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Physico-morphological Characteristics of Semen Ejaculates in *Vrindavani* Crossbred Bulls

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ABSTRACT

In crossbred cattle bulls, poor seminal quality and freezability are major constraints. The present study was conducted to determine semen quality parameters of *Vrindavani* crossbred bulls. In this study 130 semen samples were collected from five *Vrindavani* crossbred bulls. The volume, mass motility, concentration and individual progressive motility, viability and abnormality of spermatozoa were examined. Then ejaculates were categorized into four groups on the basis of spermatozoa concentration and individual progressive motility. The percentage of normozoospermia, asthenozoopsermia, oligozoospermia and oligoasthenozoospermia ejaculates were 44.62%, 29.23%, 11.54% and 14.61%, respectively. The overall mean of volume, mass motility, concentration of spermatozoa, individual progressive motility, viability and sperm abnormality in semen ejaculates of crossbred *Vrindavani* bulls were 4.64±0.63 mL, 2.48±0.09, 772.20±27.95 million/mL, 65.08±1.47%, 79.94±1.07% and 7.82±0.44%, respectively. Ejaculate mass motility, concentration, progressive motility and abnormality of spermatozoa varied significantly ($p<0.05$) among the semen ejaculate categories. The mean of the mass motility was significantly higher in normozoospermia as compared to other ejaculates categories. The normozoospermia and asthenozoospermia groups had significantly higher spermatozoa concentration than oligozoospermia and oligoasthenozoospermia groups. The normozoospermia ejaculates had significantly higher progressive motile spermatozoa as compared to other three ejaculates categories. Percent livability of spermatozoa in normozoospermia and oligozoospermia groups was significantly higher than asthenozoospermia and oligoasthenozoospermia groups. Percent abnormal spermatozoa on the other hand, were significantly lower in normozoospermia and oilgozoospermia groups than asthenozoopsermia and aligoasthenozoospermia groups.

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Introduction

Crossbred bulls are prone for many reproductive problems and possess higher culling rate due to sub-fertility (Khatun et al., 2013). Semen rejection rates are particularly high in crossbred cattle bulls due to poor semen quality and poor freezability (Soni et al., 2019). It has been reported that the incidence of poor-quality ejaculates in crossbred bulls is as high as 50% and could not be used for cryopreservation thereby hindering the crossbreeding program (Vijetha et al., 2014). Furthermore, the percentage of crossbred bulls that have their ejaculate rejected due to poor semen quality ranges from 10% to 100% (Vijetha et al., 2014; Gopinathan et al., 2016). Moreover, post-thaw semen rejection rate in crossbred bulls is still very high (Mandal et al., 2015). In crossbred bulls increase in exotic inheritance and increase in the numbers of breed components decreased the proportion of freezable quality semen producer bulls (Tyagi et al., 2006). On account of these factors, there is a significant disparity between supply and demand for frozen semen (Rao et al., 2017).

Volume, mass motility, concentration, individual progressive motility, viability and percentage of abnormal spermatozoa, and other parameters related to semen quality are usually examined after semen collection (Kumar et al., 2018). Different physical properties of spermatozoa can be examined to determine better fertility potential because no single test can reliably predict the quality of sperm (Cenariu et al., 2018). For bulls used for production of frozen semen for in artificial insemination, detailed enumeration on semen quality parameters, semen production efficiency and fertility are of paramount importance for which data on *Vrindavani* crossbred is lacking. Therefore, the present study was conducted to determine semen quality parameters in different ejaculates categories in *Vrindavani* crossbred bulls.

Materials and methods

Study area

The proposed study was conducted at Germ Plasma Center (GPC), Animal Reproduction Division, ICAR-Indian Veterinary Research Institute (IVRI); Bareilly, Izatnagar, (UP). The institute is situated approximately 564 feet above sea level. During the summer and winter seasons, the research area experiences alternating extremes of hot and cold weather. The relative humidity in the research location ranges from 15 to 85%.

Experimental animals

For this study five apparently healthy *Vrindavani* crossbred bulls (crosses of *Holstein Friesian, Jersey, Brown Swiss* with indigenous *Hariana* breed*)* aged 5-7 years maintained at the Germ Plasm Center of the Animal Reproduction Division, ICAR-Indian Veterinary Research Institute, Izatnagar were utilized to collect semen. The study was conducted from August, 2021 to July, 2022. The bulls were kept in optimal managemental conditions throughout the experiment. All these experimental bulls were under routine semen production programme.

Extender preparations

The Tris-Fructose-Egg-Yolk-Glycerol (TFEYG) extender composed of 3.028 g of Tris, 1.675 g of citric acid, 1.25 g of fructose and 10 mL of egg yolk was used for this study.

Semen collection

For this study, 130 ejaculates were collected from five *Vrindavani* bulls using artificial vagina (AV) as per the standard method. The collection was conducted twice a week in the early morning and two times a day at intervals of 15-30 minutes. After collection, a tube containing ejaculated semen was kept at 34°C in a water bath for the duration of the semen examination.

Semen volume

The volume of ejaculated semen was recoded directly from the graduated semen collecting tube.

Mass motility

Mass motility was examined on a 5-point scale basis ranging from 0–5 using phase contrast warm stage microscope (MT-6300, Meiji Techno, Japan) (Herman et al., 1994).

Sperm concentration

The concentration (million per mL) of spermatozoa of each ejaculate was determined using the photometer (Accucell Photometer, IMV technologies, France).

Individual progressive motility (IPM)

The IPM was evaluated using automated sperm quality analyzer-Vision for bulls (SQA-Vb, Medical Electronic Systems, Caesarea Industrial Park, Israel).

Grouping of ejaculates

The ejaculates were categorized into four groups on the basis of spermatozoa concentration and IPM based on the method described by Mandal et al. (2012) with slight modification as Gr. 1, normozoospermia (n=58): (concentration ≥500 million/mL and IPM≥70%); Gr. 2, oligozooserpmia (n=15): (concentration <500 million/mL and IPM ≥70%), Gr. 3, asthenozoospermia (n=38): (IPM <60% and concentration ≥500 million/mL) and Gr. 4, oligoasthenozoospermian (n=19): (IPM <60% and concentration <500 million/mL).

Spermatozoa viability and abnormality

The spermatozoa viability and abnormality were evaluated using a vital staining method employing Eosin-Nigrosin stain (Swanson and Bearden, 1951). The Nigrosin and Eosin satins were mixed in a ratio of 3:1 on pre-warmed (at 37 ^{0}C) clean greasy free glass slide and a drop of diluted semen was added to stain and mixed thoroughly. After 30 seconds a thin smear was prepared and air dried. Then the air-dried smear was examined under oil immersion magnification (1000X) using a phase contrast microscope (MT-6300, Meiji Techno, Japan). A minimum of 200 spermatozoa were counted and percent viability and abnormality were determined for each slide.

Statistical analysis

The data generated from the study was analyzed by ANOVA using SPSS software program, version 20 and the results were expressed as mean ± standard error. The mean difference among semen categories were compared using Tukey's multiple comparisons.

Results and discussion

The collected semen ejaculates were divided into four categories based on their concentration and progressive motility (IPM). In the current study, normozoospermia and oligoasthenozoospermia ejaculates constituted 44.62% and 14.61%, respectively of the semen samples that were collected, whereas oligozoospermia and asthenozoopsermia comprised 11.54% and 29.23%, respectively of the collected ejaculates. These results are in agreement with the findings of Mandal et al. (2012) in Frieswal crossbred bulls. The overall quality parameters of semen ejaculates characterized as normozoospermia and oligozoospermia, asthenozoopsermia and oligoasthenozoosperia in *Vrindavani* crossbred bulls have been presented in Table 1. The overall average of volume, mass motility (0-5 scale), concentration of spermatozoa, individual progressive motility, viability and sperm abnormality in semen ejaculates of *Vrindavani* crossbred bulls were 4.64±0.63 mL, 2.48±0.09, 772.20±27.95 million/mL, 65.08±1.47%, 79.94±1.07% and 7.82±0.44%, respectively.

In crossbred cattle bulls, poor seminal quality and freezability are key constraints, which are mostly caused by low sperm concentration, inadequate mass activity, and progressive motility of spermatozoa (Mukhopadhyay et al., 2010). In this study the overall semen quality of the *Vrindavani* crossbred bulls was in agreement with above mentioned reports. The mass motility documented in this study is lower than that reported by Kumar et al. (2015) in crossbred Jersey and Mukhopadhyay et al. (2010) in Karan Fries bulls. The overall ejaculate volume in this study is in agreement with earlier report on ejaculates volume of Karan Fries bulls (Mukhopadhyay et al., 2010) but lower than that reported in crossbred Jersey (Vijetha et al., 2014; Kumar et al., 2015). In the present study the overall spermatozoa concentration is lower than spermatozoa concentration reported by others (Vijetha et al., 2014; Rehman et al., 2016; Kumar et al., 2015) in different crossbreds. The overall individual progressive motility recorded in this study is lower than earlier reports (Rehman et al., 2016; Kumar et al., 2015) but it is higher than individual progressive motility reported by Rao et al. (2017) in different crossbreds. These variations might be due to breed differences. The overall per cent live spermatozoa in this study is in accordance with the reports of (Vijetha et al., 2014; Kumar et al., 2015) in crossbred bulls. Furthermore, the percent abnormal spermatozoa in this study are in agreement with the previous reports in crossbred bulls (Kumar et al., 2015; Perumal et al., 2016).

Table 1. The overall parameters of semen ejaculates characterized as normozoospermia and oligozoospermia, asthenozoopsermia and oligoasthenozoosperia in *Vrindavani* crossbred bulls.

Parameter	Mean ± SE
Mass motility (0-5 scale)	2.48±0.09
Volume (mL)	4.64±0.63
Concentration (10^6/ml)	772.20±27.95
IPM (%)	65.08±1.47
Abnormality (%)	7.82±0.44
Viability (%)	79.94±1.07

Mean ± S.E. of semen quality parameters in different semen categories of *Vrindavani* crossbred bulls is given in Table 2. Ejaculate mass motility, concentration, progressive motility and abnormality of spermatozoa varied significantly ($p<0.05$) among normozoospermia and asthenozoopsermia, oligozoospermia and oligoasthenozoosperia semen ejaculates. Mass motility (0-5 scale) of the ejaculates was significantly ($p<0.05$) higher in normozoospermia as compared to other semen categories. However, it was not significantly varied among asthenozoopsermia, oligozoospermia and oligoasthenozoosperia semen categories. No significant difference in mean ejaculate volume among all semen groups. Nevertheless, the difference in mass motility among these four groups has so far not been studied in this crossbred bull. However, the higher mass motility is associated with semen freezability (Kumar et al., 2018).

Spermatozoa concentration is an essential semen parameter which aids in calculating the amount of extender that desired to be added to neat semen so as to attain standardized doses for AI (Bjorndahl, 2013). Furthermore, the number of semen straws produced, the maximum number of inseminations covered, and/or the spermatozoa fertility level are all determined by the spermatozoa concentration (Kumar et al., 2018). In the current study, normozoospermia and asthenozoospermia groups had significantly ($p<0.05$) higher spermatozoa concentrations than oligozoospermia and oligoasthenozoospermia groups (Fig. 1).

However, in this crossbred the variation in concentration among these ejaculates groups has not yet been studied. But, the mean concentration of spermatozoa in normozoospermia and asthenozoospermia groups were well within the range of overall spermatozoa concentration reported by others in good freezable ejaculates of different crossbreds (Vijetha et al., 2014; Pande et al., 2015; Perumal et al., 2016). However, in *Vrindavani* crossbred bulls, the spermatozoa concentration differences in the oligozoospermia and oligoasthenozoosperia groups have yet to be reported. The mean spermatozoa concentration in oligozoospermia and oligoasthenozoosperia groups in *Vrindavani* crossbred bulls was comparable to that of Frieswal crossbred bulls (Mandal et al., 2010). In fertile bull the spermatozoa concentrations range between 800-2000 x 10^6 mL^- (Garner and Hafez, 2000). The spermatozoa concentration in bulls is affected by semen collection frequency, season and age of the bull (Garner and Hafez, 2000).

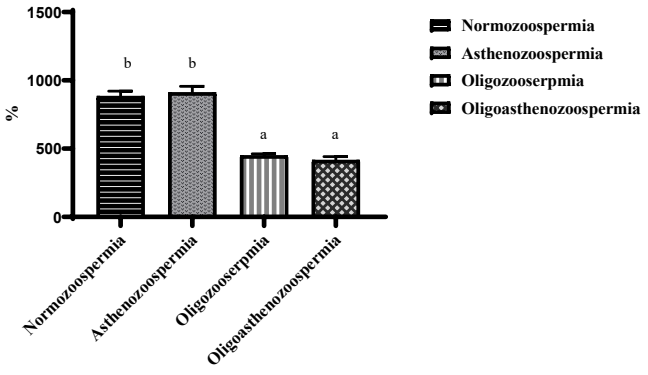

Fig. 1. The mean value of concentration of spermatozoa in normozoospermia, asthenozoospermia, Oligozooserpmia and Oligoasthenozoospermia ejaculates in *Vrindavani* crossbred bulls

Table 2. Physico-morphological characteristics of semen ejaculates characterized as normozoospermia and oligozoospermia, asthenozoopsermia and oligoasthenozoosperia in *Vrindavani* crossbred bulls.

Parameter	Oligoasthenozoospermia (n = 19)	Oligozooserpmia (n = 15)	Normozoospermia (n = 58)	Asthenozoospermia (n = 38)
Volume (mL)	4.64±0.63^a	5.15±0.44^a	5.06±0.63^a	3.78±0.31^a
Mass motility (0-5 scale)	1.63±0.18^b	2.00±0.17^b	3.19±0.11^a	2.03±0.14^b
Concentration (10^6/mL)	415.94±25.66^b	448.97±12.60^b	881.66±37.10^a	910.84±45.29^a
IPM (%)	46.84±2.30^c	70.00±0.98^b	78.97±0.97^a	51.05±2.02^c
Abnormality (%)	10.04±1.49^a	6.34±0.37^b	6.34±0.59^b	9.56±0.84^a
Viability (%)	72.56±3.12^b	82.65±2.10^a	87.42±0.91^a	71.14±1.89^b

Means bearing different superscripts (a, b and c) in a row differ significantly ($p<0.05$)

Progressive motility is spermatozoa ability of featuring rapid and forward progression at an acceptable rate and it is the most proper form of motility evaluation (Seidel, 2012). Individual progressive motility assessment is the most often applied test to determine the quality of spermatozoa at both fresh and post-thaw stages (Shukla and Mishra, 2005). It is used to evaluate the quality of freshly ejaculated semen before it is chosen for use in AI stations to produce frozen spermatozoa (Yamada et al., 2018). Spermatozoa motility evaluation allows identifying infertile bulls as their spermatozoa commonly appear less motile (Cenariu et al., 2018).

In this study, normozoospermia ejaculates had significantly higher progressive motile spermatozoa as compared to asthenozoopsermia, oligozoospermia and oligoasthenozoosperia semen categories (Fig. 2). Also, oligozoospermia ejaculates had significantly more progressive motile spermatozoa than the asthenozoopsermia and oligoasthenozoosperia groups. We have categorized ejaculates on the basis of concentration of spermatozoa and progressive motility, which has resulted in these significant differences in concentration and progressive motility of spermatozoa. As a result, there will be differences among the groups. Similar findings were reports in Frieswal crossbred bulls (Mandal et al., 2012).

Ejaculates having individual progressive motility of 70% or more are considered as freezable. In the current study, the mean progressive motility of spermatozoa was significantly higher in normozoospermia ejaculates, which is comparable with the findings of Kumar et al., 2015, Pande et al., 2015 and Perumal et al., 2016. But it was higher than the mean progressive motility of spermatozoa reported by Singh (2007) and Vijetha et al. (2014) in different crossbreds. Mandal et al. (2010) reported a much lower average progressive motility of spermatozoa as compared to the finding of this study in Holstein Friesian x Sahiwal crossbred bulls. The means of progressive motility of spermatozoa recorded in the asthenozoopsermia and oligoasthenozoosperia groups which are considered as a non-freezable ejaculate were comparable to individual spermatozoa motility in poor Karan Fries bulls (Vijetha et al., 2014) but it was lower than the report of Perumal et al. (2016) in poor freezable ejaculates of Jersey crossbreds. These discrepancies might be due to factors such as age of animals, season and semen collection procedures which affect progressive motility of spermatozoa (Javed et al., 2000). Semen sample contamination because of inappropriate handling practices can readily impede progressive motility of spermatozoa. Spermatozoa lose their vigour of forward movement if semen samples are not examined for motility soon after collection (Barth, 2007).

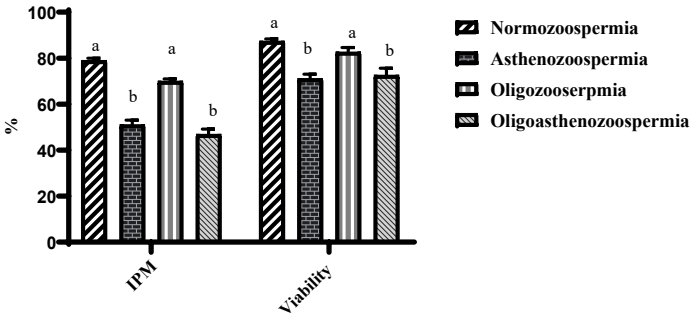

Fig. 2. The mean value of individual progressive motility and viability in normozoospermia, asthenozoospermia, Oligozooserpmia and Oligoasthenozoospermia ejaculates in *Vrindavani* crossbred bulls**.**

The percentage of viable spermatozoa determines the ejaculate's quality. According to reports, percentage of viable spermatozoa in the semen and fertility of bull are positive correlated (Januskauskas et al., 2003). Ejaculates with more than 30% initial dead spermatozoa may not be suitable for freezing and generally discarded (Januskauskas et al., 2003). In the current study, normozoospermia and oligozoospermia groups recorded significantly ($p<0.05$) higher percentages of viable spermatozoa than asthenozoopsermia and oligoasthenozoosperia categories (Fig. 2). There is no data available to compare the percentage of viable spermatozoa among these four groups of crossbred bulls. However, the reports on percentage of live spermatozoa in ejaculates of different crossbred bulls are available. The ranges of percent live spermatozoa in normozoospermia and oligozoospermia groups are in accordance with the reports of Vijetha et al. (2014), Perumal et al. (2016) and Rehman et al. *(*2016) but higher than the reports of Rao et al. (2017) and Mandal et al. (2010) in different crossbreds. The percent live spermatozoa depend on the factors such as age of the bulls, temperature, frequency of semen collections and sexual excitement before collection (Rao et al., 2017).

Morphological abnormalities of spermatozoa and their frequency may help to predict semen fertilizing capacity (Ostermeier et al., 2001; Saacke, 2008). Though abnormality of spermatozoa was significantly ($p<0.05$) higher in asthenozoopsermia and aligoasthenozoospermia than in normozoospermia and oilgozoospermia groups (Fig. 3) but it was well within the permissible range (Kumar et al., 2015; Perumal et al., 2016). In conclusion, the present study was undertaken to have a better understanding of types of semen ejaculates in *Vrindavani* crossbred bulls. The 44.62% (58/130) of the collected ejaculates were normozoospermia ejaculates, which indicate that almost half of the ejaculates from *Vrindavani* crossbred bulls are not

suitable for frozen semen production and it is needed to be studied in future.

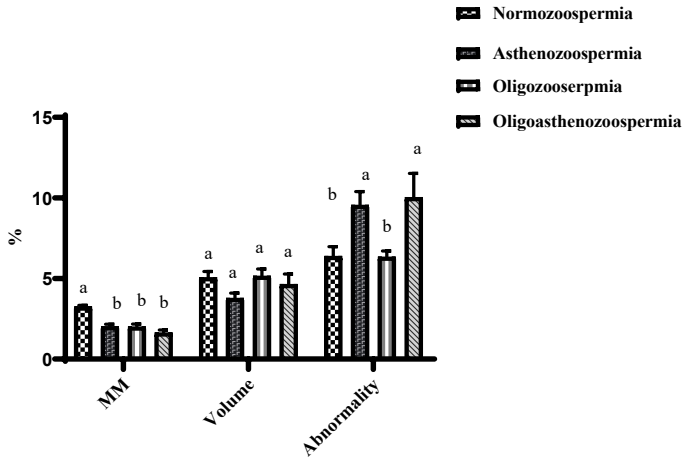

Fig. 3. The mean value of mass motility, volume and abnormality in normozoospermia, asthenozoospermia, Oligozooserpmia and Oligoasthenozoospermia ejaculates in Vrindavani crossbred bulls

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Conflict of Interest

The authors declare that they have no conflict of interest

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