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Effect of breed, age and period of production on bovine semen quality used for artificial insemination

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Various factors are believed to influence quality of semen in breeding bulls. The main objective of this study was to evaluate how breed, age and time of semen production are relevant in the production of good quality semen used for Artificial Insemination. A total of 16,827 ejaculates from 187 bulls (Holstein Frisian = 114, Jersey = 35, Borena = 12, 50%HF × 50%Borena = 5, 75%HF × 25%Borena = 15 and Fogera = 6) were collected and examined at the National Animal Genetic Improvement Institute (NAGII). All these factors (breed, age and period of production) had significant ($p \leq 0.05$) effect on semen volume, colour, concentration, mass activity, individual motility and production doses/ejaculate. All these semen quality measures were significantly ($p \leq 0.05$) minimal in 50%HF × 50%Borena crosses and all of them except concentration were observed superior in HF. Excluding ejaculate volume and production doses, all the semen quality measures were recorded better in the first two age classes (less than 2 and 2 to 4 years of age classes). Significance differences in ejaculate volume, concentration, mass activity, and individual motility were observed among production periods; these disparities could be due to the variation in managemental practice and efficiency of implementing the protocol in selecting candidate bulls for the time period. In conclusion, breed, age and period of semen production have significant effect on bovine semen characteristics.

Key words: Borena, cross breeds, Fogera, Holstein Frisian, Jersey, semen characteristics.

INTRODUCTION

Ethiopia has abundant (61.59 Million) cattle population reared under diverse agro climates. However, of this population97.66% are unimproved local breeds and only 2.34% are considered to be cross (2.0%) and exotic (0.34%) breeds (CSA, 2020). Although, the country has this huge livestock potential and is being ranked to be the first in Africa; yet its contribution to the economy is limited and remained to be quantitative boosting (Amha, 2008). This limited contribution is associated with a number of complex and inter-related factors like poor genetic potential of local breeds, inadequate feed and its seasonality, widespread diseases and inefficiency of livestock development services with respect to credit, extension, marketing, and infrastructure(Jabbar et al., 2007; Negassa et al., 2011). In Ethiopia, Holstein Frisian (HF), Crosses of 75% HF × 25% Borena and 50% HF ×

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50% Borena, Jersey, Borena and Fogera breeds have been used to improve dairy productivity through artificial insemination (AI) and nearly a million of semen doses are produced annually at the National Animal Genetic Improvement Institute (NAGII) for dairy genetic improvement. As it was stated by Desalegn et al. (2009), although there are some encouraging achievements of AI in the country, the success rate was not as it is expected; hence, constrained by a number of factors which in turn have effects on the quality of semen produced and its fertility during application. Therefore, this study was designed to investigate the effects of breed, age and period of production on semen quality parameters which could be some of the factors for the low success rate of AI in the country.

MATERIALS AND METHODS

Study animals and management

Records of 187 bulls (Holstein Frisian = 114, Jersey = 35, Borena = 12, 50%HF × 50%Borena = 5, 75%HF × 25%Borena = 15 and Fogera = 6) were considered and a total of 16,827 ejaculates (HF = 11172, Jersey = 2955, Borena = 424, 50%HF × 50%Borena = 690, 75%HF × 25%Borena = 1473 and Fogera = 113) collected from these breeding bulls for fifteen years (2005 - 2019) at the National Animal Genetic Improvement Institute (NAGII) were used in this study. The information recorded were bull identification number, breed, date of birth, date of collection, ejaculate volume (ml), semen colour, mass activity (scale: 1-4), concentration (10^9 spz/ml), percentage of motile sperm at fresh, pre freeze and post freeze of 0 and 24 h and production doses/ejaculate. The bulls were kept indoor and under identical conditions of management, feeding and watering. They received hay, green forage and concentrate fortified with vitamins and minerals. Water was given *ad libitum*. They were allowed to exercise on running track weekly. Teaser bull was used during the collection of semen using Artificial Vagina (AV).

Age of the bulls were classified into four age classes (A1, < 2 years; A2, ≥ 2 to < 4 years; A3, ≥ 4 to < 6 years and A4, ≥ 6 years) and the factor production period (years of semen collection) was grouped into five (period-1=2005-2007, period-2=2008-2010, period-3=2011-2013, period-4=2014-2016 and period-5=2017-2019).

Semen preparation and evaluation

Semen was collected in the morning once a week from the bulls using sterilized artificial vagina (IMV; France) maintained at 45°C. Immediately after collection, volume of the ejaculate was measured with the aid of graduated collection tube. Colour of individual bull semen was evaluated by visual appraisal and recorded as creamy, milky, yellowish and watery. Sperm concentration was determined by using spectro photometer method. Evaluations of mass activity and individual motility were performed as described by Hafez (1993). Mass activity assessment was conducted by putting 5 µl of undiluted semen on pre warmed slide and examining it with stage warmed microscope at 37°C using a low magnification (100x). It was scored into 1 - 4 scales: 1= weak motion without forming any wave; 2= small, slow moving wave; 3= vigorous movement with moderate rapid waves and eddies and 4= dense, very rapidly moving waves and eddies. Fresh individual motility was estimated by placing 5 µl of undiluted semen on pre warmed slide and covering it with pre warmed coverslip (37°C) as percentage of spermatozoa. The sperm cells exhibiting progressive movements under stage warmed (37°C) phase contrast microscope at a magnification of 200X scored 0 to 100%. After the approval for further process, the extended semen was filled and sealed into 0.25 ml straws that were marked with the proper identification, and the straws were allowed to lay on ranks placed in a stabilizer-refrigerator at +4°C for 4 h of equilibration. After which the semen straws were transferred to a bio-freezer that gradually cools the semen to -140°C. Finally, before the semen is plunged into liquid nitrogen for long-term storage, post freezing individual motility was checked by randomly thawing 0.25 ml frozen semen straw of each bull at 37°C for 30 s in a water bath.

Post freezing individual motility evaluation was conducted at 0 and 24 h. Any sample showing below 40% individual motility was discarded. The semen straws passing this evaluation were kept in goblets and stored in liquid nitrogen at -196°C until dispatched for AI.

Statistical analysis

The data obtained were entered to Microsoft Excel sheet and Statistical Package for Social Science (SPSS) for windows version 20 (SPSS, 2011) was used for analysis. The data of ejaculate volume, concentration, mass activity, individual motility and Production doses/ejaculate were analysed using General Linear Model (GLM) for factorial experiments. Duncan's Multiple Range Test (DMRT) and Chi square test were used to make specific comparison for significantly different fixed effects (breed, age and period of production) at a probability level of 5%. In the analysis, $p \leq 0.05$ was set for level of significance. To evaluate the effect of breed, age and period of production on semen quality traits, the following model was fitted for analyses.

$$Yijk = \mu + Bi + Aj + Pk + eijk$$

where Yijk - observed semen characteristics; µ - overall mean; B_i - fixed effect of the i^{th} breed (i=1- 6); Aj - fixed effect of the j^{th} age class (j=1- 4); P_l - fixed effect of the l^{th} period at collection (l=1- 5); and e_{ijk} - a random residual effect.

RESULTS

Effect of breed

As illustrated in Table 1 significant ($p \leq 0.05$) differences for ejaculate volume, concentration, mass activity, individual motility and production doses/ejaculate were recorded among breeds. All these semen quality measures were significantly ($p \leq 0.05$) minimal in 50%HF × 50%Borena crosses. Significantly ($p \leq 0.05$) higher ejaculate volume was recorded in HF and 75%HF × 25%Borena crosses. In line with this, significantly ($p \leq 0.05$) higher production doses of semen were recorded in HF.

In contrast, significantly ($p \leq 0.05$) higher sperm concentration was detected in Borena semen. Significantly ($p \leq 0.05$) higher mass activity and fresh individual motility percentage were observed in local zebu (Borena and Fogera) and exotic (HF and Jersey) breeds as compared to cross breeds. However, post freezing individual motilities at 0 and 24 h were significantly ($p \leq 0.05$) higher for exotic (HF and Jersey) breeds.

Table 1. Effect of breed on bull semen quality.

Semen parameter	Breed type of bulls					
	Borena (N= 424)	50%HF×50% Borena (N=690)	75%HF×25% Borena (N=1473)	Fogera (N=113)	HF (N=11172)	Jersey (N=2955)
Volume (ml)	7.11±.18^{b}	6.39±.18^{a}	10.34±.16^{d}	7.33±.32^{b}	10.44±.15^{d}	9.60±.16^{c}
Concentration (10^{9} spz/ml)	1.25±.02^{c}	1.09 ±.02^{a}	1.05±.02^{a}	1.14 ±.04^{b}	1.16±.02^{b}	1.15 ±.02^{b}
Mass activity	3.48±.03^{b}	3.27±.03^{a}	3.28±.03^{a}	3.46 ±.05^{b}	3.47 ±.02^{b}	3.46 ±.03^{b}
Fresh motility (%)	78.92±.17^{c}	76.12±.17^{a}	78.37±.15^{b}	79.20±.29^{c}	79.11±.14^{c}	79.30 ±.14^{c}
Pre freeze motility	76.73±.26^{cd}	74.37±.26^{a}	75.97±.23^{b}	76.50 ±.44^{bc}	77.19±.21^{d}	76.83±.22^{cd}
Post freeze motility at 0 h	51.75 ±.37^{a}	52.37±.37^{a}	52.21 ±.33^{a}	52.26±.64^{a}	53.37±.30^{b}	53.40 ±.31^{b}
Post freeze motility at 24 h	51.75±.37^{a}	52.01 ±.37^{a}	51.90±.33^{a}	51.33±.64^{a}	53.08 ±.30^{b}	53.09±.32^{b}
Production doses/ejaculate	257±8^{b}	191±8^{a}	314 ±7^{c}	259±14^{b}	349±6^{d}	318±7^{c}

Mean ± SE values across rows with different super scripts are significantly different (P < 0.05).

Table 2. Effect of age on bull semen quality.

Semen parameter	Age class of bulls (years)			
	< 2 (N=1037)	2 ≥to<4 (N=9350)	4 ≥ to <6 (N=4686)	≥6 (N=1754)
Ejaculate Volume (ml)	8.75±0.10^{a}	9.72±0.10^{b}	10.88±0.10^{d}	10.04±0.12^{c}
Concentration(10^{9} spz/ml)	1.14 ±0.01^{ab}	1.16 ±0.01^{b}	1.12 ±0.01^{a}	1.16±0.01 ^{b}
Mass activity	3.48±0.02^{b}	3.46 ±0.02^{b}	3.41 ±0.02^{a}	3.42±0.02^{a}
Fresh motility (%)	79.36 ±0.09^{c}	79.01±0.09^{b}	78.88 ±0.09^{b}	78.55±0.11^{a}
Pre freeze motility (%)	77.33 ±0.14^{b}	76.93 ±0.14^{a}	76.76±0.14^{a}	76.76±0.16 ^{a}
Post freeze motility at 0 h (%)	53.69 ±0.20^{c}	53.21 ±0.20^{b}	52.76±0.21^{a}	53.88±0.24^{c}
Post freeze motility at 24 h (%)	53.50±0.20^{c}	53.00±0.20^{b}	52.46 ±0.21^{a}	53.10±0.24^{b}
Production doses/ejaculate	275 ±0.4^{a}	319 ±4^{b}	360±4^{d}	349±5 ^{c}

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

Effect of age

The present study demonstrates that age had significant ($p \leq 0.05$) effect on all semen quality measures: ejaculate volume, concentration, mass activity, individual motilities of all stages of production and production doses/ejaculate (Table 2).

Effect of Production period

The factor period of production significantly ($p \leq 0.05$) affected ejaculate volume, mass activity, all stages of individual motilities and production doses/ejaculate (Table 3). Both mass activity and all stages of individual motilities were significantly ($p \leq 0.05$) low for production years of 2017 to 2019. Whereas, significantly higher (*P < 0.05*) values of these semen quality measures were recorded for production years of 2008 to 2010. On the contrary, ejaculate volume and production doses/ ejaculate were significantly ($p \leq 0.05$) minimal in these years of production. Ejaculate volume and production doses/ejaculate were significantly ($p \leq 0.05$) immense in semen production years of 2014 to 2016. Sperm concentration was significantly ($p \leq 0.05$) low for two consecutive periods (2011 - 2016) of semen production. Significantly ($p \leq 0.05$) high sperm concentration was recorded in 2005 to 2007 production years. As presented in Table 4, though significant difference of semen colour among breeds, age classes and periods of semen production were recorded; in all breeds, age classes and periods of semen production, milky was the predominant frequently observed semen colour followed by creamy. In contrast, watery semen colour was observed less frequently for all fixed factor comparisons. Higher percentages of 46.2 and 47.8% creamy semen colour were observed in Borena and Fogera breeds, respectively. This creamy colour was less frequent (17.2% and 21.6%) in 50%HF × 50%Borena and 75%HF × 25%Borena crosses, respectively. Regarding age of the bulls, the minimal creamy semen colour percentage (26.3%) was recorded for semen producing bulls older

Table 3 . Effect of period of semen production on bull semen quality.

Semen parameter	Period of semen production				
	2005-2007 (N= 636)	2008-2010 (N=3733)	2011-2013 (N=5010)	2014-2016 (N=5111)	2017-2019 (N=2337)
Ejaculate Volume (ml)	8.45±0.13^{b}	7.03±0.13^{a}	9.32±0.13^{c}	12.56±0.13^{e}	11.12±0.13^{d}
Concentration(10^9 spz/ml)	1.40±0.02^{d}	1.24±0.02^{c}	1.08±0.02^{a}	1.09±0.02^{a}	1.19±0.02^{b}
Mass activity	3.67±0.02^{d}	3.65±0.02^{d}	3.44±0.02^{c}	3.37±0.02^{b}	3.24±0.02^{a}
Fresh individual motility (%)	78.03±0.12^{a}	78.88±0.12^{b}	78.98±0.12^{b}	79.50±0.12^{c}	78.06±0.12^{a}
Pre freeze motility (%)	76.33±0.18^{b}	78.17±0.18^{d}	77.47±0.18^{c}	76.20±0.18^{b}	75.25±0.18^{a}
Post freeze motility at 0 h (%)	57.73±0.26^{d}	57.59±0.26^{d}	53.86±0.26^{c}	51.11±0.26^{b}	48.00±0.26^{a}
Post freeze motility at 24 h (%)	56.79±0.26^{e}	54.74±0.26^{d}	53.86±0.26^{c}	51.11±0.26^{b}	47.99±0.27^{a}
Production doses/ejaculate	367 ±6^{c}	258 ±6^{a}	285±5^{b}	395±5^{d}	396±6^{d}

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

Table 4. Semen colour variation (in %) among breeds, age classes and periods of semen production.

Evaluation parameter	Semen colour type (%)				χ^2
	Creamy	Milky	Watery	Yellowish	
Breed					
Borena (N= 424)	46.2	53.8	0.0	0.0	
HF × Borena (N= 690)	17.2	62.1	0.1	20.6	
HF × HF × Borena (N= 1473)	21.6	78.1	0.1	0.2	0.000
Fogera (N=113)	47.8	52.2	0.0	0.0	
HF (N= 11172)	33.1	58.6	0.1	8.2	
Jersey (N= 2955)	33.2	66.4	0.0	0.4	
Age class (years)					
< 2 (N=1037)	30.2	62.7	0.1	7.0	
2 ≥ to < 4 (N= 9350)	32.9	57.8	0.1	9.2	0.000
4 ≥ to < 6 (N= 4686)	32.3	65.1	0.1	2.5	
≥ 6 (N=1754)	26.3	72.7	0.1	0.9	
Period of semen production					
2005-2007 (N= 636)	36.2	46.1	0.2	17.6	
2008-2010 (N= 3733)	26.0	63.3	0.3	10.4	
2011-2013 (N= 5010)	28.3	64.4	0.0	7.3	0.000
2014-2016 (N= 5111)	33.6	62.5	0.0	3.9	
2017-2019 (N= 2337)	44.0	55.8	0.0	0.2	

N= number of ejaculates.

than 6 years. Relatively higher yellowish semen colour of 17.6% observed 2005 to 2007 production years.

DISCUSSION

In this study, breed affected significantly ($p \leq 0.05$) semen ejaculate volume, concentration, mass activity, individual motilities and production doses/ejaculate. These semen quality measures were significantly ($p \leq 0.05$) minimal in 50%HF × 50%Borena crosses. Although significant differences among certain breeds for some semen quality measures were not recorded, all semen quality measures except concentration were observed superior in HF breed. Breed differences on several semen parameters have been previously reported (Hafez, 1993; Brito et al., 2002). As it was stated by Bang (2008), Holstein Friesian breed is known to give best results compared to other breeds which were also in close agreement with the current findings. Previously, different authors (Hunderra, 2004; Sinishaw, 2005; Desalegn et al., 2009; Demeke, 2010; Lemma, 2011;

Lemma and Shemsu, (2015) have also reported the semen characteristics difference among cattle breeds at the same AI center. But the finding of Belayneh (2018) did not state this breed difference which might be due to short-term study period and a smaller number of observations in his study.

Like breed, age had also significant ($p \leq 0.05$) effect on all semen quality measures: ejaculate volume, concentration, mass activity, individual motilities and production doses/ejaculate. Except ejaculate volume and production doses, all the semen quality measures were recorded better in the first two age classes (less than 2 and 2 to 4 years of age classes). Volume and production doses per ejaculate tend to increase with age of the bull up to the third class of age (6 years). Better semen quality in mature bulls compared to younger and older bulls may probably be due to scrotal circumference and the heat regulation mechanisms, which increases with age until the rate of broken down for testicular tissues become faster than being replaced (King, 1993; Brito et al., 2002). Moreover, effect of age is also influenced by managemental conditions. As artificial insemination centers are highly interested in producing a maximum number of straws, less demanded bulls might not reach at a higher age. Therefore, it can be emphasized that bull management in the younger age also had significant effect in quality semen productivity. The effect of age also attributed to fat deposition in the scrotum, which increases with age and can lower the efficiency of scrotal thermo-regulation by reducing the amount of heat that can be radiated from the scrotal neck; and hence inferior semen quality will be produced in old bulls (Barth and Oko, 1989; Coulter et al., 1997; Kommisrud and Berg, 1996). Time of sexual preparation also have significant effects on ejaculate volume and number of doses per ejaculate. This finding is in close agreement with the findings of Hunderra (2004), Sinishaw (2005), Desalegn et al. (2009), Demeke (2010), Lemma (2011), Lemma and Shemsu (2015).

In this study period of production had also significant ($p \leq 0.05$) effect on ejaculate volume, mass activity, all stages of individual motilities and production doses/ejaculate. The significant difference of ejaculate volume, concentration, mass activity, and individual motilities among different production periods might be due to variation in managemental practice and efficiency of implementing the protocol in selecting candidate bulls for the time period.

CONCLUSION AND RECOMMENDATION

The results of this study clearly demonstrated that breed, age and period of semen production have significant effect on bovine semen characteristics. Holstein Frisian semen was best almost for all semen quality measures followed by Jersey; on the other hand, semen quality of cross breed bulls (in particular 50% HF × 50% Borena) was significantly inferior ($P < 0.05$) in all semen quality measures. The mid two age classes (matured bulls up to 6 years of old) were able to produce better quality semen which might be due to better testicular and preferential managemental conditions. Therefore, to produce good quality bovine semen, NAGII shall work tough giving emphasis for breeds of HF and Jersey, matured bulls up to 6 years of age.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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