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Abstract. An elite, three-generation family from the USDA Meat Animal Research Center twinning population was examined for evidence of ovulation rate quantitative trait loci (QTL). This work was both a continuation of previously reported results suggesting evidence for ovulation rate QTL on bovine Chromosome (Chr) 7 and an extension of a genome-wide search for QTL. Additional markers were typed on Chr 7 to facilitate interval mapping and testing of the hypothesis of one versus two QTL on that chromosome. In addition, 14 other informative markers were added to a selective genotyping genome screening of this family, and markers exhibiting nominal significance were used to identify chromosomal regions that were then subjected to more exhaustive analysis. For Chr 7, a total of 12 markers were typed over a region spanning the proximal two-thirds of the chromosome. Results from interval mapping analyses indicated evidence suggestive of the presence of QTL (nominal $P < 0.00077$) within this region. Subsequent analysis with a model postulating two QTL provided evidence ($P < 0.05$) for two rather than one QTL on this chromosome. Preliminary analysis with additional markers indicated nominal significance ($P < 0.05$) for regions of Chrs 5, 10, and 19. Each of these regions was then typed with additional markers for the entire three-generation pedigree. Significant evidence ($P < 0.000026$) of ovulation rate QTL was found for Chrs 5 and 19, while support on Chr 10 failed to exceed a suggestive linkage threshold ($P > 0.00077$).

Introduction

Development of highly polymorphic markers and medium density linkage maps (Kappes et al. 1997; Barendse et al. 1997) has made identification of bovine quantitative trait loci (QTL) feasible. Among the traits for which marker-assisted selection will be most useful are those which are sex-limited in expression or very costly to measure. For these reasons, twinning rate in cattle is precisely the type of trait for which identification of marker–QTL linkage should prove a useful adjunct for selection programs or as an aid in QTL introgression. The current study seeks to identify QTL for ovulation rate, a trait with high genetic correlation with twinning rate (r_G of 0.75–0.9; Van Vleck et al. 1991; Gregory et al. 1997). The ultimate objective of this effort is to elucidate information that will facilitate selection for or against alleles conferring increased ovulation rate and twinning rate.

In our previous efforts (Blattman et al. 1996), 77 informative genetic markers were used in an initial genomic screening of three elite families from the USDA Meat Animal Research Center (MARC) bovine twinning population (43–45 informative markers per sire family). The strongest evidence for QTL was observed on Chr 7 in one of the three families; however, questions of QTL location and number were not unequivocally answered. The objectives of this study were twofold. First, as a follow-up to our earlier results, Chr 7 was subjected to further scrutiny to refine estimates of QTL location and number. Second, the search for QTL was broadened by expanding the genome screen with additional genetic markers, and potential QTL were examined by additional marker typing and interval mapping analysis in the regions of interest.

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Materials and methods

The USDA Meat Animal Research Center (MARC) twinning herd is a unique genetic resource whose twinning rate at greater than 30% (Gregory et al. 1997) is 5–20 times the levels commonly observed in various breeds of beef and dairy cattle (Rutledge 1975). The foundation of this herd was developed by collecting animals with records of multiple twin births from within MARC herds and private herds (Gregory et al. 1990). Subsequent selection for ovulation rate and twinning rate has led to continuing increases in each trait. Young sires are evaluated by progeny test and evaluations of ovulation rates in daughters over six to ten estrous cycles. Young sires are mated to produce approximately 10 daughters for progeny test, and consequently individual half-sib families are typically too small for effective marker segregation analysis. However, elite sires with the highest genetic merit for ovulation rate are used more extensively such that larger, extended families are available in some cases for marker analyses. The results reported here come from an extended family headed by a Swedish Friesian bull (sire 839802); available for analysis from this family were 34 daughters, 9 sons, and 122 paternal granddaughters.

Estimated breeding value for ovulation rate rather than raw ovulation rate was used as the dependent variable in QTL mapping analyses. Use of estimated breeding values permits the inclusion of sons in the analyses. Ovulation rate breeding values were estimated with best linear unbiased prediction (BLUP) statistical methodology within a complete animal model, fitting significant effects of heifer birth-year-season, age of heifer when ovulation rate was observed, and month of observation. Heritability of ovulation rate averaged over eight consecutive estrous cycles was 0.38 (Echternkamp et al. 1990). The computer program used to estimate breeding value for ovulation rate was the Prediction and Estimation package, PEST (Groeneveld et al. 1990).

For genome screening, QTL mapping was performed in a sequential approach starting with selective genotyping of a subset of the offspring. Roughly the top and bottom 25% of offspring for ovulation rate breeding value were utilized in the initial selective genotyping (20 daughters and two sons). Markers exhibiting nominal significance ($P < 0.05$) in the initial statistical analysis (described below) were then typed on the remaining sons and daughters. If nominal significance was maintained in the analysis of all offspring, then the same marker and additional flanking markers were typed for the entire three-generation pedigree (that is, granddaughters added to the analysis), and interval mapping analysis was performed.

All markers employed (Fig. 1) were microsatellites (Kappes et al. 1997; Barendse et al. 1997). Marker typing for preliminary screening used polymerase chain reaction (PCR) amplification of microsatellites with unlabeled primers, size separation of PCR products on non-denaturing acrylamide gels, and visualization of DNA by ethidium bromide staining. Marker typing for the full three-generation pedigree was performed by PCR amplification with fluorescently labeled primers and DNA detection with an Applied Biosystems 310 genetic analyzer. Only one primer of each

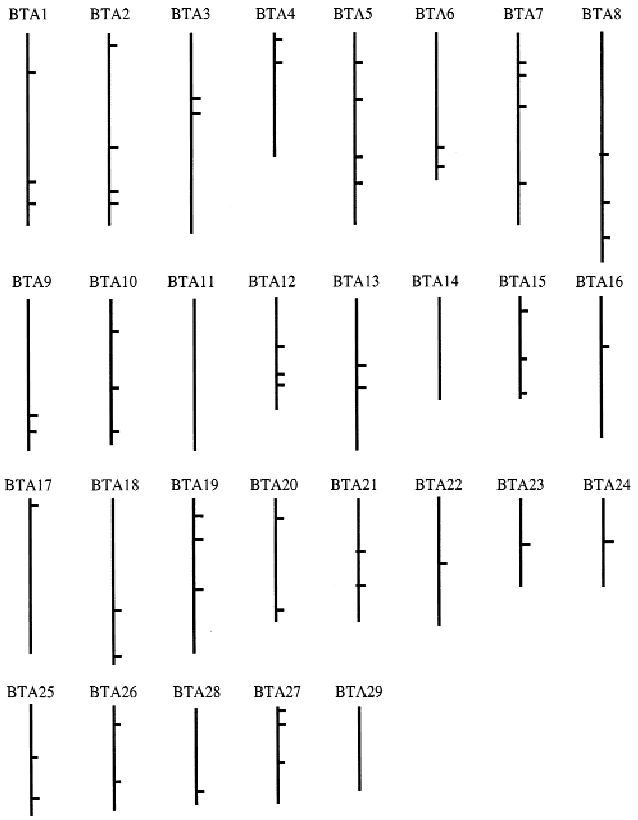

Fig. 1. Approximate locations of genetic markers used in preliminary screening in this study and a previous study (Blattman et al. 1996). Marker locations are shown with horizontal hash marks, and the top of each vertical line corresponds to the centromeric end of the chromosome.

pair was fluorescently labeled; the unlabeled 5′ end of the second primer of the pair was modified (Brownstein et al. 1996) to further promote the common addition by *Taq* polymerase of an adenosine to the 3′ end of the labeled DNA strand. Genotypes were scored with the aid of the Applied Biosystems Genotyper version 1.1 software program.

Statistical analyses of marker-QTL association were performed by regression analysis. Analysis of data from offspring in preliminary screening used the following model:

$$Y_i = a + b(x) + e_i$$

where

Y_i = estimated ovulation rate breeding value for the ith individual,
a = regression intercept,
b = chromosome substitution effect,
x = probability of inheriting sire allele 1 at the marker locus in question,
e_i = residual deviation.

In most cases, DNA was unavailable for offspring's dams. Consequently, for cases of ambiguous paternal allele inheritance (when sire and offspring had identical heterozygote genotypes), the probability of inheriting paternal allele 1 (arbitrarily designated) was determined by use of marker allele frequencies from the population of dams (Dentine and Cowan 1990). Since sire allele inheritance is treated as a probability in this approach, data from all offspring can be utilized. This was preferable to deleting data from individuals whose paternal allele inheritance was ambiguous.

Interval mapping analyses with the full three-generation family likewise employed the regression of ovulation rate breeding value on genotypic probability. In contrast to the analysis described above for genome screening, which was performed one marker at a time, genotypic probabilities for interval mapping were determined by simultaneously using marker allele frequency, map information, and pedigree information to determine the most likely haplotype inherited by a given son, daughter, or granddaughter. Haplotype inheritance was determined with the Genehunter software program (Kruglyak et al. 1996). Marker haplotypes predicted from Genehunter were then used to determine conditional probabilities of sire allele inheritance centimorgan by centimorgan (cM) across a linkage group. Probability of sire allele inheritance is conditional on genotypes at marker loci flanking the interval of interest or on the nearest informative marker for individuals where the last marker in the linkage group was uninformative. The approach is analogous to that outlined by Knott and coworkers (1996); as in their case, an assumption of no interference in recombination events is made so that Haldane's mapping function is employed.

Interval mapping analyses of marker-QTL association in the full three-generation family were performed by a weighted regression analysis. Varying amounts of information contributed to individual breeding value estimates, and to account for this, reliabilities corresponding to individual breeding value estimates were used as weights. In contrast to the preliminary regression analysis, analyses were repeated cM by cM across an entire linkage group rather than being limited to a specific marker locus. Additionally, the initial interval mapping analysis employed a multiple regression model that considered the effects of both patriarchal haplotypes:

$$Y_i = a + b_1(x_1) + b_2(x_2) + e_i$$

where

Y_i = estimated ovulation rate breeding value for the ith individual,
a = regression intercept,
b_1 = contrast of patriarch haplotype 1 versus all others,
x_1 = probability of inheriting patriarch haplotype 1,
b_2 = contrast of patriarch haplotype 2 versus all others,
x_2 = probability of inheriting patriarch haplotype 2,
e_i = residual deviation.

In an analysis limited to offspring, the only relevant contrast is the effect of patriarch haplotype 1 versus patriarch haplotype 2 as outlined for the preliminary analysis. However, granddaughters present an additional alternative—inheritance of neither patriarchal haplotype. Consequently, regression coefficient b_1 in the above model is a contrast of patriarchal haplotype 1 versus patriarchal haplotype 2 plus all other haplotypes represented in granddaughters not inheriting haplotype 1. There is a degree of colinearity between variables x_1 and x_2 and dependence between regression coefficients b_1 and b_2 given that the contrast of haplotypes 1 and 2 in offspring is represented in each. This model was used for initial testing of significance of QTL effect (based on the F-test for the full, multiple regression model). When significant effects were observed with the full model, a reduced model with only the more significant haplotype effect retained was analyzed to re-estimate QTL effect and location, thus eliminating the colinearity problem.

Thresholds for statistical significance were evaluated using the approach of Lander and Kruglyak (1995). Values used in calculating the thresholds include autosomal genome size of 30 Morgans, 29 autosomal chromosomes and ρ, the constant reflecting crossing-over rate between genotypes under comparison, equal to 1.5. The appropriate value of ρ is somewhat uncertain. For data from half-sib offspring, a value of 1 is appropriate (Lander and Kruglyak 1995), but for grandoffspring the appropriate value is not clear, though presumably higher given the accumulation of recombination events across generations. For two numerator and 163 denominator degrees of freedom (multiple regression model as outlined above), F-test and pointwise *P*-values corresponding to one expected false-positive result per genome-wide scan (suggestive linkage) were 7.49 and 0.00077, respectively. F-test and pointwise *P*-values corresponding to one expected false-positive result per 20 genome-wide scans (significant linkage) were 11.26 and 0.000026.

The possibility of multiple QTL in the same linkage group was evaluated with a multiple regression model that accounted for two separate QTL. Probabilities of sire allele inheritance based on the more significant haplotype effect were used in this analysis. In contrast to the preceding multiple regression model, where regressors were probabilities for sire allele inheritance for both patriarchal haplotypes at the same point in the linkage group, the regressors in this case are probabilities for sire allele inheritance for the same patriarchal haplotype evaluated at two different points in the linkage group. This analysis was performed as a grid search that considered all possible pairs of potential QTL locations within a linkage group, excepting the specification of two QTL at the same exact location. Evidence for two QTL versus a single QTL within a linkage group was evaluated by comparing full (two QTL) and reduced (single QTL) models for most likely QTL locations. Significance was tested with an F-test composed of

Table 1. Results of preliminary analysis from offspring of sire 839802.

Marker	Chromosome	Nominal *P*-value	
		Selective Subset[a]	All Offspring[b]
uw46	1	0.733	–
bm2113	2	0.139	–
bm4440	2	0.397	–
huj246	3	0.948	–
uw48	5	0.003	0.018
bm315	5	0.275	–
bm1853	7	0.530	–
uw29	8	0.601	–
uw36	9	0.698	–
cssm46	10	0.037	0.016
rm004	15	0.569	–
uw32	19	0.046	0.030
bm3517	20	0.698	–
bm804	26	0.270	–

[a] A selected sample of offspring including approximately the top and bottom 25% of individuals.
[b] All sons and daughters of 839802.

the ratio of sums of squares error for reduced minus full model (numerator) divided by the mean square error for the full model (denominator).

Results

Preliminary analysis with 14 microsatellite markers identified three chromosomal regions with nominal significance ($P < 0.05$) in both the selective subset of offspring and in all available offspring (Table 1). These markers were selected to fill some of the gaps in the previous genomic coverage for sire 839802 and bring the total number of informative markers used in preliminary screening to 59. Including results from the previous preliminary analysis, four of the 59 markers were nominally significant, a result little different from that expected by chance alone. With the results of this preliminary analysis, regions of Chrs 5, 10, and 19 were identified along with Chr 7 as targets for further analysis.

Interval mapping analyses of the complete three-generation family revealed evidence of QTL for three of the four targeted genomic regions (Fig. 2). Analysis with the full, multiple-regression model yielded significant ($P < 0.000026$) evidence of QTL linkage for regions of Chr 5 and Chr 19, and evidence suggestive of linkage ($P < 0.00077$) for regions of Chr 7. Results for Chr 10 (not shown) failed to exceed the threshold for suggestive linkage. Analysis with a reduced, simple linear regression model that focused on the most significant haplotype difference generally sharpened the peak of the QTL plot. The results for Chr 7 were the exception in that the QTL plots from both multiple regression and simple linear regression yielded a broad pattern of peaks, suggesting the possibility of multiple QTL. For all three chromosomes the most significant haplotype contrast was the patriarch haplotype associated with higher ovulation rate versus other.

Grid search for best fitting, two-QTL models (two QTL within the same linkage group) provided little evidence of a second QTL when comparing single and two-QTL models ($P > 0.10$) for Chrs 5 and 19. In contrast, comparison of best two-QTL and single QTL models for Chr 7 suggested a better fit ($P < 0.05$) for the two-QTL model. The grid search for two QTL suggested most likely locations at 5 and 57 cM on Chr 7. Given the support for a QTL on Chr 7 at the suggestive linkage level, the evidence for two versus one QTL should be considered preliminary.

Estimates of QTL effect on Chrs 5, 7, and 19 with the reduced, simple linear regression model indicate that the chromosome substitution effects correspond approximately to increases of from 5% to 10% in frequency of double ovulations (Table 2). Estimates of QTL effect are reported for both patriarchal haplotype contrasts; comparison of these estimates provides some suggestion of patriarchal QTL allele effects relative to the average of other alleles in the population. For Chrs 5 and 7, estimates of QTL effect from the contrast of haplotype 1 versus other and haplotype 2 versus other were of comparable magnitude but opposite sign. This suggests that the patriarch's allele 1 is superior to the average allele, while allele 2 is inferior to the average allele in the population. In contrast, for Chr 19, the estimate of QTL effect from the contrast of haplotype 1 versus other was far greater than the estimate from the contrast of haplotype 2 versus other. This suggests that allele 1 is a vastly superior allele, and allele 2 has an effect similar to that of the average allele in the population.

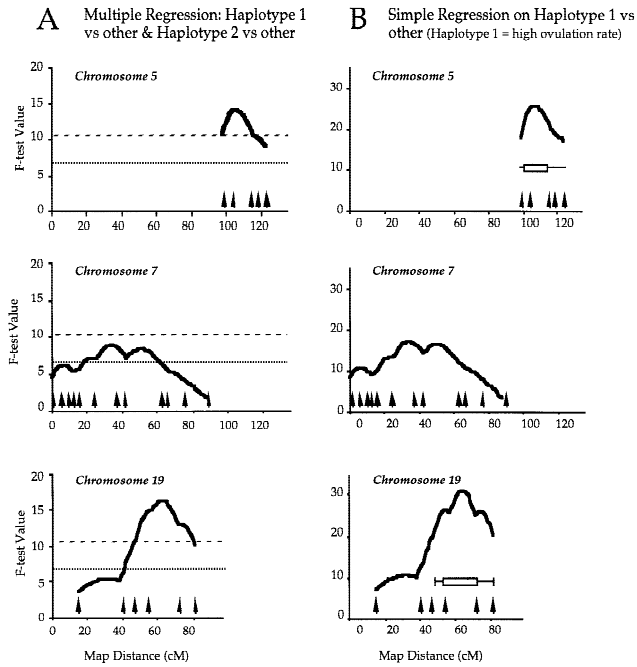

Fig. 2. Plots of F values from interval mapping analyses versus map distance on Chrs 5, 7, and 19. **A:** Results from multiple regression analysis (used for hypothesis testing), which included contrasts of haplotype 1 versus other and haplotype 2 versus other. Dotted horizontal lines show the F-test level corresponding to the suggestive linkage threshold. Dashed horizontal lines show the F-test corresponding to the significant linkage threshold. Locations of markers typed for the interval mapping analyses are denoted with arrowheads (▲). Length of the x-axis corresponds to the length of the chromosome linkage map (Kappes et al. 1997). **B:** Results from simple linear regression analysis considering only the more significant haplotype, which corresponded with the haplotype associated with increased ovulation rate in all cases. The equivalent of one-LOD (open box) and two-LOD (line extending from box) support intervals are indicated. The two-LOD support interval for Chr 5 would extend beyond the linkage group used in the interval mapping analysis.

Table 2. Estimates of ovulation rate QTL effect.

Chromosome	Location from centromere (cM)	Contrast[a]	QTL effect (breeding value)
5	107	Haplotype 1 vs. other	0.083 ± 0.016
		Haplotype 2 vs. other	−0.072 ± 0.019
7	5	Haplotype 1 vs. other	0.042 ± 0.018
		Haplotype 2 vs. other	−0.036 ± 0.019
7	57	Haplotype 1 vs. other	0.060 ± 0.019
		Haplotype 2 vs. other	−0.031 ± 0.022
19	65	Haplotype 1 vs. other	0.103 ± 0.018
		Haplotype 2 vs. other	−0.017 ± 0.022

[a] Estimates are from a simple linear regression model with the contrast shown. For the contrast of haplotype 1 versus other, "other" includes the effect of haplotype 2 and all haplotypes not inherited from the patriarch. The converse is true for the contrast of haplotype 2 versus other. For Chr 7, QTL effects are for the given QTL after first accounting for the effects of haplotype 1 at the other Chr 7 QTL location.

Discussion

The QTL effects observed here are large, corresponding to approximately four-tenths to one standard deviation of estimated ovulation rate breeding value (standard deviation = 0.108). Expressed in another way, single QTL models for the various QTL described here explain from 6% to 16% of the variation in estimated ovulation rate breeding value. There is little indication of epistatic interaction between QTL loci, as a multiple QTL model including all four QTL accounted for 32% of the variation in estimated ovulation rate breeding value, nearly the sum of their individual estimates from separate single QTL models.

QTL locations reported here can be compared with corresponding regions of the human genome in an effort to identify suitable candidate genes. Analysis of this type must be tempered with the recognition that support intervals for the location of QTL reported here are quite broad. Even in the most significant case, that of the QTL on Chr 19, the equivalent of a two-LOD interval for QTL location would span an area of 33 cM. The region of Chr 5 covered in the interval mapping analysis corresponds most closely to part of the distal region of human Chr 22q. No obvious candidate genes for ovulation rate were identified on examination of human genome information. The regions of bovine Chr 7 potentially containing ovulation rate QTL correspond to the p arm of human Chr 19 and the q arm of human Chr 5. There are no obvious candidate genes for ovulation rate, per se, though one gene of known reproductive effect maps to the corresponding region of human Chr 5, that being the gene for anti-Mullerian hormone. The bovine Chr 19 QTL represent the best case for identification of a potential candidate gene. The peak test statistic occurs at 65 cM distal to the most centromeric marker on this chromosome, and based on mapping results directly from cattle, the gene for bovine growth hormone maps to a position approximately 66 cM distal to the most centromeric marker on Chr 19 (Kappes et al. 1997). Growth hormone can be considered as a potential candidate gene for bovine ovulation rate. As additional information on genome organization and gene expression becomes available from the human genome project, additional candidate genes for the QTL regions identified here may become apparent.

Physiological evidence lends support to the consideration of growth hormone as a candidate gene for ovulation rate. Included in this evidence is the effect of exogenous growth hormone on follicular development, ovulation rate, and twinning. Research examining the effects of recombinant bovine growth hormone (rbGH) on reproductive function has demonstrated the potential for growth hormone, dependent on route of administration, to modify twinning rate. Cole and associates (1991) reported a twinning frequency of 17.42% for rbGH-treated cows (n = 155) vs. a frequency of 5.00% for untreated cows (n = 140) when rbGH was administered intramuscularly ($P < 0.01$). In other reports (Gong et al. 1991, 1993a, 1993b) administration of rbGH to heifers during the estrous cycle has resulted in increased numbers of small antral follicles but inconsistent effects on ovulation rate, suggesting that growth hormone is stimulatory to follicular recruitment. Physiological studies with the MARC twinning herd identified differences between twinning and non-twinning cows in levels of insulin-like growth-factor I (IGF1) in serum and follicular fluid. Given that IGF1 is produced in response to growth hormone, a difference in IGF1 level would be consistent with an effect of growth hormone.

Both genetic and physiological studies can be conducted to examine the hypothesis of growth hormone as a candidate gene for the BTA19 QTL. An examination of the growth hormone gene region of sire 839802 will be conducted to identify polymorphisms within coding sequences or regulatory regions. Further analyses would examine the association between these and ovulation rate phenotype. Knowledge of QTL location and flanking marker haplotype will permit the production of heifers with specified QTL genotypes. These heifers could then be used to compare growth hormone levels and gene expression at relevant points in the estrous cycle.

An important follow-up to this report will be the effort to replicate these QTL effects, particularly those observed for Chr 7. Related family material is available within the MARC twinning herd, and efforts have been initiated to examine this family for supporting evidence of QTL. The Swedish Friesian sire in question comes from a breed ancestrally related to North American Holstein-Friesian cattle; thus, there may be the possibility of replicating QTL effects by utilizing twinning rate data from the North American dairy cattle population. Efforts have likewise been initiated to replicate QTL effects by utilizing dairy cattle data and germplasm.

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