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*Short communication*

# Single nucleotide polymorphism within arylsulfatase D gene (ARSD) is associated with selected kinematic parameters of sperm motility in Holstein-Friesian bulls

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## Abstract

The aim of the study was to find out whether the single nucleotide polymorphism (SNP) within arylsulfatase D (ARSD) gene is associated with kinematic parameters of sperm motility in Holstein-Friesian bulls. 367 Holstein-Friesian bulls kept in one AI center were included in the study. Point mutation C/T at position 139037255 on chromosome X (rs42207167) was identified by PCR-RFLP method (Pflm I). Significant associations were found between ARSD genotypes and CASA-derived sperm motility parameters: average TM (Total Motility), average VSL (Straight Velocity), average VCL (Curvilinear Velocity) and for fraction of sperms showing progressive motility (a) of sperms (VSLa, VCLa and BCFA -Beat Cross Frequency). Most significant differences were observed between alternative homozygotes (CC vs TT). Our results suggest new role of arylsulfatase D gene as being involved in sperm motility.

**Key words:** bull, sperm, motility, arylsulfatase, polymorphism

## Introduction

Comparison of Holstein-Friesian and Belgian Blue bulls proved that kinematic parameters of sperms have genetic background (Hoflack et al. 2007). Based on GWAS (Genome-Wise Association Study), we found that genetic markers located on chromosome X in the close neighbourhood of arylsulfatase multilocus (Zimin et al. 2009) are significantly associated with motility of sperms (unpublished data).

Therefore we hypothesize that arylsulfatase D gene, a member of arylsulfatase family of genes, may be considered as new candidate gene involved in sperm motility.

## Materials and Methods

The analyzed data set originates from the Polish Holstein-Friesian dairy cattle population and consis-

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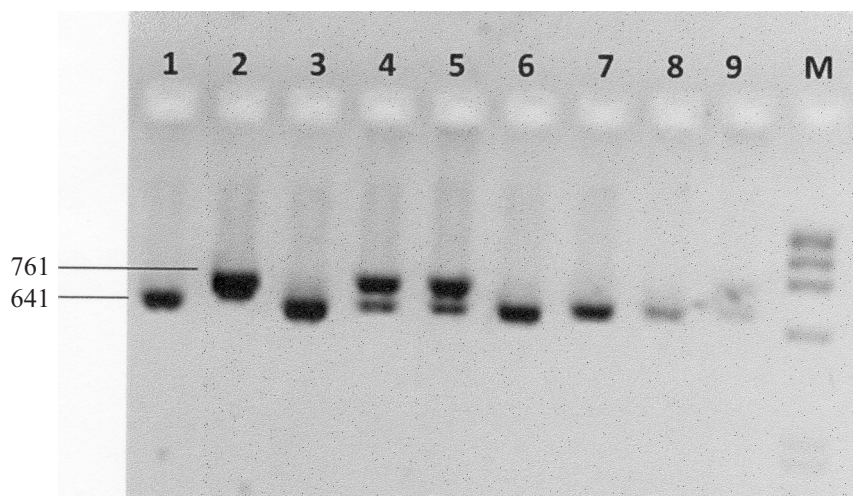


Fig. 1. Genotyping of ARSD C/T mutation by Pflm I restriction enzyme. Path 10: DNA size marker 0X174 DNA/HaeIII; paths 4,5,9 heterozygotes CT; path 2: homozygote TT; paths 1,3,6,7,8: homozygotes CC, Band of 120 bp is not visible on the gel (diffused).

Table 1. Means (X) and standard deviations (SD) for sperm kinematic parameters in bulls with particular ARSD genotypes.

Sperm kinematic parameters	All bulls N=369		Bulls with genotype						p-value
	X	SD	CC=127		CT=191		TT=51		
			X	SD	X	SD	X	SD	
TM									
average	62.60	7.36	<b>63.68<sup>a</sup></b>	<b>7.31</b>	<b>62.31</b>	<b>7.48</b>	<b>60.97<sup>b</sup></b>	<b>6.72</b>	<b>0.04</b>
A	22.03	6.90	22.57	6.83	21.96	6.99	20.95	6.70	0.34
B	31.12	6.43	31.35	6.28	31.02	6.45	30.92	6.81	0.85
C	9.45	3.61	9.77	3.58	9.33	3.57	9.11	3.85	0.34
VSL									
average	27.79	4.40	<b>27.05<sup>A</sup></b>	<b>4.23</b>	<b>27.66<sup>A</sup></b>	<b>3.78</b>	<b>30.17<sup>B</sup></b>	<b>5.99</b>	<b>0.007</b>
A	62.03	13.39	<b>60.01<sup>A</sup></b>	<b>12.09</b>	<b>61.49</b>	<b>11.57</b>	<b>69.11<sup>B</sup></b>	<b>19.49</b>	<b>0.01</b>
B	14.01	2.48	13.91	2.63	14.05	2.33	14.12	2.70	0.88
C	7.34	2.07	7.22	2.17	7.42	1.94	7.29	2.26	0.96
VCL									
average	54.77	6.86	<b>53.34<sup>A</sup></b>	<b>6.52</b>	<b>54.91</b>	<b>6.35</b>	<b>57.85<sup>B</sup></b>	<b>8.44</b>	<b>0.004</b>
A	79.09	14.12	<b>77.15<sup>a</sup></b>	<b>11.68</b>	<b>78.32<sup>a</sup></b>	<b>12.50</b>	<b>86.80<sup>b</sup></b>	<b>21.35</b>	<b>0.03</b>
B	32.13	6.27	31.88	6.48	32.49	5.94	31.39	6.94	0.29
C	53.10	14.38	51.00	14.06	53.90	13.50	55.35	17.69	0.40
BCF									
average	7.57	0.39	7.58	0.39	7.56	0.37	7.60	0.46	0.17
A	6.94	0.27	6.92 <sup>a</sup>	<b>0.25</b>	<b>6.91<sup>a</sup></b>	<b>0.27</b>	<b>7.04<sup>b</sup></b>	<b>0.33</b>	<b>0.03</b>
B	7.45	0.27	7.47	0.28	7.43	0.26	7.47	0.29	0.29
V	8.33	1.08	8.34	1.15	8.33	0.98	8.28	1.25	0.90

Total motility (TM), curvilinear velocity (VCL), straight velocity (VSL), beat cross frequency (BCF), A – progressive motility, B – non-linear motility, C – non-progressive motility

Significant associations were marked by bold. Means marked by different upper-case letter differ at  $p < 0.01$ ; marked with different lower-case letter differ at  $p < 0.05$ .

ted of 367 bulls from one AI station. Thawed semen samples (straws) were used to evaluate the sperm motility using the computer-assisted semen analysis (CASA). A 761 bp fragment of the ARSD gene was amplified by PCR with primers: forward: 5' TAG-GATGCAATCGGTCTGCC 3' and reverse: 5'

CTAGAAGCTCCAGCG GAACC 3' at annealing temp, at 61°C. Genotyping of the point mutation C/T at position X; 139037255 (Zimin et al. 2009) (rs42207167) was performed using Pflm I restriction enzyme. Kruskal-Wallis (Statistica v. 10.0) test was used to assess associations between ARSD C/T muta-

tion and kinematic parameters of sperm motility. Taking into account similar bull age (12-18 months), standardized semen collection procedures, similar feeding and welfare conditions as well as no seasonal effect (data not shown), the model used did not require any corrections for additional fixed or random effects.

## Results and Discussion

Genotyping of ARSD C/T mutation allowed to obtain three genotypes (Fig. 1) in numbers sufficient for reliable statistical analysis. Significant associations were found between ARSD genotypes and average TM, VSL, VCL and for fractions of sperms showing progressive motility (VSLa, VCLa and BCfa) (Table 1). Moreover, the most significant differences were found between alternative homozygotes (CC vs TT) with heterozygotes having intermediate values which suggest distinct allele substitution effect. The literature on bovine ARSD is very poor. The enzyme was found in cauda epididymal fluid from mature Holstein bulls (Moura et al. 2010) but its specific function is rather unknown since the substrate of human ARSD was not identified (Urbitsch et al. 2000). Our results suggest that ARSD might be involved in new role – motility of sperms, although in unknown manner. Biochemical studies are necessary to develop the

method for arylsulfatase D activity which should open more possibilities to interpret our findings.

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