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Association of single nucleotide polymorphisms in candidate genes previously related to genetic variation in fertility with phenotypic measurements of reproductive function in Holstein cows

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ABSTRACT

Many genetic markers related to health or production traits are not evaluated in populations independent of the discovery population or related to phenotype. Here we evaluated 68 single nucleotide polymorphisms (SNP) in candidate genes previously associated with genetic merit for fertility and production traits for association with phenotypic measurements of fertility in a population of Holstein cows that was selected based on predicted transmitting ability (PTA) for daughter pregnancy rate (DPR; high, ≥ 1, n = 989; low, ≤ -1.0, n = 1,285). Cows with a high PTA for DPR had higher pregnancy rate at first service, fewer services per conception, and fewer days open than cows with a low PTA for DPR. Of the 68 SNP, 11 were associated with pregnancy rate at first service, 16 with services per conception, and 19 with days open. Single nucleotide polymorphisms in 12 genes (*BDH2*, *BSP3*, *CAST*, *CD2*, *CD14*, *FUT1*, *FYB*, *GCNT3*, *HSD17B7*, *IBSP*, *OCLN*, and *PCCB*) had significant associations with 2 fertility traits, and SNP in 4 genes (*CSPP1*, *FCER1G*, *PMM2*, and *TBC1D24*) had significant associations with each of the 3 traits. Results from this experiment were compared with results from 2 earlier studies in which the SNP were associated with genetic estimates of fertility. One study involved the same animals as used here, and the other study was of an independent population of bulls. A total of 13 SNP associated with 1 or more phenotypic estimates of fertility were directionally associated with genetic estimates of fertility in the same cow population. Moreover, 14 SNP associated with reproductive phenotype were directionally associated with genetic estimates of fertility in the bull population. Nine SNP (located in *BCAS*, *BSP3*, *CAST*, *FUT1*, *HSD17B7*, *OCLN*, *PCCB*, *PMM2*, and *TBC1D24*) had a directional association with fertility in all 3 studies. Examination of the function of the genes with SNP associated with reproduction in more than one study indicates the importance of steroid hormones and immune function as determinants of reproductive function. All but 1 of the 68 evaluated SNP were variable in 11 breeds besides Holstein, indicating the potential effects of these SNP on reproductive function across breeds of cattle.

Key words: reproduction, candidate genes, Holstein

INTRODUCTION

The use of genomics has improved response to selection for functional traits with low heritability such as daughter pregnancy rate (**DPR**) and productive life (García-Ruiz et al., 2016). Much of the work on fertility traits has been performed through use of genome-wide association studies (**GWAS**) to identify genetic loci associated with reproductive traits (Cole et al., 2011; Minozzi et al., 2013; Nayeri et al., 2016). One outcome has been the identification of haplotypes affecting fertility in dairy breeds (VanRaden et al., 2011; Larkin et al., 2012; Sahana et al., 2013; Cooper et al., 2014; Cuyabano et al., 2014) and identification of loss-of-function mutations that are embryo lethal (Fritz et al., 2013; Sonstegard et al., 2013).

The basis for GWAS is the assumption that the SNP on the panel are in linkage disequilibrium with causative mutations. In many cases, identification of the causative mutation is difficult because an associated genetic marker can often be located in an intergenic region and can be in linkage disequilibrium with vari-

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ants in several nearby genes. Another approach is to identify the causative SNP in the regulatory or coding region of a gene that is responsible for genetic variation in biological function. The causative allelic variant is expected to be more strongly associated with a trait than other SNP in linkage disequilibrium. Moreover, the allelic association between a functional mutation and a genetically controlled trait would be more likely to extend across breeds than a genetic marker based on linkage disequilibrium (Zhu and Zhao, 2007; Weller and Ron, 2011). Understanding the biological basis of genetic variation could also lead to insights into the underlying physiology controlling a trait. One approach to identify causative mutations is the candidate gene approach. Among the genes with SNP associated with reproductive traits in cattle are *DGAT1*, *CAST*, *GHR*, and *LEPR* for services per conception and DPR (Garcia et al., 2006; Schneider et al., 2013; Hill et al., 2016); *IGF1* for resumption of ovarian cyclicity (Nicolini et al., 2013); and *HSPA1L*, *STAT1*, *STAT3*, *PARM1*, and *WBP1* for fertilization and embryonic development during the preimplantation period (Khatib et al., 2009; Cochran et al., 2013b).

For many genetic markers, SNP have not been independently evaluated in separate populations. When they are, replication of the effects is often poor (Ioannidis et al., 2001; Siontis et al., 2010; Littlejohn et al., 2012). Confidence in the relationship between a genetic mutation and phenotype is increased by replication of the allelic relationship in separate populations and by demonstrating that phenotype is also associated with the mutation. Here we evaluated the effect of 68 SNP in candidate genes previously associated with genetic merit for fertility and production traits in Holstein cattle (Cochran et al., 2013a; Ortega et al., 2016) on phenotypic measurements of fertility and production in a population of Holstein cows. A fraction of the SNP was similarly associated with fertility traits in both studies (Cochran et al., 2013a; Ortega et al., 2016). The majority of the 68 SNP (64 of 68) are located in coding regions of genes and result in a change in the amino acid sequence of the encoded protein. We also evaluated whether the SNP were variable only in Holsteins or were common among multiple cattle breeds.

MATERIALS AND METHODS

Phenotypic Measurements for Fertility and Milk Production

Collection of Phenotypic Data from Genotyped Animals. Details of the animals included in the study and methods for genotyping were detailed in Ortega et al. (2016). Briefly, Holstein cows with a high (≥1.5) or low (≤−1.0) PTA for DPR and located on 6 dairies in Florida and 5 in California were used. The high DPR group had 989 cows, and the low DPR group had 1285. Phenotypic data were collected for up to 5 lactations from each farm and combined with records from the national genetic evaluation system. Data for pregnancy rate at first service, services per conception, and days open (i.e., interval from calving to conception) were evaluated. Cows were genotyped for each of 68 SNP using a Sequenom MassARRAY system (iPLEX GOLD; Sequenom, San Diego, CA). The SNP were also previously described by Ortega et al. (2016). Of the 68 SNP, 48 were associated with 1 or more fertility traits [DPR, cow conception rate (**CCR**) or heifer conception rate (**HCR**)] by Cochran et al. (2013a), and the remaining SNP were associated with milk production traits by Cochran et al. (2013a).

Data Analysis. The association of each genetic variant with phenotypic traits was performed by ANOVA using the Statistical Analysis System v 9.4 (SAS Institute Inc., Cary, NC). Days open and pregnancy rate were analyzed with the MIXED procedure. Days open were log-transformed before analysis to establish normality. The number of services per conception was analyzed with the GLIMMIX procedure using a negative binomial distribution for the responses and a logarithmic link function (Dobson, 2001).

In all analyses, genotype was considered a categorical variable. The full model was as follows:

$$Y_{ijkl} = \mu + a_i + g_j + l_k + f_l + e_{ijkl},$$

where Y_{ijkl} is the value of the trait of interest for the *i*th cow ($i = 1, 2, . . ., \text{n}$), a_i is the random polygenic effect (including all available pedigree information) for the *i*th cow, g_j is the fixed effect of SNP genotype ($j = 1,\ldots, 3$ such that g_1 is the genotypic value of AA homozygotes, g_2 is the genotypic value of AB heterozygotes, and g_3 is the genotypic value of BB homozygotes), l_k is the fixed effect of lactation number ($k = 1,\ldots, 5$), f_l is the fixed effect of farm ($l = 1,\ldots, 6$), and e_{ijkl} is the random residual effect. We assume that random polygenic effects $a \sim N(0, \mathbf{A}\sigma_a^2)$ and residuals $e \sim N(0, \sigma_e^2)$, where $\mathbf{A}$ is the numerator relationship matrix, σ_a^2 is the additive genetic variance of the trait of interest, and σ_e^2 is the residual error variance. All of the available pedigree information for each cow was used to generate $\mathbf{A}$, which models the covariance among the polygenic effects. Following Falconer and MacKay (1996), we estimated the a and d parameters for each locus as $(g_3 - g_1)/2$, and $g_2 - (g_1 + g_2)/2$, respectively. Effects of $P < 0.05$ were considered significant.

Table 1. Phenotypes for fertility and production for animals classified based on predicted transmitting ability for daughter pregnancy rate (DPR)

Trait	Lactation	Records, no.			Least squares means (SEM)		
		Total	High DPR	Low DPR	High DPR	Low DPR	*P*-value
Pregnancy rate at first service, %	1	2,245	960	1,285	53.1 (1.69)	28.6 (2.32)	<0.0001
	2	2,118	928	1,190	43.9 (1.77)	23.0 (2.38)	<0.0001
	3	1,743	821	922	41.0 (1.88)	25.0 (2.53)	<0.0001
	4	1,067	589	478	38.8 (2.11)	25.0 (2.40)	<0.0001
	5	484	305	179	38.3 (2.94)	15.1 (4.00)	<0.0001
Services per conception, no.	1	2,274	989	1,285	1.93 (0.06)	3.26 (0.07)	<0.0001
	2	2,118	928	1,190	2.09 (0.07)	3.30 (0.07)	<0.0001
	3	1,743	821	922	2.20 (0.08)	3.20 (0.10)	<0.0001
	4	1,067	589	478	2.34 (0.12)	3.22 (0.28)	0.0125
	5	484	305	179	2.56 (0.12)	3.55 (0.20)	0.0960
Days open, d	1	2,274	989	1,285	98 (2.59)	163 (2.94)	<0.0001
	2	2,118	928	1,190	112 (2.80)	167 (3.13)	<0.0001
	3	1,743	821	922	110 (3.24)	158 (3.81)	<0.0001
	4	1,067	589	478	123 (3.43)	170 (3.90)	<0.0001
	5	484	305	179	133 (5.12)	174 (6.90)	<0.0001

Concordance of SNP Effects Across Studies

Results on significant SNP effects associated with phenotype from the current study were compared with results from 2 other studies examining the effects of these 68 markers on estimates of genetic merit for reproductive and production traits. One study involved an independent population of 550 Holstein bulls (Cochran et al., 2013a), and the other evaluated genetic merit from the same cow population as in the present experiment (Ortega et al., 2016).

Marker Frequencies in Other Breeds

The variability of the 68 SNP evaluated in the present study was determined in other cattle breeds. The frequency of the genetic variants was determined in a total of 203 sequenced animals from 11 *Bos taurus* breeds: 109 Angus, 10 Beefmaster, 12 Charolais, 8 Gelbvieh, 18 Hereford, 3 Jersey, 9 Limousin, 5 Maine-Anjou, 14 Red Angus, 4 Romagnola, and 11 Simmental.

RESULTS AND DISCUSSION

Effect of PTA for DPR on Phenotypic Measurements of Reproductive Function

Pregnancy rate at first service, services per conception, and days open were all affected by DPR ($P < 0.0001$, Table 1). In particular, cows with a high PTA for DPR had a higher pregnancy rate at first service, fewer services per conception, and fewer days open than cows with a low PTA for DPR. Note that the effect of DPR class was observed on pregnancy rate at first service, services per conception, and days open for each of the 5 examined lactations. These results confirm the usefulness of selection for DPR for improving reproductive function despite the low heritability associated with reproductive traits (VanRaden et al., 2004; Cochran et al., 2013a). Similar results have been obtained for a pasture-based production system (Cummins et al., 2012a).

The proportion of animals at later lactations was higher for the high DPR group than for the low DPR group. For example, the proportion of records represented from cows in fifth lactation was 305/3,603 (8.5%) for the high DPR group versus 179/4,054 (4.4%) for the low DPR group. This result probably reflects culling for reproductive reasons, which has been estimated to represent 13 to 20% of all culling decisions (Hadley et al., 2006). Indeed, the genetic correlation between productive life and DPR is positive (Cochran et al., 2013a; VanRaden et al., 2014).

SNP Associated with Fertility Traits

Twenty-six SNP were associated with one or more phenotypic measures of fertility, with 11 SNP being associated with pregnancy rate at first service (Table 2), 16 being associated with services per conception (Table 3), and 19 being associated with days open (Table 4). Most SNP effects indicated a difference between homozygotes ($a > 0$) or else both a and d effects were significant. Genes for which heterozygote deviations were found were *FSHR*, *IBSP*, and *SERPINE2* for pregnancy rate at first service; *FYB* for services per conception; and *FYB* and *IBSP* for days open.

For 12 SNP, the same allele was positively and significantly associated with 2 fertility traits (*BDH2*, *BSP3*, *CAST*, *CD14*, *CD2*, *FUT1*, *FYB*, *GCNT3*, *HSD17B7*,

Table 2. Single nucleotide polymorphisms associated with pregnancy rate at first service

				Copies of minor allele[1]				*P*-value[2]	
SNP ID	Chromosome	Location	Gene	0	1	2	Effect	A	D
rs41857027	18	2783606	*CFDP2*	0.34 (0.01)	0.35 (0.02)	0.43 (0.04)	0.04	0.0306	0.1718
rs109443582	14	33342060	*CSPP1*	0.35 (0.01)	0.35 (0.02)	0.68 (0.10)	−0.17	0.0011	0.0019
rs109137982	3	8308678	*FCER1G*	0.34 (0.01)	0.36 (0.02)	0.55 (0.06)	0.11	0.0007	0.0178
rs43745234	11	31176783	*FSHR*	0.33 (0.01)	0.36 (0.01)	0.34 (0.02)	−0.01	0.6069	0.0346
rs109830880	10	50709147	*GCNT3*	0.36 (0.01)	0.33 (0.02)	0.25 (0.05)	−0.05	0.0450	0.4352
rs110789098	6	3809790	*IBSP*	0.37 (0.01)	0.33 (0.01)	0.35 (0.02)	−0.01	0.3735	0.0168
rs41256848	11	30824442	*LHCGR*	0.36 (0.01)	0.36 (0.01)	0.30 (0.02)	−0.03	0.0057	0.0224
rs109629628	25	7716425	*PMM2*	0.33 (0.01)	0.34 (0.01)	0.38 (0.02)	0.02	0.0415	0.1719
rs43321188	2	112900094	*SERPINE2*	0.34 (0.01)	0.36 (0.01)	0.32 (0.03)	−0.01	0.3268	0.0429
rs41912290	19	35248180	*SREBF1*	0.33 (0.01)	0.36 (0.01)	0.37 (0.02)	0.02	0.0230	0.8057
rs110660625	25	2007163	*TBC1D24*	0.32 (0.01)	0.36 (0.01)	0.40 (0.02)	0.04	0.0021	0.7620

[1] Values are least squares means (SEM).

[2] A = one-half difference between homozygotes; D = deviation of heterozygotes from homozygote midpoint.

IBSP, *OCLN*, and *PCCB*), and 4 SNP had significant allelic associations with each of the 3 traits (*CSPP1*, *FCER1G*, *PMM2*, and *TBC1D24*).

SNP with Effects Concordant in More than One Study

Results from the current experiment were compared with results from earlier studies by Cochran et al. (2013a) and Ortega et al. (2016) to identify genes containing SNP in which (1) a significant association between the SNP and 1 or more reproductive traits was observed in at least 2 studies and (2) the allele associated with superior reproduction was the same in each case. Results are shown in Table 5, and 26 SNP met these criteria. A total of 13 SNP associated with phenotypic measurements of fertility were also found to be directionally associated in genetic estimates of fertility using the same study population (Ortega et al., 2016). Moreover, 14 SNP associated with genetic estimates of fertility in the cow population (Ortega et al., 2016) were directionally associated with genetic estimates of fertility in an independent population of bulls (Cochran et al., 2013a). Nine SNP (located in *BCAS*, *BSP3*, *CAST*, *FUT1*, *HSD17B7*, *OCLN*, *PCCB*, *PMM2*, and *TBC1D24*) had a directional association with fertility in all 3 studies.

The agreement of SNP effects between 2 independent populations compares favorably with other SNP associated with reproduction in which the degree of replication of SNP effects from one population to another ranged from 18% (Höglund et al., 2014) to 0% (Pryce et al., 2010). In a recent study, 93 of 245 QTL regions identified by GWAS as being related to cow fertility

Table 3. Single nucleotide polymorphisms associated with services per conception

				Copies of minor allele[1]				*P*-value[2]	
SNP ID	Chromosome	Location	Gene	0	1	2	Effect	A	D
rs133674837	6	23051485	*BDH2*	2.71 (0.05)	2.75 (0.05)	2.54 (0.06)	0.03	0.0220	0.0268
rs110217852	18	51919757	*BSP3*	2.66 (0.05)	2.73 (0.05)	2.87 (0.10)	0.04	0.0325	0.5794
rs137601357	7	98485273	*CAST*	2.63 (0.06)	2.78 (0.05)	2.79 (0.07)	0.03	0.0331	0.2267
rs109621328	7	53448291	*CD14*	2.72 (0.04)	2.59 (0.07)	2.18 (0.22)	−0.11	0.0310	0.3017
rs133747802	3	26593448	*CD2*	2.75 (0.05)	2.93 (0.21)	2.60 (0.06)	−0.03	0.0213	0.2112
rs109443582	14	33342060	*CSPP1*	2.70 (0.04)	2.74 (0.08)	1.84 (0.33)	−0.19	0.0320	0.0268
rs109137982	3	8308678	*FCER1G*	2.75 (0.05)	2.69 (0.07)	2.04 (0.20)	−0.15	0.0018	0.0176
rs41893756	18	55831611	*FUT1*	2.68 (0.05)	2.78 (0.06)	2.99 (0.16)	0.05	0.0393	0.5256
rs109262355	20	35249040	*FYB*	2.75 (0.05)	2.64 (0.05)	2.86 (0.09)	0.02	0.2322	0.0081
rs109830880	10	50709147	*GCNT3*	2.70 (0.05)	2.79 (0.07)	3.10 (0.21)	0.07	0.0441	0.3481
rs110828053	3	6630548	*HSD17B7*	2.74 (0.05)	2.67 (0.06)	2.35 (0.12)	−0.08	0.0029	0.0938
rs111015912	28	41679976	*LDB3*	2.71 (0.05)	2.73 (0.06)	3.07 (0.16)	0.06	0.0150	0.0704
rs134264563	20	10167825	*OCLN*	2.78 (0.05)	2.70 (0.05)	2.56 (0.08)	−0.04	0.0191	0.6011
rs109813896	1	134130474	*PCCB*	2.78 (0.06)	2.69 (0.05)	2.56 (0.08)	−0.04	0.0073	0.6954
rs109629628	25	7716425	*PMM2*	2.79 (0.06)	2.71 (0.05)	2.61 (0.07)	−0.03	0.0231	0.8280
rs110660625	25	2007163	*TBC1D24*	2.74 (0.05)	2.72 (0.05)	2.56 (0.08)	−0.03	0.0376	0.2119

[1] Values are least squares means (SEM).

[2] A = one-half difference between homozygotes; D = deviation of heterozygotes from homozygote midpoint.

Table 4. Single nucleotide polymorphisms associated with days open

				Copies of minor allele[1]				P-value[2]	
SNP ID	Chromosome	Location	Gene	0	1	2	Effect	A	D
rs109669573	13	82164839	*BCAS1*	141.5 (2.3)	142.7 (2.2)	147.7 (3.2)	3.08	0.0478	0.4033
rs133674837	6	23051485	*BDH2*	143.7 (2.5)	146.0 (2.3)	137.1 (3.3)	−3.33	0.0387	0.0416
rs110217852	18	51919757	*BSP3*	141.4 (2.1)	144.5 (2.3)	150.2 (4.0)	4.38	0.0481	0.7073
rs137601357	7	98485273	*CAST*	141.0 (2.6)	145.7 (2.4)	150.2 (3.2)	4.62	0.0066	0.8920
rs109621328	7	53448291	*CD14*	144.7 (2.1)	158.3 (9.5)	139.1 (3.0)	−2.80	0.0089	0.0769
rs133747802	3	26593448	*CD2*	144.8 (2.0)	156.8 (8.4)	139.0 (2.8)	−2.90	0.0382	0.0811
rs109443582	14	33342060	*CSPP1*	141.4 (1.7)	146.5 (3.3)	111.5 (17.1)	−4.22	0.0193	0.0069
rs109137982	3	8308678	*FCER1G*	144.7 (2.3)	143.5 (2.4)	140.7 (4.1)	−14.94	0.0018	0.0199
rs41893756	18	55831611	*FUT1*	142.4 (2.1)	146.3 (2.7)	157.2 (7.6)	−2.00	0.0474	0.2983
rs109262355	20	35249040	*FYB*	143.1 (2.1)	139.9 (2.1)	152.2 (3.6)	7.42	0.2029	0.0255
rs110828053	3	6630548	*HSD17B7*	144.2 (2.1)	144.3 (2.7)	130.8 (6.4)	4.54	0.0388	0.1411
rs110789098	6	3809790	*IBSP*	141.2 (2.5)	147.8 (2.3)	140.5 (3.6)	−6.73	0.8626	0.0180
rs109383758	18	62241722	*NLRP9*	137.9 (2.9)	144.5 (2.2)	145.5 (3.1)	−0.34	0.0428	0.3361
rs134264563	20	10167825	*OCLN*	147.4 (2.3)	143.7 (2.2)	135.8 (3.7)	3.81	0.0108	0.4494
rs109813896	1	134130474	*PCCB*	146.0 (2.5)	142.7 (2.3)	134.8 (3.7)	−5.79	0.0200	0.9398
rs109629628	25	7716425	*PMM2*	145.1 (2.2)	141.3 (2.0)	135.4 (2.8)	−5.60	<0.001	0.0667
rs133729105	25	26182660	*RABEP2*	141.1 (2.4)	144.8 (2.2)	149.6 (3.2)	−4.85	0.0252	0.9928
rs110660625	25	2007163	*TBC1D24*	145.1 (2.4)	143.8 (2.4)	132.3 (3.8)	4.24	0.0008	0.1144
rs132789482	3	28420362	*TSHB*	143.7 (2.2)	147.6 (3.1)	116.7 (10.4)	−6.39	0.0267	0.0114

[1] Values are least squares means (SEM).

[2] A = one-half difference between homozygotes; D = deviation of heterozygotes from homozygote midpoint.

Table 5. Single nucleotide polymorphisms associated with fertility traits in more than one study[1]

		Cow phenotype[2]			Cow genotype[2]			Bull genotype[3]		
SNP ID	Gene	PR	SPC	DO	DPR	HCR	CCR	DPR	HCR	CCR
rs109967779	*ACAT2*				C		C	C		C
rs41766835	*APBB1*				G			G	G	G
rs133700190	*AP3B1*				T	T	T	T	T	T
rs109669573	*BCAS1*			C	C				C	
rs110217852	*BSP3*			A	A		A	A		
rs109332658	*C7H19orf60*				C		C	C		
rs135744058	*CACNA1D*					G		G	G	
rs137601357	*CAST*		T	T	T		T	T		T
rs109621328	*CD14*		C	C				C	C	
rs41711496	*CD40*					G	G	G		
rs133449166	*CSNK1E*				C	C		C	C	C
rs109137982	*FCER1G*	A	A	A	A					
rs43745234	*FSHR*	C							C	
rs41893756	*FUT1*		A	A	A		A	A		A
rs109262355	*FYB*		A	A					A	
rs109830880	*GCNT3*		T			T				
rs109711583	*HSD17B12*				G	G	G	G		
rs110828053	*HSD17B7*		C	C	C	C	C	C	C	C
rs110789098	*IBSP*	T				T	T			
rs111015912	*LDB3*		T					T	T	T
rs41256848	*LHCGR*		G		G					
rs134264563	*OCLN*		G	G	G		G	G		G
rs109813896	*PCCB*		C	C	C		C	C		
rs109629628	*PMM2*	G	G	G	G		G	G		G
rs133729105	*RABEP2*			G				G		G
rs110660625	*TBC1D24*	A	A	A	A			A		A

[1] Shown are genes containing SNP in which a significant association between the SNP and one or more reproductive traits was observed in at least 2 studies. The letter represents the allele associated with superior reproduction. SNP significant in more than one study but where different alleles were associated with superior reproduction are not included in the table. CCR = cow conception rate; DO = days open; DPR = daughter pregnancy rate; HCR = heifer conception rate; PR = pregnancy rate; SPC = services per conception.

[2] Based on a population of 2,273 Holstein cows [Ortega et al. (2016) and present study].

[3] Based on a population of 550 Holstein bulls (Cochran et al., 2013a).

Table 6. Frequency of the SNP in selected breeds of cattle

		Study		Breed (no.)										
SNP ID	Gene	Minor allele[1]	Frequency	Angus (109)	Beef-master (10)	Charolais (12)	Gelbvieh (8)	Hereford (18)	Jersey (3)	Limousin (9)	Maine-Anjou (5)	Red Angus (14)	Romagnola (4)	Simmental (11)
rs109967779	*ACAT2*	G	0.37	0.48	0.45	0.25	0.44	0.25	0.83	0.33	0.30	0.35	0.75	0.41
rs133700190	*AP3B1*	T	0.23	0.35	0.60	0.08	0.25	0.25	0.33	0.50	0.00	0.31	0.38	0.27
rs41766835	*APBB1*	G	0.21	0.00	0.05	0.09	0.00	0.19	0.00	0.00	0.10	0.04	0.25	0.05
rs110541595	*ARL6IP1*	A	0.39	0.08	0.25	0.00	0.25	0.50	0.33	0.33	0.50	0.12	0.25	0.14
rs110127056	*ASL*	G	0.44	0.10	0.05	0.04	0.19	0.22	0.00	0.17	0.30	0.00	0.00	0.14
rs43114141	*AVP*	A	0.45	0.09	0.22	0.38	0.19	0.25	0.00	0.75	0.30	0.00	0.00	0.20
rs109669573	*BCAS1*	T	0.40	0.32	0.55	0.50	1.00	0.92	1.00	0.50	0.00	0.31	0.33	0.41
rs133674837	*BDH2*	A	0.41	0.58	0.35	0.33	0.31	0.28	0.33	0.39	0.70	0.12	0.00	0.59
rs109032590	*BOLA-DMB*	T	0.29	0.72	0.95	0.50	0.44	1.00	1.00	0.75	0.60	0.85	0.37	0.23
rs110217852	*BSP3*	G	0.29	0.06	0.10	0.13	0.25	0.06	0.83	0.72	0.00	0.04	0.25	0.27
rs133455683	*C17H22orf25*	A	0.43	0.60	0.70	0.13	0.50	0.39	0.00	0.50	0.90	0.54	0.50	0.05
rs109332658	*C7H19orf60*	T	0.28	0.50	0.25	0.25	0.69	0.50	0.00	0.40	0.60	0.27	0.50	0.50
rs135744058	*CACNA1D*	G	0.30	0.52	0.70	0.42	0.56	0.50	0.00	0.19	0.10	0.42	0.00	0.55
rs137601357	*CAST*	C	0.43	0.20	0.25	0.50	0.56	0.42	0.00	0.19	0.20	0.21	0.00	0.50
rs109447102	*CCDC86*	C	0.20	0.24	0.55	0.33	0.63	0.36	0.50	0.75	0.40	0.38	0.75	0.64
rs137673698	*CCT8*	T	0.32	0.99	1.00	1.00	1.00	1.00	1.00	0.94	1.00	0.88	1.00	1.00
rs109621328	*CD14*	C	0.08	0.09	0.00	0.04	0.44	0.00	0.00	0.06	0.10	0.12	0.00	0.05
rs41711496	*CD40*	A	0.47	0.22	0.80	0.33	0.37	0.53	1.00	0.42	0.40	0.23	0.17	0.55
rs41857027	*CFDP2*	T	0.16	0.00	0.10	0.25	0.31	0.17	0.17	0.11	0.00	0.00	0.00	0.14
rs133449166	*CSNK1E*	C	0.37	0.61	0.45	0.55	0.25	0.36	1.00	0.30	0.80	0.81	0.75	0.36
rs109443582	*CSPP1*	A	0.06	0.40	0.15	0.29	0.00	0.11	0.00	0.31	0.20	0.23	0.25	0.14
rs110270752	*DEPDC7*	A	0.24	0.42	0.25	0.54	0.19	0.22	0.17	0.44	0.40	0.38	0.25	0.32
rs110629231	*DNAH11*	T	0.33	0.70	0.40	0.33	0.37	0.69	0.50	0.44	0.30	0.19	0.87	0.45
rs109503725	*DSC2*	C	0.45	0.24	0.30	0.92	0.50	0.64	0.50	0.89	0.60	0.35	0.75	0.41
rs109561866	*DYRK3*	T	0.12	0.16	0.60	0.58	0.38	0.25	0.33	0.75	0.50	0.27	0.38	0.41
rs133175991	*DZIP3*	C	0.20	0.77	0.90	0.71	0.81	0.92	0.83	0.94	1.00	0.85	1.00	0.64
rs43676052	*EPAS1*	G	0.26	0.06	0.15	0.08	0.12	0.11	0.00	0.19	0.10	0.04	0.25	0.09
rs135071345	*FAM5C*	C	0.11	1.00	0.95	1.00	0.94	0.78	1.00	1.00	1.00	0.92	0.75	0.91
rs109137982	*FCER1G*	A	0.10	0.33	0.10	0.42	0.19	0.47	0.00	0.17	0.30	0.04	0.25	0.14
rs110937773	*FGF2*	A	0.31	0.49	0.75	0.46	0.87	0.75	0.50	0.78	0.50	0.54	0.75	0.64
rs43745234	*FSHR*	G	0.32	0.34	0.70	0.63	0.81	0.47	0.50	0.44	0.70	0.42	0.88	0.82
rs109247499	*FST*	C	0.45	0.00	0.05	0.04	0.06	0.00	0.00	0.06	0.10	0.00	0.00	0.05
rs41893756	*FUT1*	G	0.20	0.12	0.15	0.50	0.38	0.58	0.50	0.43	0.20	0.12	1.00	0.23
rs109262355	*FYB*	G	0.34	0.80	0.95	0.75	1.00	1.00	0.50	1.00	0.80	0.58	1.00	1.00
rs109830880	*GCNT3*	C	0.14	0.07	0.00	0.38	0.25	0.06	0.17	0.00	0.10	0.19	0.38	0.05
rs42339105	*GOLGA4*	G	0.08	0.20	0.50	0.42	0.06	0.25	0.33	0.11	0.50	0.19	0.37	0.32
rs109516714	*GPLD1*	C	0.38	0.59	0.65	0.63	0.56	0.42	1.00	0.83	0.60	0.69	0.75	0.36
rs109711583	*HSD17B12*	G	0.43	0.31	0.30	0.54	0.25	0.75	0.17	0.39	0.10	0.46	0.25	0.36
rs43079452	*HSD17B3*	C	0.13	0.95	0.95	0.96	1.00	0.72	1.00	1.00	1.00	1.00	1.00	1.00
rs109769865	*HSD17B6*	T	0.13	0.92	0.95	0.79	0.63	0.25	0.67	0.86	1.00	0.81	0.88	0.55
rs110828053	*HSD17B7*	T	0.18	0.70	0.45	0.58	0.50	0.33	0.50	0.17	0.50	0.69	0.37	0.05
—	*HSPA1L*	Deletion	0.29	0.01	0.15	0.21	0.19	0.19	0.17	0.00	0.00	0.00	0.00	0.14
rs110789098	*IBSP*	C	0.40	0.25	0.25	0.04	0.00	0.08	0.50	0.17	0.20	0.08	0.00	0.05
rs111015912	*LDB3*	T	0.19	0.29	0.00	0.50	0.94	0.33	1.00	0.25	0.10	0.35	0.33	0.36
rs41256848	*LHCGR*	T	0.44	0.78	1.00	0.92	1.00	0.97	1.00	0.89	1.00	0.96	1.00	0.95
rs41859871	*MON1B*	T	0.13	0.95	0.95	1.00	0.94	0.89	1.00	1.00	1.00	1.00	1.00	1.00
rs109248655	*MRGPRF*	T	0.04	0.17	0.20	0.21	0.06	0.22	0.00	0.20	0.00	0.15	0.00	0.27

Continued

Table 6 (Continued). Frequency of the SNP in selected breeds of cattle

		Study		Breed (no.)										
SNP ID	Gene	Minor allele[1]	Frequency	Angus (109)	Beef-master (10)	Charolais (12)	Gelbvieh (8)	Hereford (18)	Jersey (3)	Limousin (9)	Maine-Anjou (5)	Red Angus (14)	Romagnola (4)	Simmental (11)
rs43703916	*MRPL48*	G	0.40	0.63	0.45	0.29	0.31	0.14	0.00	0.67	0.50	0.73	0.75	0.36
rs109761676	*MS4A8B*	G	0.25	0.17	0.25	0.33	0.25	0.06	0.00	0.28	0.10	0.04	0.38	0.14
rs133497176	*NFKBIL1*	T	0.15	0.03	0.15	0.17	0.00	0.08	0.00	0.25	0.20	0.12	0.33	0.18
rs109383758	*NLRP9*	C	0.47	0.56	0.85	0.71	0.50	0.36	0.67	0.36	0.30	0.73	1.00	0.68
rs134264563	*OCLN*	G	0.32	0.08	0.35	0.04	0.00	0.17	0.17	0.06	0.00	0.04	0.25	0.09
rs111027720	*PARM1*	G	0.48	0.76	0.50	0.29	0.31	0.86	1.00	0.64	1.00	0.81	0.00	0.32
rs109813896	*PCCB*	C	0.36	0.38	0.20	0.29	0.69	0.42	1.00	0.00	0.00	0.46	0.50	0.23
rs109629628	*PMM2*	G	0.39	0.51	0.70	0.46	0.12	0.56	0.83	0.56	0.30	0.88	0.62	0.05
rs133729105	*RABEP2*	A	0.38	0.60	0.65	0.50	0.88	0.22	0.00	0.70	0.60	0.42	0.00	0.91
rs43572154	*ROR2*	A	0.15	0.86	0.90	1.00	0.75	0.50	1.00	0.60	1.00	0.65	0.50	0.77
rs136746215	*SEC14L1*	G	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
rs43321188	*SERPINE2*	T	0.26	0.11	0.45	0.00	0.06	0.28	0.00	0.13	0.40	0.04	0.50	0.14
rs110365063	*SLC18A2*	A	0.17	0.14	0.20	0.25	0.13	0.03	0.17	0.29	0.20	0.12	0.00	0.18
rs41912290	*SREBF1*	C	0.39	0.57	0.95	0.95	0.75	0.75	1.00	0.42	0.80	0.65	1.00	0.82
rs42158454	*SYTL2*	A	0.02	0.15	0.05	0.08	0.13	0.39	0.00	0.06	0.20	0.21	0.13	0.05
rs110660625	*TBC1D24*	A	0.35	0.95	0.75	0.67	0.88	0.81	1.00	1.00	0.60	0.81	1.00	0.68
rs110805802	*TDRKH*	T	0.12	0.01	0.15	0.17	0.00	0.00	0.33	0.00	0.00	0.04	0.00	0.00
rs132789482	*TSHB*	T	0.16	0.08	0.20	0.33	0.31	0.17	0.00	0.44	0.30	0.04	0.75	0.41
rs134031231	*TXN2*	G	0.40	0.50	0.85	0.12	0.25	0.86	0.00	0.58	0.50	0.50	0.67	0.27
rs137248155	*VCAN*	T	0.27	0.18	0.15	0.00	0.13	0.03	0.25	0.22	0.20	0.42	0.13	0.00
rs110883602	*ZP2*	C	0.40	0.13	0.00	0.29	0.56	0.31	0.17	0.11	0.10	0.35	0.25	0.27

[1] Based on a population of 2,798 Holsteins [combined data of Cochran et al. (2013a) and Ortega et al. (2016)].

were repeatable in 2 independent GWAS (Moore et al., 2016). The consistency of the effect of SNP described in Table 5 across studies provides confidence that associations between many of the SNP and reproductive function reported by Cochran et al. (2013a) and Ortega et al. (2016) are real and not false positives. Moreover, a few of the genes found to be associated with reproductive traits across studies evaluated here have also been reported to be associated with reproductive traits in other reports. The SNP in *CAST* has been associated with genetic merit for DPR, days open, and productive life (Garcia et al., 2006; Hill et al., 2016). The SNP in *PCCB*, *PMM2*, and *TBC1D24* have previously been associated with percentage of cleaved embryos that develop to the blastocyst stage (Cochran et al., 2013b).

Functional Ontology of SNP

Examination of the function of genes that were repeatedly associated with reproductive traits (Table 5) provides an indication of physiological processes important for variation among cows in reproductive function. In earlier studies of these SNP (Cochran et al., 2013a; Ortega et al., 2016), pathway analysis of genes associated with fertility traits using Ingenuity Pathway Analysis indicated that 14 of these genes were regulated by estradiol and 6 were regulated by progesterone. Most of these steroid-regulated genes were among the list of 26 genes found to be associated with reproductive function in more than one study. The list included 9 estradiol-regulated genes (*APBB1*, *BCAS1*, *CAST*, *HSD17B12*, *HSD17B7*, *LHCGR*, *OCLN*, *PMM2*, and *RABEP2*) and 4 progesterone-regulated genes (*CD40*, *LHCGR*, *PMM2*, and *RABEP2*). Besides steroid hormones being essential for reproduction in mammals, data indicate the importance of variation in their circulating concentrations for cow fertility. In beef cattle, preovulatory concentrations of estradiol are related to subsequent fertility (Perry et al., 2005; Jinks et al., 2013). For Holstein cows on pasture, those with high genetic merit for fertility had a larger corpus luteum and higher circulating concentrations of progesterone than cows with lower genetic merit (Cummins et al., 2012b; Moore et al., 2014). Progesterone concentrations on d 4 to 7 after AI were positively associated with pregnancy rate in Holstein heifers (Parr et al., 2012). Steroid hormones may be an especially important determinant of reproduction in lactating cows because of increased catabolism of steroids associated with lactation (Wiltbank et al., 2006, 2014).

The other function that was well represented in genes containing SNP identified earlier (Cochran et al., 2013a; Ortega et al., 2016) and in the present study was immune function. Of the 10 genes containing SNP related to reproductive traits that were involved in immune function as identified by Cochran et al. (2013a) or Ortega et al. (2016), 6 (*CD14*, *CD40*, *FCER1G*, *FUT1*, *GCNT3*, and *RABEP2*) were found to be related to fertility traits in more than one study (Table 5). Immune function is an important determinant of reproductive function, and dairy cows that experience one or more diseases postpartum have reduced reproductive function (Santos et al., 2010). Genes involved in inflammation are among those whose expression in endometrium, liver, and muscle differed between Holstein cows that were genetically divergent in fertility (Moran et al., 2015, 2016).

Variability of the SNP in Other Breeds

The variability of the 68 SNP studied here was evaluated in 11 *B. taurus* breeds (Table 6). All but one SNP was variable in each of the examined breeds. The exception was for *SEC14L1*, which was variable only in Holstein. The variability of these SNP in other breeds indicates that they arose before the Holstein separated from other *B. taurus* breeds and that the SNP could be associated with reproductive function in breeds other than Holstein.

CONCLUSIONS

This study demonstrated that genetic differences in fertility are associated with differences in phenotype. This finding was true in comparisons of cows that diverged in predicted transmitting ability for DPR or in examination of the effects of specific SNP on reproductive function. A total of 26 SNP were identified for which the same allele was associated with increases in at least one reproductive trait in 2 separate studies. Examination of the function of genes represented by the 26 SNP with concordant effects indicates the importance of steroid hormones and immune function as determinants of reproductive function. All but one of the studied SNP were variable in 11 breeds besides Holstein, indicating the potential association of these SNP with reproductive function in cattle in general.

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