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QTL detection for male fertility traits in dairy cattle

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Abstract - A QTL detection experiment has been implemented in France to search for QTLs related to male fertility in dairy cattle. Ten families, involving in total 515 bulls, were measured for ejaculate volume and spermatozoa concentration, number of spermatozoa, motility, velocity, percentage of motile spermatozoa after thawing and spermatozoa abnormalities. 148 microsatellite markers were used to realize a genome scan. First, genetic parameters were estimated for all the traits. Production traits presented moderate heritabilities (from 0.15 to 0.30) while some of the quality traits such as motility had high heritabilities (close to 0.60). Genetic correlations among traits showed, for instance, strong negative relationships between volume and motility or between velocity and spermatozoa abnormalities. Applying a chi-square test with one d.f., only three QTLs were significant at $P<0.001$, all related to spermatozoa abnormalities. In addition 11 QTL ($P>0.01$) and 18 QTLs ($P>0.05$) were detected. However, due to lack of power of the design further analyses are required to confirm these QTLs. Multitrait techniques such as Discriminant Analysis were applied to increase the power of detection of these QTLs. The LRT test was increased for most QTLs and even new QTLs were detected.

Objective - In the French Holstein population a QTL detection project involving several traits related to semen production or quality has been implemented with 10 sire families. The data of this project is particularly valuable and it offers the possibility to have original phenotypes for a consequent number of animals. These records, costly to measure, offer also an opportunity to estimate the genetic parameters among all these traits. The objectives of this study were therefore 1) to estimate the genetic parameters among the traits measured in this QTL detection program and 2) to perform a genome scan for these traits.

Material and Method

Animal material - Data were collected on 515 Holstein bulls from 10 sire families including from 40 to 66 bulls organised in a "daughter design" (performances were directly recorded on the sires and not obtained from their daughters as with a grand-daughter design). The pedigree file included 2131 animals.

Traits recorded were grouped in two categories: semen production and semen quality. Production traits included volume (**VOL**), sperm concentration (**CONC**) and sperm number (**NSP**) for the first ejaculate of the day. Sperm number per ejaculate was calculated by multiplying the concentration of sperm by total volume of semen. These traits were recorded between 12 and 18 months of age by the semen production centres and the mean number of

recorded ejaculate per bull was 9.1. The 6 semen production centres are attached to four breeding companies within which semen collection strategies are rather homogenous.
Twelve quality traits were observed on a semen sample after thawing. Two straws were mixed and analysed for each animal. Individual motility was measured as the percentage of motile sperm (**MOT**), motility score (**MSCO**) was appreciated on a scale from 0 to 5 based on the movement of each spermatozoa, percentage of living sperm (with an intact cytoplasmic membrane) (**LIV**) was first observed after thawing. The same measure was also recorded after osmotic stress in hyposomic solutions (**RES**) corresponding to 30, 40 and 50 % of the isosmolality. This trait measures the sperm resistance to osmotic stress and is an indicator of the quality of the plasma membrane. The trait was measured twice for each solutions and the mean of the 6 measures was used in statistical analyses. These first four quality traits were assessed by microscope evaluation.
Sperm was also analyzed with an HTM-IVOS sperm motility analyzer (www.hamiltonthorne.com): percentage of motile spermatozoa (**MOTH**), average path velocity (**VAP**), amplitude of linear head displacement (**ALH**) and percentage of progressive spermatozoa (**PROG**) were determined via computer assisted sperm analysis (**CASA**). VAP was measured twice for each animal and the mean was used as trait for evaluation of genetic parameters.
Finally, 4 traits related to counts of abnormal sperms (in %) were measured: abnormal sperm percentage (**ABNO**) and number of sperms with head (**HEAD**), tail (**TAIL**) or cytoplasmic droplet (**DROP**) abnormalities, respectively.

Genotyping data - The genetic markers were 148 microsatellites previously selected in two different experimental designs. The mean number of markers per chromosome was 5.1 and this number ranged from 2 to 10. The size of the region covered by the markers varied from 11 cM to 127 cM with a mean of 86.7 cM. The proportion of heterozygous sires averaged over all the markers of a chromosome had a mean of 0.63 and was comprised between 0.47 and 0.72. The genetic maps were based on the international genetic map published.

Method - For semen production traits, the model was:

$$y_{ijklmn} = spc_i * ys_j + bc_k * age_l + p_m + u_m + e_{ijklmn} \quad [1]$$

where y_{ijklmn} is the n^{th} record associated to bull m from semen production centre i from the breeding company k recorded in season-year j at age l, spc_i*ys_j is the fixed effect of the interaction between the semen production centre, the season and the year, bc_k*age_l is the fixed effect of the interaction between the breeding company and the age class, p_m is the permanent environment associated to animal m, u_m is the polygenic effect of animal m and e_{ijklmn} is the residual effect associated to the record ijklmn.
For quality traits measured at the laboratory, the model was:

$$y_{iom} = spc_i + dl_o + p_m + u_m + e_{iom} \quad [2]$$

where dl is the diluter used for semen preservation in the straw.
A multitrait model was applied for all the traits with the following (co)variance structure: var(**u**) = **A** $\otimes$ **G**, where **u** = ($\mathbf{u}_1$, $\mathbf{u}_2$, …, $\mathbf{u}_n$) and $\mathbf{u}_i$ is the vector of random polygenic effects for trait i, **A** is the additive relationship matrix and **G** is the matrix of genetic variances and covariances between traits; var(**p**) = **I** $\otimes$ **P**, where **p** = ($\mathbf{p}_1$, $\mathbf{p}_2$, …, $\mathbf{p}_n$) and $\mathbf{p}_i$ is the vector of random permanent environment effects for trait i, **I** is an identity matrix and **P** is the matrix of variances and covariances between traits for the permanent environmental effects; var(**e**) =

$\mathbf{I} \otimes \mathbf{R}$, where $\mathbf{e} = (\mathbf{e}_1, \mathbf{e}_2, \ldots, \mathbf{e}_n)$ and $\mathbf{e}_i$ is the vector of random residual effects for trait i and **R** is a matrix of residual variances for all traits.

For QTL detection, gametic effects were added to the models:

$$y_{ijklmn} = spc_i * ys_j + bc_k * age_l + p_m + u_m + v_m^p + v_m^m + e_{ijklmn} \quad [3]$$

and

$$y_{iom} = spc_i + dl_o + p_m + u_m + v_m^p + v_m^m + e_{iom} \quad [4]$$

where v_m^p and v_m^m are the effects of the paternal and the maternal alleles for animal m. The variance of the vector of QTL allelic effects (**v**) is equal to var(**v**) = $\mathbf{Q}\sigma_v^2$ where **Q** is the gametic relationship matrix and σ_v^2 is the allelic variance. At a given position, the presence of one QTL can be tested by comparing the maximum likelihood estimated by REML under a polygenic model with no QTL fitted ($L(H_0)$) with the maximum likelihood under the one-QTL model ($L(H_1)$). The resulting likelihood ratio test statistic is (George et al., 2000):

$$? = -2\ln\frac{L(H_0)}{L(H_1)} \quad [5]$$

The distribution of this test is not known but Grignola et al. (1996) showed that this distribution is intermediate between the 1 – and the 2-d.f. chi-square distribution. This test was performed for all positions with a 1 cM step.

Discriminant analysis – the method described by Gilbert and Leroy (2003) was used to produce new performances which are linear combinations of traits which maximize the QTL variance in comparison with the residual variance. QTL detection is then applied on these new traits.

Results

Heritabilities and correlations for all traits are presented in Table 1 while QTL detected at (P<0.05) are presented in Table 2 and 3.

Table 1. Heritabilities and genetic correlations among semen production and quality traits.

	VOL	CONC	NSP	MOT	MSCO	PROG	ALH	MOTH	LIV	VAP	RES
VOL	0.22	-0.55	0.47	-0.20	-0.17	-0.53	0.09	-0.46	-0.47	-0.00	-0.33
CONC		0.19	0.46	0.12	-0.01	0.35	0.27	0.23	0.29	0.28	0.14
NSP			0.09	-0.12	-0.24	-0.22	0.34	-0.28	-0.18	0.24	-0.23
MOT				0.44	0.88	0.74	-0.21	0.76	0.58	-0.27	0.38
MSCO					0.51	0.72	0.05	0.75	0.61	-0.15	0.44
PROG						0.13	-0.23	0.94	0.89	-0.05	0.79
ALH							0.52	-0.31	-0.23	**0.64**	-0.26
MOTH								0.27	0.86	-0.31	0.68
LIV									0.21	-0.16	0.78
VAP										0.27	0.18
RES											0.59

From Table 2 and 3, it appears that many QTLs detected with a test with a chi-square distribution with 1 d.f. are no longer detected with 2 d.f. The power of the design is relatively low and multi-trait techniques are indicated to increase the power of the design and to confirm the detected QTLs. Therefore, a discriminant analysis was performed where linear combinations of different traits were used as records. Figures 1 and 2 show examples of QTL detection curves where the significance level was clearly increased thanks to the discriminant analysis.

Table 2 and 3. QTLs detected for semen production, semen quality and abnormalities counts with a LRT test based on a chi-square distribution with one and two d.f., respectively.

	P<0.05	P<0.01	P<0.001	TOTAL
Semen production	4	+2	+0	6
Semen quality	10	+5	+0	15
Abnormalities counts	4	+4	+3	11
TOTAL	18	+11	+3	32

	P<0.05	P<0.01	P<0.001	TOTAL
Semen production	2	+0	+0	2
Semen quality	5	+2	+0	7
Abnormalities counts	4	+2	+1	7
TOTAL	11	+4	+1	16

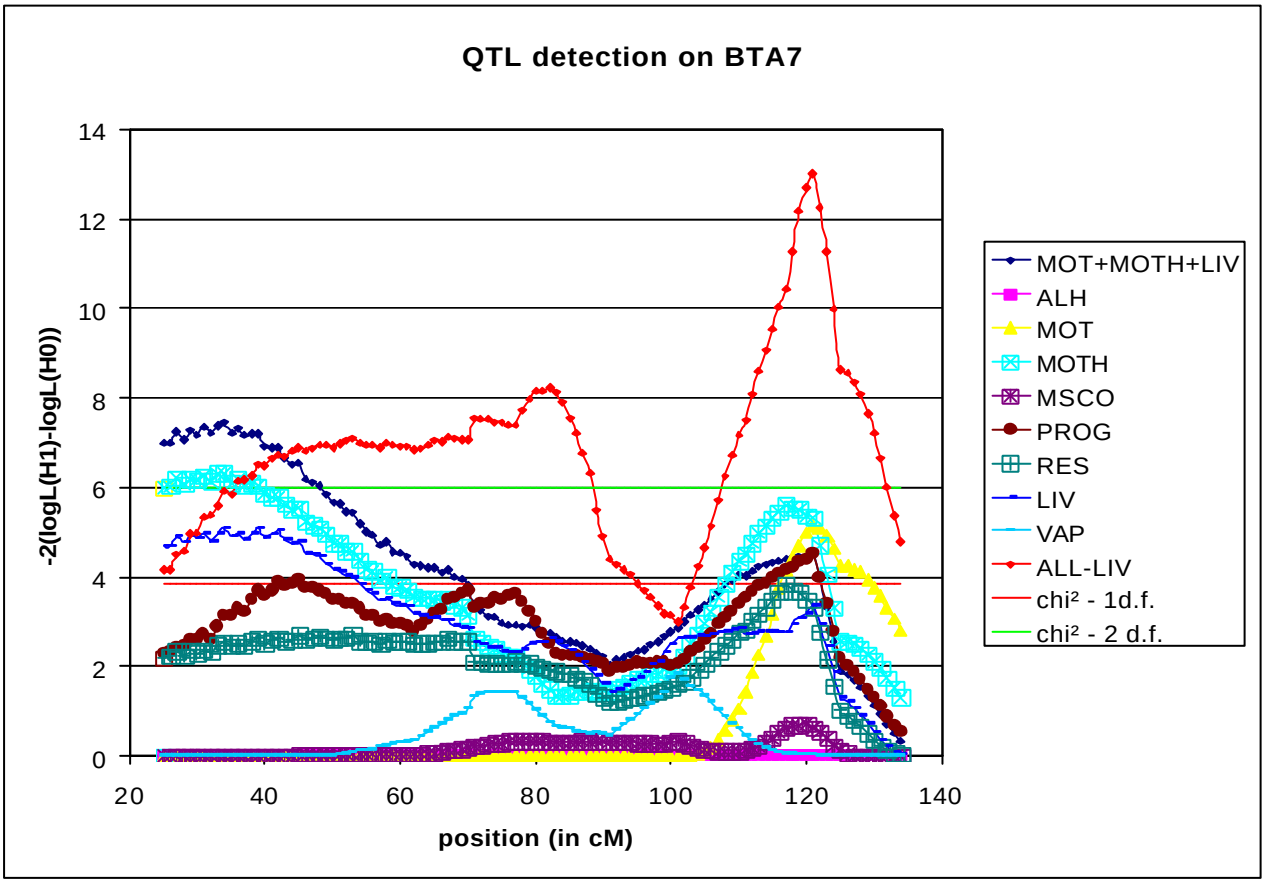

Figure 1. The red curves represents the QTL detection curve obtained with a linear combination of all semen quality traits while the other curves represent QTL detection curve obtained for individual traits.

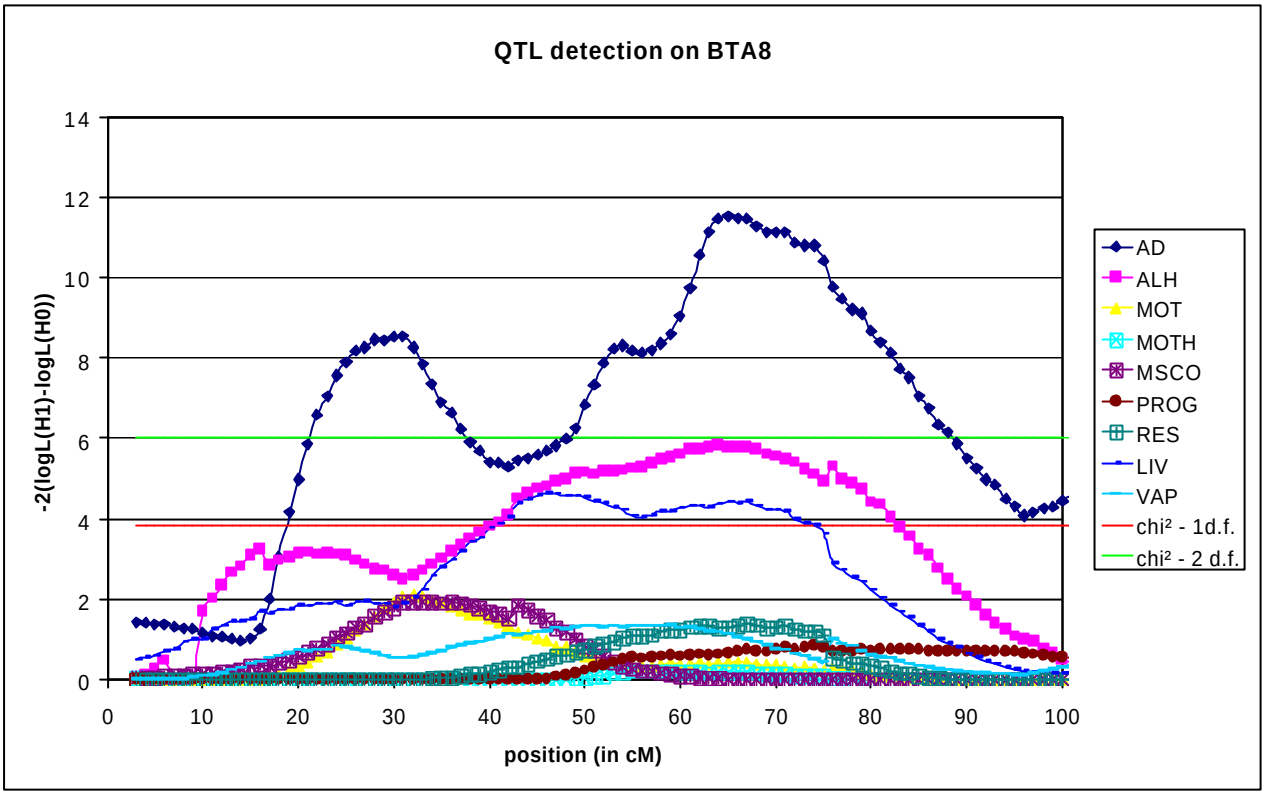

Figure 1. The red curves represents the QTL detection curve obtained with a linear combination of all semen quality traits while the other curves represent QTL detection curve obtained for individual traits.

Conclusions – Genetic correlations among semen production and quality traits showed a negative relationship between semen volume and concentration and a favourable relationship between semen concentration and most quality traits.
Several QTLs were detected with this design however, significance of these QTL was relatively low and the power of the design must be increases. Discriminant analysis offered the possibility to increase the power of the design and was a useful tool to confirm QTLs detected in single trait analysis.
The results presented in this study will be published soon and more complete results and discussion will be available.

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