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# Studies on Seminal Attributes of Neat Semen of Hariana Bulls

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**A B S T R A C T**

The mean ± S.E. values of seminal attributes evaluated for assessing the quality of freshly ejaculated neat semen from four Hariana bulls has been estimated. In the present study mean ejaculate semen volume ranges between 3.35 ± 0.14 to 5.38 ± 0.20 ml. Semen mass motility (0-5 scale) ranges between 3.13 ± 0.08 to 3.69 ± 0.09. Sperm concentration varies significantly among the four Hariana bulls and ranges between 1633.50 ± 33.58 to 1806.25 ± 31.56 millions/ml. Present study indicated that the percent sperm progressive motility amongst four Hariana bulls range between 72.0 0 ± 1.21 to 78.13 ± 1.28. Percent live sperm in the ejaculate varies significantly among the four Hariana bulls and ranges between 80.12 ± 1.70 to 87.13 ± 1.49. The mean percent total morphological sperm abnormalities of the four Hariana bulls varies between 3.50 ± 0.42 to 4.13 ± 0.35 indicating that all bulls are within the permissible limit of abnormalities and is suitable for cryopreservation.

## Introduction

India is predominantly an agricultural country with about 70% of its population dependent on income from agriculture. Animal husbandry is an adjunct to crop agriculture and Cattle, buffaloes are kept for milk production. Cattle farming are one of the major sources of income for majority of population in India. Cattle farming have played a very important role in growth of Indian economy. India has achieved top position in milk production of world producing about 109 millions tones of milk with 4.5% annual growth rate of milk (Economic Survey 2009-10). Cattle contribute about 45% of the country total milk production which have been possible due to

availability of many milch breeds. India continues to be the largest milk producing nation in the world with close to 17% of global production in 2010-11. As per the figures of 18th Livestock Census, India has 199.07 million Cattle (33.06 million crossbred cattle and 166.01million indigenous breed). India contributes its 12.7% of total world Cattle population and 2nd rank in Cattle population in the world.

Hariana Cattle (*Bos indicus*) derived their name from the tract most probably "hari (green)". Hariana breed of Cattle is known for its dual purpose, among the Zebu Cattle. The native tract of this breed is Rohtak, Hisar, Gurgaon, Karnal, Delhi. This breed also exists in more or less pure form in Jind, Patiala, Jaipur, Jodhpur and western U.P. Uttar Pradesh has maximum number of Hariana Cattle than any other breed (18th Livestock Census, India; Uttar Pradesh Census). The breed averages 1700 kg of milk per lactation but high producing animals has produced over 2500 kg in a single lactation. The males (Bullock) of this breed have been used as a powerful tool for transportation and ploughing. Depleting source of petroleum products being used in agriculture sector, there is a need for conservation of this breed.

Artificial insemination has been used exhaustively to facilitate the best use of elite bulls with proven fertility. It has tremendously improved the genetic makeup and ultimately productivity of low productive indigenous breeds in India. The results of artificial insemination depend upon the quality of semen produced and it is mainly dependent on the managemental practices adopted at bull semen collection centre (Corona *et al.,* 2006). The prime objective of artificial insemination is to improve breeds to obtain optimum milk production and it is possible only by quality semen. Keeping it in view, the present study was planned to assess seminal attributes on Neat semen of Hariana bulls.

## Materials and Methods

Present study was conducted on four Hariana bulls of the age group between 4.5 to 5.5 years and weighing more than 400 kg body weight, reared at the University Instructional Livestock Farm Complex (ILFC), College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura.

## Evaluation of semen

Semen was evaluated based on the following parameters.

## Volume

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

## Colour

Semen sample were observed for colour and consistency by direct visualization with naked eyes and any abnormalities in colour or consistency were treated as abnormal and the sample were discarded.

## Mass motility

It was assessed by method of Nazir, (1988) reported in Nili-ravi buffalo that motility was rated according to the vigour wave motion on grade scale of 0 to 5.

## Progressive motility

The progressive motility of the spermatozoa calculated as per method of Ahmad, (1994).

### Sperm concentration

Sperm concentration was estimated using Improved Neubauer's chamber method (Salisbury *et al.,* 1978).

### Live and dead spermatozoa count

Method described by Bloom (1950) and Hancock (1951) was followed.

### Morphological Abnormalities

Live and dead count was also used for enumerating abnormalities. The classification suggested by (Lasley, 1951) was used for the study.

### Hypo-osmotic swelling test (HOST)

The hypo-osmotic swelling test was performed according to the methods described by Correa and Zavos (1994). A pinch of nigrosin was added in both HOST and Control solution to provide background to facilitate counting. These spermatozoa were classified in four different classes according to presence of swelling pattern (Takahasi *et al.,* 1990).

### Acrosome integrity

Staining was carried out as described by Hancock (1952).

## Results and Discussion

The mean ± S.E. values of seminal attributes evaluated for assessing the quality of freshly ejaculated neat semen from four Hariana bulls has been estimated. The ejaculated semen collected from four Hariana bulls were evaluated for their volume (ml), mass motility (0-5 scale), sperm concentration (millions/ml), progressive motility (percent), live sperm (percent) and total sperm abnormalities (percent). The relevant data has been illustrated in Table 1.

### Semen volume

Semen volume is an important parameter as the number of breeding doses of A.I. depends on the ejaculate semen volume and sperm concentration. In the present study indicated that the mean ejaculate semen volume varies significantly among the four Hariana bulls and ranges between 3.35 ± 0.14 to 5.38 ± 0.20 ml. In a similar study (Kumar, 2011 and Singh, 2012) in Hariana bulls the mean ejaculate volume was reported to range between 2.85±0.21 to 5.65±0.39 ml. The results for this parameter confirm the earlier report and are in the reported range. Further, other reports suggest the range of ejaculate semen volume to vary between 3.3 ml to 5.3 ml for Hariana bulls (Shukla and Bhattacharya, 1949; Tomar *et al.,* 1966; Tomar and Kanaujia, 1970; Tomar and Gupta, 1984) which is in agreement with our reported values.

Variation in semen volume has been reported among different species and within a species and even the same male at different time of collection. The volume of semen was reported to vary with breed, age (Rao and Shreemanarayana, 1996), body size, size of accessory sex gland (White, 1975) , frequency of collection, exercise, teasing, level of nutrition, effect of season (Saxena and Tripathi, 1981 and Dhami *et al.,* 1998), and method of collection. In the present studies the other factors responsible for variation of semen volume are same for all bulls except that level of teasing could have been a factor for variation in semen volume.

### Mass motility

Sperm motility gives the first hand information regarding the fertilizing capacity

of semen. Sperm motility is crucial in facilitating passage through the cervix and utero-tubal junction and much more important in actual penetration of the cumulus cells and zona pellucida of the ovum (Hafez, 1987). However, Erb *et al.,* (1956) demonstrated that it is more closely related with other seminal traits than fertility. The mass motility of spermatozoa is a wholesome effect of sperm concentration and individual motility (Zemjanis, 1970).

Present study indicated that semen mass motility (0-5 scale) varies significantly among the four Hariana bulls and ranges between 3.13 ± 0.08 to 3.69 ± 0.09. In a similar study (Kumar, 2011 and Singh, 2012) in Hariana bulls the semen mass motility ranges between 3.20 ± 0.08 to 4.35±0.07. The results for this parameter mostly similar the earlier report and are in the reported range. Further, other reports suggest the range of semen mass motility was in accordance with the values reported in others cattle bulls by various authors (Tomar *et al.,* 1966; Bhosrekar and Nagpaul, 1972; Rao *et al.,* 1996). Tomar and Gupta (1984) reported mass motility in Hariana bull semen between 3.8 ± 0.2 and 4.3 ± 0.2 in summer and winter season. These values are in close proximity with our results although we have not reported our findings in season. The present study was carried out between February to August.

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.

## Sperm concentration

Accurate determination of the number of spermatozoa per milliliter of semen is extremely important. The sperm concentration is highly variable semen characteristics and when combined with ejaculated semen volume it determines number of females that can be inseminated with optimal number of sperm cells (Hafez, 1987).

Present study indicated that the sperm concentration varies significantly among the four Hariana bulls and ranges between 1633.50 ± 33.58 to 1806.25 ± 31.56 millions/ml. In a similar study (Kumar, 2011 and Singh, 2012) in Hariana bull semen the sperm concentration (millions/ml) ranges between 938.60 ± 41.39 to 2890.0 ± 118.51 which was differ compare to the present investigation. These results differ due to effect of age as well as of the season (Das *et al.,* 2007). Sperm concentration (millions/ml) in Hariana bulls different authors are reported as 1150 ± 53.44 (Tomar *et al.*,1966), 1185 ± 65.11(Tomar and Kanaujia, 1970) ,1187 ± 87.81(Kerur, 1971), 1089 ± 102.14 (Singh and Pangawkar,1990), 1225 ± 29.75 (Veerapandian *et al.,* 1992) and 1232.5 ± 41.4 (Dabas *et al.,* 1982). Our results for sperm concentration are quiet superior compare to these reports. A slight difference in semen concentration may be due to degree of sexual stimulation.

## Individual progressive motility

A drop of ejaculated semen was diluted immediately after collection to observe the progressive motility of spermatozoa. When normal semen has been handled properly and staining is carried out correctly, the percentage of live sperm is highly correlated with percent individual progressive motility, but the percentage of progressive motility is usually lower than the percentage of live spermatozoa as many of the live sperm may not have motile (Hafez, 1987). Individual sperm progressive motility is an important criterion of semen quality (Lasley, 1951) and

is an important determinant of success rate of the fertilization. Progressive sperm motility, though variable and subjective methods of semen evaluation has been found to be correlated with various semen characteristics (Tomar, 1984). However, Bloom (1950) also reported that motility below 50 percent was often associated with low conception rate and poor fertility. Erb *et al.,* (1956) also demonstrated that it is more closely related in other seminal traits than fertility and suggested that, the bovine semen with 40 to 50 percent motile sperm should be considered as subnormal and was associated with infertility. Branton *et al.,* (1951) opined a minimum initial sperm motility of 70 percent required for an effective breeding. However, a decrease in sperm motility is observed in disease condition, change of environment and temperature variations and it fluctuates between breeds, individuals, age group and technique employed for observation.

Present study indicated that the percent sperm progressive motility amongst four Hariana bulls varies significantly and was in the range between 72.0 0 ± 1.21 to 78.13 ± 1.28. Our finding agreement with earlier reports which suggested percent progressive motility ranges between 73.00 ± 1.11 to 80.00 ± 0.00 (Kumar, 2011 and Singh, 2012).The present result showed that semen samples suitable for further processing of cryopreservation.

## Percent live sperm

Tomar, (1984) reported that live sperm can be predicted by initial motility of semen yet actual evaluation of live sperm is desirable. Semen sample having less than 50 percent live sperm are of questionable fertilizing capacity where as samples containing 50-90 percent live spermatozoa showed no difference in fertilizing capacity. Pant *et al.,* (2002) reported that semen with more than 30 percent initial dead spermatozoa is not good for preservation. Keeping these reports in consideration, in the present study all semen samples which were having more than 70 percent live sperm was subjected to cryopreservation. The percentage of stained spermatozoa is dependent on various factors like duration, type of diluents used, stain integrity and technique employed. Moreover, it gives vital information regarding the livability of both neat and extended semen.

Present study indicated that the percent live sperm in the ejaculate varies significantly among the four Hariana bulls and ranges between 80.12 ± 1.70 to 87.13 ± 1.49. The neat semen viability in the present experiment was within the optimum range. Our finding is in agreement with earlier reports where percent live sperm ranges between 85.89 ± 1.16 to 90.03 ± 0.83 (Kumar, 2011 and Singh, 2012). Further, other reports were in accordance with the values reported in others cattle bulls as percent live sperm 79.86 ± 0.98 in cross breed bull (Vyas *et al.,* 1992), 86.20 ± 1.40 in Murrah bulls (Meena *et al.,* 2010), 80.70 ± 1.20 to 87.30 ± 2.50 in Sahiwal bull (Ansari *et al.,* 2011), 82.75 ± 0.02 to 82.92 ± 0.01in Cattle (Amrendra *et al.,* 2011).

Comparing this result with the result of percent progressive motility of spermatozoa clearly reflects that some of the live spermatozoa was not motile.

## Total morphological sperm abnormalities

Semen from most males contains some abnormally formed spermatozoa which are not associated with lower fertility rates until the proportion of abnormal sperm exceeds 20 percent (Hafez, 1987). Moreover, the sperm abnormality may vary due to method of collection, temperature shock (Hancock, 1952) and technique employed (Bishop *et al.,* 1954).

**Table.1** Seminal attributes of Hariana bull spermatozoa in neat semen (Mean ± S.E., n = 8)

| Bull No. | Volume (ml) | Mass Motility ( 0 -5 scale) | Concentration (millions/ ml) | Progressive motility (%) | Live sperm (%) | Total morphoological sperm Abnormalities (%) |
|---|---|---|---|---|---|---|
| **H-623** | $5.38^{b}$±0.20 (4.5-6.2) | $3.69^{b}$±0.09 (3.5-4) | $1806.25^{b}$±31.56 (1700-1970) | $78.13^{b}$±1.28 (72-82) | $86.13^{b}$±1.27 (78-90) | 3.50±0.42 (2-5) |
| **H- 580** | $5.04^{b}$±0.34 (3.2-6.2) | $3.63^{b}$±0.08 (3.5-4) | $1791.25^{b}$±27.02 (1700-1940) | $77.63^{b}$±1.26 (71-83) | $87.13^{b}$±1.49 (78-92) | 3.75±0.49 (2-6) |
| **H-609** | $3.70^{a}$±0.18 (3.0-4.5) | $3.19^{a}$±0.09 (3-3.5) | $1641.75^{a}$±26.43 (1490-1720) | $73.63^{a}$±1.13 (70-80) | $80.89^{a}$±1.70 (75-90) | 4.13±0.35 (2-5) |
| **H-470** | $3.35^{a}$±0.14 (3.0-4.2) | $3.13^{a}$±0.08 (3-3.5) | $1633.50^{a}$±33.58 (1500-1780) | $72.00^{a}$±1.21 (68-78) | $80.12^{a}$±1.70 (75-90) | 4.00±0.26 (2-5) |
| **F-Value** | $18.48^{**}$ | $11.23^{**}$ | $9.79^{**}$ | $6.00^{**}$ | $5.11^{**}$ | $0.50^{NS}$ |
| **Overall** | 4.37±0.19 (3-6.2) | 3.40±0.06 (3-4) | 1718.18±20.27 (1490-1970) | 75.34±0.75 (68-83) | 83.56±0.93 (75-92) | 3.84±0.19 (2-6) |

Means bearing different superscripts in a column (a,b,c) differ significantly.

**: significant (P<0.01).

NS: Non significant.

n = no. of total ejaculates.

In the present study, the overall sperm abnormality includes head, mid piece and tail abnormality. The mean percent total morphological sperm abnormalities of the four Hariana bulls varies between 3.50 ± 0.42 to 4.13 ± 0.35 indicating that all bulls are within the permissible limit of abnormalities and is suitable for cryopreservation.

The analysis of variance of seminal traits among all bulls revealed non-significant difference with regards to percent total morphological sperm abnormalities. Whereas other seminal parameters like volume, mass motility, sperm concentration, percent individual progressive motility and percent live sperm had a significant ($P < 0.05$) difference in all Hariana bull semen.

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