Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at www.cibtech.org/sp.ed/jls/2015/01/jls.htm
2015 Vol.5 (S1), pp. 1975-1978/Farhad et al.
Research Article

EFFECTS OF GLYCERALDEHYDE 3-PHOSPHATE DEYDROGENASE GENE ON THE MOTILITY OF HOLSTEIN BULL'S SPERMATOZOA

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ABSTRACT
Reproductive efficiency is a major determinant of cow-calf profitability. Motility is generated by the extremely long flagellum that comprises 90% of the length of the mammalian sperm. Glycolysis is a major pathway for ATP production in mammalian sperm. To determine the percentage of sperms motility, frozen semen of 55 bulls were analyzed by a Video Computer Aided Semen Analysis System (CASA). The SSCP method and sequencing were used to determined mutations, after DNA extraction and polymerase chain reactions. Four haplotypes were observed and there was a significant difference between the motility of Haplotype one and the others.

Keywords: *Glyceraldehyde 3-Phosphate Dehydrogenase Gene, Holstein Bulls Spermatozoa*

INTRODUCTION
Reproductive efficiency is a major determinant of cow-calf profitability. Fertility of the male is a major contributor to overall reproductive performance in mating systems that use natural service. High bull fertility is crucial to successful breeding and high pregnancy rates. The bull fertility, depends on sperm cell output, percentage of normal sperm cells produced, motility or ability of forward progressive movement of sperm cells, male reproductive tract condition, ability of the bull to complete an insemination, libido, age of the bull and body condition of the bull (Whittier *et al.,* 2009; Walker *et al.,* 2008).

Most mammalian testicular sperm are inactive initially and maturation be occur in the epididymis (Martin *et al.,* 2010). Sperm in the cauda epididymis are activated to move on ejaculation, where they are mixed with accessory gland secretions. Activated sperm still cannot fertilize the ovum without capacitation, in which the motility extensively changes to hyper activation. So motility can be estimated by observing the mass movement of a concentrated sample of semen (Martin *et al.,* 2010).

Motility is generated by long flagellum that comprises 90% of the length of a mammalian sperm. The process requires substantial ATP to support coordinated movement of the central axoneme and surrounding flagella structures (Nishimune *et al.,* 2006). The ATP is hydrolyzed by ATPases, which function as force generating molecular motors along the axoneme. Although quiescent in the epididymis, mammalian sperm display vigorous forward movement, termed activated or progressive motility, immediately upon ejaculation or collection into physiological medium. The motility waveform changes in the female reproductive tract, with increases in both the amplitude and asymmetry of flagella bending. These changes result in a whiplash-like motion, termed hyper activated motility, which facilitates sperm transport in the oviduct and penetration of the Zona pellucida surrounding the oocyte (Nishimune *et al.,* 2006).

The Glycolysis is a major pathway for ATP production in mammalian sperm. In several mammals, glycolysable substrates are present in seminal fluid (Katherine *et al.,* 2010) and enzymatic activity of glycolysis is detected in mammalian sperm (Force *et al.,* 2004). It seems that the Glyceraldehyde 3-phosphate dehydrogenase gene play an important role on this subject.

Researching about, the effect of Glyceraldehyde 3-phosphate dehydrogenase gene (exon 6) and its effect on the sperm motility of the Holstein bulls were the main object of this article.

MATERIALS AND METHODS

To estimate the percentage of sperms motility, the frozen semen of 55 bulls were analyzed by a Video Computer Aided Semen Analysis System (CASA). Videotapes were determined using Hamilton Thorne Biosciences software (Animal Version 12.3 H).

The DNA extraction of semen was performed by high pure PCR Template preparation kit (Roche company, Registration Code: 11796828001). The quality of the extracted DNA was checked by electrophoresis on the agarose gel.

Primers were designed in order to use in PCR reactions of Glyceraldehyde 3-phosphate dehydrogenase gene (exon6, NCBI Accession no: AY197340.2) according to the table number 1.

Table 1: Primers using in PCR reaction of Glyceraldehyde 3-phosphate dehydrogenase gene

Primers	secneuqeS	TM	GC %	Length
Forward	5'- CACTGGGGTCTTCACTACCATG -3'	60.24	55%	22
Reverse	5' – GCGTGGACAGTGGTCATAAGTC – 3'	60.04	55%	22

The PCR reaction were conducted in a 20 µL reaction mixture, which include 0.3 µM of each primer, 250 µM of deoxynucleoside triphosphate, 2 mM $MgCl_2$, 2 IU of Taq DNA polymarase enzyme, 1X PCR buffer and 150 ng of template DNA. PCR was performed for initial denaturation in 94°*C* for 5 minutes, 30 cycle of denaturation in 95°*C* for 40 seconds, Annealing in 55°*C* for 30 seconds and Extension in 72°*C* for 30 seconds and final extension in 72°*C* for 5 minutes. The Quantity and quality of PCR products were checked by spectrophotometry and agarose gel electrophoresis respectively.

The PCR products were exposing to 95°*C* for 10 minutes in order to denaturate amplicons before SSCP. The SSCP method was used in order to compare amplicons (Figure 1). DNA sequencing was performed to compare the DNA sequences of the observed haplotypes.

Haplotypes sequences were compared by BLAST program in NCBI database and the SNP mutations were detected.

Statistical Analysis

The percentage of motility was detected by CASA for each sample. Normal distribution of the data was checked by Anderson-Darling test by MINITAB software.

To determine the significant difference between haplotypes, GLM were used as a following:

$Y_i=\mu+Gi+b(A_i)_+e$

That Y_i is the i^{th} observation, μ is overall mean, G_i is the ith SNP mutation, A_i show the age of the i^{th} bull that provide sample and e_i shows the residual of the observation Y_i .

The SPSS *(version 20)* was used to analysis data.

RESULTS AND DISCUSSION

Four haplotypes were detected after doing SSCP method (Figure 1). Haplotypes were sequenced and after alignment them by BLAST software in NCBI, nine SNP were detected. Table 2 shows all SNP and the exact position of them at the Glyceraldehyde 3-phosphate dehydrogenase gene.

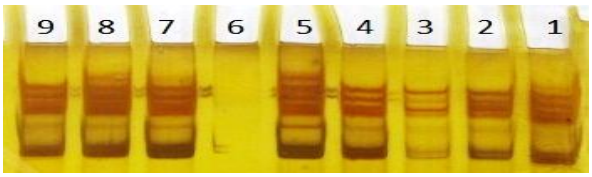

Figure 1: Result of SSCP for PCR products of Glyceraldehyde 3-phosphate dehydrogenase gene

Results show that, the haplotype two has the highest frequency between all haplotypes also the number of SNPs on this haplotype was the highest. The average of motility in haplotype one (71.02 ± 0.60) is significantly ($P<0.05$) higher than haplotype four (67.71 ± 1.71) according to table 2.

There was no significant difference for the average of motility in haplotype two and haplotype tree *(P ≥ 0.05)*.

Table 2: Detected SNPs with the average of motility in observed Haplotypes

SNP position	Kinds of mutation	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 4
21	T/C			*	
22	G/A	*	*	*	
56	A/G		*		
64	T/C		*		
97	G/A		*		
125	-/G		*	*	*
135	A/C		*		*
136	-/C		*	*	
164	A/C	*	*	*	
Frequency (%)		30.20	45.30	11.30	13.20
Average of motility ± SE		71.02 ± 0.60 a	70.19 ± 0.59 ab	68.83 ± 1.61 ab	67.71 ± 1.71 b

All SNPs characteristics were shown in table 3. Just SNP number 22 and 164 showed the significant substitution effect on the average of motility between all SNPs (*P < 0.05*). Other SNPs has not a significant effect on the average of motility (*P ≥ 0.05*). The SNP number 22 was a transition mutation while the SNP number 164 was the transversion mutation. It understood that the significant effective SNPs (SNP number 22 and 164) just observed in haplotype 1, 2 and 3 and haplotype 4 was free from them.

Table 3: Observed SNPs characteristics and its effect on the average of sperm motility

SNP position	Substitution	SNP frequency (%)	Average of motility (%)	P Value
21	T	88.6	70.1 ± 3.1	0.428
	C	11.3	68.8 ± 2.9	
22	G	11.3	67.6 ± 4.9	0.013
	A	88.7	70.2 ± 2.9	
56	A	69.8	69.5 ± 3.4	0.115
	G	30.2	71.0 ± 2.4	
64	T	69.8	69.5 ± 3.4	0.115
	C	30.1	71.0 ± 2.4	
97	G	69.8	69.5 ± 3.4	0.115
	A	30.1	71.0 ± 2.4	
125	-	30.1	71.0 ± 2.4	0.115
	G	69.8	69.5 ± 3.4	
135	A	30.2	71.0 ± 2.4	0.115
	C	69.8	69.5 ± 3.4	
136	-	58.5	69.6 ± 3.4	0.115
	C	41.5	70.4 ± 2.9	
164	A	11.3	67.6 ± 4.9	0.013
	C	88.6	70.2 ± 2.9	

Result showed that, sperm motility can be changed by mutation in coding region of Glyceraldehyde 3-phosphate dehydrogenase. It has been demonstrated that, this enzyme has a major role in glycolysis

pathway. However it's suggested to compare the production of wild type and mutant gene in a large population. This research just showed many new mutations and most of them were silent mutations. We need to evaluate the post transcriptional and post translational modification and find out if there is some change in enzyme shape and compositions on the next research.

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