Polish Journal of Veterinary Sciences Vol. 21, No. 2 (2018), 419–421

DOI 10.24425/122611

Short communication

Influence of splicing mutation within the lysophosphatidic acid receptor 1 gene (LPAR1) on semen quality in Holstein-Friesian bulls

S. Kamiński[1], D.M. Hering[1], W. Kordan [2], M. Lecewicz[2], A. Sazanov[3]

[1] University of Warmia and Mazury, Department of Animal Genetics, Oczapowskiego 5, 10-719 Olsztyn, Poland
[2] University of Warmia and Mazury, Department of Animal Biochemistry and Biotechnology, Oczapowskiego 5, 10-719 Olsztyn, Poland
[3] Department of Molecular Biotechnology, Saint Petersburg State Technological Institute (Technical University), Saint-Petersburg, 190013, Moskovsky prospect, 26, Russia

Abstract

Effect of single nucleotide polymorphism (SNP) in splicing site of the *LPAR1* (lysophosphatidic acid receptor 1) gene on selected quality traits was investigated in frozen-thawed semen of Holstein-Friesian bulls. Splicing mutation A/G in the *LPAR1* gene (rs43581860) was identified in 120 Holstein-Friesian bulls using PCR-RFLP technique (Hph I). Heterozygotes AG were the most frequent (37.5%) compared with AA (30.8%) and GG (31.7%) homozygotes. Observed differences in total motility (TM), sperm membrane integrity (SYBR-14/PI) and ATP content were significant between homozygotes AA or GG and heterozygotes AG. For all three traits disadvantageous effect of heterozygotes AG was detected. This means that *LPAR1* splicing mutation has significant effect on semen quality and should be considered as a new marker of semen quality in Holstein-Friesian bulls.

Key words: bull, sperm, quality, LPAR1, polymorphism

Introduction

Lysophosphatidic acid receptors (LPAR) are all cell-surface, seven transmembrane-spanning G protein-coupled receptors (Yung et al. 2014). Out of 6 types LPARs, LPAR1-3 are highly expressed in the mouse and human testis (Choi et al. 2010). Deletion of these receptors in mice led to a reduction in mating activity and sperm count, and an increase in azoospermia in aging animals (Ye at al. 2008). One of our genome-wide association study showed significant markers for sperm motility in fresh semen on chromosome 8 in the close neighborhood of bovine LPAR1 gene (Hering et al. 2014). Because in dairy cattle reproduction, frozen-thawed semen is commonly used it seemed to be interesting to know whether mutations within LPAR1

Correspondence to: S. Kamiński, e-mail: stanislaw.kaminski@uwm.edu.pl

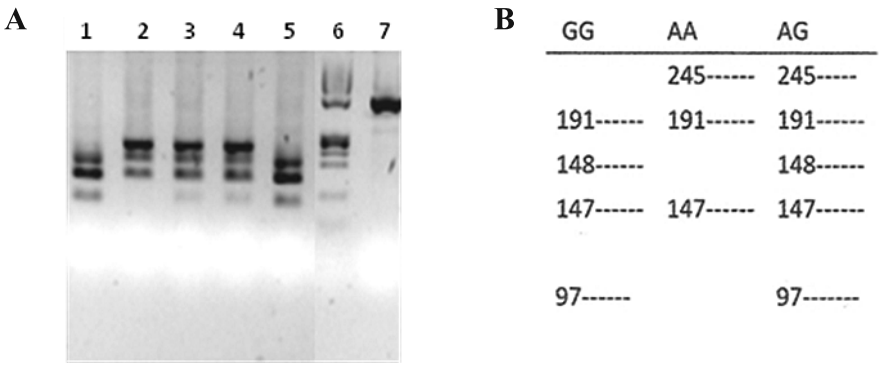

Fig. 1. Genotyping of LPAR1 A/G splicing mutation by HphI restriction enzyme. A: Lines 1, 5 – homozygote GG; line 2 – homozygote AA; lines 3,4 – heterozygote AG; line 6- DNA size marker ØX174 DNA/HaeIII; line 7 – undigested PCR product; B – theoretical pattern of restriction fragments obtained after digestion with HphI.

Table 1. Means $\bar{x}$ and standard deviations (SD) for selected semen quality traits of bulls with particular *LPAR1* genotypes. CASA Total Motility (TM), sperm membrane integrity (SYBR-14/PI), sperm mitochondrial function (JC-1/PI) and sperm ATP content (ATP).

Semen quality traits	All bulls (N=120)		AA (N=37)		AG (N=45)		GG (N=38)		p-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
TM	62.50	6.93	64.29[a]	7.44	60.38[b]	6.55	63.28[a]	6.36	0.012
SYBR-14/PI	72.20	2.71	73.24[a]	2.71	71.42[b]	2.45	72.13	2.76	0.013
JC-1/PI	71.31	1.85	71.56	1.77	71.13	1.85	71.28	1.94	0.648
ATP	15.58	2.16	16.22[a]	2.11	14.92[b]	2.21	15.73	1.96	0.035

Means marked by different lower-case letters differ at $p<0.05$

gene may affect sperm motility and other parameters of semen produced by artificial insemination (AI) bulls. Therefore the aim of the study was to find out whether the splicing mutation A/G within the lysophosphatidic acid receptor 1 gene (rs43581860) is associated with selected quality traits of frozen-thawed semen in Holstein-Friesian bulls.

Materials and Methods

The analyzed data set originates from the Holstein-Friesian dairy cattle population and consisted of 120 bulls from one AI station. The bulls included in the analysis were at a similar age (12 to 18 months) and were kept in uniform feeding and housing conditions. Commercial straws of frozen-thawed semen were used for the evaluation of sperm quality. Sperm motility was assessed by the use of a computer-assisted semen analysis (CASA) system. Sperm plasma membrane integrity was evaluated using dual fluorescent staining (SYBR-14 and PI) as described by Garner and Johnson (1995). Sperm mitochondrial function was assessed according Dziekońska et al. (2009). ATP content was measured in the supernatants using a Bioluminescence Assay Kit CLSII (Roche Diagnostics, GmbH, Basel, Switzerland). To identify LPAR1 genotype, genomic DNA was isolated from commercial semen straw as described by Hering et al. (2014). A 583 bp fragment of the LPAR1 gene was amplified by PCR with forward primer 5' TCAACACAGGGCCCAATACT 3', and reverse primer 5' GATGCCTTG TCCCCTTGTAA 3. Genotyping of the splicing mutation AG was performed using Hph I restriction enzyme. A Kruskal-Walis (Statistica v 10.0) test was used to find associations between LPAR1 A/G mutation and semen quality traits. Average of three replicates were calculated for each bull and each semen quality trait. Significance of differences between genotyping groups (AA, AG, GG) for each semen trait was calculated by the use of Tukey post-hoc test.

Results and Discussion

The genotyping of LPAR1 A/G splicing mutation is illustrated in Fig. 1. Among all bulls investigated, heterozygotes AG were the most frequent (37.5%), in comparison to AA (30.8%) and GG (31.7%) (Table 1). Splicing mutations occur in conservative short sequences in the boundary of exon and introns, and are involved in correct excision of introns from an RNA transcript. Substitutions within these sequences may disturb RNA

splicing and affect the structure of the encoded protein. A allele (being Adenine in the third position after the exon 2) is more conserved than Guanine (allele G) (Brown 2007). Therefore, one can assume that *LPAR1* RNA transcript could be processed more efficiently in bulls with the AA genotype. Indeed, we showed that bulls with AA genotype had the highest value of several semen quality traits. Among all standard CASA kinematic parameters, only total motility (TM) of sperm was found to be associated with the *LPAR1* genotype. Remaining CASA kinematic parameters showed no significant differences between bulls of different *LPAR1* genotype (data not shown). Significant differences were observed between AA or GG homozygotes and AG heterozygotes, the latter showed the lowest value of TM. For sperm membrane integrity (SYBR-14/PI) both homozygotes AA had significantly higher values than heterozygotes AG. *LPAR1* genotype influenced also ATP content which showed the lowest value in heterozygote bulls (AG) in comparison with that found in both homozygotes. For 3 traits analyzed in this paper (TM, SYBR-14/PT and ATP; Table 1) we observed disadvantageous effect of heterozygotes AG. Such allelic interaction is very rare and has not yet been explained at molecular level. The only hypothetical explanation for such phenomenon is the existence of the linkage between *LPAR1* alleles and unknown genes decreasing analyzed traits, but only in situation when the bull inherited different alleles from parents. Therefore, genes being in linkage disequilibrium with LPAR should be studied in future research to find out whether they interplay with LPAR1 to influence semen quality traits. The differences between genotypic groups for mitochondrial membrane integrity were very subtle and did not reach the level of significance, which suggests that *LPAR1* genotypes influence the plasma membrane but do not affect the integrity of the mitochondrial membrane. This observation might be due to the fact that the entire pool of ATP in spermatozoa is not synthesized by oxidative phosphorylation in the mitochondria. There are studies indicating that a significant amount of ATP is synthesized by glycolysis (Nascimento et al. 2008, Dziekońska et al. 2014). In this study, the association between total ATP content and *LPAR1* gene seems to confirm the hypothesis on different sources of ATP production in spermatozoa.

References

Brown TA **(2007)** Genomes, 3rd ed., Garland Science Publishing, New York

Choi JW, Herr DR, Noguchi K, Yung YC, Lee CW, Mutoh T, Lin ME, Teo ST, Park KE, Mosley AN, Chun J **(2010)** LPA receptors: subtypes and biological actions. Annu Rev Pharmacol Toxicol 50: 157-186.

Dziekońska A, Fraser L, Koziorowska-Gilun M, Strzeżek J, Koziorowski M, Kordan W **(2014)** Seasonal-dependent variations in metabolic status of spermatozoa and antioxidant enzyme activity in the reproductive tract fluids of wild boar/domestic pig hybrids. Pol J Vet Sci 17: 307-313.

Dziekońska A, Fraser L, Strzeżek J **(2009)** Effect of different storage temperatures on the metabolic activity of spermatozoa following liquid storage of boar semen. J Anim Feed Sci 18: 638-649.

Garner DL, Johnson LA **(1995)** Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. Biol Reprod 53: 276-284.

Hering DM, Olenski K, Kaminski S **(2014)** Genome-wide association study for poor sperm motility in Holstein-Friesian bulls. Anim Reprod Sci 146: 89-97

Nascimento JM, Shi LZ, Tam J, Chandsawangbhuwana C, Durrant B, Botvinick EL, Berns MW **(2008)** Comparision of glycolysis and oxidative phosphorylation as energy sources for mammalian sperm motility, using the combination of fluorescence imaging, laser tweezers, and real-time automated tracking and trapping. J Cell Physiol 21: 745-751.

Ye X, Skinner MK, Kennedy G, Chun J **(2008)** Age-dependent loss of sperm production in mice via impaired lysophosphatidic acid signaling. Biol Reprod 79: 328-336.

Yung YC, Stoddard NC, Chun J **(2014)** LPA Receptor Signaling: Pharmacology, Physiology, and Pathophysiology. J Lipid Res 55: 1192-1214.