

Effect of the *FGF2* SNP11646 on milk production and fertility traits of Holstein cattle

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ABSTRACT: The objective of this study was to estimate the effect of a single-nucleotide polymorphism (SNP11646) in the *FGF2* (Fibroblast Growth Factor 2) gene on the deregressed proof of breeding values (DRP) of Holstein sires ($n = 149$) for milk traits (milk yield, fat yield and percentage, and protein yield and percentage) and fertility (relative breeding value for own fertility – conception rate of heifers, cows, all females inseminated by the sperm of the sire; relative breeding value for conception rate of daughters – heifers, cows, all females). The differences between genotypes for milk performance were not significant. The lowest DRP for milk performance were found for AA sires. For fertility, sires with this genotype had the best values both for the maternal genetic effect (conception rate of the daughters) and for the direct genetic effect (fertility of the sire). For conception rate of the daughters, in some cases, the differences reached the threshold of significance. Thus, the results indicate coincidentally with other studies the potential opposing effects on milk performance and fertility. *FGF2* SNP11646 is still of interest for future cattle breeding studies.

Keywords: dairy cow; fibroblast growth factor; single nucleotide polymorphism; linear mixed model

INTRODUCTION

Dairy cattle are bred intensively to increase milk production, so the polymorphisms that affect milk production traits are of great importance. An antagonistic relationship exists between high milk production and fertility (e.g. Khatib et al. 2010). Intensive genetic selection for high milk production in dairy cattle is associated with a disruption in hormonal balance and a reduction of estrous intensity, which, in turn, contributes to declining fertility. Dairy cow infertility is a problem that causes worldwide economic losses (e.g. Royal et al. 2000), but the heritability of fertility is low (e.g. Dobson et al. 2007), and therefore, the efficiency of traditional selection is limited (Guillaume et al. 2007; Druet et al. 2008). Thus, improvements in the reproductive performance of dairy cows may be possible through the application of gene-assisted selection.

Fibroblast Growth Factors (FGFs) are a family of proteins which represent a large group of autocrine and paracrine factors that control various biological functions in many mammal species (Ornitz and Itoh 2001). Bovine *FGF2* gene with the total length of more than 59 kb is located in chromosome 17 between *BBS12* and *NUDT6* genes. Three exons encode 155 amino acid protein (<http://www.meta-life.com/Genbank/163049>).

Wang et al. (2008) have reported the expression of the *FGF2* gene during various developmental stages in mammary glands in cattle. This could indicate the importance of the *FGF2* gene during mammary gland development, because it is supposed that *FGF2* is involved in the stimulation of cell growth in mammary gland (Hironaka et al. 1997). *FGF2* regulates expression of interferon- τ (IFNT), a key member of a signal transduction pathway involved in milk production and thus affect-

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ing the milk performance, composition, and other parameters.

FGF2 is expressed also by the uterine endometrium throughout the estrous cycle (Wang et al. 2008) and early pregnancy (Michael et al. 2006). *FGF2* is a candidate gene for fertility in cattle (Oikonomou et al. 2011) and has been implicated in ovarian function (Portela et al. 2010), embryonic development (Jackson et al. 1997), and mortality (Khatib et al. 2010). *FGF2* is also known as mitogen, morphogen, and angiogenic factor (Michael et al. 2006). The endometrium is probably a source of *FGF2* for a fetus during bovine embryo development. Their study has shown that *FGF2* is a weak mediator of trophectoderm proliferation but is a strong regulator of IFNT production in bovine trophectoderm cells and blastocyst-stage bovine embryos. IFNT plays a key role in regulating the expression of genes involved in embryo implantation and in protecting the conceptus against maternal rejection (Martal et al. 1997), the effect of *FGF2* in this context has been proven by Khatib et al. (2010).

The objective of this study was to investigate the effect of polymorphism of the *FGF2* SNP11646 on milk production and fertility in a population of Czech Holsteins.

MATERIAL AND METHODS

Animals, DNA extraction, and genotyping. The association of the single nucleotide polymorphism *FGF2* SNP11646 (GenBank Acc. No. NC_007304) with milk production and fertility was examined in Holstein sires ($n = 149$) born in the period 2000–2006 in the Czech Republic. Young sires were selected randomly from two large Holstein lines NEA and NXA. Genomic DNA was extracted from whole blood by the proteinase K method. The quality of genomic DNA including concentration and purity was verified using a NanoDrop1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). The PCR conditions and genotyping of SNP11646 (*A* to *G* alleles substitution) were as described in Oikonomou et al. (2011).

Milk production and reproductive traits. The estimated breeding values (EBV) of the Holstein sires were taken from the national genetic evaluation, and they were evaluated by BLUP Animal Model, involving the relationship matrix. The EBVs were calculated by PLEMDAT s.r.o. (a company providing service for breeders including the evaluation

of breeding data, participation in Interbull etc.). There were two separate evaluations for male fertility: first, the conception rate of heifers and cows inseminated by the sperm of the sire and, second, the female fertility, i.e., the conception rate of the sire's daughters. The fertility was always evaluated separately for heifers and cows, and then together for all females. In our analysis, the milk production was evaluated by breeding values of absolute performance, whereas the fertility by relative breeding value. Therefore, the numerals of relative breeding values of fertility range also above 100%.

For our statistical analysis, the following breeding data on milk production and fertility traits were taken from PLEMDAT records: milk yield in kg; fat percentage and fat yield in kg; protein percentage and protein yield in kg; relative breeding value of the sire in per cent for the trait conception rate of the heifers, cows, all females inseminated by the sperm of the sire; relative breeding value of the sire in per cent for the trait conception rate of daughters of the sire (heifers, cows, all females).

Basic information on the material under study is given in Tables 1 and 2.

Statistical analysis. Only breeding values of evaluated traits were known, therefore we have calculated deregressed breeding values (DRP) to compensate for the fact that performance records were not available. Using DRP instead of EBV, we could separate more properly the influence of genetic background in animal effect (Bauer et al. 2015). The breeding values of the sires were deregressed according to Rozzi et al. (1990) as follows:

$$\text{DRP} = \text{EBV}/r^2$$

where:

DRP = deregressed proof of breeding value

EBV = estimated breeding value

r^2 = reliability of estimated breeding value

Statistical analyses were performed using a linear mixed model. Deregressed proofs of breeding values of the analyzed traits were dependent variables. The impact of *FGF2* SNP11646 on milk yield was estimated with the use of the following model, where each trait of milk production and fertility was analyzed separately. The effect of *FGF2* SNP11646 genotypes on the DRP for milk and fertility traits was analyzed using the least squares method of the GLM procedure of SAS software (Statistical Analysis System, Version 9.4, 2015):

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Table 1. Descriptive statistics for breeding values of sires for all traits investigated

Trait	Sires <i>n</i>	EBV				Number of daughters per sire			
		mean	SD	min	max	mean	SD	min	max
Milk yield (kg)	149	328.38	488.53	–1 164	1 701	163.89	473.90	31	5 363
Milk fat content (%)	149	–0.10	0.24	–0.65	0.74	163.89	473.90	31	5 363
Milk fat yield (kg)	149	5.72	20.35	–61	92	163.89	473.90	31	5 363
Milk protein content (%)	149	–0.06	0.12	–0.32	0.28	163.89	473.90	31	5 363
Milk protein yield (kg)	149	6.60	15.40	–40	39	163.89	473.90	31	5 363
Conception rate of daughters-heifers ¹	149	100.77	11.34	69	125	329.09	941.28	53	9 424
Conception rate of daughters-cows ¹	149	97.43	11.97	46	124	591.59	1 928.92	102	22 768
Conception rate of daughters-females ^{1,2}	149	97.81	11.98	45	121	920.68	2 800.75	155	32 192
Ability to impregnate heifers ¹	107	100.55	11.75	69	125	324.38	1 326.37	28	12 570
Ability to impregnate cows ¹	147	98.10	11.69	61	124	737.10	1 985.70	60	18 572
Ability to impregnate females ^{1,2}	147	98.44	11.56	60	121	978.88	3 088.03	75	31 142

EBV = estimated breeding values, SD = standard deviation

¹relative breeding value²females include both heifers and cows

$$y_{ijk} = \mu + \text{gen}_i + \text{anim}_j + e_{ijk}$$

where:

 y_{ijk} = deregressed proof of breeding value of the sire for each trait of milk yield or fertility

 μ = population mean

 gen_i = fixed effect of genotype of *FGF2* SNP11646 ($i = 1, 2, 3$), where $AA = 1$ (homozygous), $AG = 2$ (heterozygous), $GG = 3$ (wild type) of the i^{th} bull

 anim_j = random additive polygenic effect of the j^{th} bull

 e_{ijk} = residual random error

Differences of Least Squares Means were applied to test the significance of differences between

deregressed breeding values by the MIXED procedure of SAS (Statistical Analysis System, Version 9.4, 2015). The Hardy-Weinberg equilibrium was tested by the χ^2 test.

RESULTS AND DISCUSSION

The frequencies of alleles *A* and *G* of the Czech Holstein sire population were of 0.35 and 0.65, respectively, and the genotype frequencies were 0.13, 0.43, and 0.43 for genotypes *AA*, *AG*, and *GG*. The population was not in Hardy-Weinberg equilibrium. Wang et al. (2008) reported the same

Table 2. Descriptive statistics for deregressed proof of breeding values of sires for investigated traits

Trait	Sires <i>n</i>	DRP mean	SD	Min	Max
Milk yield (kg)	149	366.22	543.49	–1 322.73	1 718.18
Milk fat content (%)	149	–0.11	0.27	–0.76	0.94
Milk fat yield (kg)	149	6.42	23.30	–69.32	116.46
Milk protein content (%)	149	–0.07	0.13	–0.37	0.31
Milk protein yield (kg)	149	7.33	17.23	–44.44	42.17
Conception rate of daughters-heifers ¹	149	213.24	49.92	86.76	367.59
Conception rate of daughters-cows ¹	149	144.20	21.37	92.29	201.68
Conception rate of daughters-females ^{1,2}	149	130.18	16.63	85.84	170.95
Ability to impregnate heifers ¹	107	301.57	116.20	99.49	552.24
Ability to impregnate cows ¹	147	142.82	41.21	59.90	344.72
Ability to impregnate females ^{1,2}	147	134.20	31.00	57.84	307.07

DRP = deregressed proof of breeding values, SD = standard deviation

¹relative breeding value²females include both heifers and cows

allelic frequency in a Holstein population in the USA.

Other authors have reported different allelic frequencies, for example Oikonomou et al. (2011) of 0.42 for allele *A* in a Holstein population in northern Greece, Wang et al. (2008) of 0.45 in a Holstein population in Israel, and Khatib et al. (2008) of 0.53 in the USA.

In the group studied, genotypes *GG* and *AG* reached higher EBVs for milk performance and the deregressed proof of breeding values as well (Table 3).

As for the fertility (Table 4), sires with the *AA* genotype fathered the daughters of higher fertility (maternal genetic effect) compared to the sires with *AG* or *GG* genotypes. In the *AG* genotype group, the EBVs were the lowest both for the estimated breeding values and the deregressed proof of breeding values, as well as in heifers and cows and all daughters together. The fertility of the sires (direct genetic effect) was the best in the sires with the *AA* genotype as well.

The higher Least Squares Means estimates of the *GG* and *AG* genotypes than those of the *AA* genotype for milk performance are in agreement with Wang et al. (2008). However, no significant associations were found in our research, as shown in Table 3, and the pair tests showed non-significance among genotypes as well (data not shown).

Considering different levels of *FGF2* expression during different developmental stages of cattle mammary glands and the fact that *FGF2* is a member of the IFNT signal transduction pathway affecting milk production, *FGF2* gene has been found as a suitable marker associated with milk production and mammary health traits (Wang et al. 2008).

FGF2 stimulates trophoblast cell migration and adhesion and embryogenesis in mammals (Yang et al. 2011a, b). It is a key regulator of angiogenesis during placentation and has been proven to have an influence on IFNT production (Reynolds and Redmer 2001). IFNT prevents cyclical demise of the primary corpus luteum and thereby helps maintain pregnancy (de Ruijter-Villani et al. 2013). Together with other studies *in vitro* (e.g. Larson et al. 1992; Xie et al. 2015), the effect of *FGF2* on fertility is supposed (Wang et al. 2009; Fields et al. 2011).

In our study, the fertility of daughters was better in those cattle fathered by the sires of the *AA* genotype, significantly so in some cases (Table 4). The pair tests showed significance between *AG-GG* genotypes in cows ($P = 0.0337$), and between *AA-AG* ($P = 0.0208$) and *AG-GG* ($P = 0.0273$) genotypes in females, respectively. The evaluating of differences in EBV resulted in the same significance. Khatib et al. (2010) reported that the *AA* genotype was associated with higher estimated

Table 3. Descriptive statistics for breeding values of sires grouped by their *FGF2* SNP11646 genotypes for milk performance traits¹

Trait	Genotype of sires	Sires <i>n</i>	EBV mean	DRP mean	LSM	<i>P</i> -value
Milk yield (kg)	<i>AA</i>	19	302.6	335.95	345.21	0.7895
	<i>AG</i>	65	304.6	340.56	337.76	
	<i>GG</i>	65	359.7	400.74	400.83	
Milk fat content (%)	<i>AA</i>	19	-0.17	-0.19	-0.20	0.3040
	<i>AG</i>	65	-0.09	-0.10	-0.10	
	<i>GG</i>	65	-0.09	-0.10	-0.10	
Milk fat yield (kg)	<i>AA</i>	19	-1.32	-1.69	-1.59	0.2534
	<i>AG</i>	65	5.92	6.70	6.67	
	<i>GG</i>	65	7.59	8.51	8.51	
Milk protein content (%)	<i>AA</i>	19	-0.09	-0.10	-0.10	0.5790
	<i>AG</i>	65	-0.06	-0.06	-0.06	
	<i>GG</i>	65	-0.06	-0.07	-0.07	
Milk protein yield (kg)	<i>AA</i>	19	3.89	4.21	4.53	0.6774
	<i>AG</i>	65	6.42	7.16	7.06	
	<i>GG</i>	65	7.57	8.40	8.41	

EBV = estimated breeding values, DRP = deregressed proof of breeding values, LSM = Least Squares Means

¹reliability of EBVs was 0.89

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Table 4. Descriptive statistics for relative breeding values of sires grouped by their *FGF2* SNP11646 genotypes for maternal genetic effect (fertility of daughters) and direct genetic effect (fertility of the sire)

	Genotype of sires	Sires <i>n</i>	EBV mean	DRP mean	r ²	LSM	<i>P</i> -value
Conception rate of daughters-heifers	AA	19	102.2	217.4	0.49	217.38	0.8330
	AG	65	99.4	210.5	0.50	210.57	
	GG	65	101.8	214.7	0.50	214.70	
Conception rate of daughters-cows	AA	19	102.7	150.0	0.69	150.04	0.0469*
	AG	65	94.4	139.4	0.68	139.38	
	GG	65	99.0	147.3	0.68	147.33	
Conception rate of daughters-females ¹	AA	19	102.7	136.1	0.75	136.10	0.0223*
	AG	65	94.8	126.1	0.76	126.11	
	GG	65	99.3	132.5	0.76	132.51	
Ability to impregnate heifers	AA	15	104.9	305.9	0.38	305.94	0.7630
	AG	49	99.4	292.6	0.40	292.59	
	GG	43	100.4	310.3	0.38	310.27	
Ability to impregnate cows	AA	19	102.6	147.4	0.71	147.34	0.8518
	AG	64	94.4	141.2	0.71	141.24	
	GG	64	98.8	143.1	0.72	143.08	
Ability to impregnate females ¹	AA	19	102.8	137.9	0.75	137.88	0.5928
	AG	64	94.9	131.3	0.75	131.27	
	GG	64	99.2	136.0	0.75	136.04	

EBV = estimated breeding values, DRP = deregressed proof of breeding values, r² = reliability of estimated breeding values, LSM = Least Squares Means

¹females include both heifers and cows

*significant at $P < 0.05$

relative conception rates from Holstein sires compared to the sires with AG or GG genotypes. Their indicator was comparable to our direct genetic effect (fertility of the sires). Their results and the prevalence of the AA genotype in this study are in agreement.

Some of the sires had to be excluded due to the low reliability of their EBV for the direct genetic effect (Table 4). Otherwise, when all sires were included, the deregressed proofs of breeding values (DRPs) were distorted. This phenomenon could be seen in the breeding values for fertility traits of the sires as measured in the conception rate of heifers. Here, the reliability is still lower than in the breeding values for conception rate in cows and all females. It resulted in a different rank of genotypes according to EBV (AA = 104.9, AG = 99.4, and GG = 100.4) and DRP (AA = 305.9, AG = 292.6, and GG = 310.3) in heifers.

In the material analyzed, the sires of the AA genotype were found to have the lowest breeding values for milk performance indicators, even though non-significantly, and the best EBVs for fertility. Thus, the results indicate the potential opposing effects

on milk performance and fertility. The antagonism has been a fundamental question in dairy cattle breeding for decades. Our results are coincident with previous studies. Jointly with other candidate loci (Rychtarova et al. 2014), *FGF2* SNP11646 continues to be of interest for future cattle breeding studies.

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