

Detection of genomic regions associated with fertility traits in Mexican Holstein females

A. García-Ruiz¹, F.J. Ruiz-López^{1,2}, C.G. Vásquez Peláez², S.I. Román-Ponce¹

¹ National Institute of Forestry, Agricultural and Livestock Research, Ajuchitlán 76680 Querétaro, México.

garcia.adriana@inifap.gob.mx (Corresponding author)

² National Autonomous University of Mexico, Centro Universitario 04010, Cd de México., México

Summary

Fertility is a key functional trait in dairy production systems, because calving determines the beginning of milk production. The objective of this study was to detect genomic regions associated with four fertility parameters: days at first service (D1S), calving interval (CI) number of services per conception (NSC) and days open (OD) to provide tools for improvement of the selection process. Phenotypes were the breeding values of D1S, CI, NSC and OD with reliabilities greater than 0.30 of Mexican Holstein females born from 2008 to 2013. After the genotype's quality control, a total of 20,407 SNP and 3,242 animals were used to perform Genome Wide Association Studies (GWAS) through a single linear regression model, previously adjusting for population composition and counting for false discovery rate and Bonferroni's correction. Quality control and GWAS were performed with SVS Golden Helix V.8.6. Hundreds of significant SNP ($<1 \times 10^{-5}$) were associated to fertility traits in the Mexican Holstein population. Because several genomic regions were related with many traits, the 100 most significant SNPs for each trait were pooled together and the SNP associated with more than two traits were referenced in the genomic databases. Six of the nine SNP associated in this study with more than two traits, were previously reported in other populations. Three of them are related directly with fertility (The SNP ARS-BFGL-NGS-101016 with calving index and calving ease, Hapmap31053-BTA-111664 with number of inseminations and non-return rate and ARS-BFGL-NGS-103755 with calf size). Another three SNP have not been linked to fertility, but instead are related with traits for which a negative relationship with it has been reported like some production traits (for example, the marker ARS-BFGL-NGS-24522, SNP is related with caseins yield, BTB-01869907 with milk, fat, protein and lactose yield and Hapmap44709-BTA-70915 with mature weight). Markers BTB-02009158, ARS-BFGL-NGS-115837 and ARS-BFGL-NGS-113691 from chromosomes 8, 14 and 22 respectively, have not been previously associated to traits or genes that explain a possible interaction with fertility. This study shows the presence of novel genomic regions associated with fertility parameters in Holstein cattle.

Keywords: Genomic regions, fertility traits, Mexican cattle.

Introduction

Fertility plays a significant role in dairy cattle because calving determines the beginning of the lactation and if reproductive parameters do not get adjusted on time and properly, the farmer could have considerable economic losses. Selection was focus on improving milk

yield for many years and, as result and due to unfavourable genetic correlations between these traits fertility declined (Pryce *et al.*, 2004). Additionally, fertility is difficult to improve due to the low heritability (González-Recio & Alenda, 2005).

The inclusion of genomic data in the genetic improvement programs potentialize the genetic gain, especially for traits with low heritability (García *et al.*, 2016), because this technological tool helps to detect genomic regions explaining the genetic variation of different traits (Pryce *et al.*, 2010) and makes more feasible the genetic selection. With single nucleotide polymorphisms or SNP as DNA markers it is possible to apply genome wide association analysis (GWAS) to understand the genome architecture, describe structure of genetic variation, perform QTL mapping accurately and identify causal genes even with modest effects (Bush *et al.*, 2012). Although thousands of genomic regions have been associated to many important traits in dairy populations, it is necessary to know the genetic underlying structure of a specific trait if one wants to include it in a genetic selection program because the same trait may have different genetic architecture and polygenic nature in different populations (Sharma *et al.*, 2015).

The genetic improvement program of dairy cattle in Mexico has not included traits related with fertility due to the fact that records were not collected routinely. The objective of this study was to detect genomic regions associated with fertility parameters: days at first service (D1S), calving interval (CI) number of services per conception (NSC) and days open (OD).

Material and methods

Phenotypes and genotypes.

The phenotypes were estimated breeding values of fertility traits with reliability greater than 0.30 of Mexican Holstein females born from 2008 to 2013. D1S was defined as the number of days between calving and the first registered service. NSC was calculated as the number of services required for a cow to become pregnant, and CI as the number of days between two calvings. Animals were genotyped previously to this study with different platform densities, so imputation to 44,244 SNP using FindHap V2 software was performed (VanRaden *et al.*, 2011).

Quality control and GWAS.

Genotype's quality control excluded markers with MAF <0.05, with low call rate (< 85%) or a P-value for Fisher's exact test for Hardy-Weinberg Equilibrium ≤ 0.001 . Individuals with low call rate (< 90%) were also excluded. A total of 20,407 SNP and 3,242 animals were used for this study. GWAS was performed for the four traits using single linear regression under an additive genetic model, adjusting for population composition through principal component analyses. Significant SNP were counted after Bonferroni's correction. Quality control analysis and GWAS were performed with SVS Golden Helix V.8.6.

Results and discussion

GWAS identified genomic regions associated to the studied traits. Due to hundreds of significant SNP ($< 1 \times 10^{-5}$) were found for each trait and because fertility parameters are highly correlated among them (González-Recio & Alenda, 2005), the 100 most significant SNP for

each trait were pooled together and SNP associated with more than two traits (Table 1) were referenced in the genomic databases.

Table 1. Significant SNP markers associated with more than two fertility traits in the Mexican Holsten females.

SNP Marker	Chromosome	Position	Traits
Hapmap44709-BTA-70915	4	15575489	CI, D1S, OD
ARS-BFGL-NGS-24522	6	87878364	D1S, NSC, OD
BTB-02009158	8	81545238	D1S, NSC, OD
ARS-BFGL-NGS-101016	8	87236608	CI, NSC, OD
Hapmap31053-BTA-111664	9	27017136	CI, NSC, OD
ARS-BFGL-NGS-115837	14	67593766	CI, D1S, OD
ARS-BFGL-NGS-103755	19	9172300	CI, NSC, OD
ARS-BFGL-NGS-113691	22	54440045	CI, NSC, OD
BTB-01869907	29	38194335	CI, D1S, OD

CI: Calving interval, D1S; Days at first service, OD: Open days, NSC: Number of services per conception.

Six of the nine SNP associated in this study with more than two fertility traits, were previously associated to other economically important traits in other populations (Holmberg & Andersson, 2006; Höglund *et al.*, 2012; Berkowicz *et al.*, 2011). Three of them (located in chromosomes and position 8:87236608, 9:27017136 and 19:9172300) were related directly with fertility, for example, the SNP ARS-BFGL-NGS-101016 which is located in a region linked with calving index and calving ease in Nordic Holstein populations (Höglund *et al.*, 2012), the SNP Hapmap31053-BTA-111664, associated with number of inseminations and non-return rate in Swedish dairy cattle (Holmberg & Andersson, 2006) and interval from first to last insemination in the Danish and Swedish female Holstein population (Höglund *et al.*, 2009), and ARS-BFGL-NGS-103755 SNP, was related with calf size in Nordic dairy cattle (Höglund *et al.*, 2012).

Three SNP have not been linked to fertility traits, but were found in regions associated with characters that keep a negative relationship with these traits: ARS-BFGL-NGS-24522 (Chromosome 6, position 87878364), SNP highly related with caseins yield (Huang *et al.*, 2012), the marker BTB-01869907 (29:38194335) associated with milk, fat, protein and lactose yield in Irish Holstein-Friesian cattle (Berkowicz *et al.*, 2011) and SNP Hapmap44709-BTA-70915 (4:15575489) located in a region linked to mature weight and height and scrotal circumference in Angus cattle (McClure *et al.*, 2010). This SNP association could be explained by the antagonistic relationship between milk yield and reproductive measures (Pryce *et al.*, 2004).

Markers BTB-02009158, ARS-BFGL-NGS-115837 and ARS-BFGL-NGS-113691 from chromosomes 8, 14 and 22 respectively, have not been previously associated to traits or genes that explain a possible interaction with fertility. This study shows evidence of novel genomic regions associated with fertility parameters in Mexican Holstein cattle.

Conclusion

This study demonstrates the presence of genomic regions associated to fertility parameters, which could help to implement a program of genomic selection in the Mexican Holstein population.

Many genomic regions were related with more than two fertility traits, which could be the origin of the genetic correlation between them. The thirty percent of the SNP with statistically significant associations with fertility traits were located in genomic regions earlier related to the studied traits. Others were near reported QTL or genes associated with production traits, which keep a negative relationship with fertility. Three SNPs, located on chromosomes 8, 14 and 22 represent novel associations in the Mexican Holstein population.

List of References

- Berkowicz, E. W., D. A. Magee, K. M. Sikora, D. P., Berry, D. J. Howard, M. P. Mullen, & D. E. Machugh, 2011. Single nucleotide polymorphisms at the imprinted bovine insulin-like growth factor 2 (IGF2) locus are associated with dairy performance in Irish Holstein-Friesian cattle. *J Dairy Res.* 78 1–8.
- Bush, W.S., & J. H. Moore, 2012. Genome-wide association studies. *PLoS Comput. Biol.* 8, e1002822.
- García-Ruiz, A., J. B. Cole, P. M. Vanraden, G. R. Wiggans, F. J. Ruiz-López, & C. P. Van Tassell, 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. *Proc. Natl. Acad. Sci. USA.* 113: E3995–E4004.
- González-Recio, O & R., Alenda, 2005. Genetic Parameters for Female Fertility Traits and a Fertility Index in Spanish Dairy Cattle. *J Dairy Sci.* 88:3282–3289.
- Höglund, J. K., B. Guldbbrandtsen, G. Su, B. Thomsen, & M. S. Lund, 2009. Genome scan detects quantitative trait loci affecting female fertility traits in Danish and Swedish Holstein cattle. *J. Dairy Sci.* 89:3664–3671.
- Höglund, J. K., B. Guldbbrandtsen, M. S. Lund, & G. Sahana, 2012. Analyzes of genome-wide association follow-up study for calving traits in dairy cattle. *BMC Genetics.* 13:71.
- Huang, W., F. Peñagaricano, K. R. Ahmad, J. A. Lucey, K. A. Weigel & H. Khatib, 2012. Association between milk protein gene variants and protein composition traits in dairy cattle. *J Dairy Sci.* 95(1), 440–449.
- McClure, M. C., N. S. Morsci, R. D. Schnabel, J. W. Kim, P. Yao, M. M. Rolf & J. F. Taylor, 2010. A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. *Ani Genet.* 41, 597–607.
- Pryce, J. E., M. D. Royal, P. C. Garnsworthy, & I. L. Mao, 2004. Fertility in high-producing dairy cow. *Livest. Prod. Sci.* 86:125–135.
- Sharma A., J.S. Lee, C.G. Dang, P. Sudrajad, H.C. Kim, S.H. Yeon, 2015. Stories and challenges of genome-wide association studies in livestock—a review. *Asian-Australas J Anim Sci.* 28:1371–9.
- VanRaden, P. M., O’Connell, J. R., Wiggans, G. R. & K. A. Weigel, 2011. Genomic evaluations with many more genotypes. *Gen. Sel. Evol.*, 43, 10.