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Influence of season on the quality of Malabari buck semen#

S.P. Vinay Sankar[1], K.Lekshmi Bhai[2]*, K. Promod[1], C.P.Abdul Azeez[1], Leeba Chacko[1], Renjith Sebastian[3]

[1]Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animals Sciences, Pookode, Wayanad, [2]University Veterinary Hospital and Teaching Veterinary Clinical Complex, Mannuthy, Thrissur, [3]Department of Veterinary Biochemistry, College of Veterinary and Animals Sciences, Pookode, Wayanad.

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Abstract

The present study evaluated the semen quality parameters of Malabari bucks during pre-monsoon (March-May), south-west monsoon (June-September), post-monsoon (October-November) and winter (December-February) seasons. The semen ejaculates (n=120) collected from five healthy adult Malabari bucks maintained at the Artificial Insemination Centre, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Pookode, Wayanad (11.5393° N and 76.0202° E) were used for the study. Average THI was found to be 74.79, 71.33, 71.29 and 68.86 in the respective seasons. The ejaculates were subjected to preliminary evaluation in terms of volume (mL), sperm concentration (millions/mL), progressive motility of spermatozoa (%), viability (%), morphological abnormalities (%), acrosome integrity (%) and functional membrane integrity (%). In all the four seasons, seminal plasma was subjected to SDS PAGE for protein profiling. There was significant decrease in the semen ejaculate volume ($p< 0.001$) during pre-monsoon season. But, no significant effect of season could be appreciated in the other quality parameters of fresh semen such as mass activity, concentration, progressive motility, morphological abnormalities, acrosome integrity and functional membrane integrity. SDS PAGE protein profile of seminal plasma revealed significant difference in terms of number of protein bands and their incidence rate. It can be concluded that seasonal variation exists in the semen ejaculate volume and seminal plasma proteins of Malabari bucks and a general trend of decrease in the quality of Malabari buck semen in pre-monsoon season could also be revealed.

Keywords: *Malabari, buck semen, south-west monsoon, post monsoon, winter and pre-monsoon*

Cryopreservation of buck semen helps in the long-term storage of spermatozoa from superior sires and their use over distance and time too. But there exist some constraints in the production of frozen semen from bucks. Among them, the most important one is the inter male variability of cryoresistance of spermatozoa (Leboeuf et al., 2000) and the same had been reported in Malabari bucks also (John, 2016; Bhai, 2023). Therefore, selection of bucks with good cryotolerance is one of the challenges faced in the semen stations.

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**Corresponding author: lekshmibhai@kvasu.ac.in, Ph.7909292304*

Meanwhile, the unpublished observations during the cryopreservation of buck semen are suggestive of discontinuous pattern of semen freezability throughout the year. This also adversely affects the production of sufficient doses of cryopreserved semen for Artificial Insemination. Even though goats are reported to be non-seasonal breeders in tropical zones, other climatic variables like ambient temperature, relative air humidity and rain fall can affect their reproductive physiology (Rosa and Bryant, 2003).

Identifying the effect of season or climatic variables on semen quality, if any, may aid in the adoption of appropriate breeding management strategy. Ascertaining the pattern of semen cryopreservability across the year may help to define the period more suitable for production of frozen semen.

As the difference in semen quality of Malabari bucks in relation to the seasons or climatic variables have not been documented yet, the present study was conducted to assess the semen quality parameters of Malabari buck semen during different seasons. SDS PAGE profiling of the seminal plasma proteins in different seasons was also carried out.

Materials and methods

The study was carried out at Artificial Insemination center, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala. The place is located about 700 meters above sea level with a latitude and longitude of 11.5393° N and 76.0202° E, respectively.

Semen ejaculates were collected during March 2024 to February 2025 from five adult healthy Malabari bucks aged two to five years and weighed 35-40 kg, maintained under uniform feeding, housing and other management conditions. Semen samples were collected during different seasons described by Krishnakumar et al. (2009) for the region, viz. pre-monsoon (March-May), south-west monsoon (June-September), post-monsoon (October-November) and winter (December-February) seasons. Weather data regarding maximum temperature, average temperature, average relative humidity, average rainfall and average THI in Wayanad during the four seasons of the study period were collected from Regional Agricultural research Station, Ambalavayal, Wayanad and the data are presented in Table 1.

The semen samples were collected from each buck using Danish type artificial vagina (12×3.8 cm) (Urmila et al., 2023). Six semen ejaculates were collected from each buck twice weekly with an interval of two- three days during the last one third of each season. In total 120 ejaculates were used for the study (six ejaculates/buck/season). Immediately after collection, the semen samples were transferred to a water bath maintained at 37 ºC within the semen evaluation laboratory and subjected to preliminary evaluation (Behera et al., 2015).

The semen ejaculate volume (mL) was recorded directly from the markings on the graduated collection vial. In order to assess the mass activity, a small drop of semen (approximately 10-25 µL) was put on a warm, clean, grease free glass slide and examined under 100 × magnification of the bright field microscope for waves and swirls. Mass activity was expressed on a scale of + to ++++ based on the thickness and speed of the waves and eddies. The sperm concentration (millions/mL) was determined using ACCUCELL ovine photometer (IMV technologies, France) calibrated for the use in goats, after diluting 10 µL of semen with 3990 µL of 0.9 per cent (w/v) sodium chloride solution.

Progressive motility of spermatozoa was assessed following initial extension with tris buffer (2.428 g tris hydroxyl methyl amino methane, 1.38 g citric acid, 1g fructose, 100000 IU Benzyl penicillin and 100 mg Streptomycin in 100 mL triple distilled water) at the rate of 1:10. The diluted semen of 10 µL was taken on a warm, clean, grease free glass slide, covered with a clean coverslip and was observed under 400 × magnification of the bright field microscope. Progressive motility of spermatozoa was recorded in per cent as the average value obtained from five different microscopic fields for each sample.

Eosin-nigrosin staining technique (Campbell et al., 1953) was employed for assessing the per cent of viable spermatozoa. Per cent of live and dead sperms were calculated after counting a minimum of 200 sperms from different microscopic fields in each smear. Completely and partially pink stained spermatozoa were considered as dead whereas the unstained spermatozoa were recorded as viable. The percent of morphological abnormalities

Table 1. Weather parameters during the four seasons from March, 2024 to February, 2025 in Wayanad (Collected from RARS, Ambalavayal)

Seasons	Maximum temperature (°C)	Average temperature (°C)	Relative humidity (%)	Rainfall (mm)	THI
Pre -monsoon	31.26	25.33	74.5	129.06	74.79
Southwest monsoon	25.67	22.38	89.5	435.7	71.33
Post monsoon	18.15	22.4	87	138	71.29
Winter	28.26	21.28	79.16	16.93	68.86

were also calculated from the same smear.

Acrosome integrity of spermatozoa was assessed using Giemsa staining (Watson, 1975). The stained smears were observed under oil immersion objective (1000 × magnification) of the bright field microscope. A minimum of 200 spermatozoa were counted from which the per cent of spermatozoa with intact acrosome was calculated.

Hypo-osmotic sperm swelling test (HOST) was carried out to assess the functional membrane integrity of spermatozoa (Jeyendran et al., 1984). The HOST test solution (100 mOsm/L) consisted of 4.9 g trisodium citrate dihydrate and 9.9 g fructose in 1000 mL triple glass distilled water. Control solution was prepared with the osmolarity of 300 mOsm/L (14.7 g trisodium citrate dihydrate and 29.7 g fructose in 1000 mL triple glass distilled water). Test solution and control solution were taken in separate microcentrifuge tubes (0.9 mL each) kept in 37 °C water bath. Semen (0.1 mL) was added to each tube, mixed well and were incubated for 30 min. Smears were prepared on clean, grease free slides from both the suspensions after mixing one drop of diluted sample with one drop of two per cent eosin. From the air-dried smears, minimum of 200 spermatozoa were counted under oil immersion objective (1000 × magnification) of the bright field microscope. Per cent of spermatozoa with functionally intact membrane was calculated by subtracting the per cent of spermatozoa with coiled tail in control solution from the per cent of HOS positive spermatozoa in HOST solution.

For the seminal plasma protein profiling with SDS-PAGE, 0.2 mL of semen from each ejaculate was aliquoted immediately after semen collection. The aliquoted semen was supplemented with antiprotease cocktail (Sigma Aldrich, USA) at a rate of 10 µL/mL and was centrifuged at 3500 × g for 20 min to remove the suspended spermatozoa. The supernatant was collected and centrifuged at 10000 × g for 30 min at 5 ºC to further clear out the cells and debris, if any. Thereafter, the supernatant was transferred to a microcentrifuge tube and kept at -80 ºC until SDS-PAGE which was carried out according to Laemmli (1970) with slight modifications in a Mini-PROTEAN® Tetra Electrophoresis System (BioRad, USA). Molecular weight marker (Sigma Marker™ wide range molecular weight 6,500 – 200,000 Da) was used to compare the molecular weight of protein bands. Finally, the protein bands were subjected to imaging in ChemiDoc ™ MP imaging System (Bio-Rad, USA) and analysed with Quantity One®1-D analysis software version 4.6.9 (Bio-Rad, USA). Stained gels were compared and analysed for any seasonal variation in the protein bands.

The data obtained were analysed statistically using Statistical package for social studies (SPSS) version 24.0 software following the principles as per Snedecor and Cochran (1994). Effect of season on different quality parameters of Malabari buck semen were compared using repeated measures ANOVA.

Results and discussion

In the present study, the semen ejaculate volume of Malabari bucks (Table 2) ranged between 0.5 to 1 mL. The mean values during south-west monsoon, post-monsoon, winter and pre-monsoon were 0.88 ± 0.04 mL, 0.81 ± 0.04 mL, 0.92 ± 0.02 mL and 0.65 ± 0.02 mL, respectively. The values obtained in all the seasons were of normal range (Behera, 2012; Urmila et al., 2023). The semen ejaculate volume obtained in the pre-monsoon season was significantly lower than that observed in all other seasons whereas the highest value was observed in winter season ($p< 0.001$). Purdy (2006) also reported that ejaculate volume in bucks could be affected by season. It was reported that heat stress begins with THI ≥ 72 (Dunn et al., 2014). As per the weather data obtained in different seasons of the present study, pre-monsoon season had THI ≥ 72 which indicated thermal stress and the same might have negatively affected the quantity of semen. Environmental stress is also a deciding factor of secretions from the accessory sex glands and complete ejaculation. This finding was also supported by the reports of Garcia-Oliveros et al. (2022) that heat stress affected the quantity of semen.

But, the mass activity of Malabari buck semen was found to be ++++ throughout the seasons. Srinivas et al. (2002) also reported that there was no significant effect of season on mass activity of buck spermatozoa.

Concentration of spermatozoa in Malabari buck semen ejaculates (Table 2) ranged between 2253 millions/mL and 3974 millions/mL and the average values observed in south-west monsoon, post-monsoon, winter and pre-monsoon were 3206.67 ± 154.18 millions/mL, 3171.63 ± 143.83 millions/mL, 3236.70 ± 155.61 millions/mL and 3149.10 ± 145 millions/mL, respectively. Though the value of sperm concentration/mL had no significant difference with season, the total number of sperms per ejaculate differed with seasons and the lowest value was observed in pre-monsoon season. Purdy (2006) and Garcia-Oliveros et al. (2022) reported the seasonal influence of sperm concentration in bucks.

The progressive motility of Malabari buck spermatozoa (Table 2) was found to be 84.05 ± 0.69, 83.95 ± 0.63, 84.95 ± 0.41 and 82.95 ± 0.91 per cent during south west monsoon, post monsoon, winter and pre-monsoon, respectively. There was no significant difference in this quality parameter in between seasons. In contradiction, Dhilon et al. (2020) reported higher values of sperm motility in Beetal bucks from Punjab during winter. The current observation could be supported with the finding of Maya-Soriano et al. (2015) that the main change induced by high temperatures was the rise in the proportion of middle-lower motile sperm subpopulations in epididymis of rabbit whereas the proportion of high motile sperm subpopulation could be maintained. Influence of season and climatic factors on buck sperm motility was

Table 2. Characteristics of Malabari buck semen during different seasons (Mean ± SE) (n = 30)

Characteristics	South west monsoon	Post monsoon	Winter	Pre- monsoon	F value (P-value)
Volume (mL)	0.88^{ab} ± 0.04	0.81^{b} ± 0.04	0.92^{a} ± 0.02	0.65^{c} ± 0.02	13.689** (<0.001)
Concentration of spermatozoa (millions/mL)	3206.67 ± 154.18	3171.63 ± 143.83	3236.7 ± 155.61	3149.1 ± 145	0.066^{ns} (0.977)
Progressive motility of spermatozoa (%)	84.05 ± 0.69	83.95 ± 0.63	84.95 ± 0.41	82.95 ± 0.91	1.423^{ns} (0.273)
Viability of spermatozoa (%)	86.28 ± 0.64	86.28 ± 0.7	86.85 ± 0.5	85.37 ± 0.55	1.046^{ns} (0.399)
Morphological abnormalities of spermatozoa (%)	9.02 ± 0.53	7.97 ± 0.72	7.88 ± 0.42	9.63 ± 0.44	2.428^{ns} (0.103)
Acrosome integrity of spermatozoa (%)	86.77 ± 0.54	86.28 ± 0.52	87.13 ± 0.24	86.67 ± 0.44	0.596^{ns} (0.627)
Functional membrane integrity of spermatozoa (%)	88.18 ± 0.37	88.52 ± 0.55	89.05 ± 0.19	87.97 ± 0.55	1.148^{ns} (0.36)
Number of protein bands in SDS PAGE of seminal plasma	18.00^{a} ± 0.50	17.60^{b} ± 0.80	18.60^{a} ± 0.30	14.60^{c} ± 1.30	21.88506^{**} (P < 0.01)

Values having different lower-case superscripts in row differ significantly.

reported previously by Roca et al. (1992) in breeds from Murcia, Spain, at 37°59'N, 1 °08'W and Perez and Mateos (1996) in breeds from Verata (40^{0} N) and Malaguena (37^{0} N). But, those animals were located in high latitude and thereby had seasonality in reproductive characteristics.

The average viable spermatozoa in the fresh semen of Malabari bucks (Table 2) were found to be of 86.28 ± 0.64, 86.28 ± 0.70, 86.85 ± 0.50 and 85.37 ± 0.55 percent during south west monsoon, post monsoon, winter and pre-monsoon seasons, respectively. Viability of spermatozoa observed in the current study showed no significant difference between seasons. This is not in agreement with the previous reports of Talebi et al. (2009) and Dhilon et al. (2020) who observed seasonal variation in buck sperm viability which might be due to seasonality in reproductive features at high latitudes. It could be possible that high THI might result in apoptosis of spermatozoa, where, the loss of membrane integrity happens only in late stage. Thus, the spermatozoa at the early stage of apoptosis released from epididymis in each ejaculate might be seen as viable in eosin-nigrosine staining (Roca et al., 2016). The total morphological abnormalities (Table 2) of Malabari buck spermatozoa (9.02 ± 0.53, 7.97 ± 0.72, 7.88 ± 0.42 and 9.63 ± 0.44 per cent, respectively) had no significant difference in between. But, Srinivas et al. (2002) reported higher per cent of sperm abnormalities in buck semen during summer.

Malabari buck semen had 86.77 ± 0.54 per cent, 86.28 ± 0.52 per cent, 87.13 ± 0.24 per cent and 86.67 ± 0.44 per cent of sperms with intact acrosomes in south west monsoon, post monsoon, winter and pre-monsoon, respectively (Table 2). There was no significant difference in the per cent of acrosome integrity of Malabari buck spermatozoa in the fresh semen between seasons and this observation is in contradictory to that of Wang et al. (2014). The findings were in par with the observations by Dhilon et al. (2020) who noted no difference in acrosome integrity between summer and winter season in the semen of Beetal bucks.

The per cent of functional membrane integrity of spermatozoa in the fresh semen ejaculates of Malabari bucks (Table 2) were 88.18 ± 0.37, 88.52 ± 0.55, 89.05 ± 0.19 and 87.97 ± 0.55 during south west monsoon, post monsoon, winter and pre- monsoon, respectively. The values were not significantly different. There exists possibility of the release of sperms still with intact membrane but programmed to be losing the membrane integrity among the cohort of sperms released from epididymis in each ejaculate (Roca et al., 2016). Golher et al. (2018) also observed that season had no influence on the functional membrane integrity of spermatozoa of bucks.

The electrophoretogram revealed a total of 20 protein bands with molecular weight ranging from 18 kDa to 156 kDa across all the four seasons but none of the bucks were reported to express all protein bands in any particular season. John (2016) also obtained similar observations regarding protein bands in Malabari bucks. There existed significant difference in the number of protein bands observed across seasons (P < 0.01). Highest number of protein bands were observed during winter and south west monsoon (18.6 ± 0.3 and 18 ± 0.5, respectively) and pre-monsoon season was found to have comparatively less number of protein bands (14.6 ± 1.3). Seasonal variation in the number of seminal plasma protein bands in SDS PAGE was also reported in buffalo (Sharma et al., 2014). But,

Teixeira et al. (2009) observed higher number of seminal plasma protein bands in Anglo-Nubian goats during the period of high THI. There was 100% incidence rate for protein bands with molecular weight of 156 kDa, 137 kDa, 88 kDa, 55 kDa and 38 kDa, across all the four seasons. During pre-monsoon season, nine protein bands were of 100 per cent incidence rate whereas in winter season 17 protein bands were of 100 per cent incidence rate. Teixeiria et al. (2009) also observed that season had no influence on the presence of proteins of molecular weight 55 kDa (osteopontin) in the buck seminal plasma. Bands with molecular weight of 151 kDa and 35 kDa had zero per cent incidence during pre-monsoon season.

From the study, it could be revealed that semen ejaculate volume of Malabari bucks was significantly lower in pre-monsoon season. Other semen characteristics such as progressive motility, viability, morphological abnormalities, acrosome integrity and functional membrane integrity of spermatozoa were not affected with change in season. The number of protein bands in SDS PAGE of seminal plasma also had significant variation with seasons. The thermal stress indicated by high THI might have affected the reproductive behaviour and libido which might be leading to low ejaculate volume. Moreover, the protein composition would also be affected with libido.

Conclusion

It could be concluded that the quantity of semen ejaculate varied with seasons and the low volume was found to be during pre-monsoon season compared to south west monsoon, post monsoon and winter seasons. The other quality parameters of fresh semen such as mass activity, concentration, progressive motility, morphological abnormalities, acrosome integrity and functional membrane integrity did not vary significantly with seasons. SDS PAGE protein profile of seminal plasma revealed significant difference in terms of number of protein bands and their incidence rate.

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Conflict of Interest

The authors declare that there is no conflict of interest in this work.

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