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Assessment of seminal attributes of neat Hariana bull semen

Avaneesh Kumar Singh, Atul Saxena, Dilip Kumar Swain, Vikas Sachan, Jitendra Agrawal and Anuj Kumar

Abstract
The present study was undertaken to evaluate the semen characteristics of Hariana bulls. In this study total 32 ejaculates from four healthy Hariana bulls was collected and estimated for various seminal attributes. The overall mean±S.E for various attributes were, ejaculate semen volume 4.81±0.16 ml, pH 6.58±0.02, sperm mass motility (0-5 scale) 3.84±0.08, sperm concentration 1783.75±51.23 millions/ml, sperm progressive motility 84.22±0.68 per cent and live sperm 90.32±0.42 per cent. A significant (P<0.05) difference was found only in the semen volume and mass motility amongst the bulls. So that, present findings suggested that, these bulls were suitable for breeding and its semen could be used for cryopreservation as the purpose of artificial insemination.

Keywords: Hariana, bull semen, sperm, ejaculates, volume, motility, concentration, live percentage

Introduction
Livestock in general and dairying in particular play a vital role in the Indian economy and also in the socio-economic development of millions of rural households. About 70 million rural households are engaged in dairying, one of every two rural households with women playing a vital role. Hariana breed of Cattle is well known dual purpose breed, having milk yield around 809 to 1,731 kg per lactation and bullocks are good for agricultural operations (Upadhyay and Madan, 1985) [2]. This breed is also famous for its well adaptation to the tropical environments. The indigenous breeds, which traditionally form an integral part of agriculture, are decreasing in number in last couple of decades due to mechanization of agriculture (Singh *et al*., 2018). Conservation of germplasm is important for the genetic improvement and AI offers good opurtunity. The genetic improvement technique requires the efficient knowledge of semen quality parameters, cryopresevation, insemination techniques etc. Neat semen evaluation has always been of great significance to assess the fertility status of the bull.

Materials and Methods
The present study was conducted on four healthy Hariana bulls, maintained under identical and optimum conditions of feeding and management during entire period of study at the University Instructional Livestock Farm Complex (ILFC), College of Veterinary Science and Animal Husbandry, DUVASU, Mathura. The institute is located at an altitude of 287 meters above the mean sea level and at latitude of 27.17° N and a longitude of 77.41° E. A total 32 ejaculates (8 ejaculates from each bull) were collected biweekly in the morning by artificial vagina.

Evaluation of semen
Immediate after semen collection, each ejaculate was placed in a water bath at 37°C and standard laboratory tests for fresh semen were recorded.

Colour and consistency
Colour and consistency of semen sample were observed by direct visualization with naked eyes as yellowish thick, thick creamy, milky white etc. and any other abnormalities in colour or consistency were treated as abnormal and the samples were discarded.

Ejaculate semen volume
The volume of semen was directly measured in milliliter (ml) from the graduated centrifuge semen collection tube.

Seminal pH
pH of semen was measured by using pH paper (B.D.H. 5570 Indicator Paper) having the range of 5.5 - 7.0 and the colour developed was matched with the colour standards provided with the strip.

Sperm mass motility
Mass motility was evaluated as per method described by Salisbury *et al*. (1985) [13]. On a thermostatically controlled warm stage (37°C) microscope, it was assessed based on the vigour wave motion on a grade scale of 0 to 5.

Sperm concentration
Sperm concentration was estimated using Haemocytometer (Improved Neubauer's chamber) adapting method of Salisbury *et al*. (1978) [14] and was expressed in millions/ml ($x10^6$/ml).

Sperm progressive motility
The progressive motility of the spermatozoa was observed under high power phase objective (40x) on a thermostatically controlled stage maintained at 37°C. The progressive motility of spermatozoa was calculated as per the method of Salisbury *et al*. (1985) [13] and epressed in per cent.

Live sperm percentage
Method described by Campbell *et al*. (1953) [2] was followed to estimate the live percentage of the fresh semen ejaculates. Sperms that white (unstained) were classified as live and those showed any pink or red colourations were classified as dead. Live spermatozoa counts were expressed in per cent.

Results and Discussion
The mean seminal attributes of four Hariana bulls namely the volume, pH, mass motility, sperm concentration, progressive motility and live sperm per cent are presented in Table-1.0.

Ejaculate semen volume (ml)
The mean semen volume of bull number HB- 1was 4.28±0.33 ml. The respective values for bull number HB- 2, HB- 3 and HB- 4 were 4.75±0.20, 4.92±0.30 and 5.30±0.40 ml. The overall mean seminal volume irrespective of the bull was recorded as 4.81±0.16 ml. A significant ($P<0.05$) difference in the mean semen volume was found amongst the bulls, however, bull number HB- 4 had significantly ($P<0.05$) higher ejaculate volume compared to other bulls (Table 1.0 and Figure 1.0).
Ejaculate volume is the most important parameter as the number of breeding doses of A.I. depends on the ejaculate volume and sperm concentration. In the present study, the ejaculate seminal volume varied significantly ($P<0.05$) among the four Hariana bulls and ranges between 4.28±0.33 ml to 5.30±0.40 ml. Earlier information for this parameter from the same lab revealed a mean volume of 4.38±0.47 ml to 6.58±0.38 ml (Sachan, 2013) [12], 6.38±0.74 ml (Patel, 2014) [24, 7], 5.91± 0.44 ml (Yadav, 2014) [24], 5.65±0.23 ml (Shah, 2016) [15], 5.54±0.21 ml (Yadav, 2017) [23], 5.86±0.17 ml (Yadav, 2018) [25] and 5.12±0.23 ml (Rathore, 2019) [10]. Our results are in concurrence with earlier reports and reflect the normal semen volume of this breed of animal. The volume of semen was reported to vary with breed, age, body or scrotal size, frequency of collection, exercise, teasing, season, level of nutrition, reproductive health and method of collection (Hafez & Hafez, 2000) [4]. The variation in semen volume in our finding could be because of the any reasons as stated vide supra.

Seminal pH
The mean seminal pH of bull number HB- 1 was 6.54±0.04. The respective values for bull number HB- 2, HB- 3 and HB- 4 were 6.52±0.40, 6.61±0.05 and 6.65±0.05. The overall mean seminal pH in the fresh ejaculate of the Hariana bull was 6.58±0.02. The seminal pH did not differ significantly amongst different bulls **(**Table 1.0 and Figure 2.0).
pH of the semen regulates the acid balance in the sperms in terms of prostate secretions. The rise in the pH is an indication that the sperms neutralizing capacity in terms of alkali/buffer secretion is poor. The activity of proton channels present in the sperms is also increased and hence an acidification of semen. The change in the pH of semen affects the sperm motility and fertility ability (Purdy, 2006) [8]. In the present study, the overall mean seminal pH of Hariana bull was found as 6.58±0.02 which did not differ significantly amongs the bulls. Earlier studies from the same lab reported a mean seminal pH of 6.84±0.04 (Sachan, 2013) [12], 6.81±0.02 (Patel, 2014), 6.82±0.01 (Yadav, 2014) [24], 6.59±0.03 (Shah, 2016) [15], 6.63±0.03 (Yadav, 2017) [23], 6.55±0.02 (Yadav, 2018) [25] and 6.58±0.03 (Rathore, 2019) [10]. Our results are within the reported range indicating normal seminal pH.

Sperm mass motility (0-5 scale)
The mean mass motility of bull number HB- 1 was 3.50±0.13. The respective values for bull number HB- 2, HB- 3 and HB- 4 were 4.16±0.19, 3.81±0.19 and 3.88±0.12. The overall mean mass motility of spermatozoa in the fresh ejaculate of Hariana bull was 3.84±0.08 (0-5 scale). A significant ($P<0.05$) difference in the mass motility was found amongst the bulls. Bull number HB- 2 had significantly ($P<0.05$) higher mass motility compared to other bulls (Table 1.0 and Figure 3.0).
In the present study, semen mass motility (0-5 scale) varied significantly ($P<0.05$) among the four Hariana bulls and ranged between 3.50±0.13 to 4.16±0.19. The overall mass motility of semen samples was reported as 3.84±0.08. Earlier studies from the same lab reported a mean mass motility of Hariana bulls as 3.38±0.13 to 3.75±0.16 (Sachan, 2013) [12], 3.88±0.14 to 3.92±0.10 (Patel, 2014; Yadav, 2014) [7, 24], 3.79 ±0.09 (Shah, 2016) [15], 3.83 ±0.08 (Yadav, 2017) [23], 3.56 ±0.06 (Yadav, 2018) [25] and 3.50±0.11 (Rathore, 2019) [10]. Our results are in concurrence with the earlier reports of the same laboratory. The semen motility is affected by improper handling procedure, contaminated glasswares (presence of soap residue), chemicals present over the fingers, cold or hot test tubes, glass slides, microscope stage, rapid drying or cooling of glass slides, prolongation during collection and examination period etc. In the present study, the motility was accessed keeping in mind of these mentioned effects.

Sperm concentration (millions/ml)
The mean sperm concentration of bull number HB- 1 was 1663.75±124.27 millions/ml. The respective values for bull number HB- 2, HB- 3 and HB- 4 were 1908.75±109.32, 1783.75±68.63 and 1778.75±100.45 millions/ml. The overall mean sperm concentration in the fresh ejaculate of Hariana bull was 1783.75±51.23 millions/ml. The sperm concentration did not differ significantly amongst different bulls **(**Table 1.0 and Figure 4.0).

Accurate determination of the number of spermatozoa per millilitre of semen is extremely important because it is highly variable semen characteristics. When combined with the volume of the ejaculate this quality of spermatozoa determines how many female can be inseminated each with optimal number of sperm cells (Hafez & Hafez, 2000) [4]. The average sperm concentration in present study was in the range of 1663.75±124.27 to 1908.75±109.32 million/ml which did not differ significantly amongs the bulls. The overall sperm concentration was reported as 1783.75±51.23 million/ml. Earlier reports from the same lab revealed the average concentration as 1262.50±41.69 to 1517.50±195.05 millions/ml (Sachan, 2013) [12], 1403.67±95.13 to 1479.42±96.32 millions/ml (Patel, 2014; Yadav, 2014) [7, 24], 1269.75±81.97 millions/ml and 1324.17±63.25 millions/ml in winter and summer seasons respectively (Singh, 2017), 825.66±27.82 millions/ml (Yadav, 2018) [25], 1867.71±72.03 million/ml (Rathore, 2019) [10]. In 1967, Tomar *et al*. reported the sperm concentration in Hariana bulls as 1150±53.44 million/ml whereas Tomar and Kanaujia (1970) reported it as 1185±65.11 millions/ml. Banerjee and Ganguli (1973) reported sperm concentration in Zebu bull as 1091.8 millions/ml whereas Roberts (1982) suggested a range between 300-2500 millions/ml with an average of 1200 millions/ml. Tomar and Gupta (1984) further reported the effect of season on the sperm concentration of Hariana bulls which ranges from 822.70±39.90 to 1193.30±51.20 millions/ml. Our result for sperm concentration is within the reported range. Sperm concentration also varies with managemental practices such as restraint before semen collection, frequency of semen collection (Singh and Sharma, 2001), age of bull (Rao and Rao, 1975) [9] etc. In the present study, all these factors have been taken into consideration and has been kept as nearly same for all the bulls except for age. The higher value could be due to this effect.

Sperm progressive motility (%)

The mean percentage of progressively motile spermatozoa in the semen of bull number HB- 1 was 83.75±1.57. The respective values for bull number HB- 2, HB- 3 and HB- 4 were 86.25±1.57, 82.50±0.94 and 84.38±1.13 per cent. The overall mean percentage of progressively motile spermatozoa in the fresh ejaculate of Hariana bull was 84.22±0.68 per cent. The progressive motility did not differ significantly amongst different bulls (Table 1.0 and Figure 5.0).

The ejaculated semen was diluted immediately after collection to observe the progressive motility. Sperm progressive motility is an important criterion of semen quality (Lasley, 1951) [5] and is an important determinant of success rate of the fertilization and ability of spermatozoa to withstand the stress of cryopreservation process. In the present study, the percentage of progressive motile spermatozoa of Hariana bulls was in the range of 82.50±0.94 to 86.25±1.57 which did not differ significantly amongs the bulls. The overall percentage of progressively motile spermatozoa was reported as 84.22±0.68. Earlier reported values of progressive motility from the same lab were 87.83±0.58 to 87.92±068 (Patel, 2014; Yadav, 2014) [24, 7], 87.50±1.15 (Shah, 2016) [15], 85.00±0.95 (Yadav, 2017) [23], 82.81±0.55 (Yadav, 2018) [25] and 83.93±1.07 (Rathore, 2019) [10]. Our results are in concurrence with these reported values and are considered to be in normal range. No significant difference in progressive motile spermatozoa were observed amongst differed bull, hence all bull behaves similar for this parameter.

Live spermatozoa (%)

The mean percentage of live spermatozoa in the semen of bull number HB- 1was 90.29±1.04. The respective values for bull number HB- 2, HB- 3 and HB- 4 were 90.96±1.00, 89.76±0.64 and 90.29±0.78. The overall mean percentage of live spermatozoa in the fresh ejaculate of Hariana bull was 90.32±0.42. The live percentage of spermatozoa did not differ significantly amongst different bulls (Table 1.0 and Figure 6.0).

The percentage of live spermatozoa determines the quality of ejaculate. When normal semen has been handled properly and staining is carried out correctly, the percentage of sperm staining alive is highly correlated with individual progressive motility, but the percentage of motility is usually lower than the percentage of live spermatozoa as many of the live sperm may not have motility (Hafez & Hafez, 2000) [4]. Viability of spermatozoa is significantly and positively correlated with initial motility, post-thaw motility and fertility of spermatozoa (Foote *et al*., 2002) [3]. In the present study, the percentage of live spermatozoa of Hariana bulls was in the range of 89.76±0.64 to 90.96±1.00 which did not differ significantly amongst the bulls. The overall percentage of live spermatozoa was reported as 90.32±0.42. Sachan (2013) [12] reported a range 84.75±2.52 to 90.38±0.91 percent. Patel (2014) [7] and Yadav (2014) [24] reported a range of 94.99±0.48 to 95.17±0.37 percent. Shah (2016) [15] reported the mean percentage of live spermatozoa as 92.65±0.85. Yadav (2017) [23] reported the mean percentage of live spermatozoa as 93.00±0.57. Yadav (2018) [25] reported the mean percentage of live spermatozoa as 87.97±0.54. Present findings lie within the range reported by Sachan (2013) [12]. Comparing these results with the result of progressive motility clearly indicates that some of the live spermatozoa were not motile. Semen samples containing initially less than 70 per cent live spermatozoa are of questionable fertilizing capacity and are not good for freezing whereas samples containing more than 70 per cent live spermatozoa showed no difference in fertilizing capacity. Pant *et al*. (2003) [6] reported that semen with more than 30 per cent initial dead spermatozoa is not good for preservation. Keeping these reports in consideration, in the present study all semen samples which were having >70 per cent live sperm were subjected to cryopreservation.

Table 1.0: Seminal attributes in the freshly collected semen of Hariana bulls (Mean±SE=8)

Bull No.	Ejaculate semen Volume (ml)	Seminal pH	Sperm mass motility (0-5 scale)	Sperm concentration (x10^6/ml)	Sperm progressive motility (%)	Live sperm (%)
HB- 1	4.28±0.33^b (3.0 - 5.6)	6.54±0.04 (6.4 - 6.7)	3.50±0.13^b (3.0 - 4.0)	1663.75±124.27 (1200.0 - 2220.0)	83.75±1.57 (80.0 - 90.0)	90.29±1.04 (86.3 - 94.0)
HB- 2	4.75±0.20^{ab} (4.0 - 5.4)	6.52±0.40 (6.4 - 6.7)	4.16±0.19^a (3.5 - 5.0)	1908.75±109.32 (1350.0 - 2310.0)	86.25±1.57 (80.0 - 90.0)	90.96±1.00 (86.7 - 94.0)
HB- 3	4.92±0.30^{ab} (3.4 - 6.0)	6.61±0.05 (6.4 - 6.8)	3.81±0.19^{ab} (3.0 - 4.5)	1783.75±68.63 (1470.0 - 2040.0)	82.50±0.94 (80.0 - 85.0)	89.76±0.64 (87.5 - 92.5)
HB- 4	5.30±0.40^a (3.8 - 7.0)	6.65±0.05 (6.4 - 6.8)	3.88±0.12^{ab} (3.5 - 4.5)	1778.75±100.45 (1230.0 - 2120.0)	84.38±1.13 (80.0 - 90.0)	90.29±0.78 (86.9 - 93.5)
Overall	4.81±0.16 (3.0 - 7.0)	6.58±0.02 (6.4 - 6.8)	3.84±0.08 (3.0 - 5.0)	1783.75±51.23 (1200.0 - 2310.0)	84.22±0.68 (80.0 - 90.0)	90.32±0.42 (86.3 - 94.0)
'F' Value	1.77*	1.91^{NS}	2.79*	0.95^{NS}	1.38^{NS}	0.31^{NS}

Means with different small letters superscript differed significantly within a column
DMRT was used for comparing means
Figures in parenthesis indicate range
NS : Non-significant
* : Significant (P<0.05)

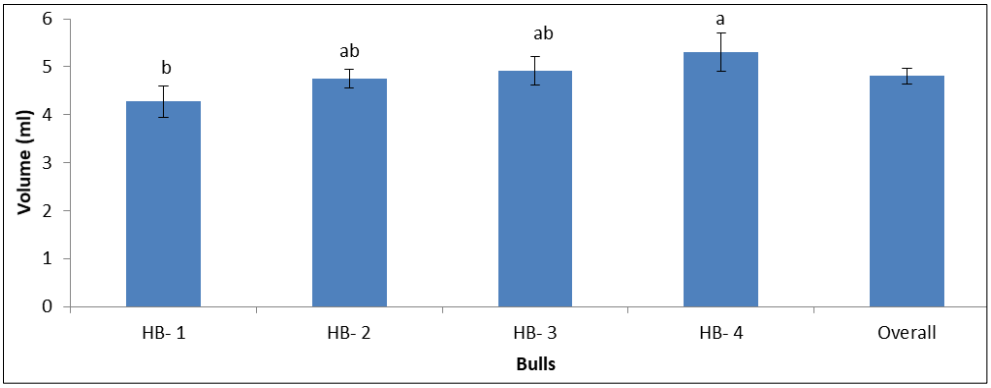

Fig 1: Graphical presentation of mean seminal volume (ml) in the freshly collected semen of Hariana bulls

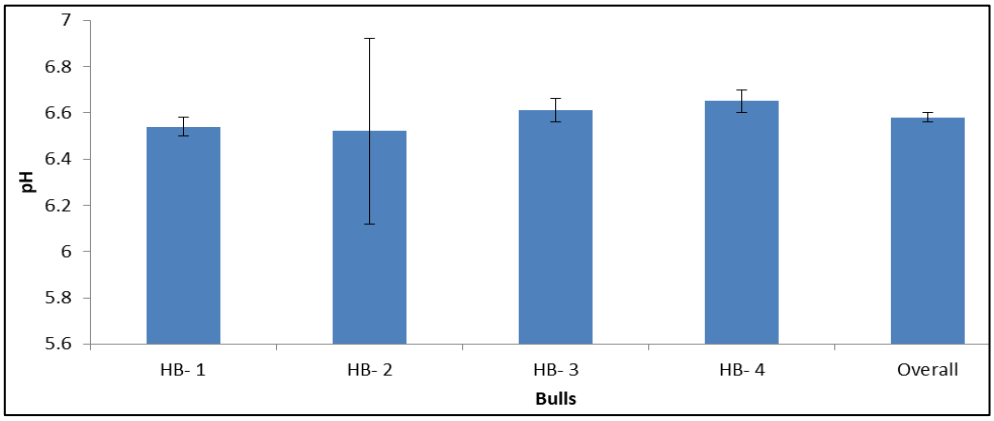

Fig 2: Graphical presentation of mean seminal pH in the freshly collected semen of Hariana bulls

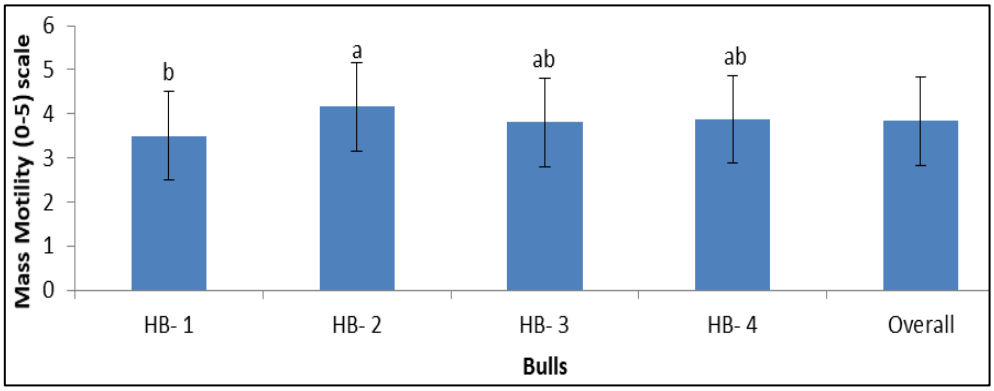

Fig 3: Graphical presentation of mean mass motility (0-5 scale) of Spermatozoa in the freshly collected semen of Hariana bulls

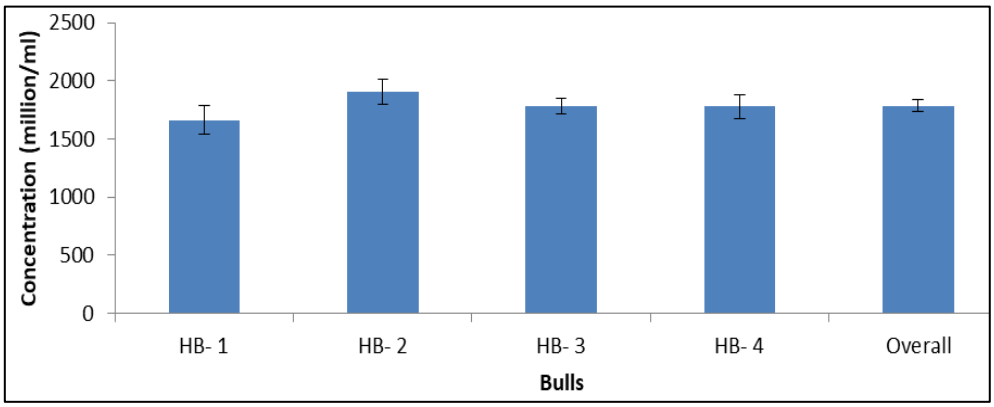

Fig 4: Graphical presentation of mean sperm concentration (millions/ml) in the freshly collected semen of Hariana bulls.

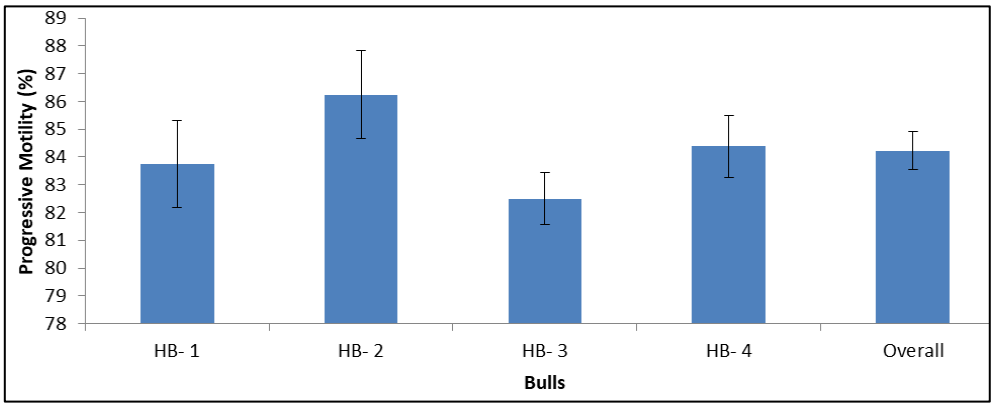

Fig 5: Graphical presentation of mean per cent progressive motility of spermatozoa in diluted semen of Hariana bulls

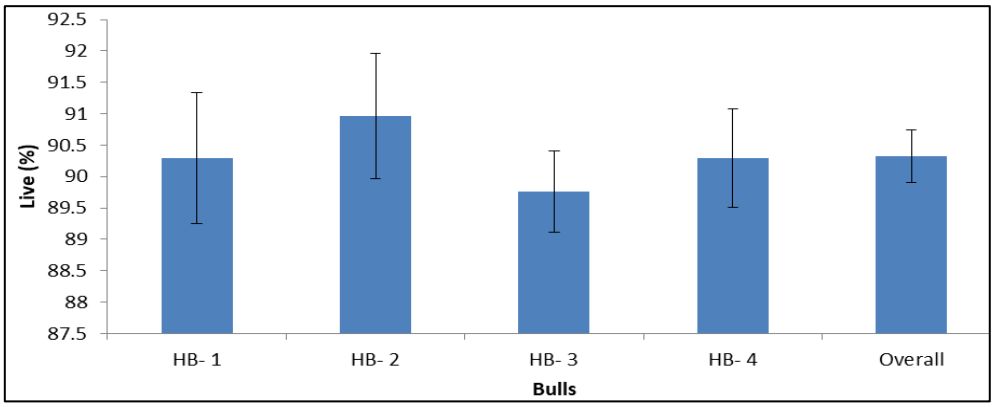

Fig 6: Graphical presentation of mean per cent live spermatozoa in the freshly collected semen of Hariana bulls

Summary and Conclusion

The analysis of variance of seminal attributes among all bulls revealed non-significant difference with regards to seminal parameters like seminal pH, sperm concentration, percent progressive motility and percent live sperm whereas semen volume and mass motility having significant (P<0.05) difference amongst all Hariana bull semen. This study indicating that all bulls having seminal physical and morphological parameters within permissible limit and is suitable for cryopreservation.

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