

Full Length Research Paper

Influence of breed on motility and motion characteristics of fresh, chilled and frozen bull spermatozoa

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At present, frozen-thawed semen is used extensively for artificial insemination (AI) in Ethiopia. However, subjective semen quality assessment is still practiced by the semen processing organizations of the country. In this study, motility and motion characteristics of spermatozoa were assessed by using integrated semen analysis system (ISAS) to diagnose breed differences in quality of semen. Semen was collected from 14 breeding bulls (Boran = 4, Crosses of 75 % Holstein Frisian × 25% Boran = 4 and Holstein Frisian = 6). After initial subjective assessment, the semen was evaluated at fresh, chilled and frozen stages for various sperm motion characteristics using integrated semen analysis system (ISAS® v1, Proiser, Spain). PH, volume and morphological defects were differed significantly ($P < 0.05$) among breeds. Significantly higher ($P < 0.05$) motility percentage (82.5%) was identified in Boran fresh semen. In contrast spermatozoa motion characteristics: Medium, medium progressive and slow types were significantly higher ($P < 0.05$) in crosses. Individual motility percentage and spermatozoa motion types (medium and slow) were significantly low ($P < 0.05$) in Boran chilled semen. In line with chilled semen significantly high motility percentage (42.9%) for frozen semen was observed in HF. The sperm kinematic parameters: Average path velocity (VAP, $\mu\text{m/s}$), straight line velocity (VSL, $\mu\text{m/s}$), curvilinear velocity (VCL, $\mu\text{m/s}$), amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz) and straightness (STR) percentage were differed significantly ($P < 0.05$) among the three breeds at all stages of semen production. The smallest kinematic values of all parameters except for LIN, STR and WOB of fresh semen were recorded in cross breed bulls at all stages of production. On the other hand significantly higher ($P < 0.05$) values for all the parameters (VCL, VAP, VSL, ALH, BCF, LIN, STR and WOB) of frozen semen were recorded in Boran breed. Thus it is possible to conclude that breed has influence on motility and motion characteristics of bull spermatozoa at different stages of semen production.

Key words: Boran, Integrated Semen Analysis System (ISAS), kinematic parameters.

INTRODUCTION

Efficiency of Artificial Insemination (AI) system in cattle invariably depends on selection of the best breed of bull. Failure of many bulls to consistently and efficiently breed

has been reported to be associated with the production of poor quality semen, seasonal changes in semen quality, high incidence of abnormal spermatozoa and problems

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in sexual behaviors that reduce their fertility (Roberts, 1971; Hafez, 1993; Blezinger, 1999). In Ethiopia, Holstein Frisian and Boran (a dual purpose Zebu) breeds have been used to improve dairy productivity. Hundreds of thousands of semen doses are produced annually at the National Animal Genetic Improvement Institute (NAGII) for dairy genetic improvement. Although efficiency problems have been previously reported for the AI system (Desalegn et al., 2009), virtually no objective data exist for evaluation of semen from Borans and their Holstein Crosses using advanced technology (Tegegne et al., 1995). Although currently there is a huge demand by the government for Boran semen, anecdotal data indicate a dismally low (<30%) pregnancy rate which has hampered its wider use both for AI and Embryo Transfer (ET) programs. It is well known that multi parametric evaluation of semen can increase the accurate estimation of fertility of a sperm. In the current study, it is thus hypothesized that breed differences in quality of semen can be diagnosed using a multiparametric semen evaluation that employ advanced technique. An integrated semen analysis system (ISAS) has been used to evaluate the influence of breed in the quality of semen.

MATERIALS AND METHODS

Study animals and management

A total of 14 breeding bulls (Boran = 4, Holstein Frisian/HF/ = 6 and HF * Boran crosses = 4) aged between 2 and 7 years were used in this study. All the bulls were kept in indoor and under identical conditions of management, feeding and watering throughout the study period. They received hay, green forage and concentrate fortified with vitamins and minerals. Water was given *ad libitum*. They are allowed to exercise on running track in weekly basis. A total of 125 ejaculates (Boran = 35, Cross = 33 and Holstein Friesian = 57) were collected once per week over three and half months period.

Semen preparation and evaluation

Bulls were given bath to remove dung from their prepuce 30 min before collection of semen. Semen was collected with the help of bovine artificial vagina (IMV; France) early in the morning between 09:00-10:00 AM as per the method described in Salisbury et al. (1978) which was also the routine practice at NAGII. The semen was grossly assessed immediately after collection and later subjected to microscopic evaluation. After the first subjective microscopic assessment for mass and progressive motility and spermatozoa concentration using spectrophotometer were done and approved for further processes, the fresh diluted semen samples were subjected to ISAS evaluation. Integrated Semen Analysis System (ISASv1®) set-up was pre-adjusted for bovine semen analysis as per the manufacturer's (prosier, Spain) recommendation. Aliquot of 100 µl of fresh diluted semen was placed into a pre warmed micro-centrifuge tube and re-diluted at a rate of 1:3 (semen to extender) to bring the concentration at 20-50 millions of spermatozoa/ml. Kinetic parameters: average path velocity (VAP, µm/s), straight line velocity (VSL, µm/s) and curvilinear velocity (VCL, µm/s) as well as other related parameters: amplitude of lateral head displacement (ALH, µm), beat cross

frequency (BCF, Hz), straightness (STR, %), linearity (LIN, %) wobble (WOB, %), spermatozoa motion characteristics, percent total motile (%TMO) and percent progressively motile (%PROG) were recorded for each sample. All these parameters were also evaluated from 0.25 ml mini straw packs after chilling and biological freezing stages of semen production.

Statistical analysis

The data obtained from semen quality parameters were entered to Microsoft excel sheet and SPSS computer statistical package for windows (Version 16, USA) was used for analysis. The data of PH, volume, concentration, mass activity, individual motility, sperm morphological defects and kinematic parameters were compared using Analysis of Variances (ANOVA) and descriptive statistics were used to describe the variables. Duncan's Multiple Range Test (DMRT) was used to compare significant difference between breeds for those means and percentages at a probability level of 5%. In the analysis, $P < 0.05$ was set for level of significance.

RESULTS

At fresh semen illustrated in Table 1 significant differences ($P < 0.05$) between breeds for fresh semen were recorded for semen quality measures of PH, Volume and morphological defects (head, tail and proximal droplets). Sperm head abnormality was significantly higher ($P < 0.05$) in Boran breed. Significantly higher ($P < 0.05$) tail abnormality was observed for Boran and HF breeds. In contrast the highest proximal abnormality was noted in crosses. In general, though the total sperm morphological abnormality for Boran and HF breeds was nearly similar; it was significantly higher in crosses.

All kinematic parameters (VCL, VSL, VAP, ALH, BCF, LIN, STR, and WOB) were significantly different ($P < 0.05$) in all stages of semen production among the three breeds. Most of the fresh semen kinematic parameters (VSL, VAP, BCF, LIN, STR and WOB) were significantly higher in HF breed compared to Boran and Crosses. On the other hand significantly smaller kinematic parametric values of VCL, VSL, VAP, ALH and BCF were recorded in Crosses (Table 2). The kinematic parameters: VCL, VSL, VAP, ALH, BCF and STR of chilled semen were significantly different ($P < 0.05$) for the three breeds. Significantly higher kinematic parameters of VCL, VSL, VAP, ALH and BCF were detected in HF compared to Boran and Crosses. In line with fresh semen, all kinematic parameters (VCL, VSL, VAP, ALH, BCF, LIN, STR, and WOB) were significantly smaller ($P < 0.05$) in Cross (Table 2).

Likewise to fresh and chilled semen, significantly lowest values ($P < 0.05$) of all kinematic parameters (VCL, VSL, VAP, ALH, BCF, LIN, STR, and WOB) for frozen semen were identified in Crosses compared to either Boran or HF. However, in contrast to fresh and chilled semen, significantly highest values ($P < 0.05$) of all kinematic parameters (VCL, VSL, VAP, ALH, BCF, LIN, STR, and

Table 1. Breed influence for fresh semen quality traits.

Semen quality measures	Breed type of the bulls			Sig.
	Boran (N=35)	Cross (N=33)	Holstein Frisian (N=57)	
PH	6.31±0.01 ^a	6.42± 0.02 ^b	6.58± 0.02 ^c	0.000
Volume (ml)	9.81± 0.40 ^a	11.29± 0.44 ^b	11.39± 0.41 ^b	0.023
Concentration	1.03± 0.05	1.16± 0.05	1.16± 0.04	0.094
Mass activity	3.29± ± 0.08	3.24± 0.08	3.35± 0.06	0.545
Individual Motility (%)	77.29 ± 0.75	77.27± 0.76	77.46± 0.55	0.974
Morphological defects				
Head	5.02 ± 0.67 ^b	4.66 ± 0.55 ^{ab}	3.42 ±0.28 ^a	0.030
Mid piece	0.31 ± 0.10	0.35 ± 0.09	0.18 ± 0.05	0.181
Tail	4.70 ± 0.60 ^b	3.16 ± 0.40 ^a	4.91 ± 0.45 ^b	0.038
Proximal droplet	0.53 ± 0.10 ^a	8.63 ± 2.62 ^b	1.01 ±0.21 ^a	0.000
Distal droplet	1.01 ± 0.21	1.30 ± 0.31	0.93 ± 0.21	0.547
Total abnormality	11.57 ± 1.05 ^a	18.10 ± 2.31 ^b	10.44 ± 0.63 ^a	0.000

Mean± SE values across rows with different super scripts are significantly different ($P < 0.05$), N= number of ejaculates.

WOB) were recorded in Boran breed for this stage of semen production (Table 2). Summary of motion characteristics of spermatozoa and motility percentages tested at different stages of semen production is given in Table 3. Fresh semen spermatozoa motion characteristics of medium, medium progressive, and slow types were significantly higher ($P < 0.05$) in Crosses as compared to Boran and HF. In contrast to this; rapid progressive was significantly minimal for this breed. On the other hand; significantly higher ($P < 0.05$) total individual motility percentage was observed in Boran and that of progressive motility in HF.

In case of chilled semen; spermatozoa motion types (medium and slow), non-progressively motile and total individual motility percentages were significantly minimal ($P < 0.05$) for Boran as compared to Cross and HF. Similar to that of fresh semen, most of the spermatozoa were rapid type and the smallest percentage was seen for slow type of motion in all the three breeds. The maximum total and progressive spermatozoa motility percentage for chilled semen was identified in HF.

In line with chilled semen, significantly high total motility percentage (42.9%) for frozen semen was observed in HF. For motion characteristics significant differences ($P < 0.05$) among the breeds were recorded for medium, rapid progressive and slow types. Likewise the fresh and chilled semen; most of the spermatozoa (10.30%) in Crosses were rapid type in their motion characteristics. whereas rapid progressive type was the maximal motion type percentage for Boran and HF. Resembling the fresh and chilled semen; the minimal motion type percentage was seen for slow type in all the three breeds.

DISCUSSION

Initial conventional semen quality assessment of PH,

volume, concentration, morphology and motility of spermatozoa was conducted before the actual ISAS evaluation for kinematic parameters and significant difference among the breeds were found for PH, Volume and morphological defects (head, tail and proximal droplets). Previously, similar significant difference between breeds for semen volume was reported by Lemma and Shemsu (2015) in the same center. But the semen volume and morphological defects (head, mid piece and tail) of this study in all the three breeds were significantly higher than the finding of Hunderra (2004) in the same center. Such variability between reports on semen quality parameters might be attributed to difference in age, breed, nutritional status, season of the year the study covers, method of the semen collection procedure and frequency (Hafez, 1993; Blezinger, 1999; Andrabi et al., 2002).

Regarding ISAS evaluation, as sperm motility is one of the most important features of fertile spermatozoa that reflects several structural and functional competences of spermatozoa and is a readily identifiable test; it was also one of the parameters which were considered for ISAS evaluation in this study. Significantly higher motility percentage (82.50%) was observed for Boran as compared to Cross and HF. Comparable percent motilities of 78.69, 79.41, 79.23 and 76.12% were reported by Lemma and Shemsu (2015) in the same center for HF, Jersey, Boran and Cross breeds of Borana X Holstein Frisian respectively. In this study, though the highest total and progressive motility percentages were been recorded in fresh semen of Boran; a drastic drop in spermatozoa motility percentages during stabilization of the semen was noticed in this breed. This drastic drop in total motility and progressive motility percentage might be due to high peroxidation of fatty acids leading to loss of membrane integrity of the spermatozoa which in turn affect the motility. In addition, the toxic effect of glycerol

Table 2. Kinematic parameters of various bull breeds at fresh, pre-freeze and post-thaw stage (Mean \pm SE, n = number of semen samples).

Semen type	Breed	N	VCL ($\mu\text{m/s}$)	VSL ($\mu\text{m/s}$)	VAP ($\mu\text{m/s}$)	ALH (μm)	BCF (HZ/s)	LIN (%)	STR (%)	WOB (%)
Fresh	Boran	45032	234.5 \pm .44 ^c	73.3 \pm .25 ^b	129.2 \pm .28 ^b	5.0 \pm .01 ^c	19.2 \pm .04 ^b	32.4 \pm .09 ^a	55.2 \pm .12 ^a	55.7 \pm .06 ^a
	Cross	44463	183.2 \pm .43 ^a	58.2 \pm .21 ^a	102.6 \pm .26 ^a	4.1 \pm .01 ^a	16.8 \pm .04 ^a	34.1 \pm .10 ^b	56.2 \pm .13 ^b	57.1 \pm .07 ^b
	HF	63500	229.1 \pm .38 ^b	78.5 \pm .20 ^c	134.7 \pm .24 ^c	4.8 \pm .01 ^b	19.9 \pm .03 ^c	36.5 \pm .08 ^c	58.8 \pm .10 ^c	59.4 \pm .05 ^c
Chilled	Boran	31589	210.7 \pm .57 ^b	61.4 \pm .23 ^b	108.4 \pm .29 ^b	4.9 \pm .01 ^b	15.9 \pm .04 ^b	31.5 \pm .10 ^b	56.6 \pm .14 ^c	53.5 \pm .07 ^a
	Cross	33927	194.0 \pm .56 ^a	52.0 \pm .21 ^a	100.9 \pm .30 ^a	4.5 \pm .01 ^a	15.4 \pm .04 ^a	29.9 \pm .11 ^a	52.4 \pm .14 ^a	53.7 \pm .08 ^a
	HF	54529	216.2 \pm .42 ^c	62.5 \pm .18 ^c	118.5 \pm .24 ^c	4.9 \pm .01 ^c	16.7 \pm .04 ^c	31.3 \pm .08 ^b	54.0 \pm .11 ^b	55.7 \pm .05 ^b
Frozen	Boran	10627	169.3 \pm .85 ^c	63.2 \pm .42 ^c	94.4 \pm .48 ^c	3.9 \pm .02 ^c	17.0 \pm .09 ^c	38.5 \pm .18 ^c	65.5 \pm .23 ^c	57.0 \pm .13 ^c
	Cross	10841	145.7 \pm .82 ^a	49.5 \pm .38 ^a	81.1 \pm .47 ^a	3.4 \pm 0.02 ^a	15.4 \pm .08 ^a	35.4 \pm .20 ^a	59.9 \pm .25 ^a	56.7 \pm .14 ^a
	HF	38680	153.7 \pm .39 ^b	55.9 \pm .20 ^b	86.3 \pm .24 ^b	3.6 \pm 0.01 ^b	16.4 \pm .04 ^b	36.6 \pm .09 ^b	62.5 \pm .12 ^b	56.7 \pm .07 ^b

Column values for each semen type bearing different superscripts are statistically significant ($P < 0.05$), N= number of spermatozoa.

Table 3. Breed influences in motion characteristics of bull spermatozoa at different stages of semen production.

Semen type	Breed	Motion Type (%)					Progressive type (%)		Motile vs Static (%)	
		Med	Med Prog	Rap	Rap Prog	Slow	Prog	Non prog	Motile	Static
Fresh	Boran (N=38)	4.0 \pm .7 ^a	3.2 \pm .6 ^a	42.9 \pm 3.2 ^b	31.5 \pm 2.5 ^b	0.8 \pm .2 ^a	34.7 \pm 2.6	47.8 \pm 3.0	82.5 \pm 1.4 ^b	17.5 \pm 1.4 ^a
	Cross (N=37)	8.9 \pm 1 ^b	7.4 \pm .9 ^b	34.8 \pm 3.3 ^a	24.4 \pm 2.1 ^a	1.3 \pm .2 ^b	31.8 \pm 2.3	45.1 \pm 2.9	76.8 \pm 1.5 ^a	23.2 \pm 1.5 ^b
	HF (N=60)	5.5 \pm .5 ^a	4.6 \pm .6 ^a	37.4 \pm 2.0 ^a	30.4 \pm 1.7 ^b	0.9 \pm .1 ^a	35.1 \pm 1.7	43.8 \pm 2.0	78.9 \pm 1.2 ^a	21.1 \pm 1.2 ^{ab}
Chilled	Boran (N=38)	5.9 \pm .6 ^a	3.8 \pm .7	31.4 \pm 2.9	15.9 \pm 1.5	0.9 \pm .1 ^a	19.7 \pm 1.7	38.2 \pm 2.6 ^a	57.9 \pm 2.8 ^a	42.1 \pm 2.8 ^b
	Cross (N=37)	9.8 \pm 1.1 ^b	5.2 \pm .7	33.8 \pm 2.8	17.0 \pm 1.6	1.3 \pm .1 ^b	22.1 \pm 1.9	44.9 \pm 2.5 ^b	67 \pm 1.7 ^b	33.0 \pm 1.7 ^a
	HF (N=60)	7.9 \pm .6 ^{ab}	3.7 \pm .4	37.9 \pm 2.2	19.7 \pm 1.4	1.1 \pm .1 ^{ab}	23.4 \pm 1.4	46.8 \pm 2.0 ^b	70.2 \pm 2.0 ^b	29.8 \pm 2.0 ^a
Frozen	Boran (N=35)	4.8 \pm .6 ^a	3.4 \pm .5	11.0 \pm 1.5	13.4 \pm 1.3 ^b	1.2 \pm .1 ^a	16.9 \pm 1.3 ^b	17.0 \pm 2.0	33.9 \pm 2.6 ^a	66.1 \pm 2.6 ^b
	Cross (N=35)	7.8 \pm .9 ^b	4.0 \pm .5	10.3 \pm 1.5	8.9 \pm 1.3 ^a	2.1 \pm .2 ^b	12.9 \pm 1.5 ^a	20.2 \pm 2.2	33.1 \pm 3.1 ^a	66.9 \pm 3.1 ^b
	HF (N=57)	6.8 \pm .5 ^b	4.6 \pm .4	13.5 \pm 1.2	16.4 \pm 1.3 ^b	1.7 \pm .1 ^b	21.0 \pm 1.3 ^c	22.0 \pm 1.5	42.9 \pm 2.3 ^b	57.1 \pm 2.3 ^a

Column values for each semen type bearing different superscripts are statistically significant ($P < 0.05$). N= number of ejaculates, Med = Medium, Prog = Progressive and Rap = Rapid.

for this breed of spermatozoa might be higher during this stage of production (Maxwell and Watson, 1996; Kadirvel et al., 2009; Muhammad

et al., 2010). Moreover, the release of phospholipids that is important for production of reactive oxygen species (ROS) which in turn could

be toxic for the normal spermatozoa might be high in this stage of production for this breed. A comparable mean percent motility of 78.49% for

fresh semen of Sahiwal breed was reported by Ulfina (2014). However, Keshav (1996) reported significantly lower (65.22%) mean motility for Sahiwal breed than the current study. On the other hand, a higher mean motility percentage of 94.3% was reported by Sundararaman et al. (2012) in Jersey bulls. In chilled semen though significant difference was not observed between Cross and HF; significantly lower motility percentage (57.90%) was observed in Boran. And to that of frozen semen; though no significant difference was recorded between Cross and Boran breeds; significantly higher motility percentage (42.90%) was observed in HF breed. As a result of accumulated cellular injuries that arise throughout the cryopreservation process the minimum spermatozoa motility percentages in frozen semen were seen in Boran and Cross breeds and these motility percentages were in agreement with the study of Amanda (2011). Whereas the total spermatozoa motility percentage of HF in this study was comparable to the study conducted by Lenz et al. (2010) but it was significantly higher than their finding at 2 and 4 h of post freezing. Though the total and progressive motility percentages of this study lies in the ranges of total and progressive motility percentages of Keshav (1996); her mean values were higher than this study finding. Sundararaman et al. (2012) also reported a higher motility percentage for chilled (89.4%) and frozen (63.0%) semen in Jersey bulls. These possible variations between studies could be due to the reasons stated above for fresh semen.

When observing the velocity parameters; all kinematic parameters (VCL, VSL, VAP, ALH, BCF, LIN, STR, and WOB) were significantly different ($P < 0.05$) in all stages of semen production among the three breeds. Significantly lowest values ($P < 0.05$) of all kinematic parameters (VCL, VSL, VAP, ALH, BCF, LIN, STR, and WOB) in all stages of semen production were identified in Crosses compared to either boran or HF. The kinematic parametric values of this study were lower than the findings of Keshav (1996) and Sundararaman et al. (2012). In contrast all the kinematic parametric values in this study were higher than the results of Amanda (2011) and Ulfina (2014) in all stages of production. The overall mean results of other kinematic parameters (ALH, BCF, LIN, STR and WOB) of this study were nearly comparable to the findings of Keshav (1996) and Sundararaman et al. (2012). However, the variation of STR, LIN and WOB between studies could also come from their functions; hence, existence of noticeable differences for velocity parameters would bring variation most likely to be seen in these ratios too. Amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) in this study were comparable to the findings of Budworth et al. (1988) and Waterhouse et al. (2010). In contrast to spermatozoa motility percentages, the kinematic parameter values of post-thawed semen were significantly higher in Boran compared to either Cross or HF breeds. This indicates

that, nevertheless the rate of drop for motility percentage was relatively high at time of stabilization for Boran breed; at last those of the spermatozoa tolerating the stages of production were more active for their kinematic activities in this breed. Numerous effects of spermatozoa cryopreservation induce range of injuries from lethal to those which merely impair their subsequent functions. For this reason, the motility and or kinematic parameter value variations between breeds might be due to variations in age, degree of sperm maturation, energy stores (ATPase), viscosity of the fluids negotiated by the sperm, the presence of surface-active agents in the cell membrane (agglutinins), osmolarity, pH, ionic composition of seminal plasma and possibly substances (Cu, Zn, Mn, Hg, hormones, kinins and prostaglandins) that stimulate or inhibit sperm motility (Farrell et al., 1998). Likewise numerous factors like temperature at which semen is analyzed, concentration of spermatozoa analyzed, type of extender used, percent motility and digitization threshold, sampling technique, method of processing, time elapsing between sampling and analysis, the accuracy of the specimen chambers used and the number of chambers, fields and spermatozoa examined could be the factors that brought variations between studies (Blasco, 1984). Based on the multi parametric objective semen quality assessment findings of this study; breed was one of the factors that influence spermatozoa motility and motion characteristics at different stages of semen production. Unexpectedly in this study, the rate of motility percentage drop at stage of chilling for Boran breed was relatively high and comparable to its freezing stage; therefore; further studies with additional tests (acrosome and plasma membrane integrity) at each stage of production are required to reformulate the equilibration protocol for this breed of semen. Moreover, as it was stated by Davis and Katz (1993) subjective spermatozoa motility is not reliable assay for the prediction of fertility. Therefore, under taking objective, repeatable, accurate and rapid tests for the semen quality assessment using ISAS for multi parametric evaluation like spermatozoa motility, motion characteristics, and swimming pattern can increase the prediction of its fertility and potentially saves considerable amounts of money for the country in screening the sub-fertile bulls in the semen production centers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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