB_P1		0.030	9.41	9.44	0.003
NBA_P1		0.050	9.05	9.11	0.005
TNB_P2		0.032	10.96	10.99	0.003
NBA_P2		0.046	10.99	11.03	0.004
TNB_P3		0.031	11.74	11.77	0.003
NBA_P3		0.069	10.33	10.40	0.007

*TNB, total number born and NBA, number born alive at parity 1-3 (P1-P3)

Table 4. Correlation between genomic prediction and phenotypic values of sow reproductive traits when training and prediction was performed in the same data set (UNL)

Trait	Parity	N	Variance of the predicted genomic merit	Correlation	R^2
AP		822	47.93	0.80	0.64
LP		550	0.07	0.65	0.42
LNBA		547	5.24	0.75	0.56
SP		757	7.03	0.91	0.83
TNB	1	622	0.10	0.64	0.40
TNB	2	433	0.28	0.76	0.57
TNB	3	365	0.49	0.76	0.57
TNB	4	208	0.06	0.81	0.66
NBA	1	620	0.15	0.65	0.42
NBA	2	433	0.34	0.77	0.59
NBA	3	365	0.55	0.80	0.64
NBA	4	208	0.02	0.78	0.61

* AP – age at puberty; LP – Lifetime total number born; LNBA – Lifetime number born alive; SP – successful parities as a categorical trait with five categories 0-4; TNB -Total number born; NBA-Number born alive; SB – Number of stillborn.

Table 5. Correlation between genomic prediction and phenotypic values of sow reproductive traits – training was performed in the UNL set while the prediction was performed in the ISU set.

Trait	Parity	N	Variance of the predicted genomic merit	Correlation	R^2
LTNB		813	0.007	-0.021	0.0004
LNBA		813	0.666	-0.027	0.0007
TNB	1	683	0.011	0.025	0.0006
TNB	2	559	0.032	-0.040	0.0016
TNB	3	442	0.052	0.018	0.0003
NBA	1	683	0.019	0.007	0.00004
NBA	2	559	0.039	-0.061	0.0037
NBA	3	442	0.056	0.037	0.0013

* LTNB – Lifetime total number born; LNBA – Lifetime number born alive; TNB -Total number born; NBA-Number born alive;

Genomic prediction. As expected, genomic prediction values of age at puberty explained an important proportion of the phenotypic differences since training and prediction was performed in the same UNL data set (r = 0.79)(Table 4). The results were similar for the litter size traits at first parity (0.59) or during lifetime (0.68). In contrast the proportion of the variance explained by SNPs is negligible in the ISU data set (discovery population) when training was performed in the UNL data set (Table 5).

Genetic relationship between age at puberty and reproductive longevity. Common sources of variation may be responsible for the variation of age at puberty and the components of reproductive longevity. Analysis of the genetic variance explained by 1 Mb window across multiple traits showed that four of the top 1% (n=26) windows that influence age at puberty (SSC12, 1.0-2.0, 2.0-3.0 Mb; SSC5, 30.0-30.9 Mb; SSC18, 60.0-60.9 Mb) ranked high (top 2%) for lifetime number of parities. Windows located at the proximal end of SSC1 (1.0-2.0, 2.0-3.0 Mb) also ranked high for lifetime productivity traits such as lifetime TNB and NBA. These windows are potential sources of the correlations between genomic predictions values of age at puberty and reproductive longevity. In contrast, only one of the top 1% windows for age at puberty ranked high for litter size traits (TNB and NBA) at parity 1.

The SNPs associated with the largest age at puberty effect in each of the top 1% (n = 26) 1 Mb GWAS-derived QTL regions described above were evaluated in single marker association analysis to investigate the presence of common sources of genetic variation with reproductive longevity. Most of these SNPs (92%) explained significant differences in age at puberty that varied between homozygote genotypes from 3.4 (ALGA0106255, SSC8, 36.5 Mb, $P < 0.05$) to 11.1 d (ASGA0105637, SSC2, 87.2 Mb, $P < 0.01$). In addition to age at puberty, BGIS0007637 (SSC5, 30.7 Mb, $p < 0.01$) and ASGA0003702 (SSC1, 94.3 Mb, $P < 0.05$) also explained important variation in the lifetime number of parities ($P < 0.05$). Suggestive trends ($P < 0.15$) that also captured the negative relationship between age at puberty and number of lifetime parities were observed for SNPs located on SSC8, SSC12 and SSC13. Additive properties of the SNPs was evaluated by integrating three SNP exhibiting additive ($P<0.10$) and effects that varied from 0.18 parities for ALGA0064320 (SSC12) and ALGA0106255 (SSC8) to 0.26 for BGIS0007637 (SSC5). An increase in the number of favorable alleles across these SNPs was associated with an early age at puberty and a positive impact on the number of lifetime parities ($P<0.001$)(Tart et al., 2013). The individuals that carried five favorable alleles across the three loci generated 1.36 more parities than individuals where the favorable alleles are absent ($P<0.05$). The distribution of the allelic combinations underlines opportunities for genetic improvement by increasing the frequency of favorable alleles.

Objective 2. Combined SNP effects of the merged UNL and ISU data set explained a relatively low proportion of the phenotypic variation of the reproductive traits that are common in both sets (Table 6). The contribution of SNP variation to the phenotypic variance of the lifetime productivity was minor (0.5 and 1 %). The contribution of the SNPs to NBA varied between parities from 1.9 % for P1 to 21.3 % for P3. Similarly the contribution of the SNPs to TNB varied from 4.7 % for P1 to 10.0 % for P3.

Table 6. Posterior means of variance components of sow reproductive traits based on 57,761 SNP effects estimated by GWAS in the combined UNL and ISU data sets.

Trait *	n	Genetic variance	Residual variance	Total variance	Proportion of phenotypic variance explained by SNPs
TNB-LT	1047	3.627	709.93	713.56	0.005
NBA-LT	1047	6.469	589.76	596.22	0.010
TNB_P1	1304	0.441	8.80	9.25	0.047
NBA_P1	1179	0.413	20.64	21.05	0.019
TNB_P2	991	0.798	10.92	11.72	0.068
NBA P2	991	0.350	11.12	11.47	0.030
TNB P3	807	1.254	11.23	12.49	0.100
NBA P3	807	2.466	9.05	11.52	0.213

Objective 3. We used the preliminary data generated by this grant to obtain additional research funding from NIFA/USDA for marker validation in different commercial swine operations. The grant titled “Translational genomics for improving sow reproductive longevity”, includes research and extension activities with a total funding of $1,166,650 for a period 3 years (12.01.2012-11.30.2015) and involves University of Nebraska, University of Missouri and USMARC. A DNA sample will be collected from maternal crossbred gilts entering different commercial systems with relatively high health status. We plan to collect samples and production data from at least 6,000 gilts beginning upon initiation of the research project. Production data such as time in the herd, number of litters produced per year and per lifetime, days from estrus to farrowing, etc., will be used as measurements of reproductive longevity and productivity. Each participant will provide records that document reasons for culling, detailed pedigree and access to sire DNA samples if available. From this industry wide resource, we will evaluate the accuracy of the SNP effects identified from our research efforts in commercial settings, thus providing an avenue for the industry to “learn by doing” ultimately aiding in technology adoption.

Discussion:

Using the UNL resource population that includes genetics from several commercial maternal lines, we uncovered several pleiotropic sources of variation of age at puberty and reproductive longevity (Tart et al., 2013). One of these sources is represented by a region located on SSC5 that harbors AVPR1A gene and carries allelic variants associated with early expression of puberty and a greater number of lifetime parities. The favorable homozygote genotype of one of the SNPs located in AVPR1A is responsible for almost 6 d earlier expression of first estrus and a half a parity more during lifetime than the alternate homozygote genotype. Our research provides evidence that this region is polymorphic in different populations including the ISU data set. Analysis of multiple SNPs showed that an increase in the number of favorable alleles had positive impact on reproductive longevity, increasing the lifetime number of parities with up to 1.36. An important percentage of genetics that contributed to the UNL resource population have an important contribution to the maternal genetics lines of the US swine industry as well as having a worldwide presence. While it is unclear if genetic

variants of AVPR1A represent the functional polymorphisms of the variation in age at puberty and reproductive longevity, the presence of a relatively large DNA block in linkage disequilibrium that is conserved in different commercial lines, could represent a potential molecular resource that could be applied to select for early expression of first estrus and reproductive longevity across populations without knowledge of the causal gene or mutation.