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Environmental and Genetic Factors Affecting on Semen Quality in Iranian Holstein Bulls

Research Article

M.V. Ghasemi[1] and A. Ghorbani[1*]

[1] Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran

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*Correspondence E-mail: dr.ghorbani@iaushab.ac.ir

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ABSTRACT

The objective of this study was to evaluate the importance of environmental and genetic factors affecting semen quality; sperm volume (SV), sperm concentration (SPCO), total sperm (TS), fresh sperm motility (FSM), total fresh motile sperm (TFMS), post thaw sperm motility (PTSM), total post thaw motile sperm (TPTMS), number of produced payout (NPP), number of fresh motile sperm in each milt ejaculation (NFMSE), motility before and after the freezing (MBATF) and number of post thaw motile sperm in each milt ejaculation (NPTMSE). We used polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) in 83 bulls belonging to two progeny test centers. The BB genotype was omitted from analysis. Season of collection had a significant effect on sperm volume (SV) (P<0.006), sperm concentration (SPCO) (P<0.0038), fresh sperm motility (FSM) (P<0.0001), post thaw sperm motility (PTSM) (P<0.001), total post thaw motile sperm (TPTMS) (P<0.0075), number of produced payout (NPP) (P<0.0247), number of fresh motile sperm in each milt ejaculation (NFMSE) (P<0.0012) and motility before and after the freezing (MBATF) (P<0.0001) but did not significantly affect TS (P<0.438), total post thaw motile sperm (TPTMS) (P<0.3606) and number of post thaw motile sperm in each milt ejaculation (NPTMSE) (P<0.1106). The interaction of seasons with follicle-stimulating hormone and semen quality traits only significantly affect sperm volume (SV) (P<0.0435), sperm concentration (SPCO) (P<0.008) and number of produced payout (NPP) (P<0.0119). Genetics and environmental factors clearly contribute to semen production in Holstein bulls.

KEY WORDS environmental factor, genetic, Iranian Holstein bulls, semen quality.

INTRODUCTION

FSH is a pituitary-expressed glycoprotein hormone that regulates gonadal function in both sexes in mammals (Moyle and Campbell, 1996). In females, the role of FSH in regulating follicular development and sex steroid production is clear and well understood, and FSH is routinely used for the treatment of female infertility (Howles, 2000; Mc Gee and Hsueh, 2000). In contrast, the role of FSH in males in regulating testicular function and spermatogenesis continues to be debated (Moudgal and Sairam, 1998; Plant and Marshall, 2001). FSH is composed of a-subunit shared with other glycoprotein hormones and a specific b-subunit encoded by the FSHB gene. Artificial insemination (AI) is a powerful biotechnology that allows producers to use superior sires, promoting faster genetic improvement and increasing profitability. The conception rate with AI depends on the characteristics of the semen provided by AI centers: AI organizations try to collect the maximum amount of spermatozoa of the highest possible quality at the lowest cost. Spermatozoa are cryopreserved in straws, and each straw contains sufficient sperm for one AI. A reduction of

sperm concentration in an AI dose would increase the number of straws produced from an ejaculate but could also reduce the conception rate of inseminated cows (Shannon and Vishwanath, 1995). Therefore, the number of motile spermatozoa per straw is chosen to balance conception rate and number of straws produced. The number of straws produced per ejaculate then depends on the volume of the ejaculate, the concentration of spermatozoa in the ejaculate, and the percentage of spermatozoa that are motile. The quantity and quality of semen produced by AI bulls are affected by environment, management, physiological status, and genetics.

The age of the bull at semen collection affects the volume of the ejaculate, its concentration, and sperm motility. In general, the literature shows that all of these ejaculate characteristics increase as bull's age (Almquist *et al*. 1976; Fuente *et al*. 1984). Most studies found evidence that season of collection significantly affects semen production (Graffer *et al*. 1988; Stalhammar *et al*. 1988). According to (Schwab *et al*. 1987) the highest volume of semen, sperm concentration, and number of spermatozoa per ejaculate are produced in winter. Cattle reproduction can be affected by heat stress; under high ambient temperature and/or humidity, body thermoregulatory mechanisms are unable to increase body heat loss and internal temperature increases above physiological limits (Chemineau, 1994). Heat stress can decrease conception rates and increase embryonic mortality in cows (Wolfenson *et al*. 2000) and decrease semen quality in bulls (Johnston *et al*. 1963; Skinner and Louw, 1966). In the tropics, sperm production and semen quality decrease during the hot season (Igboeli and Rakha, 1971; Fields *et al*. 1979; Kumi Diaka *et al*. 1981; Rekwot *et al*. 1987) however, seasonal variations cannot be attributed only to greater ambient temperature. The present study was conducted to determine environmental and genotype effects on semen quality in Holstein bulls AI in Iran.

MATERIALS AND METHODS

Animals

83 bulls of the North West AI center (Tabriz, Iran) and Progeny Test center of Jahed Co (Karaj, Iran), were included in the study. For each bull the repeated measurements of sperm quality traits of bulls were available since 1991 to 2008 (41890 records).

Genotyping

Blood and semen samples were collected from the bulls. An anticoagulant (EDTA) was added to the blood samples and then stored at -20 ˚C. Genomic DNA from whole blood was purified by standard protocol using proteinase K digestion as described by (Falconer *et al*. 1996) and from semen by DNA extraction kit (DNPTM kit Cinnagen Co. Tehran, Iran). The quality of the DNA was checked on 0.5% agarose gel and the quantity was measured by UV spectrophotometry at A260 / A280 nm. Genotyping for FSHβ polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

The PCR reaction conditions were approximately 100 ng of genomic DNA, 10 pmol of each primer, 0.2 m*M* of each dNTP, 1.5 m*M* of MgCl2, 1 × PCR buffer [50 m*M* of KCl and Tris-HCl (pH 8.4)] and 0.4 U of Taq polymerase in a total volume of 25 µL.

The PCR was conducted on Eppendorf Gradiant thermal cycler, Hot Master Mix (EPPENDORF, Germany) using a preliminary denaturation at 94 ˚C for 1.5 min, 62 ˚C for 1 min and 72 ˚C for 1 min, followed by 48 cycles of a specific temperature regime. Each temperature regime consisted of 94 ˚C for 30 s, 62 ˚C for 1 min, 72 ˚C for 30 s and a final extension at 72 ˚C for 5 min. A 313 bp fragment of FSHB consisting part of intron 2 and complete coding region of exon 3, was amplified using forward (5`CTTCCAGACTACTGTAACTCATC3`) and reverse (5GTAGGCAGCTCAAAGCATCCG`3`) primers (Dai *et al*. 2009).

PCR products were digested with 4 U of PST-I, using the supplied buffer and maintained at 37 ˚C for1 h. The resulting fragments were separated by vertical electrophoresis (110 W 40 min) in 3% agarose gel, stained with ethidium bromide and was visualized under UV light. The A (PST-I) allele had fragment sizes of 111, 202 bp, whereas the B (PST-I) allele had fragments of 111, 202 and 313 bp.

Statistical analysis

Genotype frequencies

The FSHβ allele frequencies were calculated by simple allele counting (Falconer *et al*. 1996). The possible deviations of allele and genotype frequencies from the Hardy-Weinberg equilibrium were examined with PopGene.S2 software by a Pearson's Chi-square test

Association analysis

Statistical analysis was performed using the MIXED procedure of SAS software (SAS, 1999). The following linear model was used to examine the associations between FSHβ-PSTI, polymorphisms and SV, SPCO, TS, FSM, TFMS, PTSM, TPTMS, NPP, NPTMSE, MBATF and NFMSE traits:

$$y_{ijklm} = \mu + a_i + YS_j + S_k + G_l + \sum_m b_m x_m + \varepsilon_{ijklm}$$

Where:

y_{ijklm}: the above traits.

µ: the overall trait mean.

a_i: the random effect of the i^{th} animal.

YS_j: the fixed effect of the j^{th} year-season (j=1-68).
S_k: the fixed effect of the k^{th} station (k=1-2).
G_l: the fixed effect of the l^{th} FSH genotype (l=1-3).
b_m: the regression coefficient of m^{th} covariable (e.g. age).
x_m: the fixed effect of m^{th} covariable.
ε_{ijklm} : the residual error.

FSM and PTSM traits were categorical variable, hence analyzed with logistic regression and GENMOD procedure by using the following model:

$$\eta_{iklm} = \log[p_i/(1-p_i)] = m + a_i + YS_j + S_k + G_l + \sum_m b_m x_m + \varepsilon_{ijklm}$$

η_{ijklm}: MAF and MBF traits.
m: the overall mean in logarithmic scale.
a_i: the random effect of the i^{th} animal.
YS_j: the fixed effect of the j^{th} year season (j=1-68).
S_k: the fixed effect of the k^{th} station (k=1-2).
G_l: the fixed effect of the l^{th} FSH genotype (l=1-3).
b_m: the regression coefficient of m^{th} covariable (e.g. age).
x_m: the fixed effect of m^{th} covariable.
ε_{ijklm} : the residual error.

RESULTS AND DISCUSSION

Season of collection

Least square means of sperm quality traits for Season of collection are presented in Table 1. Analysis of variance indicated significant association with season of collection SV (P<0.006), SPCO (P<0.0038), FSM (P<0.0001), PTSM (P<0.001), TPTMS (P<0.0075), NPS (P<0.0247), (P<0.0012) and MBATF (P<0.0001) but did not significantly affect TS (P<0.438), TFMS (P<0.3606) and NPTMSE (P<0.1106). Moreover age had significant effects on SPCO, TS, FSM, NPTMSE and TPTMS traits (P<0.0001).

The results show SPCO, FSM, NFMSE and PTSM traits generally higher during winter than during summer, fall and spring and TPTMS, NPTMSE in fall and winter, and TS and TFMS in fall and SV and MBATF in summer and NPS in spring were higher.

Our findings agree with those of (Sarder, 2007; Asmat, 2002; Mathevon *et al.* 1998; Everett *et al.* 1978; Schwab *et al.* 1987; Everett and Bean, 1982) who found the sperm concentration generally higher during winter than during spring, summer and fall. But other researchers found poorer results in spring and winter (Anchieta *et al.* 2005; Mostari *et al.* 2005; Li junjie *et al.* 2001; Fawzy and Rabie 1996; Shannon and Vishwanath, 1995; Usmani *et al.* 1993; Rustenev, 1989; Zafar *et al.* 1988; Fuente *et al.* 1984). Usmani *et al.* (1993) reported that seasonal stress had no effect on the number of ejaculates in crossbred (Friesian×Sahiwal) bulls. Javed *et al.* (2000) noted lowest ejaculate volume in humid summer in Nili Ravi buffalo bulls and (Fonseca, 1995; Mathevon *et al.* (1998) reported that season had no effect on ejaculate volume for Nellore and Holstein bulls, respectively. Some studies reported three genotypes for FSHβ gene AB (111, 202 and 313 bp), AA (111 and 202 bp). The allele was more frequent than B allele (0.675 *vs.* 0.325) and therefore most of the bulls (65%) were heterozygous for the B allele and only 35% were homozygous and BB genotype was not found. However reported that genotyping of FSHβ gene significantly effects on TS (P<0.0425), FSM (P<0.0001) and NPP (P<0.0302) but there was no significant association with SV, SPCO, NFMSE, TFMS, PTSM, NPTMSE, TPTMS and MBATF (P>0.05). Season can include many factors, such as temperature, photoperiod, humidity, feed quality and housing. Differences in the quantity of feed (Stalhammar *et al.* 1989) or in feed composition (Siratskii, 1990) could also affect semen output. Excessive humidity during a specific season could also reduce production of semen. Indeed, semen output reportedly increased below 50% humidity (Castillo *et al.* 1987). Everett and Bean (1982) reported that an ambient temperature between -15 and 20 ˚F had no practical effect on semen production.

The interaction of seasons with follicle-stimulating hormone in semen quality traits

Least square means of sperm quality traits for the interaction of seasons with follicle stimulating hormone in semen quality traits are presented in Table 2.

Table 1 Least squares means (±SD) of sperm quality and traits for Season of collectionin Iranian Holstein bulls

Trait	Spring	Summer	Fall	Winter	SEM	P-value
Sperm volume (mL)	4.27^{a}	4.28^{a}	4.16^{a}	3.87^{a}	0.11	0.006
Sperm concentration	1089.61^{a}	1048.41^{b}	1138.75^{b}	1150.02^{ab}	32.52	0.0038
Total sperm	4568.11	4317.61	4655.04	4366.08	242.59	0.4383
Fresh sperm motility	60.699^{a}	58.9601^{b}	60.23^{a}	60.92^{a}	0.357	0.0001
Number of fresh motile sperm in each milt ejaculation	689.72^{b}	656.38^{b}	714.73^{ab}	737.20^{a}	22.13	0.0012
Total fresh motile sperm	2962.90	2770.11	2999.46	2942.27	151.74	0.3606
Post thaw sperm motility	60.67^{a}	58.96^{b}	60.23^{a}	60.92^{a}	0.35	0.0001
Number of post thaw motile sperm in each milt ejaculation	1618.06	1430.38	1657.67	1657.67	109.53	0.1106
Total post thaw motile sperm	364.58^{ab}	326.78^{b}	388.81^{a}	388.81^{a}	32.145	0.0075
Motility before and after the freezing portion	1.76^{c}	1.96^{a}	1.84^{bc}	1.84^{bc}	0.04	0.0001
Number of produced straw	161.36^{a}	153.57^{b}	156.68^{ab}	156.68^{ab}	3.26	0.0247

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 2 Least squares means (±SD) the interaction of seasons with follicle stimulating hormone in semen quality traits

Trait	Spring		Summer		Fall		Winter			
	AA	AB	AA	AB	AA	AB	AA	AB	SEM	P-value
Sperm volume (mL)	3.8^{ab}	4.7^{a}	3.8^{ab}	4.7^{a}	3.5^{b}	4.7^{a}	3.1^{b}	4.6a	0.4	0.043
Sperm concentration	1036.4^{b}	1142.7^{a}	967.8^{c}	1128.9^{ab}	1155.1^{a}	1122.3^{ab}	1148.7^{a}	1151.3^{a}	103.7	0.008
Total sperm	3700.7	5435.5	3354.4	5280.7	3916.3	5393.7	3394.5	5337.6	678.6	0.780
Fresh sperm motility	58.4	58.8	58.3	57.0	59.8	58.3	62.2	58.9	1.7	0.206
Number of fresh motile sperm in each milt ejaculation	690.3^{b}	689.0^{b}	653.3^{b}	659.3^{b}	765.2^{a}	664.1^{b}	787.6^{a}	686.7^{b}	70.1	0.011
Total fresh motile sperm	2618.8	3306.9	2431.6	3108.5	2775.7	3223.2	2662.3	3222.1	399.7	0.852
Post thaw sperm motility	29.3	27.9	28.5	23.7	29.8	24.4	33.0	27.1	1.7	0.321
Number of post thaw motile sperm in each milt ejaculation	1642.8	1593.2	1561.5	1299.1	1671.6	1329.9	1806.2	1509.1	268.0	0.536
Total post thaw motile sperm	408.6	320.4	387.0	266.5	435.2	267.9	471.4	306.1	53.3	0.115
Motility before and after the freezing portion	1.7	1.9	1.9	2.1	1.6	2.2	1.8	2.0	0.1	0.529
Number of produced straw	144.7^{b}	171.9^{ab}	146.2^{b}	167.4^{ab}	194.9^{a}	170.8^{a}	180.8^{a}	178.2^{a}	12.7	0.011

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Analysis of variance indicated season of collection with follicle stimulating hormone gene only significantly affects SV (P<0.0435), SPCO (P<0.008), NFMSE (0.0117) and NPS (P<0.0119) but does not significantly affect TS, FSM, TFMS, PTSM, TPTMS, NPTMSE, MBATF traits.

Comparison of the characteristic sperm volume, sperm concentration, total fresh motile sperm, total sperm, and motility before and after the freezing and fresh sperm motility showed that the genotype AB in spring was higher than the AA genotype but in summer, fall and winter AA genotypes was higher than AB genotype.

For number of fresh motile sperm in each milt ejaculation traits AB genotype was higher than AA genotype but in spring, fall and winter AA genotype higher than AB genotype and for the post thaw sperm motility, number of post thaw motile sperm in each milt ejaculation and for total post thaw motile sperm traits AA genotype higher than AB genotype.

For the number of produced straw trait in spring and winter AB genotype was higher than AA genotype but in fall and summer AA genotype was higher.

CONCLUSION

In conclusion, the results of the present study showed that including season can affect reproduction traits in farm animals. This effect may be due to changes in the concentrations of reproductive hormones in animals in response to environmental conditions. The use of molecular genetics with favorable environmental conditions for each genotype can lead to successful breeding process.

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