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### Semen characteristics of hallikar bulls

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#### Abstract

The present study was undertaken with the objective of establishing the normal spermiogram in Hallikar bulls based on 11,008 ejaculates from 25 Hallikar bulls over a period of 15 years (January 1999 to December 2013). The observed overall mean ejaculate volume, mass activity (0-5 scale) and sperm concentration based on 11,008 ejaculations were  $4.66 \pm 0.01$ ml,  $4.32 \pm 0.01$  and  $1048.55 \pm 3.64$  million per ml respectively. The overall mean initial motility, post thaw motility, based on 11,008 ejaculations were  $80.26 \pm 0.11$  per cent,  $49.36 \pm 0.07$  per cent, respectively.

Keywords: Spermiogram, hallikar bulls, ejaculate volume, mass activity, sperm concentration

#### Introduction

Hallikar cattle is considered as one of the premier draught breed of India, popularly known as the champion of draught breeds and it is the pride cattle breed of Karnataka having a history of 600 years <sup>[31]</sup>. They are fast track animals used extensively for dry land agricultural operations and for transportation in rural areas. This breed is considered as the progenitor of the Amrithmahal, Khillar and Kangayam breeds <sup>[13]</sup> in the recent times, there is deterioration in the form, size, quality, growth, reproduction and production potentialities of Hallikar cattle breed due to changes in the utility and cropping pattern, breeding objectives and agro biodiversity of the breeding tract. The first step for the sustainable use of domestic animal genetic resources is the gathering of information about the genetic variability through characterization of breeds.

A proper exploitation of the genetic potential of the Hallikar bulls and their semen potential needs to be assessed. Such an assessment based on adequate data helps to know the requirement of bulls to cover the breedable cattle in a given area (Karnataka state) and also helps the Artificial Insemination programmers to plan a suitable semen production regime to cover the breedable cattle of the area and helps the management to fix the physiological norms of semen production for Hallikar bulls. Moreover, the semen quality traits of Hallikar bulls still lack a proper scientific documentation. There is no scientific data available on semen quality traits of Hallikar bulls. Hence, in view of these facts the present study was undertaken to establish the normal spermiogram in Hallikar bulls.

#### **Materials and Methods**

#### Study area

The present study was carried out by utilising the recorded data, on semen production traits of 25 Hallikar bulls for a period of 15 years (January 1999 to December 2013) at State Semen collection centre, Department of Animal Husbandry and Veterinary Services, Government of Karnataka, Hesseraghatta, Bangalore.

#### **Data Collection**

At the farm, all the bulls whose data utilized for the study were maintained under identical housing, feeding, management and health care according to minimum standard protocol (MSP) of Central Monitoring Unit, Government of India. The semen collection was done twice a week from individual bull and 2 ejaculates were obtained with an interval of 15 minutes. A total of 11,008 ejaculates data were collected during the investigation period. Records on semen production were obtained from the collection registers of individual bulls. The data on ejaculate volume, mass activity, sperm concentration, initial motility, post thaw motility were utilized.

#### **Statistical Analysis**

The data on 11,008 ejaculate samples from Hallikar bulls, during the period of investigation, were tabulated and analyzed for volume, mass activity, sperm concentration, initial motility, post thaw motility expressed as mean with standard error. The data were analyzed by method of least square analysis of variance <sup>[8]</sup>.

#### **Results and Discussion**

The mean seminal attributes of 25 Hallikar bulls namely the volume, mass activity, sperm concentration, initial motility and post thaw motility are presented in Table-1.

Table 1: Spermiogram in Hallikar bulls (N= 11008). (Mean± SE)

S. No	Semen attribute	Mean ±SE
01	Volume (ml)	$4.66 \pm 0.01$
02	Mass activity (0-5 Scale)	$4.32 \pm 0.01$
03	Sperm concentration (10 <sup>6</sup> /ml)	$1048.55 \pm 3.64$
04	Initial motility (%)	80.26± 0.11
05	Post-thaw motility (%)	$49.36 \pm 0.07$

#### Volume

The ejaculate volume is an important inherited individual and family trait <sup>[28]</sup> and its assessment is paramount significance in preparation of frozen semen straws. The overall mean ejaculate volume recorded based on 11,008 ejaculates of 25 Hallikar bulls in the present study was  $4.66 \pm 0.01$  ml (Table-1) and ranged between 0.40 to 13.00 ml. The mean ejaculate volume of Hallikar bulls observed in the present study are in close conformity with the values of  $4.43 \pm 0.04$  ml <sup>[37]</sup>, 4.28 ml <sup>[24]</sup> in Sahiwal bulls,  $4.88 \pm 0.19$  ml <sup>[36]</sup> in Ongole bulls,  $4.84 \pm 0.01$  ml <sup>[16]</sup> in Kankrej bulls,  $4.32 \pm 0.07$  ml <sup>[38]</sup> in Red Sindhi bulls,  $4.84 \pm 0.16$  ml<sup>[32]</sup> in Gir bulls. However, the mean ejaculate volume recorded in the present study was lower than those values of  $6.0 \pm 0.22$  ml <sup>[30]</sup> 9.80 ml <sup>[9]</sup> in Sahiwal bulls,  $5.77 \pm 1.75$  ml <sup>[18]</sup> in Ongole bulls, 6.62 ml <sup>[11]</sup> in Kankrej bulls and 7.03  $\pm$  0.44 <sup>[20]</sup> in Gir bulls have been reported and higher than those reported lower mean ejaculatory volume of  $3.36 \pm 0.14$  ml<sup>[19]</sup> in Sahiwal bulls,  $2.91 \pm 0.06$  ml  $^{[25]}$  3.25  $\pm$  0.15 ml  $^{[6]}$  in Punganur bulls, 2.39  $\pm$ 0.13 ml <sup>[21]</sup> in Ongole bulls,  $3.55 \pm 1.22$  ml <sup>[41]</sup> in Kangayam bulls,  $3.84 \pm 1.29$ <sup>[23]</sup> in Tharparkar bulls.  $3.60 \pm 0.40$  ml<sup>[7]</sup> in Nellore bulls.

Ejaculate volume is probably a breed characteristic, which depends upon a long list of factors such as age, body weight and size, scrotal size and weight, sexual stimulus, general health of the bulls, environmental factors, method and frequency of collection, familiarity with attendant, pooled volume, nutrition, season and management <sup>[15]</sup>. The differences in the mean volume of the ejaculate recorded in the present study may possibly attributed to the variation in the management, preparation of individual bulls prior to collection and to the functions of accessory glands which are androgen dependent. Variations in age and breed of bulls, seasonal effects and nutritional status can be attributed to these differences <sup>[28]</sup>.

#### Mass activity (mass motility)

The mean mass activity of semen (0-5 scale) estimated in the present study was  $4.32 \pm 0.01$ (Table-1) with range of 0.50 to 4.50. The mean mass activity recorded in the present study was higher than those reported the mass activity of  $3.33 \pm 0.14$  <sup>[17]</sup>  $2.32 \pm 0.01$  <sup>[4]</sup>, 3.44 <sup>[9]</sup>  $3.17 \pm 0.07$  <sup>[24]</sup> in Sahiwal bulls,  $3.12 \pm 0.16$  <sup>[17]</sup> in Redsindhi bulls,  $2.84 \pm 0.06$  <sup>[25]</sup>, 3.26

 $\pm$  0.06 <sup>[6]</sup> in Punganur bulls, 2.50  $\pm$  0.70 <sup>[36]</sup> in Ongole bulls, 3.72  $\pm$  0.02 <sup>[16]</sup> in Kankrej bulls, 3.33  $\pm$  0.11 <sup>[20]</sup> in Gir bulls, 3.50 <sup>[5]</sup> in Tharparkar bulls, 3.80 <sup>[40]</sup> in Hariana bulls, 2.65  $\pm$  1.05 <sup>[7]</sup> in Nellore bulls and the results of mean mass activity observed in the present study is lower than the value of 4.80  $\pm$  0.40 <sup>[27]</sup> in Nellore bulls.

Variations in the mean mass activity as observed in the present study when compared to other reports could possibly be attributed to the subjective nature of evaluation assessed through naked eye which can vary from person to person and the equipment used may influence the observation. The variations among results of different studies can be attributed to differences in age of bulls at the time of study along with seasonal effects<sup>[1]</sup>.

#### Sperm concentration

The mean sperm concentration for Hallikar bulls in the present study was 1048.55 ± 3.64 million per millilitre (Table-1) and it ranged from 500 to 2473 million per milliliter. This observation is in close agreement with the mean sperm concentration of  $1000.73 \pm 11.06 \times 10^{6}$ /ml<sup>[37]</sup> in Sahiwal bulls,  $1043.75 \pm 16.35 \times 10^{6}$ /ml <sup>[38]</sup> in Redsindhi bulls, 1008 x 106/ml [40] in Hariana bulls. However, several other studies have reported sperm concentrations values of  $1212.81\pm$  98.94 x 10<sup>6</sup>/ml <sup>[17]</sup> 1858.40 x 10<sup>6</sup>/ml <sup>[9]</sup> 1185.53 ± 4.17 x 10<sup>6</sup>/ml  $^{[24]}$  in Sahiwal bulls, 1545  $\pm$  42.55 x 10<sup>6</sup>/ml  $^{[25]}$  $1427.25 \pm 3.77 \text{ x } 10^{6}/\text{ml}$  <sup>[6]</sup> in Punganur bulls,  $1176 \pm 31.58 \text{ x}$  $10^{6}$ /ml <sup>[36]</sup> in Ongole bulls,  $1253.83 \pm 14.68 \times 10^{6}$ /ml <sup>[16]</sup> in Kankrej bulls,  $1298 \pm 116.08 \times 10^{6}$ /ml<sup>[17]</sup> in Red Sindhi bulls,  $1608 \pm 53 \text{ x } 10^{6}/\text{ml}^{[20]}$  in Gir bulls,  $1332 \pm 1.43 \text{ x } 10^{6}/\text{ml}^{[23]}$ in Tharparkar bulls,  $1400 \pm 0.04 \times 10^{6}$ /ml<sup>[34]</sup> in Hariana bulls, which were higher than those values recorded in the present study. The observed sperm concentration of Hallikar bulls as recorded in the present study was found to be higher than those reported values of  $766.69 \pm 5.50 \times 10^{6}$ /ml<sup>[4]</sup> in Sahiwal bulls,  $497.04 \pm 22.37 \text{ x } 10^{6}/\text{ml}^{[21]}$  in Ongole bulls, 894.16 ± 112.66 x  $10^{6}$ /ml<sup>[12]</sup> in Tharparkar bulls.

The variations in the sperm concentration reported by other studies when compared to those observed in the present study may probably subscribed to the variations in age of the bull, scrotal circumference, sexual maturity, health status of the reproductive tract, plane of nutrition, season, frequency and method of semen collection and degree of sexual excitement etc <sup>[2, 10]</sup>.

#### **Initial motility**

The overall mean initial motility recorded for Hallikar bulls in the present study was 80.26  $\pm$  0.11 per cent (Table-1) and ranged from 70.00 to 90.00 per cent. These findings are in accordance with the findings of 81.62  $\pm$  3.94 per cent <sup>[23]</sup> in Tharparkar bulls, 81.40  $\pm$  0.01 per cent <sup>[35]</sup> in Sahiwal bulls. Whereas, observed values were lower than that of 86.15  $\pm$ 0.30 per cent <sup>[16]</sup> in Kankrej bulls, 86.41  $\pm$  0.66 per cent <sup>[33]</sup> in Hariana bulls. The observed sperm concentration was found to be higher than 76.73  $\pm$  0.43 per cent <sup>[24]</sup>, 65.53  $\pm$  0.31 per cent <sup>[37]</sup>, 68.80 per cent <sup>[9]</sup> in Sahiwal bulls, 72.85  $\pm$  0.71 per cent <sup>[6]</sup> in Punganur bulls, 68.40  $\pm$  1.33 per cent <sup>[20]</sup> 77.27  $\pm$ 0.66 per cent <sup>[36]</sup> in Ongole bulls, 66.89  $\pm$  0.37 per cent <sup>[38]</sup> in Red Sindhi bulls, 71.50  $\pm$  0.89 per cent <sup>[20]</sup> in Gir bulls, 70.00 per cent <sup>[39]</sup> in Hariana bulls.

The variations in the mean initial motility as reported by others studies when compared to the values obtained for Hallikar bulls in the present study may possibly discounted for the subjective nature of evaluation, which is influenced by human elements involved in the evaluation. The difference in individual motility in various reports can be due to variations in handling of semen, number of bulls studied and environmental influences <sup>[3]</sup>. Moreover, the results can also vary from person to person, as sperm motility is usually assessed under microscope through naked eye.

#### Post thaw motility

The mean post thaw motility recorded in Hallikar bulls was  $49.36 \pm 0.07$  per cent (Table-1) with a range of 30.00 to 60.00 per cent. These findings are in close agreement with those reported mean values of  $50.14 \pm 0.02$  per cent <sup>[37]</sup> in Sahiwal bulls,  $50.11 \pm 0.03$  <sup>[38]</sup> in Redsindhi bulls. The observed values were higher than those reported values of  $42.19 \pm 2.67$  per cent <sup>[32]</sup> in Gir bulls,  $42.73 \pm 1.68$  per cent <sup>[14]</sup> in Sahiwal bulls,  $44.50 \pm 13.50$  per cent <sup>[27]</sup> in Nellore bulls. The observed mean was lower than those reported value of  $57.85 \pm 1.61$  per cent <sup>[36]</sup> in Ongole bulls,  $53.58 \pm 0.59$  and  $57.21 \pm 0.66$  per cent <sup>[33]</sup> in Hariana bulls and Sahiwal bulls, respectively, 63.60 per cent <sup>[9]</sup> in Sahiwal bulls.

The variations in the post thaw motility reported by others when compared to the present study might be due to various freezing techniques employed during different stages of cryopreservation, since, the degree of cryoinjuries can vary with breed and the laboratory module employed for freezing besides age of the bull can affect post thaw motility. Further, freezing of semen reduced sperm motility irrespective of the breed <sup>[26]</sup>. Both freezing and thawing implicate tremendous alterations in cell water volume, which result considerable mechanical stress on the membrane and consequently reduce sperm motility (Hammerstedt *et al.*, 1990). Cryopreservation of bovine semen involves many critical steps such as extension, cooling, freezing, storage and thawing, which can also affect the sperm both physiologically and structurally <sup>[3]</sup>.

#### **Summary and Conclusion**

The overall mean for seminal attributes like ejaculate volume, mass activity (0-5 scale) and sperm concentration based on 11,008 ejaculations were  $4.66 \pm 0.01$ ml,  $4.32 \pm 0.01$  and  $1048.55 \pm 3.64$  million per ml, respectively. The overall mean initial motility, post thaw motility, based on 11, 008 ejaculations were  $80.26 \pm 0.11$  per cent,  $49.36 \pm 0.07$  per cent, respectively.

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