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Genomewide association and identification of candidate genes for ovulation rate in swine[1,2]

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ABSTRACT: Reproductive efficiency has a great impact on the economic success of pork production. Ovulation rate is an early component of reproduction efficiency and contributes to the number of pigs born in a litter. To better understand the underlying genetics of ovulation rate, a genomewide association study was undertaken. Samples of DNA were collected and tested using the Illumina Porcine SNP60 BeadChip from 1,180 females with ovulation measurements ranging from never farrowed to measurements taken after parity 2. A total of 41,848 SNP were tested using the Bayes C option of GenSel. After the Bayes C analysis, SNP were assigned to sliding windows of 5 consecutive SNP by chromosome-position order beginning with the first 5 SNP on SSC1 and ending with the last 5 SNP on SSCX. The 5-SNP windows were analyzed using the Predict option of GenSel. From the Predict analysis, putative QTL were selected having no overlap with other 5-SNP window groups, no overlap across chromosomes, and the highest genetic variation. These putative QTL were submitted to statistical testing using the bootstrap option of GenSel. Of the putative QTL tested, 80 were found to be statistically significant ($P < 0.01$). Ten QTL were found on SSC1, 12 on SSC2, 4 on SSC3, 8 on SSC4, 3 on SSC5, 3 on SSC6, 3 on SSC7, 4 on SSC8, 2 on SSC9, 4 on SSC10, 1 on SSC12, 4 on SSC13, 2 on SSC14, 4 on SSC15, 4 on SSC16, 6 on SSC17, 4 on SSC18, and 1 on SSCX. Sixteen QTL were found to be statistically significant at the $P < 0.001$ level. Six additional QTL were significant at the $P = 0.001$ level. These 22 QTL accounted for 71.10% of the total genetic variance. The most compelling candidate genes in these regions include *Estrogen receptor 1*, *growth differentiation factor 9*, and *inhibin βA*. These QTL, when combined with information on genes found in the same regions, should provide useful information that could be used for marker assisted selection, marker assisted management, or genomic selection applications in commercial pig populations.

Key words: Bayes, genomewide association studies, genomic, ovulation rate, single nucleotide polymorphism, swine

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INTRODUCTION

Litter size is known to be important to swine producers. Litter size has been shown to have a low heritability of 0.09 for both total number born (**TNB**) and number born alive (**NBA**; Schneider et al., 2012a). Direct selection has historically resulted in slow progress in litter size and this project was designed to identify markers to supplement direct selection, although many industry lines are now achieving or nearing achievement of 30 pigs per sow per year. This level of production does not negate the need to fully understanding the underlying genetics of litter size. A major genetic component associated with litter size is ovulation rate (**OR**; Bennett and Leymaster, 1989). Foxcroft et al. (2006) observed that OR has increased more than litter size and therefore created uterine crowding with negative consequences. These negative consequences include reduced survival of the fetuses, impaired development of the surviving fetuses, a reduction in muscle fiber development, lower birth weights, and decreases in finishing gain and carcass merit. Foxcroft et al.'s (2006) observations provide further justification for understanding OR and they also suggested opportunities for using marker information to control OR at least in certain lines where OR is not balanced with uterine capacity.

To date, genomewide association studies (**GWAS**) have relied heavily on microsatellites to identify regions of interest. The advent of the Porcine SNP60 BeadChip (Illumina, San Diego, CA; Ramos et al., 2009) has offered advances for identifying QTL. Recent use of this genomic platform has identified novel QTL for litter trait components that do not overlap previously discovered regions generated through the use of microsatellite marker components (Onteru et al., 2012).

The objective of the current study was to perform a GWAS using marker data from the Porcine SNP60 BeadChip in Landrace–Duroc–Yorkshire composites to identify QTL for OR.

MATERIALS AND METHODS

The experimental procedures were approved and performed in accordance with the U.S. Meat Animal Research Center's (**USMARC**) Animal Care and Use committee and the *Guide for Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010).

Animals and Data

A composite population was developed in 2001 at the USMARC. Twelve terminal Landrace males and 12 terminal Duroc males were selected from commercially available seedstock. Each sire within breed was arbitrarily assigned a sire code of 1 to 12. Semen from these boars was used to inseminate females (n = 220) from an existing maternal Landrace–Yorkshire composite population developed at the USMARC. One son and 10 daughters from each boar were randomly selected to produce the next generation. Breeding Landrace-sired animals to Duroc-sired animals formed the second generation. Matings of gilts from a sire code were made to a boar from the corresponding sire code of the opposite breed. Selection of parents in subsequent generations was based on sire code, where 1 boar and 20 gilts from each sire were selected. Boars were selected annually from the first season and used across all seasons of that year. Matings were random except full- and half-sib matings were avoided. Batch farrowing was used with inseminations taking place during a 3-wk period of every 2-mo season. Matings for this population were restricted to 5 of the 6 farrowing seasons produced annually. The first 3 seasons contained gilts and the remaining 2 seasons contained second-parity sows and a smaller, varied number of gilts. Twelve original sire lines were maintained and semen from all sire lines was used to produce approximately 600 litters per year. Gilts and sows available to be inseminated for farrowing during 2005 through 2009 were genotyped and used for this study. To the extent possible, all animals were managed as a single cohort with the same nutrition, etc.

The phenotypic trait of OR defined as the number of corpora lutea found in the ovary was evaluated at the abattoir on 1,180 females that never farrowed (parity 0; n = 300), farrowed once (parity 1; n = 516), or farrowed 2 or more times (parity 2; n = 364). Eighty percent of animals that never farrowed were sent to the abattoir 4 to14 d after their first estrus. The remaining 20% were sent to the abattoir at 4 to 14 d after estrus after an unknown number of estrous cycles or postinsemination. Similarly, 75% of parity 1 and parity 2 sows were sent to the abattoir 4 to 14 d after their first estrus after weaning. The remaining sows were not able to be slaughtered before a second estrous cycle; therefore, they were sent to the abattoir 4 to 14 d after their second estrous cycle. Ovulation rate data only included counts of corpora lutea and corpora hemorrhagica recorded during the luteal phase; data from cystic ovaries were not used. Summary statistics of the raw data are shown in Table 1. The OR data included counts of 42 and 82. Both measurements were confirmed at slaughter and it was decided that 2 actual but unusually high measurements were unlikely to have an adverse affect on an analysis of 1,180 animals. In addition to OR, age at slaughter determination of OR (ovulation age), lactation length, and parity distribution are shown. Lactation length was used to adjust parity 1 and parity 2 data and parity distribution is shown for information purposes only.

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Table 1. Number of animals and summary statistics of the raw data

Trait and factors	Number of animals	Mean	SD	Minimum	Maximum
Ovulation rate	1,180	16.39	4.18	1	82
Ovulation age in days	1,180	432.5	144.9	165	1,207
Lactation length in days	880	18.25	2.29	0	24
Parity 0 ovulation rate	300	14.29	2.84	6	27
Parity 1 ovulation rate	516	16.32	3.44	1	30
Parity 2 ovulation rate	364	18.2	5.14	1	82

Deoxyribonucleic Acid Isolation, SNP Array Genotyping, and Quality Control

Genomic DNA was extracted from frozen tail tissue using the Wizard SV Genomic DNA Purification kit (Promega, Madison, WI) for all phenotyped pigs. Samples of 300 ng at a concentration ≥75 ng/μL of DNA were genotyped using the Illumina Porcine SNP60 BeadChip containing 64,232 SNP (Illumina; Ramos et al., 2009). Genotypic reactions were completed at the USMARC (Clay Center, NE) and then scanned at the USDA, ARS, Bovine Functional Genomics Laboratory (Beltsville, MD). Scan results were interpreted at the USMARC using Illumina's BeadStudio Genotyping software. Genotypes were called for 59,895 SNP spanning the entire porcine genome. Chromosome and position locations from the *Sus scrofa* genome assembly 10.2 (www.ncbi.nlm.nih.gov/mapview/) were used.

Any SNP located on SSCY, with unknown chromosome positions in swine genome 10.2, or with call rates <95%, or minor allele frequencies <0.05 were excluded from the data set. Animals were eliminated for genotypic call rates averaging <95% or for failing a Mendelian segregation (parentage) test. After using these quality control measures, 41,848 SNP out of a total of 64,232 SNP and 1,175 of the original 1,180 females qualified for GWAS.

Genomewide Association Analyses

Bayes C methods found in GenSel software (http://bigs.ansci.iastate.edu) were used in the GWAS analyses. Except where noted, the methods of Onteru et al. (2012) were followed. The basic model of Bayes C (Kizilkaya et al., 2010) was modified to incorporate fixed effects as follows:

$$\mathbf{y} = \mathbf{Xb} + \sum_{j=1}^{k} \mathbf{z_j}\alpha_j\delta_j + \boldsymbol{\mu},$$

in which $\mathbf{y}$ is the phenotypic vector, $\mathbf{X}$ is an incidence matrix relating fixed factors to phenotypes, $\mathbf{b}$ is a vector of fixed factors, k is the total number of SNP, $\mathbf{z_j}$ is the column vector of genotypic covariates for SNP_j, α_j is the allele substitution effect for SNP_j, and δ_j is an indicator for whether SNP_j was included ($\delta_j = 1$) or excluded ($\delta_j = 0$) in the model for a given Markov chain. The prior probability ($\boldsymbol{\pi}$) that $\delta_j = 0$ was set equal to 0.997. The allele substitution effect α is conditional on σ^2_α and is considered to be normally distributed N (0, σ^2_α). The vector of random residual effects $\boldsymbol{\varepsilon}$ is assumed to be normally distributed N (0, σ^2_ε).

A fixed classification effect used in this statistical model was insemination–year–season defined as the year and season when females would have been inseminated. An additional fixed effect was the covariate of age at OR determination. Ovulation rate of parity 1 and parity 2 females was adjusted for lactation length using linear adjustments generated from the data set. The fitting of age when number of corpora lutea were counted removed much of the variation of parity, causing parity to be not significant and therefore not included in the final model.

Initial priors were taken from residual and additive genetic variance (**GV**) components from a preliminary analysis using ASReml (Gilmour et al., 2006, 2009). The model fitted was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e},$$

in which $\mathbf{y}$ represented a vector of observations; $\mathbf{b}$ was a vector of fixed effects; $\mathbf{a}$ was a vector of random additive genetic effects of animals, which was assumed to be distributed N (0, $\mathbf{A}\sigma^2_\mathbf{a}$) where $\mathbf{A}$ was the numerator relationship matrix among animals; and $\mathbf{e}$ was a vector of residual effects, which was assumed to be distributed N (0, $\mathbf{I}\sigma^2_\mathbf{e}$), where $\mathbf{I}$ was the identity matrix.

Incidence matrix $\mathbf{X}$ related records to fixed effects and incidence matrix $\mathbf{Z}$ related records to additive genetic random effects. The fixed effects were those used in the previous Bayesian model. The pedigree file included 292 sires and 1,136 dams without phenotypic data, 610 dams with phenotypic data, and 565 females with phenotypic data but no phenotyped progeny.

A preliminary run of the Bayes Cπ option of GenSel used the variance priors from ASReml to estimate marker variance and also estimated π to be 0.997 where π is the probability that any SNP would have a 0 effect. Bayes C used the marker variance prior and the estimate of π from Bayes Cπ for the analysis of SNP effects with a burn-in of 1,000 iterations and a total of 51,000 iterations.

After the Bayes C analysis, the Predict option of GenSel was used to estimate the GV of sliding windows

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of 5 consecutive SNP assigned by chromosome-position order beginning with the first 5 SNP on SSC1 and ending with the last 5 SNP on SSCX. There were 8,354 nonoverlapping 5-SNP windows available for statistical testing. The expected proportion of GV accounted by 1 nonoverlapping 5-SNP window is 1/8,354 or 1.197×10^{-4} (Onteru et al., 2011, 2012). One hundred forty-two 5-SNP windows that exceeded 1.197×10^{-4} proportion of GV were defined as putative QTL and were submitted for statistical testing using the Bootstrap option of GenSel.

Bootstrap Analysis for Hypothesis Testing

Bootstrap samples were produced using the posterior means of the 41,848 SNP to construct the distribution of the test statistic (GV of a 5-SNP window) for each putative QTL. One thousand bootstrap data sets were created (Fan et al., 2011; Onteru et al., 2011, 2012). The null hypothesis was that no QTL existed in the identified 5-SNP window. Construction of the bootstrap began with the results of the Bayes C analysis including the SNP effects of all markers and the estimates of the fixed effects relevant to each animal's phenotypic record. The SNP that are within the region of the putative QTL were set to 0. Residual effects are sampled according to the residual variance previously found and added to each record. The only difference between bootstrap data replicates was due to different residual effects being sampled in different replicates. The same Bayes C model that was used for the initial data was used to analyze the bootstrap samples and each Bayes C run was followed by a Predict Option identical to the initial analysis. Genetic variances from the 1,000 bootstrap samples for each putative QTL were accumulated for comparison with the test statistic (GV of a 5-SNP window) generated in the initial analysis of the data. If just 1 bootstrap statistic from the 1,000 that were simulated exceeded the test statistic from the real data, the comparison-wise *P*-value was determined to be $0.001 < P < 0.002$ (Davidson and Hinkley, 1997).

The proportion of false positives (Fernando et al., 2004) was used to take into account multiple testing in this study. The proportion of false positive conclusions across all the tests undertaken is controlled by that approach, rather than the probability of making 1 mistake over all tests, as would be the interpretation of an experiment-wise error correction. The proportion of false positives is calculated as a function of the average comparison-wise Type I error rate, the proportion of true null hypotheses tested among all hypotheses tested, and the power of the test. This experiment and previous swine reproduction experiments (Onteru et al., 2011, 2012) used 0.99 as an estimate of the proportion of true null hypotheses among all hypotheses. This experiment differs from the others because it is based on larger numbers of parity 1 females, higher heritability, and a higher proportion of variance explained by markers. These factors would normally support a higher power of the test. The USMARC previously developed an unpublished set of figures used to estimate power of the test. Those figures were used to make 3 estimates of the power of the test. These estimates were averaged and used to estimate the probability of false positives to be 0.13 for $P = 0.001$, 0.42 for $P = 0.005$, and 0.60 for $P = 0.01$.

RESULTS AND DISCUSSION

In the present study, a GWAS using the Porcine SNP60 BeadChip was performed by means of Bayes C model averaging with random SNP effects for OR. The variances, heritability, and proportion of total variance explained by the markers are shown in Table 2. Using ASReml, heritability was found to be moderate at 0.33. Bidanel (2011) summarized 18 OR heritability estimates and determined a mean of 0.32 with a range of 0.10 to 0.59. The proportion of total variance explained by the markers was estimated by GenSel to be 0.28 and indicates that the SNP explain a comparable amount of genetic variation similar to the infinitesimal model. Supplemental Fig. 1 is a Manhattan plot of percent total GV explained by SNP for OR. A total of 142 putative QTL were identified for statistical testing using bootstrap analyses.

Table 3 presents information about the QTL identified in this study and includes percent of GV explained by each QTL, bootstrap *P*-values, and previously reported QTL found in overlapping positions with OR QTL. Many of the previously reported QTL are for traits which have been shown to be genetically correlated with OR (Table 4). Eighty QTL regions were significantly ($P < 0.01$) associated with OR after bootstrap analysis. Statistical testing identified 22 QTL with $P \leq 0.001$, 36 QTL with $0.001 < P < 0.005$, and 22 QTL with $0.005 < P \leq 0.010$. The QTL were identified on SSCX and all 18 autosomes except SSC11 and explained 88.3% of the GV identified by the 8,354 5-SNP windows tested. Sixteen additional putative QTL with $P \leq 0.057$ located within 1 Mb of a significant QTL or associated with previously identified candidate genes are also listed in Table 3.

Many of the QTL detected were within 1 Mb of another QTL and these adjacent pairs of QTL may actually be due to a single QTL in the region. To determine if these adjacent QTL were independent, linkage disequilibrium (**LD**) between SNP were computed as r^2 and presented in Supplemental Table 1. Linkage disequilibrium values above 0.5 are shown. Genetic variance of QTL was identified using the SNP sliding windows computed in the Predict option, which accounts for LD between SNP within the QTL. It is the LD between SNP of closely positioned significant ($P < 0.01$) QTL that is of greatest

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Table 2. Summary statistics for ovulation rate

Method	Number of animals	Heritability/marker h^2 [1]	Genetic variance	Residual variance	Total variance
ASReml	1,180	0.32 ± 0.06	4.93 ± 1.14	10.56 ± 0.83	15.57 ± 0.77
GenSel Bayes C	1,175	0.282	4.19	10.54	14.73

[1]Marker h^2 is the proportion of total variance explained by the markers.

importance in Supplemental Table 1 (LD among SNP within QTL are not shown). Based on these results, 12 different groups of QTL including 5 pairs, 6 trios, and 1 quad exhibited LD above the threshold of 0.5 between markers in different QTL. These groups of QTL were located on SSC1, SSC2, SSC3, SSC4, SSC6, SSC8, SSC9, SSC13, SSC16, and SSC17. The LD between SNP of closely positioned QTL was greatest for the pairs of QTL detected on SSC1, SSC2, SSC4, and SSC6. Interpretation of adjacent QTL with significant LD among SNP should not assume that there are 2 unique quantitative trait nucleotides segregating for each QTL. The LD between the 2 QTL of SSC1 with positions of 202,763,865 and 203,690,819 are nearly perfect examples of the high LD.

Quantitative Trait Loci of Moderate Effects

Thirteen QTL located on 7 different chromosomes individually accounted for >1% of the GV. A single QTL on SSC17 covers 64.39 to 64.70 Mb and explains 23.78% of the GV but has no LD above the threshold between markers of close QTL. A possible candidate gene to study for this region is *bone morphogenetic protein 7* (*BMP7*), located at 64.75 Mb (Table 5). *BMP7* has been shown to increase granulosa cell mitosis and decrease ovarian progesterone production and ovulation in the rat (Lee et al., 2001). Within the USMARC population, a QTL for NBA was discovered in this region (Schneider et al., 2012b) that may be a result of this chromosomal region's effect on OR.

Chromosome 2 had 2 QTL explaining a total GV (26.88%) located at 137.29 to 139.99 Mb. These QTL appear to be independent based on LD but both QTL are located near a viable biological candidate gene. *A disintegrin and metalloproteinase with thrombospondin motifs 19* (*ADAMTS19*), located at 137.46 Mb on SSC2, has previously been implicated in premature ovarian failure in humans (Knauff et al., 2009) and is highly expressed in the developing ovary. *Growth/differentiation factor 9* (*GDF9*; 140.00 Mb), which is a member of the transforming growth factor beta (**TGF-β**) superfamily, is located near the second QTL. *GDF9* is coregulated with bone morphogenetic factor 15 (*BMP15*) in a species-specific manner to control OR (Crawford and McNatty, 2012). Variants in *GDF9* have been associated with ovarian disorders as well as mothers giving birth to dizygotic twins in humans (Palmer et al., 2006).

Chromosome 1 had 4 QTL explaining more than 1% of the GV. The region on SSC1 (16.14 to 16.22 Mb) explained 3.61% of the GV and was located near *estrogen receptor 1* (*ESR1*) at 16.78 Mb. Variation in *ESR1* has been associated with differences in litter size in Meishan crossbred (Rothschild et al., 1996) and commercial swine (Short et al., 1997) populations. The second QTL on SSC1 explained 2.26% of the GV and was located at 107.22 to 107.28 Mb near *SMAD2* (*mothers against decapentaplegic homolog 2*). Deletion of *SMAD2* (and *SMAD3*) is associated with disruption of folliculogenesis and ovulation in mice (Li et al., 2008). A third region (185.72 to 185.78 Mb) containing the transcriptional coregulator *TLE3* explained 2.51% of the GV and was found to be associated with NBA (Coster et al., 2012). The fourth QTL explained 1.77% of the GV and was located on SSC1 at 202.76 to 203.05 Mb near *BMP4*, which has been shown to promote primary follicular development (Nilsson and Skinner, 2003).

The 6 remaining QTL explaining over 1% of the GV were located on 4 chromosomes. One QTL located on SSC3 at 100.47 to 100.59 Mb explained 1.23% of the GV. Two QTL were found on SSC4 and explained 2.40 and 1.25% of the GV. The first was located at 74.18 to 74.36 Mb and had been associated with OR (Bidanel et al., 2008). The second QTL was found at 137.30 to 137.55 Mb and had been associated with mummified fetuses (**MUM**; Onteru et al., 2012). A QTL on SSC6 was found at 147.48 to 147.69 Mb that explained 1.53% of the GV. This QTL had previously been associated with MUM (Holl et al., 2004; Onteru et al., 2012). A second QTL on SSC6 was identified at 153.38 to 153.52 Mb explained 1.68% of the GV; the nearest candidate gene is a direct target gene of EGR-1 (*TOE1*) at 153.5 Mb. *TOE1* acts as a growth suppressor protein through *EGR1* (de Belle et al., 2003), which is necessary for LH production and signaling (Topilko et al., 1998).

The final QTL explaining >1% of the GV was found on SSC15 at 121.73 to 122.30 Mb within *cAMP responsive element binding protein 1* (*CREB1*) and overlapped QTL for OR identified by Rohrer et al. (1999) and Wilkie et al. (1999) as well as a gestation length QTL (Wilkie et al., 1999). *CREB1* is a transcriptional coactivator involved in steroidogenesis and FSH responsiveness (Sher et al., 2007).

Associations with Other Reported QTL

To the best of our knowledge, results in the literature from other GWAS using the Porcine SNP60 BeadChip

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Table 3. Information about QTL associated significantly ($P < 0.01$) after bootstrap analysis with ovulation rate

SSC	Position (Start–end)	5-SNP window (Start–end)	Genetic variance, %	Bootstrap *P*-values	Previously reported QTL at the 5-SNP window [1,2]
1	15.05–15.23	ALGA0001112–DRGA0000110	0.18	$P = 0.011$	
1	15.98–16.10	ALGA0001160–ASGA0001179	0.33	$P = 0.001$	MUM[7]
1	16.14–16.22	MARC0057601–MARC0023380	3.61	$P < 0.001$	MUM[7]
1	107.23–107.28	ASGA0003984–ALGA0005129	2.26	$P = 0.002$	
1	184.92–185.12	ALGA0006843–ASGA0005190	0.08	$P = 0.034$	TNB[5]
1	185.72–185.78	ALGA0006891–ALGA0006895	2.51	$P = 0.002$	TNB[5]
1	202.76–203.05	MARC0045620–ALGA0007195	1.77	$P < 0.001$	
1	204.99–205.31	ALGA0007253–ALGA0007263	0.12	$P = 0.005$	
1	205.58–205.70	ALGA0007272–ALGA0007278	0.14	$P = 0.005$	
1	286.01–286.17	H3GA0004412–ASGA0006940	0.11	$P = 0.009$	AP[8] and GL[12]
1	288.31–288.42	ALGA0009672–ALGA0009685	0.49	$P = 0.002$	AP[8] and GL[12]
1	301.03–301.13	ALGA0010487–H3GA0004945	0.39	$P = 0.006$	
2	21.00–21.41	H3GA0006250–ALGA0012412	0.30	$P = 0.010$	GL[7]
2	86.51–86.65	ASGA0010629–ALGA0014115	0.08	$P = 0.003$	
2	128.87–128.98	H3GA0007563–INRA0009686	0.27	$P < 0.001$	
2	133.41–133.59	ASGA0011790–ASGA0011793	0.28	$P = 0.001$	MUM[7]
2	137.05–137.14	ALGA0015991–ALGA0016007	0.09	$P = 0.014$	NSB[7]
2	137.29–137.63	ALGA0016016–ISU10000003	16.73	$P < 0.001$	
2	139.84–139.99	ALGA0016191–H3GA0007803	10.15	$P < 0.001$	
2	144.35–144.55	ASGA0104950–ALGA0109544	0.10	$P = 0.007$	MUM[7]
2	144.57–144.81	ALGA0113220–DIAS0003220	0.26	$P = 0.002$	MUM[7]
2	144.89–145.14	ALGA0121603–H3GA0053178	0.11	$P = 0.005$	MUM[7]
2	152.61–152.69	ALGA0016849–ASGA0012724	0.88	$P < 0.001$	
2	153.00–153.13	ASGA0012738–MARC0024052	0.15	$P = 0.004$	GL[7]
2	156.07–156.21	MARC0058435–ASGA0099432	0.21	$P = 0.004$	
3	3.03–3.11	DRGA0003754–H3GA0008443	0.06	$P = 0.016$	OR[8]
3	99.54–99.74	M1GA0004494–H3GA0010068	0.18	$P = 0.002$	
3	100.48–100.59	ASGA0015392–MARC0038494	1.23	$P < 0.001$	
3	100.79–101.22	H3GA0010134–ASGA0015465	0.27	$P = 0.005$	
3	107.58–107.68	DRGA0004115–MARC0012871	0.16	$P = 0.003$	
4	74.18–74.36	H3GA0012878–ALGA0025611	2.40	$P = 0.001$	OR[3]
4	75.25–75.39	ASGA0020045–ALGA0025658	0.08	$P < 0.001$	OR[3]
4	75.45–75.56	ALGA0025665–ASGA0020060	0.28	$P = 0.004$	OR[3]
4	120.73–121.05	ALGA0028070–M1GA0006435	0.17	$P = 0.010$	
4	131.56–131.92	ALGA0028847–ALGA0028853	0.15	$P = 0.009$	GL[7]
4	135.39–135.63	ALGA0029239–DRGA0005348	0.07	$P = 0.010$	OR[3]
4	137.02–137.10	MARC0091857–H3GA0014773	0.18	$P = 0.004$	MUM[7]
4	137.30–137.55	DRGA0005371–MARC0077249	1.25	$P < 0.001$	MUM[7]
5	5.67–5.86	ALGA0030106–ASGA0024035	0.36	$P = 0.004$	NSB[7]
5	9.89–10.24	H3GA0015604–ASGA0024321	0.22	$P = 0.001$	GL[7] and NSB[7]
5	68.41–68.57	ASGA0025985–H3GA0016584	0.28	$P = 0.006$	NSB[7]
6	147.48–147.69	ALGA0114066–H3GA0019192	1.53	$P < 0.001$	MUM[6,7]
6	152.82–152.91	ASGA0030214–MARC0058375	0.10	$P = 0.010$	
6	153.11–153.26	ALGA0037706–H3GA0019269	0.11	$P = 0.012$	
6	153.38–153.52	H3GA0019279–ALGA0037743	1.68	$P < 0.001$	
7	10.90–11.14	SIRI0000391–ASGA0031167	0.36	$P = 0.002$	GL,[7] NBA,[11] and TNB[7]
7	32.79–32.88	ASGA0032270–H3GA0020625	0.37	$P = 0.002$	AP[4]
7	121.91–122.11	H3GA0023245–M1GA0010841	0.09	$P = 0.005$	GL[7] and UHL[12]
7	124.04–124.15	ALGA0045253–MARC0073407	0.11	$P = 0.008$	GL[7] and UHL[12]
8	9.03–9.15	ASGA0037687–ASGA0037704	0.10	$P = 0.022$	OR[8] and TNB[7]
8	10.72–10.99	MARC0051752–ALGA0112294	0.19	$P = 0.007$	
8	11.13–11.23	ASGA0037801–ALGA0046431	0.05	$P = 0.031$	
8	11.46–11.57	H3GA0024295–ASGA0037818	0.15	$P = 0.007$	
8	13.41–13.63	CASI0003674–DRGA0008317	0.46	$P = 0.002$	

continued

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Table 3. (cont.)

SSC	Position (Start–end)	5-SNP window (Start–end)	Genetic variance, %	Bootstrap *P*-values	Previously reported QTL at the 5-SNP window [1,2]
8	22.90–23.19	MARC0082286–MARC0058613	0.11	$P = 0.006$	
9	48.79–48.89	ASGA0042880–M1GA0012952	0.47	$P = 0.002$	OR[8]
9	50.20–50.63	ALGA0108618–ASGA0042960	0.05	$P = 0.022$	OR[8]
9	61.78–61.96	ASGA0043259–ASGA0043272	0.51	$P = 0.011$	OR[8]
9	62.69–62.75	H3GA0027510–MARC0059326	0.07	$P = 0.057$	OR[8]
9	63.42–63.64	M1GA0024594–ASGA0043336	0.11	$P = 0.013$	OR[8]
9	135.81–136.01	MARC0043119–ASGA0044615	0.16	$P = 0.006$	OR,[8] RTW[9] and UHW[9]
10	6.34–6.65	ASGA0095895–ALGA0056551	0.17	$P = 0.008$	
10	17.38–17.95	MARC0004259–ASGA0046818	0.12	$P = 0.008$	
10	63.52–63.63	H3GA0030479–MARC0114199	0.43	$P < 0.001$	OR[8]
10	65.88–66.21	MARC0064111–H3GA0030570	0.30	$P = 0.002$	OR[8] and FSH[8]
12	56.16–56.36	ASGA0055114–ASGA0055110	0.07	$P = 0.003$	
13	33.84–34.09	ALGA0069378–MARC0033504	0.17	$P = 0.010$	OR,[3] AP,[3] and UHL[9]
13	34.48–34.60	ASGA0057090–H3GA0036129	0.36	$P = 0.002$	OR,[3] AP,[3] and UHL[9]
13	132.50–132.82	ALGA0071863–MARC0084645	0.45	$P = 0.003$	OW,[9] TNB,[7] and UHL[9]
13	133.71–134.02	ASGA0058771–H3GA0037239	0.05	$P = 0.028$	TNB[7] and UHL[9]
13	150.71–151.11	ALGA0108778–ALGA0072255	0.09	$P = 0.006$	
14	0.10–0.44	H3GA0038237–ALGA0074152	0.46	$P = 0.004$	
14	17.57–17.87	ALGA0075620–ALGA0075815	0.06	$P = 0.008$	
15	83.70–84.11	MARC0058320–ALGA0085877	0.05	$P = 0.014$	OR[8] and MUM[7]
15	89.10–89.22	ALGA0086091–ASGA0094816	0.07	$P = 0.036$	OR[8]
15	89.71–89.84	ALGA0086110–DRGA0015245	0.71	$P < 0.001$	OR[8]
15	120.34–120.49	MARC0063927–H3GA0044820	0.13	$P = 0.004$	OR[8,12] and GL[12]
15	121.73–122.30	ALGA0086718–ASGA0093298	2.73	$P < 0.001$	OR[8,12] and GL[12]
15	138.73–139.05	ALGA0087194–ASGA0070698	0.06	$P = 0.006$	OR,[8,12] AP,[6] GL,[12] and NBA[7]
16	36.47–36.75	ALGA0090273–H3GA0056624	0.34	$P = 0.003$	MUM[7]
16	61.31–61.61	MARC0103861–DRGA0016231	0.09	$P = 0.012$	
16	62.25–62.68	MARC0025628–ASGA0073666	0.12	$P = 0.003$	MUM[7]
16	62.70–63.22	MARC0025861–ALGA0091053	0.21	$P = 0.002$	
16	67.43–67.86	H3GA0046863–MARC0010374	0.16	$P = 0.003$	
17	59.31–59.52	MARC0085465–H3GA0049524	0.51	$P = 0.003$	
17	60.14–60.26	MARC0104763–H3GA0049554	0.65	$P = 0.001$	
17	60.49–60.62	ALGA0095910–ALGA0095920	0.08	$P = 0.013$	
17	60.89–61.01	ALGA0095927–MARC0051474	0.32	$P = 0.002$	NSB[7]
17	63.58–63.93	H3GA0049700–M1GA0022377	0.64	$P = 0.002$	MUM[7] and NBA[10]
17	64.39–64.47	ASGA0078020–ALGA0096230	23.78	$P < 0.001$	MUM[7] and NBA[10]
17	66.46–66.79	M1GA0022687–MARC0002156	0.30	$P < 0.001$	NBA[10]
18	46.63–46.76	DBWU0000182–DIAS0000538	0.05	$P = 0.008$	NBA[11]
18	53.64–53.78	H3GA0055288–H3GA0051108	0.30	$P = 0.008$	NSB[7]
18	57.03–57.16	ASGA0080381–ALGA0098856	0.05	$P = 0.004$	
18	57.91–58.06	MARC0056150–MARC0025527	0.19	$P = 0.002$	
X	15.51–15.98	INRA0056528–ASGA0080878	0.10	$P = 0.001$	

[1]Most QTL information was obtained from PigQTLdb using the pig genome build 10.2, accessed Sept. 4, 2013 (www.animalgenome.org/cgi-bin/gbrowse/pig/). Additional references are noted.

[2]AP = age at puberty; GL = gestation length; MUM = mummified fetuses; NBA = number born alive; NSB = number still born; OR = ovulation rate; OW = ovary weight; RTW = reproductive tract weight; TNB = total number born; UHL = uterine horn length; UHW = uterine horn weight.

[3]Bidanel et al., 2008.

[4]Cassady et al., 2001.

[5]Coster et al., 2012.

[6]Holl et al., 2004.

[7]Onteru et al., 2012.

[8]Rohrer et al., 1999.

[9]Rosendo et al., 2012.

[10]Schneider et al., 2012b.

[11]Tribout et al., 2008.

[12]Wilkie et al., 1999.

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Table 4. Literature estimates of genetic correlations of ovulation rate with other female reproductive traits

	Trait[1]									
Author	AP	TNB	NBA	NSB	MUM	OW	RTW	UC	UHL	UHW
Long et al., 1991		0.62–0.73								
Irgang et al., 1993									0.23	
Bidanel et al., 1996	–0.36									
Nielsen et al., 1996								0.43–0.68		
Johnson et al., 1999		0.24	–0.02	0.34	0.27					
Rosendo et al., 2007	0.24		0.42	0.20						
Bidanel, 2011	–0.08	0.08	0.25							
Rosendo et al., 2012						0.47	0.31		0.24	0.35

[1]AP = age at puberty; TNB = total number born; NBA = number born alive; NSB = number still born; MUM = mummified fetuses; OW = ovary weight; RTW = reproductive tract weight; UC = uterine capacity; UHL = uterine horn length; UHW = uterine horn weight.

are not available for OR. Limited OR results based on microsatellites and SNP found on regions overlapping these OR QTL have been published (Table 3). To assist in the validation of QTL found in this analysis, we decided to expand the search to QTL found in overlapping locations associated with other correlated female reproduction traits. Table 4 presents a summary of genetic correlations of OR with other female reproductive traits referenced in Table 3. Bidanel et al. (1996) demonstrated a moderate negative genetic correlation with age at puberty. Bidanel (2011) presented small correlations with both age at puberty (negative) and TNB (positive) and a moderate positive correlation with NBA. Johnson et al. (1999) showed moderate positive correlations with TNB, number still born (**NSB**), and MUM but a very small negative correlation with NBA. Rosendo et al. (2007) found modest positive correlations with age at puberty, NBA, and NSB. Due to the moderate genetic correlations reported among these traits, we decided to search for QTL associated with female reproductive traits by using PigQTLdb (www.animalgenome.org/cgi-bin/QTLdb/SS/ontrait?trait_ID = 722). A total of 38 of the 80 OR QTL (Table 3) overlap 1 or more QTL for 1 of 11 additional female reproduction traits.

Previously reported QTL mapping using linkage analysis resulted in QTL regions with extremely broad confidence intervals, often spanning 20 cM or more. A comparison of results from linkage analyses versus GWAS is therefore difficult. These results and other microsatellite results are presented in Table 3 for completeness but will not be discussed.

Several QTL for additional traits have been reported that have been found to overlap OR QTL reported in Table 3 from this study and other studies. The QTL at SSC15 138.73 to 139.05 Mb overlapped a NBA QTL (Onteru et al., 2012). The existence of QTL for traits that overlap with OR QTL may suggest that genes that contribute to OR may also contribute to differences in the overlapping traits. For example, uterine development is known to become ovarian dependent after d 90 of age (Bartol et al., 1993). Alternatively, QTL for traits could overlap OR QTL simply by chance.

Numerous OR QTL reported from this study in Table 3 overlap published QTL for correlated female reproductive traits. The OR QTL on SSC1 were associated with QTL found at 15.98 to 16.22 Mb for MUM (Onteru et al., 2012) and at 185.72 to 185.78 Mb for TNB (Coster et al., 2012). Chromosome 2 QTL at 21.00 to 21.41 Mb and 153.00 to 153.13 Mb overlaps previously reported gestation length QTL, and QTL at 133.41 to 133.59 Mb and 144.35 to 145.14 Mb overlaps previously reported QTL for MUM (Onteru et al., 2012). Onteru et al. (2012) reported QTL on SSC4 for gestation length (overlaps OR QTL at 131.56 to 131.92 Mb) and for MUM (overlaps OR QTL at 137.02 to 137.55 Mb). Quantitative trait loci were also reported on chromosome 5 for NSB (overlaps OR QTL at 5.67 to 5.86 Mb, 9.89 to 10.24 Mb, and 68.41 to 68.57 Mb) and gestation length (overlaps OR QTL at 9.89 to 10.24 Mb). On SSC6 an OR QTL at 147.48 to 147.69 Mb overlaps a previously reported QTL for MUM (Onteru et al., 2012), which is near the candidate gene *zinc finger FYVE domain containing-9* (*ZFYVE9*). Chromosome 7 has 3 overlapping QTL. The first at 10.90 to 11.14 Mb overlaps a QTL for gestation length and TNB (Onteru et al., 2012) and the final region (121.91 to 124.15 Mb) overlaps QTL for gestation length (Onteru et al., 2012). Chromosome 16 (62.25 to 62.68 Mb) overlaps a QTL for MUM reported by Onteru et al. (2012). Chromosome 17 QTL (60.89 to 61.01 Mb) overlaps a QTL for NSB (Onteru et al., 2012), and a QTL at 63.58 to 64.47 Mb overlaps a previously reported QTL for MUM (Onteru et al., 2012). The chromosome 17 QTL at 63.58 to 66.79 Mb overlaps a previously reported QTL for NBA (Schneider et al., 2012b). Chromosome 18 OR QTL at 46.63 to 46.76 Mb overlaps a QTL for NBA (Schneider et al., 2012b) and the OR QTL at 53.64 to 53.78 Mb overlaps a QTL for NSB (Onteru et al., 2012). As for uterine traits, these overlapping QTL may be due to the effects of OR on these reproductive traits. Increased OR is associated with intrauterine crowding,

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Table 5. Candidate genes in QTL regions

SNP_ID[1]	SSC	Position (9.2)[2]	Position (10.2)[3]	Symbol	Gene name
MARC0023380	1	14,771,447	16,221,205	*ESR1/VIP*	*Estrogen receptor 1*
MARC0057601	1	14,857,638	16,135,014	*ESR1*	*Estrogen receptor 1*
H3GA0003414	1	192,690,906	204,625,574	*SOCS4*	*Suppressor of cytokine signaling 4*
MARC0023454	1	192,891,205	204,799,137	*SOCS4*	*Suppressor of cytokine signaling 4*
ASGA0007537	1	277,812,680	296,854,248	*RABGAP1*	*Rab GTPase-activating protein 1*
M1GA0001554	1	278,249,247	297,217,239	*RABGAP1*	*Rab GTPase-activating protein 1*
ASGA0007653	1	281,320,139	300,830,758	*FAM125B*	*Multivesicular body subunit 12B*
H3GA0004939	1	281,485,575	301,038,913	*FAM125B*	*Multivesicular body subunit 12B*
ALGA0015991	2	119,440,494	137,046,877	*ADAMTS19*	*ADAM metalloproteinase 19*
ALGA0016007	2	119,536,976	137,143,359	*ADAMTS19*	*ADAM metalloproteinase 19*
ALGA0016062	2	120,421,426	137,910,267	*ADAMTS19*	*ADAM metalloproteinase 19*
CASI0009217	2	120,537,883	138,026,728	*ADAMTS19*	*ADAM metalloproteinase 19*
ALGA0016212	2	122,384,184	140,002,186	*GDF9*	*Growth differentiation factor 9*
MARC0004958	2	122,915,957	140,474,431	*GDF9*	*Growth differentiation factor 9*
ALGA0016255	2	123,022,711	140,580,800	*GDF9*	*Growth differentiation factor 9*
H3GA0008038	2	128,911,823	147,854,152	*CXXC5*	*CXXC finger protein 5*
M1GA0024750	2	129,147,662	148,083,435	*CXXC5*	*CXXC finger protein 5*
ASGA0012712	2	132,432,573	152,297,333	*NR3C1*	*Glucocorticoid receptor*
ALGA0016849	2	132,747,410	152,612,171	*NR3C1*	*Glucocorticoid receptor*
ALGA0016913	2	133,055,033	153,202,449	*NR3C1*	*Glucocorticoid receptor*
H3GA0008193	2	133,089,376	153,079,388	*NR3C1*	*Glucocorticoid receptor*
MARC0035741	6	113,581,999	147,456,862	*ZFYVE9*	*Zinc finger FYVE domain containing 9*
H3GA0019193	6	113,765,118	147,582,955	*ZFYVE9*	*Zinc finger FYVE domain containing 9*
ALGA0096230	17	59,840,056	64,468,858	*BMP7*	*Bone morphogenetic protein 7*
H3GA0051240	18	51,570,495	58,034,747	*INHBA*	*Inhibin βA*

[1]SNP marker identifier provided in the manifest sheet of the Porcine SNP60 BeadChip.

[2]SNP position in the swine genome based on sus scrofa build 9.2

[3]SNP position in the swine genome based on sus scrofa build 10.2.

reducing fetal and birth weights and leading to late gestation fetal losses (increased MUM) and increased NSB (Johnson et al., 1999). Many of the studies listed did not measure OR in their populations so the associations observed with birth traits could be effects due to variation in OR. Alternatively, the overlapping regions could occur by chance, since many previously reported QTL include large regions with numerous genes.

Promising Candidate Genes for QTL of Minor Effect

A portion of the QTL identified in this experiment are located near putative candidate genes based on our current knowledge of OR and the swine genome. Three of these QTL are located near genes in the FSH signaling pathway. The QTL on SSC2 at 33.75 to 34.18 Mb is near the gene for the *β subunit of FSH* (*FSHβ*) while the other 2 QTL (SSC5 at 18.01 to 18.27 Mb and SSC18 at 58.03 to 58.43 Mb) are located near the FSH regulator genes *activin receptor type-1B* (*ACVR1B*) and *inhibin subunit βA* (*INHBA*), respectively (Knight et al., 2012). In addition to QTL potentially associated with FSH signaling, several other QTL are located near possible candidate genes that could affect ovarian function or OR. The QTL on SSC1 at 204.63 to 204.80 Mb is located near *suppressor of cytokine signaling 4* (*SOCS4*), which modulates the activation of primordial follicles (Sutherland et al., 2012). One QTL on SSC2 at 147.85 to 148.08 Mb is near *CXXC finger protein 5* (*CXXC5*) and a nearby QTL at 152.61 to 152.69 Mb is close to the glucocorticoid receptor *NR3C1*. *CXXC5* expression is reduced in ovaries of monotocous vs. polytocous goats (An et al., 2012) and patients with diminished ovarian reserve (May-Panloup et al., 2012). Greater glucocorticoid receptor protein is associated with polycystic ovary syndrome (Milutinović et al., 2011) and administration of glucocorticoid will block ovulation in sows (Gee et al., 1991). A QTL on SSC6 at 147.48 to 147.69 Mb is located in the candidate gene *ZFYVE9*. ZFYVE9 interacts with SMAD2 and SMAD3, which are TGF-β family members essential for normal follicular development and ovulation (Li et al., 2008).

Comparison with Other Study Designs

Differences between these results and the results of previous studies are likely due to differences in the genetics of the population under experimentation. Early marker work was often completed using synthetic or

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multigenerational populations based on crosses of exotic breeds with conventional breeds. Bidanel et al. (2008) studied Meishan by × Large White crossbred populations. Wilkie et al. (1999) experimented with Meishan × Yorkshire populations. Rohrer et al. (1999) evaluated Meishan crossed with a 4-breed white composite line.

A Large White × Landrace population was evaluated by Cassady et al. (2001) and Holl et al. (2004). Coster et al. (2012) used a Large White purebred population whereas Onteru et al. (2012) examined 2 populations: a Large White and a Large White × Landrace cross. Tribout et al. (2008) used a Large White × French Landrace population.

Bidanel et al. (2008), Rohrer et al. (1999), and Wilkie et al. (1999) included OR in their studies and found 6, 9, and 3 QTL respectively that overlapped with QTL found in this study. They also found OR chromosomal regions that did not overlap, but these regions could have originated from the Meishan breed that was included in their populations. Cassady et al. (2001), and Holl et al. (2004) included OR in their studies. Cassady et al. (2001) found an OR QTL but this region did not overlap any QTL from this study. All of these studies predated the Porcine SNP60 BeadChip and GenSel and therefore were using alternative methodologies.

Comparable results were limited because only 5 reports analyzed OR in commercial swine. Ten other experiments used from 119 to 57,814 markers with 9 of those analyses in the range of 137 to 309 markers in exotic crosses of pigs.

Summary

Sixteen QTL were found to be statistically significant at the $P < 0.001$ level. These QTL explained 67.1% of the GV. Six QTL significant at the $P = 0.001$ level totaled 4.0% of GV for a grand total of 71.1%. Chromosomes 2 and 17 contributed greatly to both the number of highly significant QTL as well as explaining GV. Neither chromosome appears to have been well studied in the past and should be ripe for exploration using newer technologies such as gene sequencing, etc. Quantitative trait loci on SSC15, SSC1, SSC16, SSC4, and SSC7 have been previously reported and include both overlapping OR markers and markers overlapping other traits such as age at puberty, gestation length, NBA, and uterine horn length. The QTL found on these chromosomes are worthy of additional study.

Direct selection for OR has been shown to increase litter size; however, uterine capacity would soon become the limiting factor. If QTL can be found for uterine capacity, it should be possible to increase litter size while maintaining or improving proper fetal development, piglet birth weight, and piglet viability. The population studied has litter size and piglet birth weights available for 890 sows. Future research will attempt to address genomic factors for uterine capacity by evaluating birth data from these sows while accounting for the sow's genomic EBV for OR. This approach would assume that if the genomic potential for OR is considerably greater than the number of piglets born, then uterine capacity was limiting. Data from sows where the OR genomic potential was similar to litter size would conclude the sow's uterine capacity was at least as large as the number born. Factors such as average birth weight and piglet vigor can enhance the analysis if one assumes that birth weights and vigor scores will decrease as the number of piglets born approaches the maximum capacity.

In the absence of uterine capacity QTL, index selection could be practiced where a genomic EBV for OR could be used in combination with TNB QTL (Onteru et al., 2011; Schneider et al., 2012b) and a measure of piglet viability to improve sow productivity. This would be similar to the successful index selection practiced by Johnson et al. (1999) but enhanced with genetic markers. Some producers have already exceeded their goals for litter size and may have created uterine crowding and other problems associated with large litters. In those and similar cases, selecting against OR may be beneficial. Further work with candidate genes, gene sequencing, and other technologies will prove to be beneficial.

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