

# Quantitative trait loci for fertility traits in Finnish Ayrshire cattle

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**Abstract** – A whole genome scan was carried out to detect quantitative trait loci (QTL) for fertility traits in Finnish Ayrshire cattle. The mapping population consisted of 12 bulls and 493 sons. Estimated breeding values for days open, fertility treatments, maternal calf mortality and paternal non-return rate were used as phenotypic data. In a granddaughter design, 171 markers were typed on all 29 bovine autosomes. Associations between markers and traits were analysed by multiple marker regression. Multi-trait analyses were carried out with a variance component based approach for the chromosomes and trait combinations, which were observed significant in the regression method. Twenty-two chromosome-wise significant QTL were detected. Several of the detected QTL areas were overlapping with milk production QTL previously identified in the same population. Multi-trait QTL analyses were carried out to test if these effects were due to a pleiotropic QTL affecting fertility and milk yield traits or to linked QTL causing the effects. This distinction could only be made with confidence on BTA1 where a QTL affecting milk yield is linked to a pleiotropic QTL affecting days open and fertility treatments.

**QTL / fertility / dairy cow**

## 1. INTRODUCTION

High fertility in cows is economically important for dairy farmers. Low fertility leads to higher replacement costs, veterinary costs, labour costs and costs due to reduced milk production. The proportion of fertility treatments represents 21% [36] of all the veterinary treatments in Finland. Also, 20% of the involuntary culling cases in Finland are due to fertility disorders (Rautala, personal communication, 2004).

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Fertility traits have a low heritability and are often difficult to measure [31]. Genetic progress by traditional breeding can therefore be slow and the negative correlations with production traits are of special concern [34]. Pösö and Mäntysaari [34] have reported that a genetic improvement of 500 kg milk yield would increase cases of ovulatory disorders by 1.7%-units and days open by 4.2 days. These are traits for which marker-assisted selection could increase genetic progress compared to traditional breeding schemes [25, 38].

Attempts have been made to map loci affecting fertility. QTL have been detected for ovulation rate [4], twinning [26], days open [39], non-return rate and stillbirth [24], fertility treatments [15], and pregnancy rate [2]. In Finland, mapping fertility traits is feasible because there is a good health data recording system with a database maintained by the Agricultural Data Processing Centre Ltd.

Several studies have found unfavourable associations between milk production traits and fertility traits [23, 34, 37]. Cows with high milk yield records tend to have poorer fertility performances than cows with moderate or low milk production. Selection for high milk yield has led to longer intervals between calving and the following pregnancy and an increase in fertility disorders. In order to use marker information to select for better fertility without compromising improvement in milk production, more knowledge on the chromosomal regions affecting both milk and fertility traits and the underlying genes is needed. Milk production traits and fertility traits are correlated genetically. This genetic correlation may be due to pleiotropic QTL affecting both traits simultaneously and/or to linked QTL each affecting one trait. For effective marker-assisted selection, it is necessary to distinguish between a pleiotropic QTL and a linked QTL to avoid undesirable correlated responses. The standard way of deciding how many QTL (marginal effects) and their interaction effects should appear in the final model relies on comparing several models, *e.g.* single-trait analysis with one or multiple QTL models followed by multi-trait analysis with pleiotropic or linked QTL models. There are two limitations of this approach: first, it allows the comparison of nested models only; second, it is not clear how to adjust the significance threshold for each consecutive test [5]. Akaike information criterion (AIC) [1] or Schwarz Bayesian information criterion (BIC) [41] are two criteria that do not require that the compared models be nested and they have often been employed to choose marker covariates for multiple QTL mapping [16, 17] or to directly estimate QTL number *e.g.* [3, 5, 7, 30, 42]. Piepho and Gauch [33] have investigated model selection criteria *via* simulation. Their results suggest that out of the considered

criteria BIC has the best properties and can be used for the estimation of the number of QTL with main effects.

The objectives of this study were (i) to use the Finnish granddaughter design data to map QTL for fertility traits (days open, fertility treatments, paternal non-return rate, and calf mortality in the Finnish Ayrshire population); (ii) to distinguish between pleiotropy and linked QTL when a region is affecting more than one fertility trait or at least one fertility trait and milk trait identified previously by Viitala *et al.* [46].

## 2. MATERIAL AND METHODS

### 2.1. Traits and population

Days open (DO) is calculated as the number of days from calving to the following pregnancy. Fertility treatments (FT) include information about fertility treatments done by a veterinarian within 150 days after calving and information about culling due to fertility problems. Non-return rate (NRR) indicates the ability of a bull to make cows pregnant. Its evaluation is based on the insemination of the bull's semen to a random set of cows and in this study, is measured as the non-return rate within 60 days from insemination with the first 500 inseminations of a bull included in the data. Calf mortality (CM) is measured here as a trait of the sire of the cow. It indicates the mortality at birth of the offspring of the daughters. The response variables used in QTL mapping were breeding values obtained from the Finnish Animal Breeding Association mainly from the evaluation carried out in autumn 2000. For NRR, the breeding values from the evaluation carried out in spring 1996 were used because there was not enough data for the six oldest grandsires in the year 2000 evaluation for NRR.

Breeding values for DO were estimated using a repeatability animal model and for FT a repeatability sire model. Records from the first three lactations were used. All bulls in the mapping population had daughter records from all three lactations. For CM a sire-grandsire model was used. CM and FT were recorded as binary traits. The heritability estimates used for calculating the breeding values were 0.05 for DO, 0.01 for FT, 0.03 for CM, and 0.03 for NRR.

The milk yield traits used for pleiotropic and linked QTL analyses were the following: milk yield 1<sup>st</sup> lactation (MY), protein yield 1<sup>st</sup> lactation (PY), fat yield 1<sup>st</sup> lactation (FY). Daughter yield deviations (DYD) originated from a test day animal model.

A granddaughter design was used for QTL mapping. Twelve Finnish Ayrshire half-sib families were genotyped. Only eleven of them could be used

for the analysis of CM because the smallest family did not have enough sons with daughter records for this trait. The number of genotyped sons per sire ranged from 21 to 82 with an average of 41 sons. The total number of sons in the population was 493. The average number of daughter records per bull was 496 for DO, 468 for FT, and 841 for CM.

## 2.2. Markers and genotypes

Markers were genotyped on all 29 bovine autosomes. All available sons of the chosen bull sires were typed. A total of 169 microsatellites and two candidate gene SNP were used. Out of these, 21 microsatellites were new compared to those reported in previous studies with the Finnish granddaughter design [40, 46]. Thus, eleven linkage maps were recalculated. The linkage maps are available at <http://www.mtt.fi/julkaisut/cattleqtl>. The number of markers per chromosome varied from 2 to 14. The average spacing between markers was 19 cM. The total length of the analysed genome was 2618 cM. ANIMAP [12] or CRIMAP [13] were used to construct the linkage maps. The methods for DNA extraction, PCR reaction protocols, and electrophoresis have been described in previous studies [10, 47].

## 2.3. Statistical analysis

QTL analyses consisted of the following steps: (1) a genome scan was carried out using multiple linear regression for four fertility related traits; (2) the significant QTL detected from (1) and milk production QTL detected by Viitala *et al.* [46] that overlapped with the fertility QTL were reanalysed with the variance component method using a single-trait model (STVC); (3) multi-trait pleiotropic ( $MT_P$ ) and linked ( $MT_L$ ) QTL models were analysed when QTL for two fertility traits or one fertility trait and one milk yield trait [46] were detected on the same chromosome.

### 2.3.1. Regression method

Associations between markers and traits were analysed using a multiple marker regression approach [22]. The model used was the following:  $y_{ij} = a_i + b_i x_{ij} + e_{ij}$ , where  $y_{ij}$  is the breeding value of bull  $j$ , who belongs to family  $i$ ,  $a_i$  is the polygenic effect for half-sib family  $i$ ,  $b_i$  is the allele substitution effect for a QTL within family  $i$ ,  $x_{ij}$  is the conditional probability for bull  $j$

of inheriting the first haplotype from sire  $i$ , and  $e_{ij}$  is the residual. Significance thresholds and  $P$ -values for the F-statistic, were obtained by permutation, which was repeated 10 000 times for each trait and chromosome separately [8]. Genome wise  $P$ -values were obtained by Bonferroni correction  $P_{\text{genome}} = 1 - (1 - P_{\text{chromosome}})^{29}$ , where 29 is the total number of chromosomes analysed.

A two-QTL model was fitted in the regression analysis for those chromosomes that had more than three informative markers if one significant QTL had been detected and if the estimated QTL positions in the individual families indicated two different positions [44, 45]. With the two-QTL model, the permutations were done to test two QTL vs. no QTL. If this result exceeded the chromosome-wise significance threshold of 5%, the  $P$ -value for two QTL vs. one QTL was obtained from a standard F table. The degrees of freedom for the F statistic were the number of grandsires as the numerator and total number of offspring minus three times the number of grandsires as the denominator.

### 2.3.2. Variance component method

Single- and multi-trait QTL mapping based on the variance component method was carried out using the method described by Lund *et al.* [27]. The traits were modelled using the following linear mixed model with  $n_q$  number of QTL:

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{Z}\mathbf{u} + \sum_{i=1}^{n_q} \mathbf{W}\mathbf{q}_i + \mathbf{e},$$

where  $\mathbf{y}$  is a vector of breeding values or DYD recorded on  $t$  traits for each genotyped son,  $\boldsymbol{\mu}$  is a vector of overall trait means,  $\mathbf{Z}$  and  $\mathbf{W}$  are incidence matrices,  $\mathbf{u}$  is a vector of random additive polygenic effect results from a combined effect of background genes,  $\mathbf{q}_i$  is a vector of the effects of the  $i^{\text{th}}$  QTL, and  $\mathbf{e}$  is a vector of random residual effects. The random variables  $\mathbf{u}$ ,  $\mathbf{q}_i$  and  $\mathbf{e}$  are assumed to be multivariate normally distributed and mutually uncorrelated. For details of the method see Lund *et al.* [27].

The variance components were estimated using the average information restricted maximum likelihood algorithm [18] implemented in the software package DMU [29]. The restricted likelihood was maximised with respect to the variance components associated with the random effects in the model. Maximising a sequence of restricted likelihoods over a grid of specific positions yields a profile of the restricted likelihood for the QTL position. The interval for QTL was estimated by one-LOD support [28].

### 2.3.2.1. IBD matrices

The elements in the IBD matrix are a function of the marker data and the position ( $p$ ) of a putative QTL on the chromosome. Here we used the most likely marker linkage phase in the sire and computed the IBD matrix using a recursive algorithm [48]. The IBD matrices were computed for every 4 cM along the chromosomes and used in the subsequent variance component estimation procedure.

### 2.3.2.2. Test statistics

Hypothesis tests for the presence of QTL were based on the asymptotic distribution of the likelihood ratio test (LRT) statistic,  $LRT = -2\ln(L_{\text{reduced}} - L_{\text{full}})$ , where  $L_{\text{reduced}}$  and  $L_{\text{full}}$  were the maximised likelihoods under the reduced model and full model, respectively. The reduced model always excluded the QTL effect for the chromosome being analysed. The two-QTL models were compared with one-QTL (null) models. Thresholds were calculated using the method presented by Piepho [32].

### 2.3.2.3. Model selection between pleiotropic and linked-QTL models

Since the pleiotropic and the linked-QTL models are not nested, the Bayesian Information Criterion (BIC) [20, 41] was used to evaluate which model was favoured. The two models in the present study entail the same number of parameters and consequently the BIC simplifies to  $2 \log \left[ \frac{p(y|\hat{\theta}_{\text{linkage}}M_{\text{linkage}})}{p(y|\hat{\theta}_{\text{pleiotropy}}M_{\text{pleiotropy}})} \right]$ . If the two models are assumed equally likely *a priori*, the results using this criteria are an approximation to the posterior probability of the pleiotropic model relative to the posterior probability of the linked QTL model (Bayes factor). We used the BIC calibration table by Raftery [35] for interpreting BIC estimates. A BIC score of  $\geq 6$  (model M1 vs. M2) indicated strong evidence for M1 over M2. Another less formal criterion used to indicate which model is more likely, is the estimated correlation between QTL effects on the two traits ( $r_{Q12}$ ) from the pleiotropic model. The rationale behind using  $r_{Q12}$  is that if the two traits are under the influence of a biallelic pleiotropic QTL the true value of  $r_{Q12}$  will be one.

### 3. RESULTS

#### 3.1. Days open

In the single-trait regression analysis, QTL for DO were detected on BTA1, 2, 5, 12, 20, 25, and 29 at chromosome-wise 5% significance (Tab. I). The single-trait model with variance component analysis (STVC) confirms QTL on BTA1 and 12 in the same region of the chromosomes (Tab. I). The two-QTL model with regression was fitted for BTA1 and 2. No support was found for this model for either chromosome. In the analysis within families there were two to five families with chromosome-wise significant F-values per chromosome. The positions of the highest F-values on the chromosomes were not consistent between families. The estimated allele substitution effects in these families ranged from 0.7 to 1.5 standard deviations of EBV, which means 5.2 to 11.1 days.

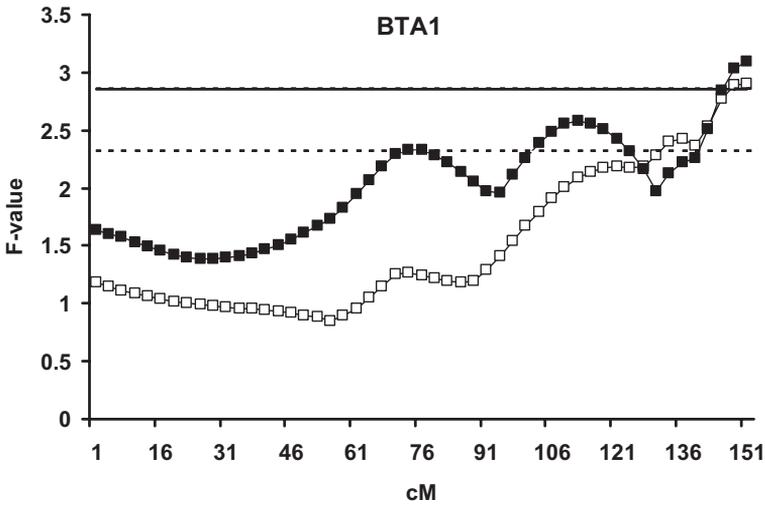
#### 3.2. Fertility treatments

With the regression analysis, QTL were detected on BTA1, 10, 15, 19, and 25 at chromosome-wise 5% significance and on BTA5 and 14 at chromosome-wise 1% significance (Tab. I). The STVC analysis confirms the QTL for FT on BTA1. The two-QTL model using regression analysis was significant for BTA1, 5, and 14 (Tab. II). The strongest evidence for two QTL was on BTA14. There were one to four families with chromosome-wise significant F-values in the analysis within families. The positions of the highest F-values differed between families. The allele substitution effects ranged from 0.6 to 2.2 standard deviations of EBV or 0.62% to 2.22% of treatments.

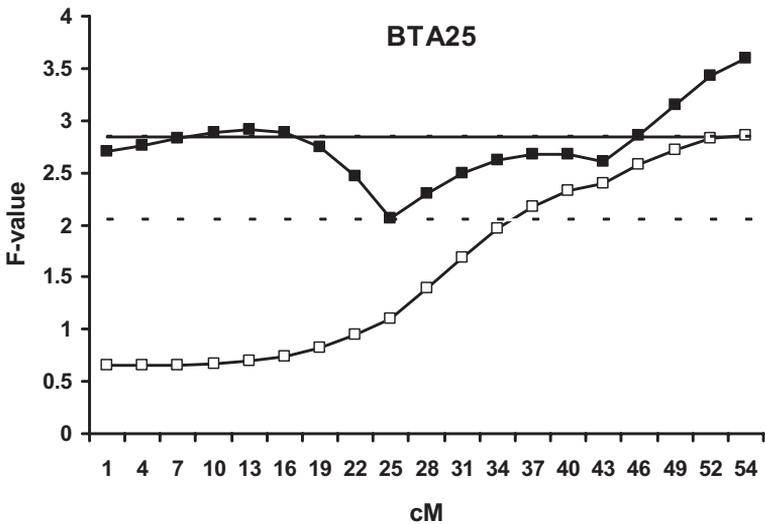
On BTA1 and BTA25 the QTL positions in the across families analysis for DO and FT were overlapping. For both chromosomes the QTL positions were at the end of the chromosome, on BTA1 close to marker *BMS4014* and on BTA25 close to marker *AF5* (Figs. 1 and 2).

#### 3.3. Calf mortality

In the single trait regression analysis, QTL for CM were detected on BTA4, 6, 11, 15, 18, and 23 at 5% chromosome-wise significance (Tab. I). The STVC analyses did not confirm any of the QTL for CM, however, the QTL on BTA4 and 15 were close to significance. The two-QTL model using regression was not supported for any of the chromosomes. In the analysis within families



**Figure 1.** Profiles of linear regression test statistics for BTA1 from single trait analysis across families. Quantitative trait loci were detected for days open □ and fertility treatments ■. The upper horizontal line indicates the chromosome-wise 5% threshold level for fertility treatments and the lower dashed line the chromosome-wise 5% threshold level for days open.



**Figure 2.** Profiles of linear regression test statistics for BTA25 from single trait analysis across families. Quantitative trait loci were detected for days open □ and fertility treatments ■. The 5% threshold levels for the traits are shown. The upper horizontal line indicates the chromosome-wise 5% threshold level for fertility treatments and the lower dashed line the chromosome-wise 5% threshold level for days open.

**Table I.** Quantitative trait loci for days open, fertility treatments, calf mortality and non-return rate with regression and variance component methods in Finnish Ayrshire cattle.

Trait	BTA <sup>1</sup>	Regression method		Variance component method	
		Pos. <sup>2</sup> (cM)	F-value	Pos. (cM)	LRT <sup>3</sup>
Days open	1	146	2.75**	144	11.29**
	2	2	2.86**	0.1	3.26
	5	108	2.86**	107	4.29
	12	47	2.34*	48	8.49*
	20	1	2.44*	2	5.80
	25	47	2.93**	45	5.19
	29	4	2.27*	45	4.90
Fertility treatments	1	151	3.09*	148	9.75*
	5	113	3.94**	84	3.83
	10	145	2.99*	2	5.83
	14	67	3.46**	50	1.37
	15	1	3.30*	120	4.09
	19	1	3.19*	1	1.78
	25	54	3.60**	–	< 1.0
Calf mortality	4	17	2.36*	1	6.60
	6	93	2.71*	85	3.9
	11	29	2.09*	16	2.75
	15	115	2.08*	120	6.33
	18	1	2.24*	–	< 1.0
	23	3	2.02*	1	2.05
Non-return rate	10	68	2.06*	144	3.54
	14	29	2.14*	30	2.85

<sup>1</sup>BTA = *Bos taurus* chromosome.<sup>2</sup>Pos. = position.<sup>3</sup>LRT = likelihood ratio test statistics.\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

there were two to four families with chromosome-wise significant F-values per chromosome. For BTA15, three families had their highest F-values close to marker *MGTG13B*. For BTA18, two families had their highest F-values at *BMS1355* and two between markers *BMS1355* and *BMS2213*. On the other chromosomes with significant QTL in the across families analysis, the positions of the highest F-values were not consistent between families. The allele substitution effects of the detected QTL ranged from 0.5 to 2.2 standard deviations of EBV, which is 0.45% to 2.0% of CM.

**Table II.** Results from the two-QTL model for fertility treatments by linear regression.

BTA <sup>1</sup>	1 vs. no QTL			2 vs. no QTL			2 vs. 1 QTL	
	F <sup>2</sup>	Pos. <sup>3</sup>	5% <sup>4</sup>	F	Pos.	5%	F	5%
1	3.09	151	2.85	2.71	71	151	2.55	1.85
5	3.94	113	2.84	3.33	21	96	2.45	1.85
14	3.46	67	2.70	3.74	46	76	2.29	1.85

<sup>1</sup>*Bos taurus* chromosome.

<sup>2</sup>F-value.

<sup>3</sup>QTL position cM.

<sup>4</sup>Threshold level for 5% significance.

### 3.4. Non-return rate

Non-return rate QTL were found on BTA10 and 14 at 5% chromosome-wise significance (Tab. I). Neither of these QTL was detected by STVC analysis. The allele substitution effects of the detected QTL ranged from 0.7 to 1.6 standard deviations of EBV. This is 2.70% to 6.16% of NRR. There was no indication of two separate QTL positions on any of the chromosomes, and the two-QTL model using regression was not applied. In the analysis within families, one to two families had 5% chromosome-wise significant F-values per chromosome and the positions of the highest F-values were not consistent between families.

### 3.5. Single-trait analysis of milk production traits

Out of the 16 chromosomes observed segregating for fertility related QTL in this study, BTA1, 2, 5, 12, 14 and 25 were analysed by STVC for milk production traits. This was done because QTL for milk production were reported on these chromosomes by Viitala *et al.* [46] in the same families. The STVC analyses detected QTL for MY on BTA1; for MY and PY on BTA5; MY, PY, and FY on BTA12; FY on BTA14 (Tab. III). None of the QTL for the production traits on BTA2 and 25 were confirmed by STVC analyses.

### 3.6. Multi-trait analysis

Multi-trait analyses were carried out on BTA1, 2, 5, 10, 12, 14, 15, and 25 using the variance component method (Tab. IV). On these chromosomes, fertility QTL were detected in the single trait regression analysis close to milk

**Table III.** QTL identified by single-trait analysis using the variance component method on *Bos taurus* autosomes (BTA) 1, 2, 5, 12, 14 and 25, which shows at least one fertility trait QTL and one QTL for milk production traits in Finnish Ayrshire cattle.

Trait	BTA1		BTA2		BTA5		BTA12		BTA14		BTA25	
	Pos. (cM)	LRT (cM)										
MY	104	8.36*	116	2.61	68	8.66*	28	8.45*	0.01	1.50	6	2.86
PY	108	4.19	120	5.06	68	8.79*	40	6.89*	–	< 1.0	57	4.79
FY	–	< 1.0	112	4.78	–	< 1.0	36	15.74**	0.01	11.12**	–	< 1.0

\* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table IV.** Multi-trait analysis with pleiotropic and linked QTL models on BTA1, 5, 12, 14 and 15 where QTL for both reproduction and milk production were identified in single-trait analysis.

BTA	Traits	Pleiotropic QTL			2 Linked QTL			Comparison BIC <sup>6</sup> (pleiotropy <i>vs.</i> linkage)
		Pos. <sup>2</sup> (cM)	LRT <sup>4</sup>	$r_Q^5$	Pos1 <sup>3</sup> (cM)	Pos2 <sup>3</sup> (cM)	LRT	
BTA1	DO and FT <sup>1</sup>	148	17.8** <sup>7</sup>	0.96	148	80	9.48	8.4
	DO and MY	144	8.1	–0.28	144	104	17.1**	–9.0
	FT and MY	144	12.1	–0.95	156	104	11.9	0.2
BTA5	FT and MY	72	11.5	–0.24	0.1	72	14.1*	–2.6
	FT and PY	68	11.2	–0.47	0	68	13.9*	–2.7
BTA12	DO and FY	31	13.9*	–0.75	52	31	14.6*	–0.7
BTA14	FT and NRR	36	4.4	0.99	48	32	4.2	0.2
	FT and FY	0.1	11.2	–0.99	52	0.1	12.1*	–0.9
	NRR and FY	4	12.8*	–0.45	32	0.1	13.8*	1.0
BTA15	FT and CM	120	13.4*	0.35	8	120	12.1	1.3

<sup>1</sup>DO = days open; FT = fertility treatments; MY = milk yield; PY = protein yield; FY = fat yield; NRR = non-return rate; CM = calf mortality.

<sup>2</sup>Pos. = position.

<sup>3</sup>Pos1 and Pos2 = position of the QTL affecting the first and second trait respectively.

<sup>4</sup>LRT = likelihood ratio test statistics.

<sup>5</sup> $r_Q$  = correlation between the QTL effects.

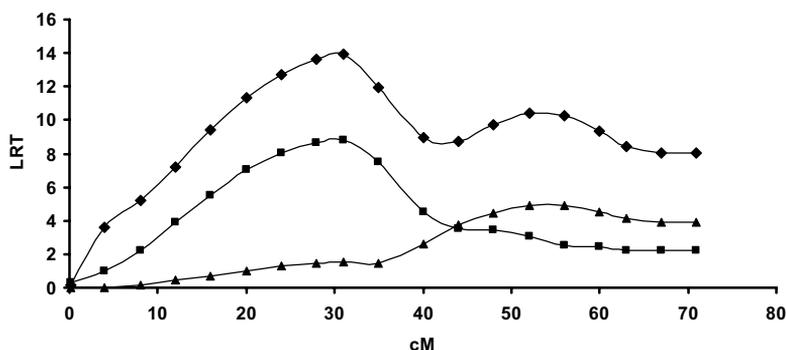
<sup>6</sup>BIC = Bayesian information criterion.

<sup>7</sup>\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ .

QTL [46] detected earlier and some of them had QTL regions affecting two different fertility traits. The analysed traits were DO, FT, CM, NRR, MY, PY, and FY. Out of the eight chromosomes analysed with multi-trait models, there was no indication of pleiotropic or linked QTL affecting fertility and milk, segregating on BTA2, BTA10 or BTA25.

**BTA1.** With the STVC analysis, a QTL for DO was detected at 1% chromosome-wise significance with the peak position at 144 cM and QTL for FT and MY at 5% significance with peak positions at 148 cM and 104 cM. The  $MT_P$  model for DO and FT was significant ( $P < 0.01$ ) and the peak QTL position was at 148 cM. The LRT for the  $MT_L$  model was not significant. The BIC (8.4) was strongly in favour of the  $MT_P$  model over the  $MT_L$  model. Besides, the correlation between the QTL effects ( $r_Q$ ) was also high (0.96). One pleiotropic QTL around 148 cM is affecting both DO and FT. The  $MT_P$  model was not significant for DO and MY, while the  $MT_L$  model for these two traits was significant ( $P < 0.01$ ). The BIC in favour of  $MT_L$  over  $MT_P$  was 9.0, which strongly favours the linked model. Additionally, the  $r_Q$  was low ( $-0.28$ ), which also supports this. The BIC and  $r_Q$  indicate that there may be two linked QTL on BTA1, one near 144 cM affecting DO and FT, and one near 104 cM affecting MY. For FT and MY, both the  $MT_P$  and  $MT_L$  model were approaching 5% significance. The BIC (0.2) estimate for  $MT_P$  over  $MT_L$  indicates that both models are equally likely. In the single-trait regression analysis, there was an indication of two QTL on BTA1 affecting FT. However, the linked QTL model affecting FT (with putative positions at 72 cM and 148 cM, LRT = 12.1) was not significant compared with the one-QTL model in the variance component method. When a multi-trait linked QTL model, with a pleiotropic QTL at 104 cM affecting both FT and MY, and the other QTL at 148 cM affecting only FT was fitted, the LRT did not exceed the significance threshold. Overall the results indicate that two linked QTL are segregating on BTA1, one QTL (at 148 cM) affecting DO and FT and the other (at 104 cM) affecting MY.

**BTA5.** With the STVC analysis, QTL for MY and PY were found at 5% chromosome-wise significance with peak LRT at 68 cM (Tab. III). Although, the STVC model for FT on BTA5 did not reach the 5% significance level, the QTL profile peak around 84–88 cM overlaps with the QTL profiles for MY and PY. Therefore, multi-trait analyses of FT and milk traits were carried out. The  $MT_P$  models, one with FT and MY, and the other with FT and PY were not significant, but the  $MT_L$  models were significant at 5% (Tab. IV). The BIC estimate was in favour of the linked QTL model but did not provide conclusive evidence for any one model over the other.



**Figure 3.** QTL profile on BTA12 from multi-trait variance component analysis of days open and fat yield. The QTL affecting both days open and fat yield  $\blacklozenge$ ; only fat yield  $\blacksquare$ ; and only days open  $\blacktriangle$ .

**BTA12.** The QTL in the STVC analysis were chromosome-wise significant at 1% for FY and at 5% for DO, MY, and PY (Tabs. IV and III). The STVC for FT was not significant. A  $MT_P$  model for the three yield traits supports a pleiotropic QTL at 31 cM. The  $MT_P$  model for DO and FT was not significant. It seems that the QTL is affecting DO but not FT. The  $MT_P$  and the  $MT_L$  models for DO and FY were significant ( $P < 0.05$ ) (Tab. IV). The BIC comparison of the models was inconclusive. Therefore, it was not possible to select one model over the other. In the STVC analyses, the highest LRT for DO was at 56 cM (close to *MB100* at 53.9 cM), while the highest LRT for FY was at 31 cM (closer to *BM6404* at 31.7 cM). There may be two linked QTL segregating on BTA12. The linked QTL model was also supported when a QTL was fitted to affect either DO or FY in a multi-trait model (Fig. 3). When the QTL was fitted to affect only FY in a multi-trait model with DO and FY the highest LRT was at 31 cM. When in a similar multi-trait model the QTL was fitted to affect only DO it did not exceed the significance threshold.

**BTA14.** The QTL at 67 cM on BTA14 affecting FT was significant at 1% and the NRR QTL at 29 cM was significant at 5% in single-trait regression analysis. In the STVC analyses, the QTL for FY was significant and located in the proximal region of BTA14. This may be the *DGAT1* gene, which has a major effect on milk composition and particularly fat content [14, 49]. The  $MT_P$  model for FT and FY approached 5% significance, while the  $MT_L$  model was significant (5%) (Tab. IV). Both the  $MT_P$  and  $MT_L$  models for FY and NRR were significant ( $P < 0.05$ ). The BIC estimate did not favour any of the competing models. In STVC analysis, the fat yield QTL was located at the proximal end of BTA14 (0.1 cM) and the QTL for FT and NRR were at 67 and 29 cM, respectively. These results indicate that the QTL located

at the centromeric region of BTA14 is affecting fat yield. None of the  $MT_P$  and  $MT_L$  models for FT and NRR were significant on BTA14.

**BTA15.** The results from the STVC analysis indicated that two QTL may be segregating for FT on BTA15, at 12 and 120 cM. The CM QTL approached 5% significance and located at 115 cM. The  $MT_P$  model for FT and CM was significant ( $P < 0.05$ ) and the peak LRT was at 120 cM. The same model also indicated another pleiotropic QTL approaching significance at 16 cM. When the QTL was fitted to affect FT in a multi-trait model, the QTL profile exceeded the 5% significance level at 120 cM and was close to the 5% significant level at 16 cM. While when a QTL was fitted to affect only CM in a multi-trait model, no evidence of the QTL at 16 cM was observed and the QTL at 120 cM approached the 5% significance threshold. It seems that the QTL segregating at the distal end of the chromosome is affecting both FT and CM and the QTL at the proximal region is only affecting FT.

### 3.7. Increased power in multi-trait models

The multi-trait analyses using the variance component method identified more QTL compared with single-trait variance component analyses. For example, on BTA5 the STVC analysis could not confirm any QTL for FT segregating on this chromosome. However, when FT was jointly analysed with MY or PY, both  $MT_L$  models identified one QTL at the centromeric region of BTA5 for FT. Similarly, on BTA14, the STVC analyses did not exceed the significance threshold for FT and NRR. While the  $MT_L$  models with FY identified a QTL for FT at 52 cM and a QTL for NRR at 32 cM. A similar phenomenon was also observed on BTA15 for FT and CM. None of the STVC analyses for these two traits was significant on this chromosome. However, the  $MT_P$  model for FT and CM identified a pleiotropic QTL at 120 cM.

## 4. DISCUSSION

A genome scan was carried out for four fertility related traits in Finnish Ayrshire cattle using the multiple linear regression method. A variance component method was used for multi-trait analysis with pleiotropic and linked QTL. Since we used VC for multi-trait analysis, we reanalysed the significant QTL models observed with the regression method by single-trait VC. The QTL for three milk production traits identified by Viitala *et al.* [46] were also reanalysed by the STVC method for the chromosomes where there was at least one fertility related QTL.

In this QTL mapping study, seven chromosome-wise significant QTL were suggested for DO, seven for FT, six for CM, and two for NRR using the regression method. When these significant QTL were reanalysed by STVC, some of the QTL were not detected. However, when STVC analyses were carried out within those families segregating for the QTL in the regression method, the QTL effects were observed (results not shown). Across family linkage analyses using the variance component method with both segregating and non-segregating families may have averaged out the QTL effect. The VC method may not detect QTL with a low allele frequency whereas regression detects these effects [9].

FT as a trait have only been analysed in one previous study [15], where possible QTL on BTA1, 3, and 22 were found. A QTL for FT was detected on BTA1 in the present study but at the opposite end of the chromosome. The other two QTL were not supported. Three additional studies have detected QTL for female fertility [2, 6, 39]. Schrooten *et al.* [39] analysed NRR in the cow and detected QTL on BTA2 and 9. Ashwell *et al.* [2] defined the trait as pregnancy rate and detected a QTL on BTA18 and putative QTL on BTA6, 14, 16, 18, 27, and 28. Boichard *et al.* [6] defined the trait as success/failure of each insemination of the daughter and found QTL on BTA1 and 7. Of these female fertility QTL only those on BTA1, 2, and 14 are supported by the present study.

In the present study, QTL for DO were suggested on seven chromosomes and two were confirmed by STVC analyses. The trait was calculated as the interval from calving to pregnancy. Schrooten *et al.* [39] defined DO as the interval from calving to first insemination. They suggested QTL for DO on BTA6 and 17, and none of them were found in the present study.

Calf mortality (stillbirth) as a maternal trait has been previously mapped by Holmberg and Andersson-Eklund [15] and Kühn *et al.* [24]. QTL were suggested on BTA4 and 19 and detected at genome-wise significance on BTA7 and 11 [15] and BTA8, 10, 18, and X/Y [24]. The QTL on BTA4, 11 and 18 were supported in the present study. The QTL on BTA4 is located near marker *HUJ673* in the Swedish and the present studies. In our study, the position of the QTL on BTA18 is at the opposite end of the chromosome close to *BMS1355* instead of *TGLA227* in the study by Kühn *et al.* [24]. Additionally, a QTL reported by Kühn *et al.* [24] for direct stillbirth, which is related to maternal CM, was supported in the present study where a QTL for CM was suggested on BTA6.

In this study, putative chromosome-wise significant QTL were found for paternal NRR on BTA10 and 14. NRR as a paternal effect was previously mapped in one study [24] in which QTL affecting NRR were found on BTA10 and 18.

The position of the QTL on BTA10 [24] was between markers *TGLA378* and *TGLA102*, which is close to our finding near the marker *ILSTS53*.

When comparing the fertility QTL with the positions of the milk trait QTL detected in an earlier study in the same families [46], several milk and fertility QTL were found on the same chromosomes. For example, on BTA25, where QTL were detected for DO and FT at the end of the chromosome, QTL for milk yield and protein yield were also detected. Furthermore, BTA1, 2, 5, and 12 harbour QTL for milk and fertility on approximately the same chromosome segments according to the regression based linkage analysis.

Multi-trait QTL analyses were carried out on eight chromosomes, which harbour QTL for more than one fertility trait or at least one fertility related trait and one milk production trait identified earlier by Viitala *et al.* [46] in the same population. We selected the chromosomes based on the significance of the QTL in the regression analysis. Though some of these QTL were not significant in the single-trait VC method, we did not put this as a precondition for selecting the chromosome and trait combinations. This was done as a multi-trait analysis for a pleiotropic QTL because it has higher statistical power of detection and a higher precision of the estimated map position compared to analysing the traits individually [19, 21, 43]. Sørensen *et al.* [43] observed that this is especially true when a second correlated trait with higher heritability (*e.g.* milk yield traits in our study) is used together with a low heritability trait (*e.g.* fertility traits in our study). Besides, a majority of the QTL identified by regression, which did not exceed the significance threshold in STVC analysis, had suggestive evidence of QTL segregation in the same region of the chromosome when analysed with STVC. Therefore, we kept a liberal entry level for the QTL to be included in the multi-trait analysis. Our results support the earlier findings of Jiang and Zeng [19], Knott and Haley [21] and Sørensen *et al.* [43] that show that multi-trait analyses have more power in detecting QTL compared to single-trait analyses.

Multi-trait QTL analyses were able to distinguish pleiotropic QTL from linked QTL only on BTA1 and not on the other chromosomes. The results also indicated linked QTL on BTA5, 12, 14 and 15 for fertility related traits and milk production traits, but it was not possible to precisely select the linked model over the pleiotropic model or *vice versa*. The QTL intervals (one-LOD support) on a single chromosome affecting more than one trait were large and overlapping. Also, the segregating families had QTL peaks spread over a considerably large region of the chromosome. The marker density used in the genome scan was sparse (average marker spacing 19 cM) and increasing marker density may help in reducing the QTL interval in linkage

mapping especially to distinguish pleiotropic/linked QTL. The traits show variable amounts of genetic correlation. A significant QTL for a given trait might be non-significant for a highly correlated trait but still have an effect on it [11]. This makes the separation between a QTL having a pleiotropic effect on two traits and a QTL affecting only one trait and showing an effect on the other trait due to a linked QTL, difficult.

## 5. CONCLUSIONS

Four traits related to bovine fertility were analysed in a QTL mapping study. A total of 22 chromosome-wise significant QTL were suggested in regression analysis and three were confirmed with the single trait variance component method. Only few of the detected QTL have been reported in earlier studies and many of the QTL of the previous studies were not supported in the present study. This could be due to a low power of detection related to the low heritability and difficulty to adequately measure these traits. Some of the fertility trait QTL are closely linked to milk production QTL or the QTL show pleiotropic effects on milk production and fertility traits. A denser marker map, larger population and linkage disequilibrium based mapping may be needed to distinguish two-linked QTL from a pleiotropic QTL.

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