Genome-wide association study of normal and atypical progesterone profiles in Holstein-Friesian dairy cows

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ABSTRACT

Female fertility has a major role in dairy production and affects the profitability of dairy cattle. The genetic progress obtained by traditional selection can be slow because of the low heritability of classical fertility traits. Endocrine fertility traits based on progesterone concentration in milk have higher heritability and more directly reflect the cow's own reproductive physiology. The aim of our study was to identify genomic regions for 7 endocrine fertility traits in dairy cows by performing a genome-wide association study with 54,000 SNP. The next step was to fine-map targeted genomic regions with significant SNP using imputed sequences to identify potential candidate genes associated with the normal and atypical progesterone profiles. The association between a SNP and a phenotype was assessed by a single SNP analysis, using a linear mixed model that included a random polygenic effect. Phenotypes and genotypes were available for 1,126 primiparous and multiparous Holstein-Friesian cows from research herds in Ireland, the Netherlands, Sweden, and the United Kingdom. In total, 44 significant SNP associated with 7 endocrine fertility traits were identified on *Bos taurus* autosome (BTA) 1–4, 6, 8–9, 11–12, 14–17, 19, 21–24, and 29. Three chromosomes, BTA8, BTA17, and BTA23, were imputed from 54,000 SNP genotypes to the whole-genome sequence level with Beagle version 4.1. The fine-mapping identified several significant associations with delayed cyclicity, cessation of cyclicity, commencement of luteal activity, and inter-ovulatory interval. These associations may contribute to an index of markers for genetic improvement of fertility. Several potential candidate genes reported to affect reproduction were also identified in the targeted genomic regions. However, due to high linkage disequilibrium, it

was not possible to identify putative causal genes or polymorphisms for any of the regions.

Key words: dairy cattle, fertility, progesterone profile, genome-wide association studies, imputation

INTRODUCTION

Female fertility has a major role in the dairy production and is an economically important trait affecting profitability in dairy cattle (De Vries, 2006). The genetic progress obtained by traditional selection can be slow, because traits such as interval from calving to first insemination (**CFS**) often show low heritability, below 5% (Pryce and Veerkamp, 2001). Low heritability together with an antagonistic relationship between the production and fertility traits complicates genetic improvement for both traits. Endocrine fertility traits, such as progesterone (**P4**) profiles, might be more useful because they have higher heritabilities and more closely reflect the biology of the cow compared with classical fertility traits. Deviations from a normal estrous cycle, termed atypical P4 profiles, have been associated with compromised fertility (Bulman and Wood, 1980; Royal et al., 2002), and have an antagonistic relationship with milk yield in early lactation (Nyman et al., 2014). Heritabilities of delayed cyclicity and commencement of luteal activity (**C-LA**) determined by P4 profiles are generally higher. Delayed cyclicity have been reported as having a heritability of 0.24 (Nyman et al., 2014), and C-LA a heritability from 0.12 to 0.18 (Berry et al., 2012; Nyman et al., 2014; Tenghe et al., 2015). A higher heritability estimate might reflect a more effectively estimated genetic variance (Pryce and Veerkamp, 2001). Progesterone-based fertility traits are free from management influences (e.g., estrus detection and insemination dates). Hence, it might be possible to identify genes underlying this genetic variation.

Many genome-wide association studies (**GWAS**) have been performed on classical fertility traits [e.g., calving to first service (Höglund et al., 2009; Olsen et al., 2011; Berry et al., 2012), pregnancy rates (Berry et al., 2012), and nonreturn rate (Olsen et al., 2011)]. Only

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a few GWAS have been performed on endocrine fertility traits. Berry et al. (2012) found clear associations with C-LA on BTA2 and on BTA21. Furthermore, Tenghe et al. (2016) reported regions on BTA2 and BTA3 (among others) that are likely to include mutations associated with C-LA, interval from C-LA to first insemination, luteal phase length (**LPL**), inter-ovulatory interval (**IOI**), and CFS (among other traits). One challenge when working with low heritability traits is that GWAS requires a large number of individuals with phenotype and genotype information (Olsen et al., 2011). Therefore, the high cost associated with data collection in larger population reflects the low number of genomic studies on fertility traits, and especially endocrine fertility traits.

By using whole-genome sequences (**WGS**), causal variants underlying QTL should be identified more effectively by GWAS as the sequence data are expected to include the causal variants. Daetwyler et al. (2014), who were the first to implement WGS data in cattle, and Druet et al. (2014) found higher precisions in detecting QTL in WGS data. Whole genome sequence data should include the causal variants and have less dependency on population linkage disequilibrium (**LD**). Furthermore, it is possible to increase the power and precision of QTL mapping by increasing the numbers of markers with imputed sequences (e.g., Höglund et al., 2014; Sahana et al., 2014; Duchemin et al., 2016).

To our knowledge, no previous GWAS have identified genomic regions for the atypical P4 profiles in dairy cows. The objective of this study was to perform a GWAS to identify genomic regions associated with normal and atypical P4 profiles in Holstein-Friesian (**HF**) dairy cows. Furthermore, we wanted to fine-map chromosomes with significant SNP using imputed sequences to identify potential candidate genes associated with normal and atypical P4 profiles.

MATERIALS AND METHODS

Animals and Phenotypes

The data consisted of P4 records from 1,126 primiparous and multiparous HF cows belonging to 4 research herds: (1) Teagasc, Moorepark (Ireland, $n = 168$), (2) Järla, Swedish University of Agricultural Science (Sweden, $n = 224$), (3) Scotland's Rural College (United Kingdom, $n = 148$), and (4) Wageningen UR Livestock Research (the Netherlands, $n = 666$). A more detailed description of the experimental treatments imposed on animals in the different experimental herd, procedures for milk sampling, and P4 analysis have been reported for the Netherlands by Veerkamp et al. (2000), for Ireland by Horan et al. (2005), for Sweden by Petersson et

al. (2006), and for the United Kingdom by Pollott and Coffey (2008).

Traits were defined using P4 records as described in Nyman et al. (2014). Three atypical P4 profiles were analyzed: (1) delayed cyclicity defined as P4 concentrations below the predefined P4 threshold for luteal phase for more than 56 d postpartum followed by a normal cycle ($n = 178$), (2) cessation of cyclicity defined as a normal start of cyclicity but interrupted for at least 14 d with P4 concentrations below the threshold for luteal phase followed by a normal cycle ($n = 94$), and (3) prolonged luteal phase defined as a normal start of cyclicity but with high P4 concentrations (>8 ng/mL) for at least 20 d followed by a normal cycle ($n = 240$). Within each lactation, 4 cycle length traits were analyzed: (1) C-LA, defined as the number of days from calving to the first time the P4 value crossed the predefined threshold for luteal phase ($n = 1,592$); (2) IOI, defined as the number of days from the increase in P4 above the predefined threshold for luteal phase in one estrous cycle to the P4 increase in the next cycle ($n = 2,068$); (3) LPL, defined as the number of days from the P4 increase above the predefined threshold for luteal phase to the time P4 diminished below the predefined threshold ($n = 3,376$); and (4) inter-luteal interval (**ILI**), defined as the period of time following ovulation in which the corpus luteum secretes P4 below the predefined P4 threshold value ($n = 2,122$).

Milk yield at 60 DIM and the classical fertility traits were also analyzed for comparison with the P4 traits. The classical fertility traits included (1) the interval from calving to first observed heat (**CFH**), (2) CFS, (3) pregnancy at first service (**PFS**), (4) the interval from first to last service, and (5) calving interval (**CInt**).

Genotypes

The DNA extracted from blood samples was used for genotyping purposes. Genotyping was performed on 1,735 HF cows with the Illumina BovineSNP50 v1 BeadChip (50K; Illumina Inc., San Diego, CA). For each trait, animals were included in the analysis if both genotypic and phenotypic data were available. Therefore, 1,191 phenotypes were available for delayed cyclicity, cessation of cyclicity, and prolonged luteal phase, 793 phenotypes for C-LA and IOI, and 1,068 phenotypes for ILI and LPL.

Significant Associations

For the 7 fertility traits, a significant association was selected at 10^{-4} . We wanted to find significant associations for the traits of interest and select the most promising chromosomes for imputation. The P -value of 10^{-4}

is considered as a more liberal/less stringent threshold, which could be used to detect associations for complex traits with low heritability and for traits controlled by many genes with small effect (Teyssèdre et al., 2012). A more stringent significance level would reduce the power to validate SNP (Streit et al., 2013), whereas too liberal *P*-values may give high false positive rates.

GWAS on 50K SNP Genotypes

The quality criteria applied before imputation was a minimum call rate of 95%, a minor allele frequency above 1%, and no extreme deviation from Hardy-Weinberg equilibrium (*P*-value > 0.01). A total of 1,661 genotyped cows and 43,582 markers passed the quality criteria and remained in the study.

All statistical analyses were performed with the GenABEL package RepeatABEL in R 2.15.0 software (R Core Team, 2015) using a linear mixed model. Single SNP analyses were performed using the following animal model:

$$y_{ijklmn} = \mu + P_i + Y_j + S_k + \text{SNP}_l + a_m + \text{pe}_n + e_{ijklmn},$$

where y_{ijklmn} was the phenotypes for a given trait, μ was the overall mean, P_i was parity within country, Y_j was calving year within country, S_k was calving season within country, SNP_l was the fixed effect of SNP genotype, a_m was the random polygenic effect of animal, pe_n was the permanent environment to account for repeated measures (i.e., if more than 1 estrous cycle, lactation, or both) within animal, and e_{ijklmn} was the random error term. More information about the effects used in the model is provided by Nyman et al. (2014).

After our initial GWAS, we focused on the most promising chromosomes. The chromosomes that showed significant ($P < 10^{-4}$) associations with several of our 7 traits were considered for further analyses. Then these chromosomes were imputed from the 50K SNP genotypes to sequence level, and a chromosome-wide association study (CWAS) was performed.

Imputations

A reference population of 547 HF cows and bulls from the sixth run of the 1,000 Bull Genomes Consortium were available (Daetwyler et al., 2014). All positions of the SNP on the 50K SNP genotypes and variants on the sequences were aligned to the bovine genome assembly UMD3.1 (Zimin et al., 2009; SNPchimp, 2017). Alignment, variant calling, and filtering of the sequence

data were performed in accordance with Daetwyler et al. (2014).

The reference population was used to impute the genotypes of our experimental population from 50K to WGS. This imputation was performed by using Beagle version 4.1 (Browning and Browning, 2016). Imputation started by checking the inconsistency between the 50K SNP genotypes and the WGS reference population of 547 HF bulls, using the Conform-gt software (<http://faculty.washington.edu/browning/conform-gt.html>). After this check, the cows were imputed from 50K to sequence level. The imputed sequences were filtered to remove poorly imputed genotypes. The accuracy of imputation (AR2) of each marker was provided by Beagle as the bi-allelic r^2 . A variant was considered as well imputed and retained for further analyses if the average accuracy of imputation was equal to or higher than 0.7.

CWAS on Imputed Sequences

The CWAS with imputed sequence data for the most promising regions was run by using the same statistical model as in the GWAS. Two CWAS were run: (1) where only variants imputed with an $\text{AR2} \geq 0.2$ were included and (2) where only variants imputed with an $\text{AR2} \geq 0.7$ were included. Single-marker analyses were run using the same model as for the GWAS. The genomic relationship matrix was based on the 50K SNP genotypes, as in the GWAS. The WGS genotypes used in the imputation were not included in the genomic relationship matrix in the CWAS.

Only results for the 50K SNP genotypes and the sequence data with polymorphic markers with an $\text{AR2} \geq 0.7$ will be shown.

Candidate Genes

Based on the CWAS results for each trait, putative candidate genes were identified based on the *Bos taurus* UMD3.1 genome assembly (Zimin et al., 2009; <http://bovinegenome.org/>). The gene information was based on the Ensembl database (version 91, accessed February 2018 at <http://www.ensembl.org/>) and the information about the function of the genes was based on the GeneCards human gene database (accessed February 2018 at <http://www.genecards.org/>).

RESULTS

GWAS on 50K SNP Genotypes

Significant SNP for the analyzed endocrine fertility traits are presented in Table 1. In total, 44 significant

SNP associated with endocrine fertility traits were detected on BTA1–4, 6, 8–9, 11–12, 14–17, 19, 21–24, and 29. Regarding the 50K SNP genotypes, the strongest association was found on BTA9 with a $-\log_{10}(P\text{-value})$ of 6.30 for cessation of cyclicity (Table 1).

Twenty-four significant SNP associated with milk yield and the classical fertility traits were identified on BTA1, 2, 4, 5, 8, 10–12, 14–15, 19, 25, and 28 (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-15418>). The strongest SNP associated with classical fertility traits was identified for CInt on BTA14 and significant at a $-\log_{10}(P\text{-value})$ of 5.59 (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-15418>).

Because SNP on BTA8, BTA17, and BTA23 showed significant associations with 4 of the studied endocrine fertility traits, we focused on these chromosomes for the CWAS. Moreover, on BTA8 we identified significant SNP for the classical fertility traits CFH and CFS.

Manhattan plots for the atypical P4 profiles and cycle length traits for the 50K SNP genotypes are presented in Supplemental Figures S1 and S2, respectively (<https://doi.org/10.3168/jds.2018-15418>). Quantile-quantile plots for the atypical P4 profiles and for the cycle length traits are presented in Supplemental Figures S3 and S4, respectively (<https://doi.org/10.3168/jds.2018-15418>).

Imputation to WGS on BTA8, BTA17, and BTA23

Table 2 presents information about the SNP and the imputed sequences on BTA8, BTA17, and BTA23. After imputation with an $AR2 \geq 0.7$, the total number of variants on BTA8 increased from 1,962 SNP on the 50K genotypes to 23,051 variants, on BTA17 from 1,338 SNP to 26,901 variants, and on BTA23 from 914 SNP to 21,376 variants. This is an increase of 12, 20, and 23 times for BTA8, BTA17, and BTA23, respectively. The average accuracy of imputation using our approach was 0.87 for BTA8, 0.86 for BTA17, and 0.86 for BTA23.

CWAS for Imputed Sequences on BTA8, BTA17, and BTA23

We ran a CWAS based on imputed sequences for BTA8, BTA17, and BTA23. Regarding imputed sequences on BTA8, 9 significant variants were associated with cessation of cyclicity, 6 with delayed cyclicity, and 2 with C-LA (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15418>). For CFS, we identified 57 significant variants on BTA8 (details not given). Regarding imputed sequences on BTA17, 10 significant variants were associated with cessation of cyclicity, 5 with IOI, and 20 with delayed cyclicity (Supplemental Table S3; <https://doi.org/10.3168/jds.2018-15418>).

We identified 5 significant variants for CFH and 67 for CFS on BTA17 (details not given). Regarding imputed sequences on BTA23, 2 significant variants were associated with C-LA, 4 with delayed cyclicity, and 1 with cessation of cyclicity (Supplemental Table S4; <https://doi.org/10.3168/jds.2018-15418>). For CFS, we identified 11 significant variants on BTA23 (details not given). Figures 1 and 2 show Manhattan plots for BTA8, BTA17, and BTA23 with the 50K SNP genotypes overlaid with the imputed variants ($AR2 \geq 0.7$).

For delayed cyclicity, we detected 2 QTL regions on BTA8 (between 38.9 and 40.9 Mbp, and between 58.9 and 60.9 Mbp), 1 on BTA17 (between 52.8 and 55.2 Mbp), and 2 QTL regions on BTA23 (between 29.90 and 31.90, and between 41.2 and 43.2 Mbp; Figure 1). For cessation of cyclicity, 1 significant variant was identified on BTA8 (at 36.22 Mbp), 1 on BTA17 (at 47.01 Mbp), and 1 on BTA23 (at 7.91 Mbp; Figure 1). For C-LA, 1 QTL region was detected on BTA8 (between 58.9 and 60.9 Mbp), 1 on BTA17 (between 52.8 and 55.2 Mbp), and 1 on BTA23 (between 41.2 and 43.2 Mbp; Figure 2). For IOI, we detected 1 QTL region on BTA17 (between 52.8 and 55.2 Mbp; Figure 2). Manhattan plots for delayed cyclicity, cessation of cyclicity, C-LA, and IOI with the imputed variants with $AR2 \geq 0.7$ on BTA8, BTA17, and BTA23 are presented in Supplemental Figure S5 (<https://doi.org/10.3168/jds.2018-15418>).

Overlapping Regions

Delayed cyclicity and C-LA had overlapping regions with significant and suggestive variants in all 5 of the detected QTL regions (Figure 3). On BTA17 the observed QTL region included significant variants associated with delayed cyclicity, C-LA (close to significance), and IOI (Figure 3).

No significant variants or overlapping regions were identified for the endocrine fertility traits, CFH and CFS (results not presented). Using the 50K SNP genotypes, we identified overlapping regions with significant SNP for cessation of cyclicity and CInt on BTA14, and for ILI and milk yield on BTA11.

Potential Candidate Genes

We used a window of 1 Mbp upstream and downstream of a significant variant for checking candidate genes on the chromosomes, except for a region on BTA17 where we used a wider window (2.4 Mbp) to include all significant variants. The 2 QTL regions on BTA8, the 1 region on BTA17, and the 2 regions on BTA23 were checked for possibly candidate genes. We

Table 1. Significant SNP [$-\log_{10}(P\text{-value}) \geq 4$] from a genome-wide association study with endocrine fertility traits based on 50K SNP genotypes

Trait ¹	BTA	SNP name	Position (Mbp)	MAF ²	Effective size (SE)	α_{marker}^3	$\alpha_{\text{marker}}^2 / \alpha_A^2$	$\alpha_{\text{marker}}^2 / \alpha_P^2$	$-\log_{10}$ (P-value)
Cessation of cyclicity	1	BTB-01330924	104.80	0.01	0.18 (0.04)	0.0008	1.78	0	5.13
Cessation of cyclicity	1	Hapmap53293-rs29016851	116.35	0.35	0.04 (0.01)	0.0006	1.44	0	4.23
C-LA	2	BTA-49769-no-rs	130.14	0.35	4.87 (1.10)	10.81	0.10	0.03	5.03
IOI	3	ARS-BFGL-NGS-28490	104.63	0.02	12.34 (3.00)	6.90	0.33	0.07	4.42
IOI	3	BTB-01405537	40.36	0.07	-4.56 (1.15)	2.65	0.13	0.03	4.13
Cessation of cyclicity	4	Hapmap52573-rs29023352	118.68	0.44	-0.04 (0.01)	0.0006	1.40	0	4.34
ILI	4	Hapmap23939-BTA-142377	91.63	0.05	1.64 (0.42)	0.26	0.37	0.03	4.11
ILI	4	BTB-01295280	92.82	0.44	-0.66 (0.17)	0.21	0.31	0.02	4.01
LPL	6	BTA-76416-no-rs	59.01	0.02	7.64 (1.70)	2.31	0.11	0.02	5.14
Cessation of cyclicity	8	ARS-BFGL-NGS-11896	59.66	0.08	0.07 (0.02)	0.0006	1.40	0	4.92
Delayed cyclicity	8	BTB-00350148	59.91	0.20	-0.08 (0.02)	0.002	0.16	0.02	4.28
C-LA	8	BTB-00350148	59.91	0.20	-5.39 (1.36)	9.14	0.03	0.03	4.14
Cessation of cyclicity	8	BTA-80935-no-rs	36.23	0.01	0.23 (0.06)	0.001	2.68	0	4.05
Cessation of cyclicity	9	BTB-01891792	57.54	0.01	0.32 (0.06)	0.002	5.30	0	6.30
C-LA	9	BTA-115307-no-rs	81.55	0.43	4.12 (1.03)	8.4	0.02	0.03	4.21
Prolonged luteal phase	9	ARS-BFGL-NGS-95470	17.07	0.37	0.07 (0.02)	0.002	0.31	0.01	4.18
C-LA	11	ARS-BFGL-NGS-37815	101.83	0.16	5.41 (1.33)	7.9	0.02	0.02	4.34
IOI	11	ARS-BFGL-NGS-25510	54.70	0.02	3.42 (0.85)	0.47	0.67	0.05	4.23
IOI	12	Hapmap39246-BTA-102737	38.64	0.03	9.49 (2.12)	4.43	0.21	0.04	5.12
IOI	12	ARS-BFGL-NGS-39021	32.26	0.01	12.34 (2.96)	3.80	0.18	0.04	4.51
ILI	12	ARS-BFGL-NGS-74429	14.53	0.30	0.72 (0.18)	0.21	0.32	0.03	4.04
IOI	14	ARS-BFGL-BAC-21440	43.65	0.10	4.41 (0.96)	3.47	0.17	0.03	5.34
IOI	14	BTA-28303-no-rs	42.95	0.14	3.62 (0.86)	3.23	0.16	0.03	4.62
IOI	14	Hapmap41211-BTA-25540	45.71	0.14	3.52 (0.86)	2.92	0.14	0.03	4.40
IOI	15	ARS-BFGL-NGS-15379	30.02	0.38	2.54 (0.59)	3.03	0.15	0.03	4.73
C-LA	15	ARS-BFGL-NGS-57325	36.85	0.25	4.84 (1.17)	8.88	0.03	0.03	4.48
Delayed cyclicity	16	ARS-BFGL-NGS-1639	58.71	0.14	0.07 (0.02)	0.001	0.12	0.01	4.14
Cessation of cyclicity	17	BTB-00678183	47.02	0.02	0.12 (0.03)	0.0006	1.33	0	6.19
Delayed cyclicity	17	ARS-BFGL-NGS-27920	55.08	0.09	0.10 (0.02)	0.002	0.16	0.02	4.65
IOI	17	Hapmap36255-SCAFFOLD281038_3360	53.20	0.38	-2.52 (0.61)	2.99	0.14	0.03	4.43
IOI	17	Hapmap47001-BTA-40676	3.68	0.28	2.68 (0.67)	2.92	0.14	0.03	4.18
C-LA	17	BFGL-NGS-111058	82.24	0.09	7.18 (1.82)	8.7	0.02	0.02	4.10
C-LA	17	ARS-BFGL-NGS-27920	55.08	0.09	6.80 (1.73)	7.8	0.02	0.02	4.07
LPL	19	ARS-BFGL-NGS-30808	7.68	0.47	2.45 (0.57)	2.99	0.14	0.02	4.69
Cessation of cyclicity	19	ARS-BFGL-NGS-29163	56.58	0.01	0.16 (0.04)	0.0007	1.66	0	4.44
IOI	21	ARS-BFGL-NGS-107681	8.78	0.01	15.41 (3.72)	5.21	0.25	0.05	4.45
Cessation of cyclicity	22	ARS-BFGL-NGS-31024	57.87	0.01	0.18 (0.05)	0.0008	1.74	0	4.18
C-LA	23	ARS-BFGL-NGS-14014	30.95	0.01	17.86 (3.97)	8.33	0.08	0.02	5.39
Cessation of cyclicity	23	BTA-10816-no-rs	7.91	0.01	0.17 (0.04)	0.0007	1.50	0	4.64
Delayed cyclicity	23	ARS-BFGL-NGS-14014	30.95	0.01	0.18 (0.04)	0.001	0.08	0.01	4.56
ILI	24	ARS-BFGL-NGS-25998	57.16	0.32	0.82 (0.19)	0.29	0.42	0.03	4.90
Cessation of cyclicity	29	Hapmap23993-BTA-140467	15.90	0.04	0.21 (0.04)	0.003	6.92	0	8.76
Cessation of cyclicity	29	Hapmap24687-BTA-152298	49.95	0.01	0.30 (0.06)	0.002	5.13	0	6.21
Cessation of cyclicity	29	ARS-BFGL-NGS-34508	2.52	0.23	0.05 (0.01)	0.0007	1.65	0	4.38

¹C-LA = commencement of luteal activity; IOI = inter-ovulatory interval; ILI = inter-luteal interval; LPL = luteal phase length measured in days.²MAF = minor allele frequency.³ α_{marker}^2 = marker variance, computed for each marker as 2 times the major allele frequency times the minor allele frequency times the square of the allele substitution effect.⁴ $\alpha_{\text{marker}}^2 / \alpha_A^2$ = proportion of genetic variance explained by a marker.⁵ $\alpha_{\text{marker}}^2 / \alpha_P^2$ = proportion of phenotypic variance explained by a marker.

Table 2. Number of SNP at the 54K SNP genotypes and the number of imputed sequences for all imputed variants (all), with an imputed accuracy (AR2) ≥ 0.2 and with an AR2 ≥ 0.7 , and the number of indels on BTA8, BTA17, and BTA23

Item	BTA8	BTA17	BTA23
Total number of SNP ¹	1,962	1,338	914
Total number of imputed variants			
All	1,798,450	1,299,024	1,119,123
AR2 ² = 0, no. (%)	1,594,607 (88.67)	1,075,177 (82.77)	937,466 (83.77)
AR2 ≥ 0.2 , no. (%)	69,207 (3.85)	79,895 (6.15)	70,055 (6.26)
AR2 ≥ 0.7 , no. (%)	23,051 (1.28)	26,901 (2.07)	21,376 (1.91)
After quality control: (AR2 ≥ 0.7), ³ no. (%)	22,506 (97.6)	26,268 (97.6)	20,821 (97.4)
Indels, ⁴ no. (%)	3,156 (4.6)	3,488 (4.4)	3,089 (4.4)

¹SNP from the Illumina BovineSNP50 v1 BeadChip (Illumina Inc., San Diego, CA).

²AR2 = imputation accuracy.

³Number and percentage of imputed variants (AR2 ≥ 0.7) after quality control.

⁴Number and percentage of indels out of all imputed variants with an AR2 ≥ 0.2 .

identified many genes in these 5 regions, but as presented in Table 3 we only considered the genes affecting reproduction (i.e., the reproduction system, embryo phenotype, hormones affecting fertility, and endocrine morphology/physiology). On BTA8 we identified 4 genes between 38.9 and 40.9 Mbp and 5 genes between 58.9 and 60.9 Mbp. In the targeted genomic region on BTA17, we identified 9 genes. On BTA23 we identified 2 genes between 29.90 and 31.90 Mbp, and 3 genes between 41.2 and 43.2 Mbp.

DISCUSSION

In this study, we performed a GWAS for 7 endocrine fertility traits, milk yield at d 60 DIM, and 5 classical fertility traits. When using imputed sequences with high imputation accuracy, GWAS can assist in fine-mapping and increase the chance of identifying a causal variant (Marchini and Howie, 2010). The number of variants increased at least 10 times, after the imputation from 50K to sequence data. With the imputed sequences, we identified potential candidate genes within 5 observed QTL regions at 3 chromosomes, which contained the significant top variants for each trait. The phenotypic and the genetic variance explained by the markers were estimated for the top SNP on the 50K genotypes (Table 1) and for the 5 QTL regions on BTA8, BTA17, and BTA23 (Supplemental Tables S2, S3, and S4; <https://doi.org/10.3168/jds.2018-15418>). For all the studied traits, the phenotypic variance explained by the markers was low. The genetic variance explained by the markers ranged between 5 and 80%. This may be an overestimation because the QTL variance, as illustrated by Ekine et al. (2014), is affecting both the genetic variance and the residual variance.

To our knowledge, this is the first GWAS detecting genomic regions associated with the atypical P4

profiles. To date, no QTL region has been reported for C-LA on BTA8, BTA17, and BTA23. Earlier studies have reported significant associations with C-LA on other chromosomes; for example, Berry et al. (2012) and Tenghe et al. (2016) detected a QTL region associated with C-LA on BTA2. Although we used different methods, we found the same significant SNP on BTA2 in this study as was found by Berry et al. (2012). The inclusion of this SNP in genetic evaluations might be of interest, thus, it deserves further investigation. Berry et al. (2012) also reported a possible QTL for C-LA at position 9.38 Mbp on BTA21. In the present study, no significant SNP on BTA21 was associated with C-LA. However, on BTA21 we found a significant SNP associated with IOI at 8.78 Mbp with a $-\log_{10}(P\text{-value})$ of 4.45. Berry et al. (2012) also found significant SNP associated with CFH on BTA8, but this was not at the same position as in the present study. Tenghe et al. (2016) reported significant SNP associated with LPL on BTA3 and BTA17, whereas in the present study we found interesting regions for IOI on the same chromosomes. As IOI combines information from LPL and ILI, this may suggest that the same genes influence both IOI and LPL. Nevertheless, on BTA3 there was a difference of 10 Mbp between these 2 SNP, and on BTA17, the distance was even greater. Discrepancies between sample size, significance levels, methods of analysis, and number of observations could explain the differences between the results of Berry et al. (2012), Tenghe et al. (2016), and our study.

Results from the GWAS of the 50K SNP genotypes showed significant SNP on BTA1 with an overlapping region for cessation of cyclicity (at 116.35 Mbp) and PFS (at 118.71 Mbp). The genetic correlation between cessation of cyclicity and PFS could not be estimated in Nyman et al. (2014) because of the low genetic variation of cessation of cyclicity. On BTA11, we found

Figure 1. Chromosome-wide association study for delayed cyclicity (turquoise) and cessation of cyclicity (red) on BTA8, BTA17, and BTA23. Bovine 50K SNP genotypes (turquoise and red circles) overlaid with imputed variants with an imputation accuracy (AR_2) ≥ 0.7 (black triangles). The black dotted line is the genome-wide significance level of $-\log_{10}(P\text{-value}) \geq 4$ based on 50K SNP genotypes.

a significant SNP associated with ILI at 54.70 Mbp and with milk yield at 60 DIM at 54.37 Mbp. In the study by Nyman et al. (2014), the genetic correlation between ILI and milk yield at 60 DIM was low (0.25) and unfavorable. Even though there was a low correlation, the genes affecting milk yield and ILI were located

close to each other. This finding suggests that breeding for higher milk yield may influence ILI, resulting in a longer period of low P4 after ovulation.

We detected many possible candidate genes in the 5 observed QTL regions on BTA8, BTA17, and BTA23. Only genes reported to affect reproduction (e.g., embry-

Figure 2. Chromosome-wide association study for commencement of luteal activity (C-LA; purple) and inter-ovulatory interval (IOI; green) on BTA8, BTA17, and BTA23. Bovine 50K SNP genotypes (purple and green circles) overlaid with imputed variants with an imputation accuracy (AR_2) ≥ 0.7 (black triangles). The black dotted line is the genome-wide significance level of $-\log_{10}(P\text{-value}) \geq 4$ based on 50K SNP genotypes.

onic and reproductive phenotypes, abnormalities in the reproductive system, and morphology and physiology of the endocrine gland) were considered in this study. Due to high LD between the imputed variants (Figure 3), it was not possible to specify genes or polymorphisms as causal factors for any of the regions. Compared with humans, long-range LD blocks are more common in

dairy cattle, especially in HF cows. This is due to the intensive use of relatively few sires for breeding the next generation, which results in a relatively small effective population size (Schmid and Bennewitz, 2017).

On BTA8 we identified 9 possible candidate genes controlling fertility functions (Table 3). Earlier studies have also reported genes on BTA8 that are controlling

fertility (e.g., Peters et al., 2013). Peters et al. (2013) identified genes affecting conception rate at first calving in Brangus cattle, and Daetwyler et al. (2014) identified a mutation in the *SMC2* gene when studying animals from the 1,000 Bull Genomes Project. The *SMC2* mutation is reported to cause embryonic loss. Kühn et al. (2003) identified genes affecting dystocia and stillbirths in Holstein cattle on BTA8. In the present study, we identified 9 interesting genes controlling fertility on BTA17. Frischknecht et al. (2018) found a QTL region for nonreturn rate at d 56, and the interval from first to last insemination on BTA8 in Brown Swiss cattle. Liu et al. (2017) reported strong associations with conception rate at first insemination and the interval to first insemination in Chinese and Nordic Holstein cows on BTA23 and for heifers on BTA17. In the present study, we identified 5 interesting genes in the 2 QTL regions

on BTA23. Blattman et al. (1996) identified genes in a QTL region on BTA23 affecting ovulation rate and Cole et al. (2011) found significant SNP associated with daughter stillbirth. Few reports have studied endocrine fertility traits, although Tenghe et al. (2016) identified significant SNP on BTA8, BTA17, and BTA23 for proportion luteal activity between d 25 and 60 postpartum, first LPL, interval from C-LA to first service, and CFS. All reports with the potential genes on BTA8, BTA17, and BTA23 suggest that these 3 chromosomes are of interest when looking for genomic markers to improve the genetic evaluation for fertility.

Fertility is complex, composed of many sub-traits, and affected by many genes. In the present study, we identified significant SNP spread over the chromosomes, with some exceptions. We identified significant SNP at different locations associated with the same

Figure 3. Fine-mapping [imputed sequences with an imputation accuracy ($AR2 \geq 0.7$) of (a1) BTA8, (b1) BTA17, and (c1) BTA23 showing an overlay of delayed cyclicity, cessation of cyclicity, commencement of luteal activity (C-LA), and inter-ovulatory interval (IOI). Fine-mapping of delayed cyclicity and C-LA between 38.9 and 40.9 Mbp (a2) and 58.9 and 60.9 Mbp (a3) on BTA8; of delayed cyclicity, C-LA, and IOI between 52.8 and 55.2 Mbp (b2) on BTA17; and of delayed cyclicity and C-LA between 29.9 and 31.9 Mbp (c2) and 41.2 and 43.2 Mbp (c3) on BTA23. The black dotted line is the genome-wide significance level of $-\log_{10}(P\text{-value}) \geq 4$ based on 50K SNP genotypes.

Table 3. Position of top variant on BTA8, BTA17, and BTA23, possible candidate genes, and start and end position of these genes

BTA	Top SNP position (Mbp)	Candidate gene	Start position	End position
8	39.93	<i>PDCD1LG2</i>	Programmed cell death 1 ligand 2	39,338,936
		<i>PLGRKT</i>	Plasminogen receptor with a C-terminal lysine	39,532,018
		<i>INSL6</i>	Insulin like 6	39,603,073
		<i>JAK2</i>	Janus kinase 2	39,632,638
	59.90	<i>SIT1</i>	Signaling threshold regulating transmembrane adaptor 1	60,238,488
		<i>CA9</i>	Carbonic anhydrase 9	60,259,473
		<i>HINT2</i>	Histidine triad nucleotide binding protein 2	60,390,945
		<i>TLN1</i>	Talin 1	60,280,812
		<i>GBA2</i>	Glucosylceramidase β 2	60,316,733
		<i>SCARB1</i>	Scavenger receptor class B member 1	53,180,927
17	53.20	<i>ABCL5A</i>	Lysine methyltransferase 5A	54,457,612
		<i>CDK2AP1</i>	Cyclin dependent kinase 2 associated protein 1	54,556,011
		<i>TCTN2</i>	Tectonic family member 2	54,245,927
		<i>CCDC62</i>	Coiled-coil domain containing 62	54,952,889
		<i>ABCB9</i>	ATP binding cassette subfamily B member 9	54,827,286
		<i>CLIP1</i>	CAP-Gly domain containing linker protein 1	55,245,219
	55.08	<i>KDM2B</i>	Lysine demethylase 2B	55,898,511
		<i>ORAI1</i>	ORAI calcium release-activated calcium modulator 1	55,858,836
	30.92	<i>HIST1H1C</i>	Histone cluster 1 H1 family member C	31,636,947
		<i>HIST1H1D</i>	Histone cluster 1 H1 family member D	31,490,462
23	42.25	<i>RANBP9</i>	RAN binding protein 9	42,736,705
		<i>JARID2</i>	Jumonji and AT-rich interaction domain containing 2	41,165,798
		<i>CD83</i>	CD83 molecule	42,335,199

traits. For example, delayed cyclicity was associated with significant SNP and variants on BTA8, BTA17, and BTA23, but also on BTA16 (only analyzed with the 50K SNP genotypes; Table 1). Genes with pleiotropic effect are genes that affect more than one trait. In our study we detected several QTL regions with the same significant SNP or variant associated with both delayed cyclicity and C-LA [e.g., SNP “ARS-BFGL-NGS-27920” on BTA17 (Table 1), a variant positioned at 30,953,561 bp, and a variant positioned at 30,954,230 bp on BTA23 (Supplemental Table S4; <https://doi.org/10.3168/jds.2018-15418>)]. This suggests that the same genes may control these traits. On the other hand, we detected other regions with variants that were only associated with one of the traits. The complexity of the traits manifested with the absence of very strong associations for either of the traits suggests that many genes with small effects are involved, which makes it difficult to identify causal genes. Further exploration of the genetic basis for endocrine fertility traits would require a much larger GWAS study in terms of many genotyped animals with P4 records. The increasing use of in-line data is one possibility. HerdNavigator (DeLaval, Tumba, Sweden) is an on-farm milk analysis tool collecting large amounts of phenotypic data. Combined with genotyping of the cows, a much larger GWAS using the same approach could be feasible in the future and be useful to validate the results in this study.

The GWAS results can increase the biological knowledge about traits, which could be of interest in the

breeding evaluation (Goddard et al., 2016) and provide information on the genetic architecture of the quantitative trait.

CONCLUSIONS

We detected a large number of potential chromosomal regions for the observed fertility traits. The fine-mapping of QTL regions on BTA8, BTA17, and BTA23, using imputed sequence variants, identified several significant associations with delayed cyclicity and C-LA that can contribute to an index of markers for genetic improvement of fertility. In the observed QTL regions, we identified several potential candidate genes reported to affect reproduction. However, due to high LD, it was not possible to specify putative causal genes or polymorphisms for any of the regions.

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