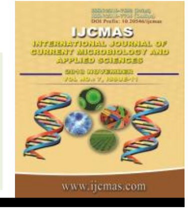
Int.J.Curr.Microbiol.App.Sci (2018) *7*(11): 3219-3229

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 11 (2018)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2018.711.370

Effect of Non-genetic Factors on Semen Quality Traits of Crossbred Holstein Friesian Bulls (*Bos taurus* X *Bos indicus)* in Organized Farming Conditions at Tamil Nadu, India

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ABSTRACT

Keywords

CBHF bulls, Semen production, Non-genetic factors and semen quality

Article Info

Accepted:
26 October 2018
Available Online:
10 November 2018

The objective of the study was to assess the effects of non-genetic factors such as farm, order of ejaculate, period, season of collection and age of the bull on semen quality traits of crossbred Holstein Friesian (CBHF) bulls. Data on a total of 22442 ejaculates from 72 CBHF bulls collected during the period from 1996 to 2014 were obtained from three organized farms in Tamil Nadu, India. The overall least-squares means for semen volume (SV), sperm concentration (SC), mass activity (MA), initial sperm motility (ISM), post-thaw motility (PTM) and number of doses per ejaculate were 4.53 ± 0.05 ml, 1081.33 ± 15.59 millions per ml, 2.54 ± 0.02, 70.00 ± 0.00 per cent, 52.48 ± 0.00 per cent and 221.82 ± 1.54 doses respectively. The fixed effects such as farm, period and age of the bull; and interaction of fixed effects of farm x season and age x season were highly significant ($P<0.01$) for all the semen quality traits. The order of ejaculate was highly significant ($P<0.01$) on PTM and number of doses per ejaculate. SC and ISM were significantly ($P<0.01$) higher during winter season. Based on the results, it could be concluded that farm I, first ejaculate, period-IV, winter season and 118 to 142 months of age, the CBHF bull produced comparatively best quality of semen.

Introduction

In India, cross breeding is practiced to improve cattle productivity by crossing the indigenous cattle (*Bos indicus*) with temperate (*Bos taurus*) breeds. In 1891, the crossbreeding was first started in dairy farms of British Indian Army with exotic cattle breeds such as Jersey, Holstein Friesian (HF) and Brown Swiss to improve native cattle breeds of Sahiwal, Hariana, Tharparkar, Sindhi, Gir and non-descript cattle population, since then the population of exotic and their crossbred cattle in India has been increasing.

As per the 19th Livestock Census (2012), the total number of cattle in the country is 190.90 million. The exotic/crossbred cattle population increased from 14.40 million in 2007 to 19.42 million in 2012, giving rise to an increase of 34.78 per cent whereas the indigenous cattle increased marginally from 48.04 to 48.12 million, an increase of 0.17 per cent only. The share of Tamil Nadu to the total cattle population in the country is 4.61 per cent (8.8 million) with the exotic/crossbred and indigenous breeds of 5.4 and 1.7 million respectively. Among exotic breeds, Jersey and Holstein Friesian (HF) have been found to be more suitable for crossbreeding in Tamil Nadu. The increase in number of exotic/crossbred cows necessitated the huge demand of quality frozen semen of purebred and crossbred bulls over the years. The semen quality parameters were influenced by breed (Mukhopadhyay *et al.*, 2010); environment (Mandal *et al.*, 2008) and age of the bull (Mandal *et al.*, 2010), but these factors are not reliable as their effects differ from one environment to another, which makes assessment of semen production difficult. Therefore, all the frozen semen stations in India are following a protocol for Minimum Standard for Production (MSP, 2012) of bovine frozen semen, which reduces the variable management and semen processing conditions and improves the quality of frozen semen. Hence, more emphasis needs to be given for continuous supply of frozen semen from CBHF bulls in required areas of the state. But, there is inadequate information on the performance of CBHF bulls even though such crossbred bulls are being used for decades for production of frozen semen in Tamil Nadu. Therefore, the present study was designed to investigate the effect of various non-genetic factors on semen quality traits in CBHF bulls. This study is first of its kind in southern India, with respect to HF inheritance, since HF bulls were predominately used in northern India.

Materials and Methods

Farms

Evaluation of semen quality traits was carried out in three farms situated in Tamil Nadu, such as

(i) District Livestock Farm

(ii) Nucleus Jersey and Stud Farm, both situated in 11^0 24’N and 76^0 42’E with an altitude of 2460 to 2662 metres above mean sea level (MSL)

(iii) Exotic Cattle Breeding Farm, Eachenkottai situated in 10^0 45’N and 79^0 29’E with an altitude of 50 metres above MSL.

Data collection and structure

From these three farms, data on a total of 22442 ejaculates from 72 CBHF bulls collected during the period from 1996 to 2014 were obtained.

The semen production data comprising of bull number, date of birth, date of semen collection, order of ejaculation, semen volume (SV), sperm concentration (SC), mass activity (MA), initial sperm motility (ISM), post-thaw sperm motility (PTM) and number of doses per ejaculate were collected.

Classification of data

The data were analyzed to study the effects of farms (I, II and III), order of ejaculate (I and II), periods (period I-1996 to 1998, II-1999 to 2001, III-2002 to 2004, IV-2005 to 2007, V-2008 to 2010 and VI- 2011 to 2014), seasons [winter (December, January and February), summer (March, April and May), southwest monsoon (June, July and August) and northeast monsoon (September, October and November)] and age of the bulls (age group I-18 to 42 months, II-43 to 67 months, III-68 to 92 months, IV-93 to 117 months, V-118 to

142 months and VI-more than 143 months).

Analysis of semen quality traits

To evaluate the effect of non-genetic factors on semen quality traits, the following model was fitted using the univariate analyses under general linear model of Statistical Package Software (SPSS Version 17; SPSS Inc. Chicago, IL).

$$Y_{ijklmo} = \mu + F_i + E_j + P_k + S_l + A_m + (FS_i)_{il} + (ES)_{jl} + (AS)_{ml} + (AE)_{mj} + e_{ijklmo}$$

Where, Y_{ijklmo} is the semen quality trait of o^{th} observation belonging to i^{th} farm, j^{th} ejaculate, k^{th} period, l^{th} season and m^{th} age effects. μ is the overall mean; F_i is the fixed effect of the i^{th} farm (i=1 to 3). E_j is the fixed effect of j^{th} ejaculate (j=1 and 2); P_k is the fixed effect of k^{th} period (k=1 to 6). S_l is the fixed effect of l^{th} season (l= 1 to 4) and A_m is the fixed effect of m^{th} age of the bull (m=1 to 6). $(FS)_{il}$ is the interaction of fixed effects between i^{th} farm and l^{th} season; $(ES)_{jl}$ is the interaction of fixed effects between j^{th} ejaculate and l^{th} season; $(AS)_{ml}$ is the interaction of fixed effects between m^{th} age and l^{th} season; $(AE)_{mj}$ is the interaction of fixed effects between m^{th} age and j^{th} ejaculate and e_{ijklmo} is a random residual effect.

The differences between the least-squares means for sub-classes under a particular effect were tested by using Scheffe test (1959) for their significance. The semen quality traits (in percentages) such as ISM and PTM were adjusted after angular transformation of the percentages as per Snedecor and Cochran (1987).

Results and Discussion

Semen quality traits

The least-squares means and the least-squares ANOVA (mean squares) for semen quality traits are given in Tables 1 and 2 respectively.

Semen volume (SV)

The overall least-squares mean for semen volume was 4.53 ± 0.05 ml, which was significantly (P<0.01) influenced by the fixed effects viz. farm, period and age of the bull; and interaction effects of farm x season; age x season and age x ejaculate. Of the three farms, farms I and II recorded the highest SV (4.61 ± 0.06 ml). SV was found be significantly more in period III (2002 - 2004) when compared to other periods which might be due to selection of superior bull calves in previous periods and improved management apart from the other environmental factors. The SV increased proportionately as the age advanced (3.34 ml to 5.81 ml). Even at the age of 12 years, the bulls were able to give the highest volume of semen. Quite interestingly, the season did not influence the SV. It is the general perception that animals with HF inheritance would perform better only at cooler climate and high altitude in India. But this age-old perception is proved wrong now in understanding that the CBHF bulls would perform equally well, even in the plains if the management is good. This explanation is also supported by significant farm x season interaction. The SV was not affected by winter and summer seasons in all the three farms, because of better summer management in farm I (plain region) and better winter management in farms II and III (hilly region).

Perusal of available literature revealed higher SV of 6.22 ml (Haque *et al.*, 2001), 4.70 ml (Andrabi *et al.*, 2002), 5.62 ml (Haq *et al.*, 2003), 4.60 ml (Sugulle *et al.*, 2006), 6.40 ml (Shaha *et al.*, 2008), 4.73 ml (Mukhopadhyay *et al.*, 2010), 4.60 ml (Mandal *et al.*, 2012), 6.68 ml (Patel *et al.*, 2012), 5.80 ml (Khatun *et al.*, 2013) and 5.60 ml (Srivastava and Kumar. 2014) in CBHF bulls. Some authors had reported lower SV of 4.06 ml (Mathur *et al.*, 2002), 4.01 ml (Nasrin *et al.*, 2008) and 3.59

ml (Mandal and Tyagi. 2009). The wider differences in SV reported for CBHF bulls in India and other neighbouring countries could be due to the type of indigenous breed or zebu cattle used for crossing with HF and to some extent the differences in bull managment.

Period effect was found to be significant on SV (Bhakat *et al.,* 2009; Chauhan *et al.,* 2010; Khatun *et al.,* 2013). Many authors reported that season of collection significantly influenced the SV (Andrabi *et al.,* 2002; Asad *et al.,* 2004; Mandal *et al.,* 2008) with favoured season being winter. Whereas the SV was not affected by the season as found in the present study (Sarder *et al.,* 2000; Mathur *et al.,* 2002 and Sarder, 2007). In consonance with the present observation, the SV was reported to have increased as the age of the bulls advanced (Sudheer, 2000; Asad *et al.,* 2004; Jain *et al.,* 2008 and Mandal *et al.,* 2010) in CBHF bulls.

Sperm concentration (SC)

The least-squares mean SC was 1081.33 ± 15.59 million per ml and this trait was affected significantly (P<0.01) by the farm, period, season and age of the bull. The interaction effects of farm x season, age x season and age x ejaculate were significant on SC while ejaculate x season was not significant. The highest SC was seen in farm I even though the mean semen volume was also high. The first ejaculate had a higher SC (1137.96 ± 21.30) than the second ejaculate (1024.69 ± 21.92) but did not differ significantly. Highest SC was noticed in period IV (1295.79 ± 18.53) and winter season (1103.59 ± 12.63). The highest SC was observed during 43 to 67 months of age (1183.37 ± 7.56) after that it decreased as age advanced and showed negative correlation with SV.

Earlier workers reported both higher SC (Mathur *et al.,* 2002; Sugulle *et al.,* 2006; Sarder. 2007; Mandal *et al.,* 2008; Patel *et al.,* 2012 and Akhter *et al.,* 2013) and lower SC (Haque *et al.,* 2001; Nasrin *et al.,* 2008; Kumar and Srivastava. 2008; Mandal and Tyagi. 2009 and Srivastava and Kumar. 2014) in CBHF bulls than that obtained in the present study. Period effect was found to be significant on SC (Bhakat *et al.,* 2009; Chauhan *et al.,* 2010 and Khatun *et al.,* 2013). Similarly season had significantly influenced the SC (Andrabi *et al.,* 2002; Asad *et al.,* 2004; Sarder. 2007 and Shaha *et al.,* 2008). However, the SC was reported to increase with the age of the bulls (Asad *et al.,* 2004 and Mandal *et al.,* 2010). On the contrary, there are few reports on age of the bull not affecting the sperm concentration in CBHF bulls (Sudheer. 2000 and Jain *et al.,* 2008).

Mass activity (MA)

The least-squares mean mass activity was 2.54 ± 0.02 and it was influenced significantly (P<0.01) by the farm, period and age of the bull, but not by the ejaculate and season. The interaction effects of farm x season and age x season were highly significant (P<0.01) on MA while age x ejaculate interaction was significant (P<0.05) and ejaculate x season did not influence this trait. The wider variation in MA values across the farms and periods could be attributed to different personnel who processed the semen over the period of 19 years and the MA is a subjective assessment which is bound to vary from person to person. The higher value of MA is the result of higher SC in the first ejaculate than in the second ejaculate. The MA score increased non-significantly from 18 to 42 months of age (2.51) to 68 to 92 months (2.56), it peaked from 93 to 117 months of age (2.78) and started decreasing significantly.

Higher MA scores of 2.94 (Mathur *et al.,* 2002), 3.30 (Sugulle *et al.,* 2006), 2.91 (Sarder. 2007), 3.06 (Kumar and Srivastava.

2008), 3.25 (Mukhopadhyay *et al.,* 2010), 2.83 (Patel *et al.,* 2012) and 3.00 (Srivastava and Kumar, 2014) were reported by earlier workers. On the other hand, lower MA scores of 2.49 (Haque *et al.,* 2001), 1.25 (Haq *et al.,* 2003) and 1.47 (Mandal and Tyagi, 2009) were also reported in CBHF bulls. The present study is in agreement with reports of earlier workers on period effect (Bhakat *et al.,* 2009; Chauhan *et al.,* 2010 and Khatun *et al.,* 2013) and age of the bull (Asad *et al.,* 2004 and Mandal *et al.,* 2010) significantly on MA. Non-significant effect of season on MA found in the present study corroborates with the report of Mathur *et al.,* (2002) in CBHF bulls.

Initial sperm motility (ISM)

The least-squares analysis brought out an overall mean of 70.00 ± 0.00 per cent of ISM in CBHF bulls. This trait was also affected by farm, period, season and age of the bull significantly ($P<0.01$). All the interaction effects except ejaculate x season significantly ($P<0.01$) influenced the ISM. Like MA, ISM is also a subjective assessment; it differs significantly between the farms and different periods under the study. The winter season (70.97 ± 0.00) produced the highest ISM when compared to other seasons. The second ejaculate had higher ISM (72.43) than the first ejaculate (67.49) due to aging of sperms, which is lesser in second ejaculate when compared to first. An increasing trend of ISM was observed between the bulls of age group from 18 to 117 months of age (65.70 to 72.68), and then it attains significantly highest ISM per cent (75.53) at 118 to 142 months of age.

There are only two reports (Patel *et al.,* 2012 and Srivastava and Kumar. 2014) which showed a higher ISM in CBHF bulls. All the other reports showed a lower ISM than that obtained in the present study (Haque *et al.,* 2001; Mathur *et al.,* 2002; Haq *et al.,* 2003; Sugulle *et al.,* 2006; Sarder. 2007; Kumar and Srivastava, 2008; Nasrin *et al.,* 2008; Mandal and Tyagi, 2009; Mandal *et al.,* 2012; Khatun *et al.,* 2013). The reports on effects of period, season and age of the bull were sparse while pursuing the literature survey with respect to CBHF bulls.

Post-thaw motility (PTM)

The overall mean value for PTM was 52.48 ± 0.00 per cent. It was affected by the farm, ejaculate, period and age of the bull significantly ($P<0.01$) but not by the season. Interaction of fixed effects such as farm x season and age x season had significant ($p<0.01$) effect on PTM. Farm I had the highest PTM followed by farm III and farm II. The second ejaculate had higher PTM than the first ejaculate. Period V showed PTM values of 56.07 per cent which was significantly higher when compared to other periods except period VI. The increasing trend of ISM and corresponding decreasing trend of PTM over the age groups of CBHF bulls exhibited the reduced sperm motility after exposure to freezing. But, the per cent reduction of sperm motility was more from 93 to >143 months of age. It indicated that up to 7.5 years of age, the sperms withstand the process of freezing as the PTM reduces thereafter. Sperms produced by the aged bulls might have impaired membrane integrity that leads to freezing injury and reduction of PTM.

All the available reports revealed that the lower PTM (Kumar and Srivastava, 2008; Mandal and Tyagi, 2009; Mandal *et al.,* 2012; Srivastava and Kumar. 2014) than the present study. The only report (Sarder, 2007) showed higher PTM of 55.09 per cent, when compared to present study.

The overall higher PTM recorded in the present study might be due to genetic merit of selected bulls and better processing conditions.

Table.1 Least-squares means along with standard error for semen quality traits

Effects	SV (ml)	SC (millions per ml)	MA (0 to 5 scale)	ISM (per cent)	PTM (per cent)	No. of doses per ejaculate
Overall	4.53 ± 0.05 (22442)	1081.33 ± 15.59 (22442)	2.54 ± 0.02 (22442)	70.00 ± 0.00 (22442)	52.48 ± 0.00 (20242)	221.82 ± 1.54 (19478)
Farm	**	**	**	**	**	**
I	4.61^{a} ± 0.06 (3697)	1129.32^{a} ± 18.31 (3697)	2.58^{b} ± 0.02 (3697)	74.71^{b} ± 0.00 (3697)	59.31^{a} ± 0.00 (3600)	261.60^{a} ± 2.51 (3592)
II	4.61^{a} ± 0.06 (14182)	1109.97^{b} ± 16.41 (14182)	3.20^{a} ± 0.02 (14182)	77.91^{a} ± 0.00 (14182)	47.85^{c} ± 0.00 (13390)	188.63^{b} ± 1.91 (12635)
III	4.38^{b} ± 0.06 (4563)	1004.70^{c} ± 16.09 (4563)	1.85^{c} ± 0.02 (4563)	56.23^{c} ± 0.00 (4563)	50.21^{b}± 0.00 (3252)	215.23^{a}± 1.95 (3251)
Ejaculate	NS	NS	NS	NS	**	**
I	4.53 ± 0.07 (13470)	1137.96 ± 21.30 (13470)	2.60 ± 0.02 (13470)	67.49 ± 0.00 (13470)	52.09 ± 0.00 (12117)	238.69 ± 1.90 (11649)
II	4.54 ± 0.07 (8972)	1024.69 ± 21.92 (8972)	2.49 ± 0.02 (8972)	72.43 ± 0.00 (8972)	52.86 ± 0.00 (8125)	204.95 ± 2.06 (7829)
Period	**	**	**	**	**	**
I (1996 - 1998)	4.42^{c} ± 0.07 (1048)	941.20^{d} ± 21.82 (1048)	2.23^{d} ± 0.02 (1048)	65.98^{f} ± 0.00 (1048)	45.40^{d} ± 0.00 (977)	152.71^{d} ± 3.87 (787)
II (1999 - 2001)	4.26^{e} ± 0.06 (7663)	1039.76^{c} ± 16.38 (7663)	2.33^{c} ± 0.02 (7663)	73.98^{a} ± 0.00 (7663)	53.05^{b} ± 0.00 (7265)	152.24^{c} ± 1.84 (7055)
III (2002 - 2004)	4.87^{a} ± 0.05 (6315)	1099.67^{bc} ± 16.25 (6315)	2.48^{c} ± 0.02 (6315)	72.71^{b} ± 0.00 (6315)	51.76^{c} ± 0.00 (5922)	203.68^{c} ± 1.78 (5637)
IV (2005 - 2007)	4.61^{b} ± 0.06 (2074)	1295.79^{a} ± 18.53 (2074)	2.61^{b} ± 0.02 (2074)	68.23^{d} ± 0.00 (2074)	53.13^{b} ± 0.00 (1703)	306.23^{a} ± 2.81 (1629)

Effects	SV (ml)	SC (millions per ml)	MA (0 to 5 scale)	ISM (per cent)	PTM (per cent)	No. of doses per ejaculate
V (2008 - 2010)	$4.34^{d} \pm 0.07$ (1605)	$1162.32^{b} \pm 20.36$ 1605)	$2.87^{a} \pm 0.02$ (1605)	$72.66^{c} \pm 0.00$ (1605)	$56.07^{a} \pm 0.00$ (1428)	$276.64^{b} \pm 3.18$ (1425)
VI (2011 - 2014)	$4.69^{b} \pm 0.06$ (3737)	$949.23^{d} \pm 17.98$ (3737)	$2.74^{b} \pm 0.02$ (3737)	$66.13^{e} \pm 0.00$ (3737)	$55.43^{a} \pm 0.00$ (2947)	$239.42^{bc} \pm 2.49$ (2945)
Season	NS	**	NS	**	NS	NS
Winter	4.70 ± 0.04 (5734)	$1103.59^{a} \pm 12.63$ (5734)	2.57 ± 0.01 (5734)	$70.97^{a} \pm 0.00$ (5734)	52.59 ± 0.00 (5292)	221.76 ± 2.72 (5058)
Summer	4.71 ± 0.04 (5905)	$1048.27^{c} \pm 11.91$ (5905)	2.54 ± 0.01 (5905)	$70.86^{b} \pm 0.00$ (5905)	52.27 ± 0.00 (5247)	217.00 ± 2.56 (5027)
Southwest monsoon	4.61 ± 0.04 (5939)	$1085.89^{ab} \pm 11.25$ (5939)	2.55 ± 0.01 (5939)	$69.53^{c} \pm 0.00$ (5939)	52.30 ± 0.00 (5249)	223.66 ± 2.53 (5091)
Northeast monsoon	4.18 ± 0.18 (4864)	$1086.67^{bc} \pm 51.44$ (4864)	2.52 ± 0.06 (4864)	$68.81^{d} \pm 0.02$ (4864)	52.72 ± 0.00 (4454)	224.88 ± 2.95 (4302)
Age	**	**	**	**	**	**
I (18 to 42 m)	$3.34^{b} \pm 0.03$ (6749)	$1067.59^{b} \pm 7.55$ (6749)	$2.51^{b} \pm 0.01$ (6749)	$65.70^{b} \pm 0.00$ (6749)	$53.38^{a} \pm 0.00$ (6113)	$162.44^{f} \pm 1.69$ (5805)
II (43 to 67 m)	$4.27^{a} \pm 0.03$ (7633)	$1183.37^{a} \pm 7.56$ (7633)	$2.59^{b} \pm 0.01$ (7633)	$66.98^{b} \pm 0.00$ (7633)	$53.35^{a} \pm 0.00$ (6920)	$210.47^{d} \pm 1.66$ (6720)
III (68 to 92 m)	$4.54^{a} \pm 0.03$ (5274)	$1151.15^{a} \pm 9.11$ (5274)	$2.56^{b} \pm 0.01$ (5274)	$66.04^{b} \pm 0.00$ (5274)	$52.42^{a} \pm 0.00$ (4693)	$223.02^{c} \pm 2.04$ (4484)
IV (93 to 117 m)	$4.73^{a} \pm 0.05$ (1499)	$1096.03^{b} \pm 15.19$ (1499)	$2.78^{a} \pm 0.02$ (1499)	$72.68^{b} \pm 0.00$ (1499)	$51.69^{b} \pm 0.00$ (1435)	$247.46^{b} \pm 3.21$ (1389)
V (118 to 142 m)	$5.21^{a} \pm 0.07$ (693)	$1026.82^{c} \pm 21.21$ (693)	$2.59^{b} \pm 0.02$ (693)	$75.53^{a} \pm 0.00$ (693)	$52.23^{a} \pm 0.00$ (622)	$279.24^{a} \pm 4.56$ (621)
VI (>143 m)	$5.81^{a} \pm 0.08$ (594)	$953.41^{d} \pm 24.88$ (594)	$2.31^{c} \pm 0.03$ (594)	$74.94^{a} \pm 0.00$ (594)	$51.78^{b} \pm 0.00$ (459)	$208.30^{e} \pm 5.80$ (459)

** - Highly significant (P<0.01) and NS- Non-significant. Figures in parentheses indicate number of observations. Means with at least one common superscript within classes do not differ significantly (P>0.05)

Table.2 Least-squares ANOVA (mean squares) for semen quality traits

Source of variation	SV		SC		MA		ISM		PTM		No. of doses per ejaculate	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Farm	2	65.27**	2	14627439.77**	2	2215.09**	2	203820.98**	2	45998.16**	2	5537303.44**
Order of ejaculate	1	0.22^{NS}	1	140362.15^{NS}	1	1.04^{NS}	1	265.98^{NS}	1	314.04**	1	1808744.20**
Period of collection	5	254.80 **	5	29041904.48**	5	134.63**	5	15360.67**	5	5960.17**	5	7464438.65**
Season of collection	3	6.87^{NS}	3	976486.68**	3	0.50^{NS}	3	337.43**	3	23.91^{NS}	3	18941.49^{NS}
Age	5	973.57**	5	8671965.99**	5	15.71**	5	5726.38**	5	315.15**	5	2537911.69**
Farm x season	6	10.62**	6	836909.53**	6	5.36**	6	676.84**	6	92.82**	6	38869.61**
Ejaculate x season	3	1.49^{NS}	3	216828.92^{NS}	3	0.82^{NS}	3	161.05^{NS}	3	9.85^{NS}	3	14356.59^{NS}
Age x season	15	4.96**	15	840321.23**	15	2.58**	15	276.97**	15	69.94**	15	31482.15**
Age x ejaculate	5	53.83**	5	1746656.25**	5	0.81*	5	2518.02**	5	21.94^{NS}	5	57102.61**
Error	22396	2.84	22396	243364.79	22396	0.30	22396	83.62	20196	17.57	19432	9934.57

** - Highly significant (P<0.01); *- Significant (P<0.05) and NS- Non-significant

Number of doses per ejaculate

The least-squares analysis produced a mean of 221.82 ± 1.54 doses of frozen semen per ejaculate. As seen in most of the other traits of semen quality, this trait was also significantly affected (P<0.01) by the farm, ejaculate, period and age of the bull. The effects of farm x season, age x season and age x ejaculate interactions were significant on the number of doses per ejaculate. Season of semen collection did not affect the number of doses produced per ejaculate.

Farm I produced the maximum of 261.60 doses per ejaculate followed by farm III (215.23 doses) and farm II (188.63 doses), since, number of doses per ejaculate depends on SV, SC and ISM. Even though, both farms II and III are in hilly regions, the variation of semen quality traits could be directly due to indigenous breed component in CBHF bulls and indirectly due to sample size, forage quality (plant – animal interaction) and source of concentrate feed.

The first ejaculate (238.69 doses) produced significantly more doses of frozen semen than the second ejaculate (204.95 doses). The highest number of doses (306.23 doses) per ejaculate was produced during period IV was reflected by highest SV, SC and ISM during that period.

The number of doses increased from 162.44 (18 to 42 months of age) to 279.24 (118 to 142 months of age) and thereafter it decreased significantly to 208.30 doses. In this study, it is proved that in older age (>143 months), the SV is increasing due to male accessory sex glands, but the real sperm producing potential of seminiferous tubules in the testes was reduced as it was witnessed by lower SC and in turn by lowest number of doses per ejaculate. As there is scarcity of literature on the number of doses per ejaculate in CBHF bulls, the results obtained in the present study could not be compared.

It is concluded that from the findings of the study farm, period and age of the bull and the interaction effects of farm x season, age x season and age x ejaculate were producing significant effect on all semen quality traits. SV per ejaculate increased with age of the bull and it exhibit negative correlation with sperm concentration over the ages. Further age of the bull interacted significantly with other fixed effects in the model, i.e. season and ejaculate. The first ejaculate had better SC, MA and number of doses per ejaculate; and second ejaculate had higher ISM and PTM. It was observed that there was no seasonal influence on semen quality parameters except SC and ISM, which were highest during winter season when compared to other seasons. The interaction effects of season with other fixed effects of farm and age of the bull were significant, which indicate the authority of season on semen quality parameters are relatively diverse, depending on location of the farm, age of the bull, feeding, ambient temperature and humidity.

Conflict of interest: The authors declare that they have no conflict of interest.

Acknowledgement

I express my gratitude to the Tamil Nadu Veterinary and Animal Sciences University for permitting me to undergo Ph.D. programme on part-time basis. I wish to extend my deep sense of gratitude to the Director of Animal Husbandry and Veterinary Services and the Managing Director, Tamil Nadu Co-operative Milk Producers' Federation Limited, Chennai, Tamil Nadu for permitting me to utilize semen production data from frozen semen stations for my research work.

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How to cite this article:

Gopinathan, A., S.N. Sivaselvam, S.M.K. Karthickeyan, K. Kulasekar, J. John Kirubaharan and Venkataramanan, R. 2018. Effect of Non-genetic Factors on Semen Quality Traits of Crossbred Holstein Friesian Bulls (*Bos taurus* X *Bos indicus*) in Organized Farming Conditions at Tamil Nadu, India *Int.J.Curr.Microbiol.App.Sci.* 7(11): 3219-3229.
doi: https://doi.org/10.20546/ijcmas.2018.711.370