Identification of Loci Associated with Fertility in US Holstein Heifers

E.M. Keuter[1], C.M. Seabury[2], M. Neupane[1], J.N. Kiser[1], J.G. N. Moraes[3], J. Dalton[4], G.W. Burns[3], T.E. Spencer[3], H.L. Neibergs[1]

[1]Department of Animal Science and Center for Reproductive Biology, Washington State University, Pullman; [2]Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX; [3]Division of Animal Sciences, University of Missouri, Columbia, MO; [4]Department of Animal and Veterinary Sciences, University of Idaho, Caldwell

ABSTRACT

Current conception rates in US Holstein heifers are estimated to be between 55-60%. The objective here was to identify genomic loci associated with fertility in Holstein dairy heifers. Breeding and health records of Holstein heifers were analyzed from a commercial dairy heifer raising facility in Southern Idaho. All heifers were bred by artificial insemination (AI) at observed estrus, and pregnancy determined at day 35 after AI via palpation. Records analysis identified 497 heifers that could be classified as highly fertile (HF) due to conceiving on first AI service, and 429 subfertile (SF) that did not conceive until after fourth AI service or were culled due to failure to conceive. DNA was extracted from blood samples and genotyped using the Illumina BovineHD BeadChip. Quality control consisted of removing animals with <90% of genotypes and removing markers with <90% of genotypes, a minor allele frequency <1%, or if they failed Hardy-Weinberg Equilibrium testing. A total of 466 HF and 368 SF heifers and 590,904 SNPs remained for the analysis. A genome-wide associated analysis (GWAA) was conducted using an additive model of the Efficient Mixed-Model Association expedited (EMMAX) statistical test with a genomic relationship matrix. Covariates used in the analysis accounted for relatedness (Identity by descent ≥ 0.2) of heifers and the AI bull the heifer was bred to as conception rates differed between AI sires ($P<6.9x10^{-13}$). The GWAA identified 153 SNPs representing 147 QTLs ($P< 5.5 \times 10^{-5}$) that were moderately associated and 34 SNPs representing 26 QTLs ($P< 5.5 \times 10^{-7}$, proportion variance explained (PVE) ranged from 0.032 to 0.115) that were strongly associated with heifer fertility. Psuedo-heritability was estimated to be 0.46 and $\lambda = 0.98$. These results indicate that there is ample opportunity to make significant gains in fertility in Holstein heifers with genomic selection.

Acknowledgement: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2013-68004-20365 from the USDA National Institute of Food and Agriculture.

Keywords: dairy, fertility, quantitative trait loci

OBJECTIVE

The objective of this study was to conduct a GWAA on a Holstein heifer population to identify genomic loci associated with fertility.

INTRODUCTION

Increased selection for higher milk production in US dairy cattle has been successful over the last 50 years, however fertility has suffered leaving Holstein heifers with estimated conception rates of 55-60% (Kuhn, Hutchison, and Wiggans, 2006). Increased occurrences of subfertility result in significant revenue losses due to costs of increased days open and replacing animals due to reproductive culling. Recent developments in translational genomics and our understanding of breeding and reproduction offer an opportunity to identify genomic loci associated with fertility in heifers.

MATERIALS AND METHODS

- DNA was extracted from blood samples collected from 497 HF and 429 SF heifers for a total of 926 heifers in the study
- HF heifers conceived after one AI service and SF heifers conceived after four AI services or were culled due to failure to conceive
- Samples were genotyped using the Illumina BovineHD BeadChip. The resulting genotype data was quality control filtered using the thresholds presented in **Table 1**.
- Nineteen heifers were removed due to phenotypic inconsistencies.
- A GWAA was performed using the EMMAX (Kang et al. 2010) statistical method with a genomic relationship matrix.
- Covariates for the model included relatedness between heifers (identity by descent ≥ 0.2) and AI bull used for breeding as conception rates differed between sires ($P<6.9x10^{-13}$).

Table 1. Quality control thresholds for animals and SNPs in the GWAA

Animals	Filtering Thresholds	# of Heifers Removed
Call rate	<90%	49
IBD	>95% IBD	24
SNPs	Filtering Thresholds	# of SNPs Removed
Call rate	<90%	50,837
MAF	< 1%	133,212
HWE	$< 1x10^{-100}$	50
	Remaining in Model	
SNPs	590,904	
Heifers	834	

Identification of Loci Associated with Fertility in US Holstein Heifers

E.M. Keuter[1], C.M. Seabury[2], M. Neupane[1], J.N. Kiser[1], J.G. N. Moraes[3], J. Dalton[4], G.W. Burns[3], T.E. Spencer[3], H.L. Neibergs[1]

[1]Department of Animal Science and Center for Reproductive Biology, Washington State University, Pullman; [2]Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX; [3]Division of Animal Sciences, University of Missouri, Columbia, MO; [4]Department of Animal and Veterinary Sciences, University of Idaho, Caldwell

RESULTS

Figure 1. Principal Component Analysis identifying heifers clustered by relatedness. Animals (n=656) within the red circle were determined to be at least half siblings (IBD value ≥ 0.2). Each dot of color represents a heifer, and each color represents offspring from a specific sire (n=50).

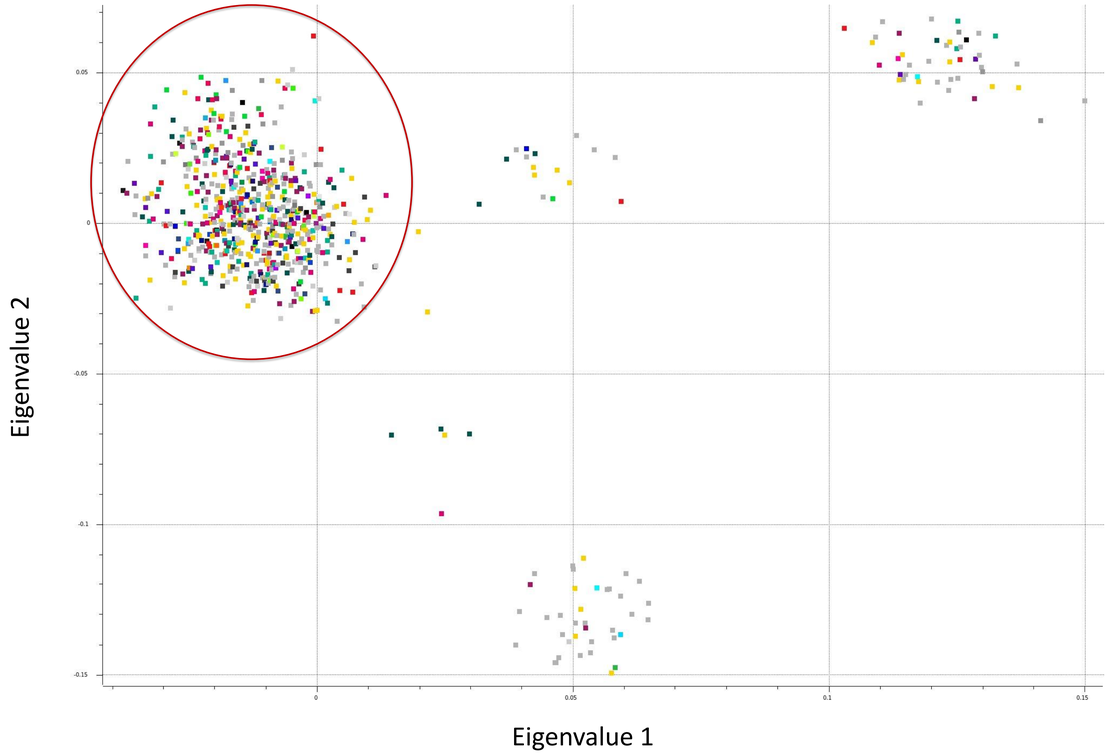
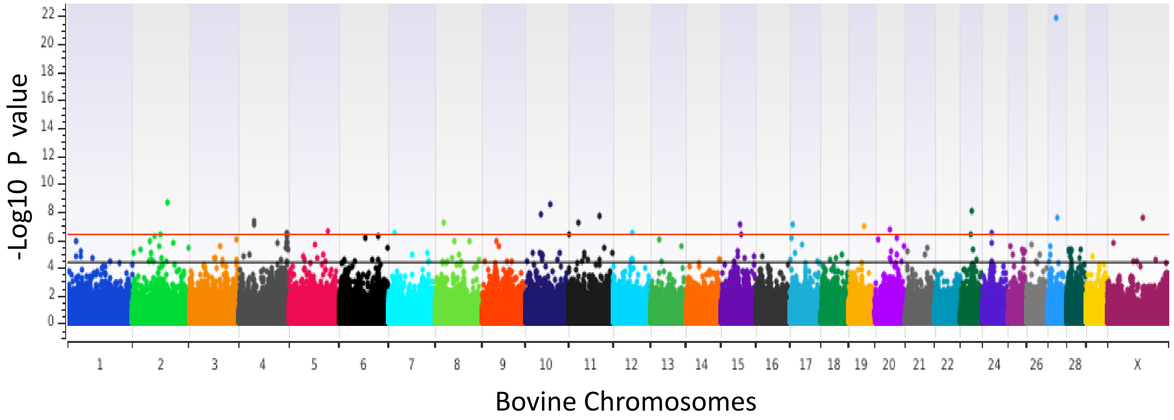


Figure 2. Manhattan plot identifying QTL associated with heifer fertility in the EMMAX additive model. SNPs located between 4.3 (black line) and 6.3 (red line) on the *y* axis (-Log10 P value) provided evidence of moderate association, and SNPs above 6.3 (red line) provided evidence for strong association based on the Wellcome Trust Consortium guidelines (2007).



- 153 SNPs representing 147 QTLs ($P< 5.5 \times 10^{-5}$) were moderately associated, and 34 SNPs representing 26 QTLs ($P< 5.5 \times 10^{-7}$) were strongly associated with heifer fertility
- The proportion of variance (PVE) explained for moderately associated SNPs ranged from 0.021 - 0.032 and the PVE for strongly associated SNPs was 0.032-0.115
- Heritability estimate for heifer fertility was 0.46
- $\lambda_{GC} = 0.98$

Identification of Loci Associated with Fertility in US Holstein Heifers

E.M. Keuter[1], C.M. Seabury[2], M. Neupane[1], J.N. Kiser[1], J.G. N. Moraes[3], J. Dalton[4], G.W. Burns[3], T.E. Spencer[3], H.L. Neibergs[1]

[1]Department of Animal Science and Center for Reproductive Biology, Washington State University, Pullman; [2]Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX; [3]Division of Animal Sciences, University of Missouri, Columbia, MO; [4]Department of Animal and Veterinary Sciences, University of Idaho, Caldwell

RESULTS

Table 2. Loci strongly associated ($P < 5.5 \times 10^{-7}$) with fertility in US Holstein heifers. **Bolded type identifies markers selected for Whole Genome Sequencing.**

SNP	BTA	BP Position	P-value	PVE	Genes within 100kb	Gene Function
rs132728892	27	21,375,791	1.37E-22	0.115	*SGCZ*	Positive regulator for estrogen
rs109342415	2	85,462,609	2.77E-09	0.044	---	---
rs110617366	10	59,882,200	3.80E-09	0.043	*TRPM7*	Deletion disrupts embryonic development
rs109111192	23	27,994,640	1.06E-08	0.041	*DDR1*	Expressed in large follicles
rs135421839	10	37,205,266	1.60E-08	0.040	***MGA***	Interacts with MYC- a null mutation of c-myc leads to early embryonic death and reduced fertility
rs109611855	11	75,222,305	2.34E-08	0.039	*ATAD2B*	Placental manganese
rs43331413	27	22,594,468	2.89E-08	0.039	---	---
rs133469168	X	85,992,720	3.20E-08	0.038	*EDA*	Multicellular organismal development
rs133417267	4	37,235,676	5.88E-08	0.037	*SEMA3E*	Dysfunctional signaling impacts GnRH neurons
rs42494516	11	23,867,860	6.75E-08	0.037	---	---
rs134858319	8	20,510,016	7.67E-08	0.036	---	---
rs43388620	4	37,618,902	8.65E-08	0.036	*PCLO*	Major Depressive Disorder
rs109396578	15	47,135,215	1.06E-07	0.036	*APBB1, DNHD1*	Daughter pregnancy rate; Male infertility
rs132777965	17	5,596,017	1.08E-07	0.035	---	---

Identification of Loci Associated with Fertility in US Holstein Heifers

E.M. Keuter[1], C.M. Seabury[2], M. Neupane[1], J.N. Kiser[1], J.G. N. Moraes[3], J. Dalton[4], G.W. Burns[3], T.E. Spencer[3], H.L. Neibergs[1]

[1]Department of Animal Science and Center for Reproductive Biology, Washington State University, Pullman; [2]Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX; [3]Division of Animal Sciences, University of Missouri, Columbia, MO; [4]Department of Animal and Veterinary Sciences, University of Idaho, Caldwell

RESULTS

Table 3. Loci strongly associated ($P < 5.5 \times 10^{-7}$) with fertility in US Holstein heifers. **Bolded type identifies markers selected for Whole Genome Sequencing.**

SNP	BTA	BP Position	P-value	PVE	Genes within 100kb	Gene Function
rs135474456	19	38,028,013	1.28E-07	0.035	***B4GALNT2***	Required for embryo implantation in mice; ovulation rate regulation in sheep
rs109846657	20	35,874,119	2.47E-07	0.033	***LIFR***	Humans- highly expressed at implantation
rs109606410	5	94060598	2.86E-07	0.033	***SLC5A5***	Congenital hypothyroidism
rs136817058	4	116,540,002	3.32E-07	0.033	---	---
rs133202371	7	14,998,140	3.55E-07	0.033	---	---
rs137778395	24	23,433,827	4.00E-07	0.032	*NOL4*	Major Depressive Disorder
rs132902673	12	47,032,066	4.16E-07	0.032	---	---
rs135051964	4	116,556,079	4.64E-07	0.032	---	---
rs133812771	11	2,125,437	5.11E-07	0.032	*GPAT2*	Testicular cancer
rs42471300	2	68,561,962	5.30E-07	0.032	---	---
rs135572576	15	49,602,802	5.33E-07	0.032	*OR51T1, OR51F2*	Spontaneous abortions at 10-20 weeks
rs109850164	23	24,254,314	5.41E-07	0.032	*PKHD1*	Polycystic kidney disease
rs133206539	4	116,554,403	5.55E-07	0.032	---	---

CONCLUSION

These results represent a preliminary analysis of ongoing work to identify loci associated with heifer fertility and suggest that there is ample opportunity to make significant gains in fertility using genomic selection. Reducing the considerable economic losses associated with poor reproductive performance in dairy cattle requires the simultaneous development of genomic tools to enable the selection of heifers with superior fertility and appropriate bull selection for daughter fertility as well as a sustained educational effort to increase the adoption of genetic management practices that improve reproductive performance, thereby increasing industry profitability.

ACKNOWLEDGEMENTS

This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2013-68004-20365 from the USDA National Institute of Food and Agriculture.

REFERENCES

Kang, H. M., J. H. Sul, S.K. Service, N. A Zaitlen, S. Kong, N. B. Freimer, C. Sabatti, E. Eskin. 2010. Variance component model to account for sample structure in genome-wide association studies. Nature Genetics 42:348-356.

Kuhn, M. T., J. L. Hutchison, G. R. Wiggans. 2006. Characterization of Holstein heifers fertility in the United States. J. Dairy Sci. 89:4907-4920.

Wellcome Trust Case Control Consortium. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447:661–678.