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ABSTRACT

Fertility is of primary economic importance in dairy cattle and the most common reason for involuntary culling. However, standard fertility traits have very low heritability that renders genetic selection slow and difficult. In this study, we explored fertility from an endocrine standpoint. A total of 1,163 crossbred Holstein-Normande females in a 3-generation familial design were studied for progesterone level measured every 10 d to determine age at puberty (PUB) and commencement of postpartum luteal activity (CPLA). Genetic parameters were estimated using REML with WOMBAT software. The heritability estimates were 0.38 ± 0.10 and 0.16 ± 0.07 for PUB and CPLA, respectively. Moreover, the 2 traits were genetically correlated (0.45 ± 0.23), suggesting a partially common determinism. Because of the family structure, a linkage disequilibrium and linkage analysis approach was preferred over standard genome-wide association study to map genomic regions associated with these traits. Ten quantitative trait loci (QTL) were detected for PUB on chromosomes 1, 3, 11, 13, 14, 21, and 29, whereas 3 QTL were associated with CPLA on chromosomes 21 and 26. Only the QTL on chromosome 21 was common to both traits. Four functional candidate genes (*NCOA2*, *GAS2*, *OVOL1*, and *FOSL1*) were identified in the detected regions. These findings will contribute to a clearer understanding of fertility determinism and enhance the value of introducing endocrinological data in fertility studies.

Key words: progesterone, age at puberty, commencement of luteal activity, fertility, dairy cow

INTRODUCTION

Reproductive efficiency is of crucial economic importance in dairy cattle. Calving is necessary to produce milk, and any delay in reproduction lengthens the unproductive period. A lack of fertility is therefore one of the most common reasons for culling (Bascom and Young, 1998; Seegers et al., 1998; Hadley et al., 2006). Past selection programs, largely devoted to milk production traits, have indirectly selected for reduced fertility, leading to a decline of reproductive efficiency over years (Pryce et al., 2004; Miglior et al., 2017). This conflict between milk production and reproductive performance has been reported extensively in the literature, although marked differences in magnitude exist between studies (e.g., Boichard and Manfredi, 1994; Veerkamp et al., 2001; Kadarmideen et al., 2003; Pryce et al., 2004; Melendez and Pinedo, 2007). Except for Nordic countries starting in the 1970s, fertility traits were included in national overall merit indices only in the late 1990s (e.g., in 1998 for France; Boichard et al., 1998). They are now considered in all large national selection schemes (Miglior et al., 2017; Cole and VanRaden, 2018).

The fertility traits used for genetic evaluation (e.g., calving interval, nonreturn rate, age at first service, calving to first service, days open, number of services) vary between countries and consider heifers and cows separately. Genetic correlations between the same traits measured in heifers and cows were found to be only moderate, indicating that fertilities in heifers and in adult cows are different traits (Jansen et al., 1987; Boichard and Manfredi, 1994; Jamrozik et al., 2005; Tiezzi et al., 2012).

However, the heritability ($\mathbf{h^2}$) of fertility traits is very low ($h^2 < 0.10$; e.g., Jamrozik and Kistemaker, 2016), which hampers genetic improvement. This considerable environmental variability is partly due to the strong influence of herd management policies (e.g., heat de-

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tection ability, timing of insemination, calving season), which do not reflect the inherent capacity of cows to establish ovulation (Darwash et al., 1997).

One way to measure fertility more objectively would be using progesterone. The onset of progesterone secretion is the sign of puberty (i.e., the start of ovarian activity in heifers; Morrow et al., 1976; Coyral-Castel et al., 2011). Progesterone levels can also be used to detect the resumption of ovarian activity postpartum (Darwash et al., 1997; Veerkamp et al., 1998). Postpartum resumption is considered to be normal if it occurs within 35 d. Abnormalities affecting postpartum cycles may lead to delayed pregnancies (Tanaka et al., 2008; Cutullic et al., 2010). A few studies based on milk progesterone allowed the estimation of heritability for the first luteal activity postpartum. These studies found a higher heritability for this trait (h^2 between 0.13 and 0.28) than for the usual fertility trait, calving to first service (Darwash et al., 1997; Veerkamp et al., 1998), thus showing that progesterone might be a direct measurement of fertility that is less affected by farmers' decisions. Because progesterone measurements in milk or blood are not performed on a regular basis in commercial farms, obtaining this rare information on a large number of experimental animals offers an important opportunity to analyze genomic regions associated with ovarian activity and hence with fertility.

In this study, data from a large crossbreeding design between Holstein and Normande breeds were used to detect QTL. A crossbreeding design increases detection power by using both within- and between-breed genetic variability and can reveal alleles that are fixed or at very low frequency in a given breed. The objectives of this study were therefore (1) to estimate the genetic parameters for the 2 previously defined fertility traits related to blood progesterone level and (2) to investigate genomic regions involved in the variability of ovarian activity in heifers and cows of both the Holstein and Normande breeds.

MATERIALS AND METHODS

Animals

This experiment was carried out in the INRA experimentation facility in Le-Pin-Au-Haras (Normandy, France) between 1992 and 2012 (and was previously described in Larroque et al., 2012). The design involved 3 generations. To procreate the first generation (**F_1**), 5 Holstein purebred founder sires were mated with 13 Normande females, and 5 Normande sires were mated with 11 Holstein dams. Then, 10 F_1 sires and 70 F_1 dams were used to procreate the second generation (**F_2**). Family size was maximized by superovulation and embryo transfer, with an average of 3 F_1 daughters per F0 dam and about 11 F_2 daughters (1 to 40) per F_1 dam. Some embryos were sexed to limit the number of recipient females. A total of 862 F_2 cows were thus obtained. Finally, a third generation (**F_3**) of 324 cows was procreated using the same 10 F_1 sires and 49 F_2 dams, chosen to be heterozygous for the K232A mutation of the diacylglycerol acyl-transferase 1 gene (*DGAT1*). Analyses were performed on F_1, F_2, and F_3 females. Heifers were managed until a calving age of 2 or 3 yr, depending on birth season and reproduction success. Herd management remained consistent throughout the experiment with respect to reproduction periods and diets.

Phenotyping and Trait Definition

The ovarian activity of heifers was monitored by blood progesterone assays every 10 d from 230 d of age until a positive assay was obtained (threshold at 1.5 ng/mL). The age at puberty (**PUB**) was considered to be the age at the first positive assay. This analysis included 1,096 heifers (856 F_2 and 240 F_3).

The ovarian activity of primiparous cows was also monitored using blood progesterone assays every 10 d from 20 d postpartum until a positive assay was obtained (threshold at 1.5 ng/mL). The commencement of postpartum luteal activity (**CPLA**) was defined as the interval elapsing between calving and the first positive assay. This analysis included 1,025 cows (64 F_1, 787 F_2, and 174 F_3).

Progesterone levels were determined by the INRA Hormone Assay Laboratory (Tours, France) from 5 mL of blood collected in heparinized tubes. All blood samples were collected by a veterinarian or under the supervision of a veterinarian. Two different methods were used for plasma progesterone assays: RIA (Terqui and Thimonier, 1974) until 2009 (for F_1, F_2, and most F_3 females) and ELISA (Canépa et al., 2008) between 2010 and 2012 (for 85 F_3 females). The laboratory switched to the second method to avoid the use of radioactive products, which are deleterious to both human health and the environment. Both methods provide very similar results, with a correlation of 0.94 in cattle (Canépa et al., 2008). Then, the same progesterone level threshold was used to determine cycle status regardless of the method.

Body weight was measured at birth, weaning, and returning to field for heifers and monthly during lactation. Weight maturity was defined as the ratio between weaning weight (86 d old on average) and adult weight (measured at 4 yr of age).

Genotyping, Quality Control, and Phasing

All animals were genotyped with the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA) from blood samples or ear biopsies. DNA extraction and genotyping were performed at Labogena (Jouy-en-Josas, France). Quality controls included the SNP call rate (>95%), animal call rate (>98%), minimum allele frequency (>3%), Hardy Weinberg equilibrium (*P*-value $<10^{-4}$), and the removal of pedigree inconsistencies. A total of 12 females were discarded because of pedigree inconsistencies or low animal call rates. Following these controls, 1,084 heifers and 1,013 cows remained, and after editing, a total of 42,238 out of 44,580 synthesized SNP were validated for further analyses. The genomic coordinates refer to the bovine genome Assembly Bos_Taurus_UMD_3.1.1 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000003055.6). Genotypes were phased using LinkPhase and DagPhase software (Druet and Georges, 2010).

Statistical Analysis

Descriptive statistics were performed with SAS software (SAS Institute Inc., 2008). The CORR and GLM procedures were used to determine variables that significantly affected the traits. Genetic parameters were estimated using Wombat software (Meyer, 2007) according to the following bivariate polygenic animal model:

$$y_i = \mu + \mathbf{x}_i\,\mathbf{f} + g_i + \varepsilon_i, \qquad [1]$$

where y_i is the performance of animal i (PUB and CPLA), μ is the overall mean, $\mathbf{f}$ is the vector of fixed effects [combination of season and year of birth (3 seasons, 12 yr) and weight maturity for PUB; interaction between season and year of calving (4 seasons, 19 yr), age at calving (2 or 3 yr), and calving difficulties (without help, easy, or difficult) for CPLA], $\mathbf{x}_i$ is the incidence vector relating cow i to fixed effects, g_i is the random polygenic effect of animal i, and ε_i is the residual. Random effects were assumed to be normally distributed, and a pedigree-based relationship matrix including 1,511 animals of 5 generations was used.

Due to the strong family structure and the limited number of purebred ancestors (10 males and 20 females), a standard GWAS approach would not have been appropriate to take all information into account. Therefore, QTL mapping was performed by linkage disequilibrium and linkage analysis, as proposed by Meuwissen et al. (2002) and implemented by Druet et al. (2008) and Tarres et al. (2009). This method was able to account for within-breed variability, across-breed differences, and within-family variability. Moreover, the use of highly informative haplotypes greatly improved the estimation of covariances between relatives so that the chromosome segments were perfectly traced all along the pedigree. The method we used, first proposed by Meuwissen and Goddard (2001), was based on an AI-REML. The analysis was performed using homemade software developed by Druet et al. (2008) from BLUPF90 software (Misztal et al., 2002), which incorporates relationship matrices among QTL allelic effects. The following animal model was used:

$$y_i = \mu + g_i + q_{is} + q_{im} + \varepsilon_i, \qquad [2]$$

where y_i is the performance of animal i adjusted for the environmental fixed effects described in Equation 1, μ is the overall mean, g_i is the random polygenic effect of animal i, q_{is} and q_{im} are allele effects for a QTL received from sire or dam, and ε_i is the residual. The same relationship matrix as for the genetic parameter estimation was used (including 1,511 animals of 5 generations). The QTL variance was estimated using the restricted maximum likelihood method from the haplotypes of 6 successive markers. The covariance structure of the QTL was built from the probability of QTL identity first between parents and progeny and second between founders (Meuwissen and Goddard, 2001). The presence or absence of a QTL at a given position was tested by comparing the H_1 model (with polygenic and QTL effects) described in Equation 1 with the H_0 purely polygenic model (i.e., without the QTL terms). The statistical test was the log of the ratio of likelihoods between the 2 models (likelihood ratio test, **LRT**). To account for multiple testing, the significance *P*-value of 1% was divided by 2,800, which was considered to be a conservative Bonferroni correction. The corresponding chi-squared test with 1 df produced a genome-wide significance threshold of 21.48. As no peak reached this level, 2 chromosome-wide thresholds were defined, one with a *P*-value of 1% and one with a *P*-value of 2%, divided by 2,800/29 (= 96.55) to account for multiplicity at the chromosomal level. The corresponding LRT thresholds were 15.07 and 13.76 at 1% and 2%, respectively. The 98% confidence intervals of the QTL locations were estimated using the logarithm of odds drop-off approach. In practice, the bounds of the interval were the 2 locations where the likelihood was equal to the maximum likelihood minus 5.41 $\left[\chi^2_{(1,0.02)}\right]$. The bounds of the QTL location were defined by the positions of the first SNP of the first haplotype and the last SNP of the last haplotype.

Table 1. Description of age at puberty (PUB) and commencement of postpartum luteal activity (CPLA) for the different crossbreed generations[1]

Trait	Generation[2]	No.	Mean	SD	Minimum	Maximum
PUB	F_2	856	306	51	219	603
	F_3	240	308	51	232	515
CPLA	F_1	64	31.5	9.6	18	53
	F_2	787	31.3	12.3	17	94
	F_3	174	29.7	14.6	18	92

[1]No. = number of heifers or cows. Mean, SD, minimum, and maximum values are in days.
[2]F_1 = first generation; F_2 = second generation; F_3 = third generation.

RESULTS

Descriptive Statistics

The descriptive statistics by generation regarding PUB and CPLA are presented in Table 1. The PUB was 307 ± 51 d, and the time of the resumption of ovarian function was 31 ± 12 d postpartum. No significant differences were observed between generations. Significant phenotypic correlations of 0.29 and −0.17 ($P < 0.0001$) were observed between PUB and season of birth or weight maturity, respectively, suggesting that animals born in winter or weaned at a lower ratio of their adult weight tended to reach puberty later. No correlation with birth weight was observed.

Similarly, CPLA was significantly associated with the calving season (r = 0.21, $P < 0.0001$), with animals calving in winter having the longest resumption period. Correlations of CPLA with calving age (r = 0.05, $P = 0.14$) and with calving difficulties (r = 0.06, $P = 0.04$) were low. This suggests a slight trend of older cows and cows with difficult calving having a longer period before resumption.

Genetic Parameters

Genetic parameters are presented in Table 2. The estimated heritability for PUB was moderate (0.38, SE = 0.10), whereas the estimate was lower for CPLA (0.16, SE = 0.07). The genetic correlation between traits was positive and moderate (0.45, SE = 0.23) despite a low phenotypic correlation (0.08, SE = 0.05).

QTL Detection

All of the significant regions detected are detailed in Table 3 (corresponding profiles are available on request). Six regions were associated with PUB at the 1% significance threshold (scattered on BTA 1, 13, 14, and 29, with 3 hits on BTA 29), and 4 additional regions reached a 2% significance threshold (on BTA 3, 11, 13, and 21). The most significant signal (LRT = 19.7) was detected at 25.9 Mb on BTA 29 with a confidence interval of 0.36 Mb (Figure 1). For CPLA, only 2 regions were detected on BTA 26 (at 20.6 and 32.8 Mb) at the 1% significance threshold, and 1 additional region on BTA 21 reached the 2% significance threshold. The regions detected for these 2 traits were essentially different, with hits on BTA 21 around 26 Mb being the only signals that overlapped.

DISCUSSION

The PUB and CPLA observed here were consistent with the values known in pure breeds. The literature reports values for an average PUB between 9 and 12 mo in Holstein (Grass et al., 1982; Troccon and Petit, 1989; McNaughton et al., 2002) and 11 to 12 mo in the Normande breed (Loisel and Clavreul, 1981), and we reported an intermediate value of 10 mo. On average, ovarian cyclicity is reported to be set at around 29 to 33 d in Holsteins (Horan et al., 2005; Cutullic et al., 2010; Ledoux et al., 2011) and 27 d in the Normande breed (Cutullic et al., 2010). The result of 31 d obtained here seems closer to the Holstein value. Variability is important for both traits so that some individuals will present high values compared with reproduction standards, thus reinforcing the importance of selection on these traits. The effect of calving season has also been observed previously in the literature (Darwash et al., 1997; Royal et al., 2002), with winter- or spring-calving animals experiencing a longer interval before resumption, in line with our results.

Table 2. Estimates (SE in parentheses) of heritability (on the diagonal), genetic correlation (above the diagonal), and phenotypic correlation (below the diagonal) for age at puberty (PUB) and commencement of postpartum luteal activity (CPLA)

Trait	PUB	CPLA
PUB	0.38 (0.10)	0.45 (0.23)
CPLA	0.08 (0.05)	0.16 (0.07)

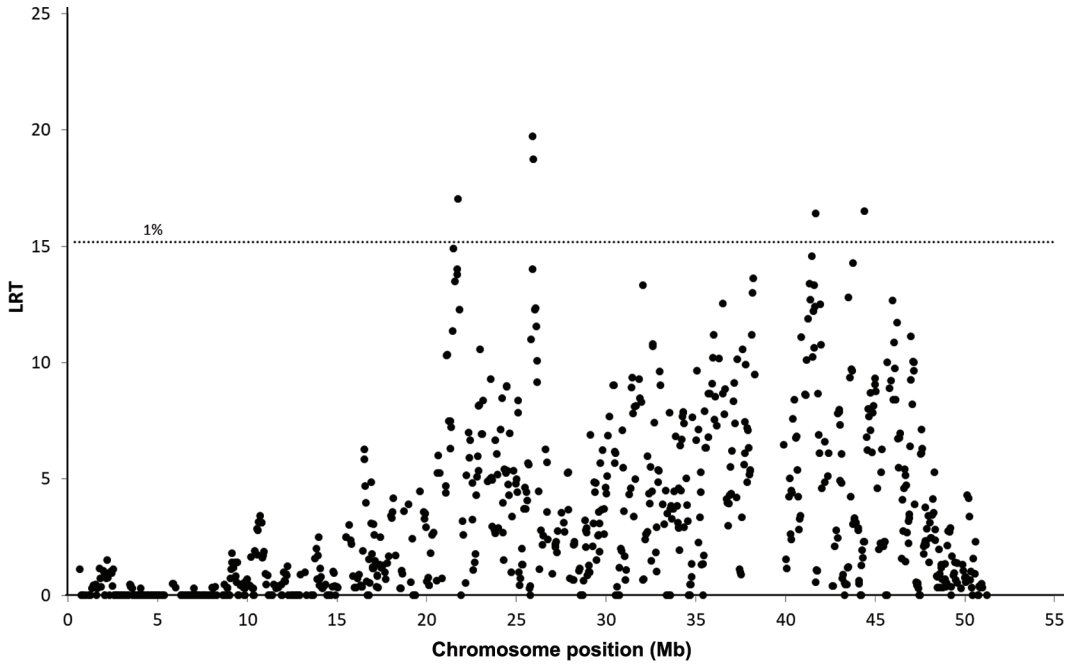

Figure 1. Detection profile for age at puberty on chromosome 29 at a 1% threshold. LRT = likelihood ratio test.

Frequency of Measurement

The question of the frequency of measurement can be discussed. In this experiment, progesterone was indeed measured every 10 d, whereas in most studies the measurement is performed more often—for example, twice a week in Berry et al. (2012) and Tenghe et al. (2016). By measuring every 10 d, we may have missed the real first cycle, especially if it was short and incomplete. Compared with daily progesterone monitoring, the 10-d frequency induced a delay of 1 to 10 d for PUB and CPLA. Reasonably assuming a uniform distribution, the average delay was 5.5 d with a variance equal to 6.75 d^2, which is a negligible value compared with the overall phenotypic variance for PUB (2,601 d^2) but substantial for CPLA (144 d^2). Then, up to 5% of the overall variability of CPLA could be imputed to measurement frequency. This fact may have influenced the heritability estimate accordingly and weakened a bit the detection power for possible QTL. However, considering the population size, the influence of the 10-d frequency on the estimated marker effects is likely very

Table 3. Significant regions for age at puberty (PUB) and commencement of postpartum luteal activity (CPLA) detected in a linkage disequilibrium and linkage analysis

Trait	BTA	*P*-value threshold (%)	QTL variance	Maximum position (Mb)	Maximum LRT[1]	Maximum MAF[2]	Significant SNP (no.)	Start (Mb)	End (Mb)
PUB	1	1	0.117	3.5	16.1	0.266	4	3.4	4.3
	3	2	0.131	85.5	14.5	0.148	9	80.2	102.5
	11	2	0.570	45.0	14.1	0.411	3	44.6	45.2
	13	1	0.293	43.5	15.7	0.200	4	43.1	43.9
	13	2	0.088	60.3	14.4	0.472	13	59.3	62.2
	14	1	0.311	37.2	16.1	0.164	5	36.6	37.6
	21	2	0.141	27.8	14.1	0.223	8	27.4	40.5
	29	1	0.093	21.8	17.0	0.300	7	21.3	22.0
	29	1	0.222	25.9	19.7	0.492	3	25.8	26.1
	29	1	0.128	44.4	16.5	0.227	22	35.8	47.1
CPLA	21	2	0.545	25.7	14.6	0.206	8	24.8	34.8
	26	1	0.311	20.6	16.5	0.473	9	16.6	21.4
	26	1	0.296	32.9	17.0	0.325	21	26.0	34.3

[1]LRT = likelihood ratio test.

[2]Minor allele frequency of SNP from the most significant haplotype of the QTL.

limited. In addition, as this study used blood samples and not milk samples, contrarily to most studies for CPLA in the literature, there was also an ethical and safety question of acceptability by the animal, as well as limited human forces on the farm. Therefore, a 10-d frequency was considered to be a good compromise between individual measurement accuracy and large population size.

Genetic Parameters

A heritability estimate of 0.16 for CPLA was consistent with those previously reported, which ranged from 0.13 to 0.28 (Darwash et al., 1997; Veerkamp et al., 2000; Royal et al., 2002; Berry et al., 2012; Nyman et al., 2014; Tenghe et al., 2015; Tarekegn et al., 2019). It is worth noting that the progesterone parameters in the literature were obtained from milk instead of blood as in the present study. Despite this important methodological difference, the genetic parameters were quite similar to those obtained in the present study. In the past, milk and blood progesterone values have been reported to be closely correlated, with r = 0.9 or higher (Dobson and Fitzpatrick, 1976; Meisterling and Dailey, 1987); this led most studies to milk sampling, which is easier and less expensive to collect. Here, we were also interested in PUB (i.e., with no milk samples available), and we opted to collect blood samples in cows as well so that the method would be the same for both measurements.

Because they require blood samples, analyses of PUB using progesterone assays are less frequently reported in the literature, especially in dairy cattle. Indeed, most studies are based on behavioral observations of first estrus in beef cattle. Reported values for heritability are highly variable in this species (ranging from 0.07 to 0.67), with an average of around 0.4 (Martin et al., 1992; Morris et al., 2000; Amyes and Morris, 2009). The heritability reported from behavioral observations of puberty in dairy heifers is lower: 0.09 according to Morris and Hickey (2004) and 0.13 for Price et al. (2017). The latter authors also estimated the heritability of puberty from blood progesterone levels in 2 fertility-related divergent lines of heifers and obtained quite high estimates of $h^2 = 0.63 \pm 0.17$ for the upper line and $h^2 = 0.49 \pm 0.16$ for the lower line. These estimates were higher than ours for PUB ($h^2 = 0.38$, SE = 0.10), although the difference was not significant.

In our study, it was not possible to obtain traditional fertility trait measurements on the same animals because all reproduction was based on embryo transfer. However, the heritability estimates obtained here for endocrine traits were higher than the ones reported for the corresponding traditional fertility traits, which are usually very low (<0.1). For example, Royal et al. (2002) reported 7 studies with estimates of heritability for calving to first service ranging from 0.02 to 0.07. Heritability estimates for age at first service are less frequent, but Jamrozik and Kistemaker (2016) reported an estimate of 0.05. Moreover, moderate to high correlations between calving to commencement of luteal activity and calving to first service or calving to first heat have been reported (Tenghe et al., 2015; Tarekegn et al., 2019). Price et al. (2017) estimated a genetic correlation of −0.3 between PUB and overall fertility breeding value. This indicates the value of adding endocrine-based traits to traditional fertility traits to improve their evaluation.

To our knowledge, the genetic correlation between PUB and CPLA estimated in our study is the first one estimated from endocrine data. This moderate and favorable genetic correlation is, however, within the same range of that previously observed for the correlation between age at first service and calving to first service (0.33, obtained by Jamrozik and Kistemaker, 2016) as well as in line with the general trend of fertility traits to be favorably correlated genetically between each other (Pryce et al., 2007; Tiezzi et al., 2012). This confirms the idea that fertility traits in heifers and cows differ but partly share a common genetic determinism.

QTL Overlapping Between Studies

Based on our QTL analysis, we were able to identify a markedly high number of signals, scattered throughout the genome and most with a minor effect on the trait considered. This suggests a polygenic determinism of the traits, as generally supported by the literature. Indeed, numerous studies have already investigated genome regions associated with fertility traits, and many regions have been highlighted. Concerning the traits most closely related to the onset of puberty and resumption of cyclicity, there have been no fewer than 358 reports to date regarding PUB in the cattle QTL database (https://www.animalgenome.org/cgi-bin/QTLdb/index), 107 on age at first calving, 1,171 for the interval to first estrus after calving, 66 for days open, and 91 for calving interval. In this study, we focused on a specific type of fertility traits, the endocrine traits. Quantitative trait loci studies regarding endocrine traits are much less common, and only a few studies have reported QTL (Berry et al., 2012; Tenghe et al., 2016; Nyman et al., 2019).

In summary, the literature has highlighted a very large number of signals but has not always been consistent across studies. In our study, all of the hits detected

were shared with, or close to, previously published findings, except for the QTL on BTA 21 for PUB and CPLA. The QTL for PUB on BTA 1 is 2 Mb away from a signal that was reported for age at first calving in a population of Hanwoo heifers (Hyeong et al., 2014). The peak observed on BTA 3 at 85.5 Mb is located between 2 peaks found by Tenghe et al. (2016): one for calving to first service at 80.5 Mb and one for luteal activity onset at 90.7 Mb, both of which were included in the large confidence interval of our signal on BTA 3. On BTA 11, the signal we detected was 4 Mb away from the observations made by Fortes et al. (2013) regarding PUB. For BTA 13, the QTL at 43.5 Mb co-localized with a QTL for the interval from calving to first insemination (Sahana et al., 2010), whereas the QTL at 60.2 Mb was consistent with a signal previously reported by Daetwyler et al. (2008). On BTA 26, we detected 2 QTL for CPLA: the first at 20.6 Mb and the second at 32.9 Mb. The first one was located close to 2 previously detected QTL: the first one for first service to conception at 17.8 Mb (Peters et al., 2013) and the second one at 22 Mb for age at first service (Hyeong et al., 2014). The second QTL, found at 32.9 Mb, was also concordant with some previous data in the literature regarding a QTL associated with calving to first service detected at 34.7 Mb (Daetwyler et al., 2008) or one with fertility treatment at 31.7 Mb (Höglund et al., 2009). As mentioned previously, the QTL on BTA 21 was the only QTL detected during our study that, to our knowledge, did not match with previously detected QTL in the literature. Optimistically, one might consider that this region was already detected by Hawken et al. (2012) for the interval from calving to first service. However, their study used a very low threshold of significance, leading to an extraordinarily large number of hits (almost 200). As this was the only QTL shared between both traits (PUB and CPLA) in our study, we believe that this region is worthy of interest. However, because of the very large confidence interval of the QTL (almost 15 Mb), no supplementary analyses have yet been performed.

A Candidate Gene on BTA 14 for PUB

The signal found on BTA 14 around 37 Mb overlapped with a region detected for age at first calving in Nellore cattle (Mota et al., 2017) and could also be related to an association reported with fertility treatments at 33 Mb (Höglund et al., 2009). This QTL was particularly interesting because of the presence of a functional candidate gene localized nearby: the nuclear receptor coactivator (*NCOA2*) gene is indeed located at 36 Mb on BTA 14. This gene has been reported to act as a transcription factor in the hypothalamus (Fortes et al., 2011) and has been described as part of the response to progesterone and the cellular response to hormone stimulus in terms of gene ontology. Moreover, Fortes et al. (2011) revealed protein–protein interactions between NCOA2 and factors involved in puberty regulation and development, suggesting an effect of the gene on the onset of puberty in Braham cattle. In addition, de Camargo et al. (2015) found associations between polymorphisms in *NCOA2* and fertility traits, including age at first calving in Nellore cattle. It is therefore likely that the *NCOA2* gene could explain our QTL on BTA 14, and further investigations are now necessary to clarify the effect of this gene on puberty in European cattle.

A Large Associated Region on BTA 29 with Several QTL

Chromosome 29 was of particular interest during this study because it is the only chromosome bearing 3 close signals (at 21.8, 25.9, and 44.4 Mb). Moreover, a Manhattan plot (Figure 1) showed that 3 different and significant QTL were defined by our statistical methods, although the entire region from 20 to 50 Mb appeared to be associated at a nonsignificant level. The literature is also very prolific, with a large number of QTL for fertility traits detected in this BTA 29 region. There have been reports of QTL for days open at 22 to 24 Mb (Müller et al., 2017), age at first calving at 24 Mb (Akanno et al., 2015), male puberty at 26 Mb (Casas et al., 2004), postpartum anestrus interval at 33 Mb (Collis et al., 2012), calving to first service at 35 Mb (Daetwyler et al., 2008), and signals for calving to first service between 42 and 44 Mb (Frischknecht et al., 2017). In addition, no fewer than 11 associations with postpartum anestrus in this region were reported by Hawken et al. (2012) according to the cattle QTL database, scattered between 26 and 49 Mb.

This study was carried out considering a marker map based on the UMD3.1 genome assembly. Unfortunately, compared with the recent ARS-UCD1.2 assembly, 2 markers initially located at positions 27529419 and 27749740 were shown to be located on another chromosome (20 and 9). These 2 markers were included in 14 wrong haplotypes likely poorly associated with the QTL and may explain the low LRT test between QTL 2 and 3. Between QTL 1 and 2, the order of all markers was confirmed. However, 2 F_3 individuals were double recombinants in the 21.29 to 22.38 Mb region, probably due to a genotyping error. The effect of these 2 individuals, compared with the 250 single recombinants observed in the region, is hard to predict.

Due to this situation and the limited resolution of our analysis, it appears difficult to estimate the number of independent signals underlying these QTL between 1 and 3. Nevertheless, under the peaks detected during our study, we were able to identify 3 candidate genes. The first one was the growth arrest-specific protein 2 (*GAS2*) gene, located on BTA 29 at 22.2 to 22.4 Mb. *GAS2* has been found to be critical to the folliculogenesis process and tissue remodeling in the mouse ovary (York et al., 2016), thus rendering this gene a strong functional candidate for an association with puberty. The second gene was putative transcription factor ovo-like 1 (*OVOL1*), located at 44.5 to 44.6 Mb. This gene, encoding a zinc finger protein that binds to DNA, has been found to be associated with oogenesis in flies and spermatogenesis in mice (Dai et al., 1998), and the corresponding locus in humans has been associated with several disorders, including urogenital abnormalities (Beales et al., 1997). The third candidate gene was Fos-related antigen 1 (*FOSL1*), located at 44.6 to 44.7 Mb. This gene has been reported to be implicated in the initiation of pregnancy in rats (Kent et al., 2011) and the response to progesterone in human cells (Mazur et al., 2015). These 3 genes are very promising candidates, and further analyses regarding their possible existing polymorphism are required.

Relationships Between Fertility Traits

During this study, we analyzed 2 different fertility traits that were measured in 2 different periods of a cow's life: PUB when she is a young heifer, and the postpartum resumption of cyclicity when she is in first lactation. It has already been widely reported that the fertility traits of heifers and cows differ and need to be treated differently in a genetic evaluation (Pryce et al., 2004; Tiezzi et al., 2012). As we already mentioned, this was also the case for our 2 endocrine traits with a genetic correlation of 0.45, within the range of what is usually found for correlations between heifer and cow fertility traits (e.g., Muuttoranta, 2015). Interestingly, despite this important genetic correlation, only 1 common QTL was detected by our analyses. However, when comparing our results with the literature, we observed that some QTL found for PUB indeed overlapped with previously reported hits for the calving to first service interval on BTA 3 and 13 (Sahana et al., 2010; Tenghe et al., 2016). Similarly, 1 QTL that we detected for CPLA (on BTA 26) had also previously been reported as being associated with age at first calving (Hyeong et al., 2014). A large number of QTL for various fertility traits was detected on BTA 29, as previously discussed. All of these elements, in addition to the general tendency of all fertility traits to be favorably correlated (e.g., Jamrozik and Kistemaker, 2016), highlighted the partially common genetic determinism of all fertility traits.

Benefits of Endocrine Traits

Traditional fertility traits are lengthy and difficult to select because of their low heritability, which, in addition to a polygenic determinism, leads to a large number of weakly associated genomic regions found by numerous studies that are not consistent with each other. Ultimately, despite an abundance of literature, the genomic control of fertility remains unclear.

Endocrine traits have the obvious benefit of being less dependent on farm management, which results in more heritable traits. Using such a trait to better understand the architecture and genetic determinism of fertility is therefore strongly recommended. Including endocrine traits in genomic evaluations would be advantageous and would ensure a more accurate estimation of traditional fertility traits when both are combined (Tenghe et al., 2015). However, its limitations include the cost of determination and the practical problems encountered with the real-time analysis of samples. This is particularly true when determining PUB that cannot be performed using milk. The usefulness of investing in a reference population remains unclear in view of both the cost and potential gains, and this point needs to be investigated further in different situations and populations.

CONCLUSIONS

Moderate to high heritability was estimated for PUB and CPLA, demonstrating that endocrine traits are more heritable than traditional fertility traits. Estimates of the genetic correlation between the 2 traits suggested a partly shared determinism, as is generally the case for all fertility traits. Ten QTL were identified for PUB and 4 for CPLA, highlighting new genome regions and confirming others, whereas several candidate genes were suggested in target regions. These findings provide further evidence in favor of including endocrine data in fertility studies.

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