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Semen Characteristics and Protein Contents in Seminal Plasma of Vechur Bulls

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ABSTRACT

The present investigation was carried out to study the semen characteristics of Vechur bulls. A total of 36 ejaculates collected from six Vechur bulls were studied. The overall mean ejaculate volume, colour, pH, mass activity, progressive sperm motility and total seminal plasma protein concentrations were analysed. The mean ejaculate volume noted was 2.26 ± 0.36 ml, colour of semen ranged from milky to thick milky, pH was 6.87 ± 0.02, density from DDD to DDDD, mass activity +++ to ++++ and sperm progressive motility was 78.1 ± 1.05 %. Significant differences in ejaculate volume, mass activity, progressive sperm motility and live sperms were observed among the bulls. The mean protein content of seminal plasma was 84.10 ± 2.73 mg/ml. On SDS-PAGE analysis, 20 proteins bands of varying intensities were observed within the range of 10-180 kDa and preponderance of lower molecular weight proteins was recorded.

Key words: Vechur, Semen, Gross semen characteristics.

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INTRODUCTION

Vechur is an indigenous cattle breed of Kerala, which has limited population. With a mean height 89.43 ± 6.55 cm and average body weight of around 170 kg for the bulls, it is one of the shortest cattle breeds in the world (Sudheer *et al.,* 2021). The breed is characterized by its adaptability to hot and humid climatic conditions and minimal feed requirement. The daily milk yield of the breed is 2.5 ± 0.5 litres. Though the breed is in high demand, the population of purebred individuals is limited. Semen collection and artificial insemination are considered as powerful biotechnological tools for conservation of Vechur cattle and dissemination of its germ plasm all over the state. The Vechur

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farm under Vechur conservation project, Department of Animal Breeding and Genetics, Kerala Veterinary and Animal Sciences University, maintains Vechur bulls for semen collection and conservation. Studies on Vechur semen characteristics are limited, with the predominant work being reported by Venkatachalapathy *et al.* (2004). The present study was undertaken to evaluate the characteristics of Vechur bull semen ejaculates with respect to the volume, colour, pH, density, mass activity, progressive motility and protein content of seminal plasma.

MATERIALS AND METHODS

Vechur bulls maintained under the Vechur conservation project of Department of Animal Breeding and Genetics, College of Veterinary and Animal Sciences, Mannuthy were used for semen collection. The bulls were screened for breeding soundness and six healthy adult Vechur bulls were selected for the study. The research was carried out as per the guidelines of the Institutional Animal Ethics Committee.

A Danish model artificial vagina was used for the semen collection, which was carried out as per the standard practice. On the day of semen collection, the prepuce of the bulls was washed with water followed by normal saline and the preputial orifice was wiped with sterile napkin. Two false mounts were given before semen collection during every time. Artificial vagina maintained at a temperature of 40-45°C with adequate pressure were used for semen collection with the other bulls maintained in the farm serving as dummy. Ejaculates were collected at a frequency of twice per week from a single bull and at least two days gap was ensured between collections from each individual bull. The collected ejaculate was immediately transferred to semen processing laboratory for evaluation. A fraction of the whole ejaculate was separated immediately post collection and supplemented with a protease inhibitor cocktail used at the manufacturer's recommended concentrations (P 8340, Sigma Aldrich, USA). This fraction was used for evaluation of protein content. A total of 36 ejaculates were collected and evaluated.

The volume of the ejaculate was measured in milliliter directly from the graduations of the glass collection vial. The colour and density of the semen sample were assessed visually, for which a drop of pooled semen sample was placed on clean and grease free glass slide and visually examined. The density was graded on a scale of 0 to DDDDD. The pH of semen was assessed using narrow range indicator paper. The mass activity of each semen sample was evaluated by examining 25 µl of the ejaculate placed on a clean, grease free glass slide under 100× magnification of a light microscope. The waves and eddies produced by the vigorous massive movement of sperms were observed and based on which the samples were graded on a scale of 0-5 (0 – no motility and 5-thick waves and eddies). Progressive motility of semen sample was assessed following extension with 2.96 % sodium citrate solution to such an extent that each high power field had 20 – 25 spermatozoa. At least five random fields were assessed for estimation of sperm progressive motility. For evaluation of motility, the slides were maintained at 37°C on a stage warmer during examination.

For the harvest of seminal plasma, the antiprotease supplemented fraction was centrifuged initially at 2000 × g for 20 min at room temperature to remove the suspended spermatozoa. The supernatant plasma was collected and further centrifuged at 10000 × g for 60 min at 5°C to clarify the seminal plasma of cells, if any. The seminal plasma was supplemented with sodium azide (0.025 %) and then stored at –80 °C until analysis. The total protein content of seminal plasma of individual ejaculates from each bull were estimated using BCA protein assay kit (EMD Millipore Corp.). Discontinuous SDS- PAGE profiling of the total proteins of Vechur seminal plasma were done according to Laemmli, (1970) in a Biorad mini vertical electrophoresis apparatus (mini PROTEAN® tetra cell, USA).

RESULTS AND DISCUSSION

Six ejaculates from each of the six bulls under study were collected for analysis. The mean values of gross semen characteristics of the collected ejaculates with respect to volume, colour, density and mass activity are presented in Table 1.

Table 1. Semen characteristics of Vechur bull ejaculates (n=36)

S.No	Semen Characteristics	Mean SE	Minimum	Maximum
1	Volume	2.26 ± 0.36 ml	1.1 ml	4.2 ml
2	Colour	-	Milky	Thick milky
3.	pH	6.87 ± 0.02	6.7	6.9
3	Density	-	DDD	DDDD
4	Mass activity	-	+++	++++
5	Progressive motility	78.1 ± 1.05 %	70%	85%

In the present study, the volume of semen ejaculate obtained from Vechur bulls was 2.26 ± 0.36 ml, with a range of 1.1 to 4.2 ml. Venkatachalapathy *et al.* (2004) and Yamini (2017) had reported ejaculate volumes of

2.8 ± 0.92 ml and 2.36 ± 0.21 ml, respectively in Vechur bulls. The semen volume recorded in Vechur bulls was much lower than that of other indigenous breeds such as Nellore bulls (4.8 ± 0.1 ml, Koivisto *et al.,* 2009) and Kankrej bulls (4.84 ± 0.01 ml, Patel and Siddiquee, 2013). Pungannur, which also is a dwarf breed of cattle, too had a higher semen volume (3.25 ± 0.15 ml, Bramhaiah *et al.,* 2013). The crossbred bull reared in the country also had a higher ejaculate volume. Sugulle *et al.* (2006) and Rahman *et al.* (2014) reported ejaculate volumes of 6.5 ± 1.32 ml and 5.81 ± 0.016 ml, respectively, in cross bred bulls.

The size of the bull and testicular size influences the volume of ejaculate (Sane *et al.,* 1994). It was also reported that the volume of *Bos indicus* semen ejaculates were lower than that of *Bos taurus* (Koivisto *et al.,* 2009). The lower body and testicular size of the Vechur breed might have contributed to the lower ejaculate volume compared to other native breeds.

Colour of the Vechur bull semen ejaculate varied from milky to thick milky, which was comparable to the previous observations made on Vechur bull semen ejaculates by Venkatachalapathy *et al.* (2004). However, Yamini (2017) had noted that the ejaculate colour varied between milky white to creamy. The colour of the ejaculate was predominantly a function of the sperm concentration and Kanchan and Matharoo (2015) suggested that when colour was creamy, the concentration of the ejaculate was at its peak. The density of Vechur semen ejaculate recorded in the present study varied from DDD to DDDD, which was similar to the observations made by Yamini (2017).

In the present study, the Mean ± SE pH of Vechur bull semen ejaculates was found to be 6.87 ± 0.02 which is in agreement with the observations of Venkatachalapathy *et al.* (2004) in Vechur bulls and also with another indigenous breed Kankrej (Patel and Siddiquee, 2013). However, the present observation was varying from Holstein bulls (Uysal *et al.,* 2007) and crossbred bulls (Rahman *et al.,* 2014) where the pH of semen is more acidic than Vechur bull semen.

The mass activity of the ejaculates observed in the present study varied from +++ to ++++, which was similar to the values reported for Vechur bulls by Venkatachalapathy *et al.* (2004). The recorded mass activity was comparable to those recorded in zebu crossbred bulls (Rahman *et al.,* 2014).

The sperm progressive motility (mean ± SE) in the present study was found to be 78.10 ± 1.05 %. These values were lower to those reported by Venkatachalapathy *et al.* (2004, 80.0 ± 2.8 %) and Yamini (2017, 85.83 ± 0.93 %). The reported sperm progressive motility in other Indian breeds were 60.00 ± 4.08 % in Kangayam (Veerapandian *et al.,* 1992), 71.50 ± 0.89 % in Gir (Raina and Dhami, 2004), 77.27 ± 0.66 % in Ongole (Talluri *et al.,* 2011), 65.53 ± 0.31 % in Sahiwal (Tiwari *et al.,* 2013) and 86.15 ± 0.03 % in Kankrej (Patel and Siddiquee, 2013).

The total protein concentration in seminal plasma of all the 36 ejaculates were studied and observations are represented in Table 2.

Table 2. Seminal plasma protein concentration (mg/ml) in Vechur bull ejaculates assessed by Bicinchoninic acid assay

S. No.	Bull No.	Mean ± SE (n=6)	Minimum	Maximum
1	V 929	93.63^{b} ± 4.53	80.59	112.19
2	V962	76.22^{a} ± 6.92	53.58	98.25
3	V 1025	71.82^{a} ± 2.14	64.84	78.26
4	V959	108.57^{c} ± 3.18	98.95	117.04
5	V 964	75.09^{a} ± 2.76	62.85	82.41
6	V974	79.28^{a} ± 4.65	65.18	90.55
Average protein concentration		84.10 ± 2.73	53.58	117.04
F value		10.743**		

***: Significant at 0.01 level, different superscript in a row differs significantly.*

Vechur bull semen was recorded to have a mean protein concentration of 84.10 ± 2.73 mg/ml with a range of 71.82 ± 2.73 mg/ml to 108.57 ± 3.18 mg/ml among individual bulls. Significant differences (P<0.01) were recorded among the bulls for total protein content. The observations suggested that the protein content of seminal plasma varied significantly between bulls and between ejaculates of each bull. The average seminal plasma protein concentration in indigenous and crossbred cattle was reported by Seshagiri and Pattabhiraman (1991). They had recorded the mean seminal plasma protein concentration in the seminal plasma of Sindhi, Jersey, Jersey × Sindhi and Sindhi × Friesian bulls as 116.0, 87.0, 103.0 and 107 mg/ml, respectively which was higher than the values recorded for Vechur bull. Roncoletta (2006) recorded a broader range in semen protein concentration among ejaculates of Nellore bull (33 to 111 mg/ml), with a mean protein content of 61.74 mg/ml. The total protein content of Holstein bull seminal plasma was found to vary from 73 to 93 mg/ml (Nauc and Manjunath, 2000). Ramteke *et al.* (2014) reported that the total seminal plasma protein concentration of buffalo bull seminal plasma ranged from 28.51 to 32.53 mg/ml.

On SDS-PAGE analysis, 20 proteins bands of varying intensities were observed in seminal plasma of Vechur bulls within the range of 10-180 kDa and preponderance of lower molecular weight proteins (Fig. 1).

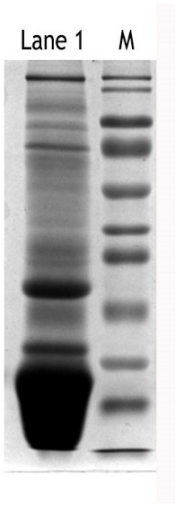

Fig. 1: SDS-PAGE profile of seminal plasma proteins from Vechur bulls, Lane 1 – seminal plasma proteins; Lane M: Molecular weight marker (Puregene, 10-180 kDa).

Karthikeyan (2015) had recorded 18 protein bands on SDS-PAGE analysis of total seminal plasma proteins in Vechur bull. Kulkarni *et al.* (1996) had recorded 20 protein bands in the molecular weight range of 11 to 92 kDa in bovine seminal plasma. Druart *et al.* (2013) had recorded that the seminal plasma of cattle had an electrophoretic profile in the 5–250 kDa range with a predominance of proteins with molecular weight below 25 kDa.

CONCLUSION

The seminal attributes of Vechur bull was found to be comparable to other indigenous cattle, except for volume, which was found to be lower in Vechur bulls. The quantity of seminal plasma protein was found to be comparable to the other cattle breeds.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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