

EFFECT OF NON-GENETIC FACTORS ON SEMEN PRODUCTION TRAITS IN SALEM BLACK BUCKS

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ABSTRACT

Selection of breeding bucks is one of the crucial decisions in both organised and field populations for genetic improvement. Using high genetic merit bucks in the breeding programme could improve the overall production of the stock, thereby augmenting the economic sustainability of the rural goat keepers with zero input system of management. Therefore, understanding the effect of non-genetic factors with respect to semen characteristics is of paramount importance among the breeding bucks within a breed for effective breeding management. In this study, a total of 164 ejaculates from six adult Salem Black bucks were analysed to determine the effect of season and order of the ejaculate on conventional semen attributes using linear mixed models with lme4 package in R software. The estimated marginal means for semen volume (ml), sperm concentration (million per ml), mass activity (0 to 5 scale), initial and pre-freeze sperm motility (per cent) were 0.69 ± 0.05 , 3253.00 ± 167.00 , 4.70 ± 0.09 , 81.80 ± 0.002 and 79.43 ± 0.003 respectively. Season had a significant effect ($p < 0.5$) on all the traits under study except for post-thaw motility, whereas, the order of ejaculation did not influence the traits except mass activity.

Key Words: Season, Order of the ejaculate, Semen production traits.

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INTRODUCTION

Among the livestock, small ruminant production, especially the goat production system is gifted with the advantages of prolificacy, low investment and minimal maintenance cost required for rearing. This provided a significant growth opportunity for goat farming among the small holders and has become a successful enterprise to augment the

rural income as well as to provide an affordable nutrition. India ranks second in terms of goat population with rich genetic resources of 37 registered breeds besides non-descript animals amassing a total of 148.88 million (Livestock Census, 2019).

It stands second in chevon production with 1097.91 thousand metric tonnes, contributing 13.53 per cent to total meat in the country. There is a huge potential to improve the productive and reproductive efficiency of the non-descript or native goat population. Indisputably, artificial insemination is one of the established and proven reproductive biotechnology tools to improve the genetic makeup of the population by means of introducing the elite germplasm, which leads to sustainable production.

The state of Tamil Nadu is endowed with three registered goat breeds, *viz.* Salem Black, Kanni Adu and Kodi Adu. Of the three autochthonous breeds, Salem Black or the *Karuppuadu* is a primary meat breed in the northwestern agro-climatic zone of Tamil Nadu. These goats are known to have good reproduction potential in terms of early sexual maturity and prolificacy. They are hardy with negligible mortality and innately resistant to harsh climatic conditions prevailing in their habitat (Thiruvankadan and Karunanithi, 2006) and they could be used as a superior indigenous breed to upgrade the non-descript goats in adjoining areas for genetic improvement.

Selection of superior bucks is the foremost step in planning the genetic improvement programmes both at organized farms and field level. This could be done by

evaluating the conventional semen production parameters such as semen volume, sperm concentration, mass activity and sperm motility etc. Semen production parameters are known to be influenced by different factors such as season (Elsheikh and Elhammali, 2015); genetic group (Bastola *et al.*, 2018, Islam *et al.*, 2019), method of collection (Memon *et al.*, 1986; Bopape *et al.*, 2015), frequency of collection (Ritar *et al.*, 1992), and plane of nutrition (Arangasamy *et al.*, 2018). Hence, determining the effect of different non-genetic factors on semen characteristics among the bucks of the same breed could plausibly assist in breeding management. Hence, the present investigation was carried out to study the effect of non-genetic factors on conventional semen production traits of Salem Black bucks, as there were no studies attempted earlier in this breed.

MATERIALS AND METHODS

Data on 164 ejaculates from Salem Black bucks (Six Nos.) over a period of seven months (March to September) were utilized in this study. The bucks were purchased from the farmers flocks at the age of 10-12 months and were unrelated to each other. They were maintained under uniform management conditions at Frozen Semen Bank, Department of Animal Genetics and Breeding, Madras Veterinary College, Chennai (Tamil Nadu). Bucks were stall-fed by providing 300 g of concentrate feed/day/head, *ad libitum* green fodder and potable water. Semen was collected by artificial vagina method, twice a week from each buck using a restrained buck as a teaser. Semen samples (first and second ejaculates) were collected in a graduated collection tube

and maintained at 37°C for macroscopic and microscopic evaluation.

The semen production data comprised of buck number, date of semen collection, order of ejaculate, volume of ejaculate (ml), concentration of spermatozoa (million per ml), mass activity (graded as 0-5) and microscopic initial, pre-freeze and post-thaw motility (per cent) and number of frozen semen doses produced per ejaculate. Other traits such as total initial motile spermatozoa (as product of volume of ejaculate, concentration of spermatozoa and initial motility of spermatozoa) and total post-thaw motile spermatozoa (as product of volume of ejaculate, concentration of spermatozoa and post-thaw motility of spermatozoa) were calculated.

Semen volume, colour and consistency of each ejaculate were recorded by visual appraisal of semen from the graduated collection tube. The sperm concentration of samples was estimated using a sperm photometer (Accucell, IMV technologies) standardized for goat semen. The mass activity of the semen was graded from 0 to 5 on the observation of sperm wave motion from a drop of semen on the glass slide under the 10x magnification of the phase contrast microscope at 37° C. For the production of frozen semen straws required for artificial insemination, neat semen was diluted to 100 million/dose concentration using Tris - Egg yolk - Glycerol diluent. The percentage of progressively motile spermatozoa after dilution, i.e., the initial sperm motility was evaluated subjectively with a magnification of 20x and 40x by covering a 10 µl drop of

diluted semen with a thin cover slip.

The data on semen production attributes were classified into random and fixed effects. The buck was considered as a random effect, while the order of the ejaculate and season were taken as fixed effects. The order of the ejaculate had two levels, first and second as the ejaculates were collected twice, whereas the season was classified as winter (December to February), summer (March to April), south-west monsoon (June to August) and north-east monsoon (September to November) and in this study, the semen production data were observed only for three seasons, except winter. The data measured as percentages were arcsine transformed before analyses.

Every attribute was subjected to exploratory analysis to understand the distribution pattern and eliminate the significant outliers. The outliers were removed using Z-score up to mean $\pm$ 4 S.D. In this study, the data consisted of there are repeated observations of semen production traits over time for the same buck, illustrating the non-independence in data. Therefore, to allow us to skirt past that independence assumption, the inclusion of the buck as random effect is essential and hence, a linear mixed model was used for analysis to resolve the non-independency. Random intercept statistical model was constructed using the lme4 package (Bates *et al.*, 2015) in R for semen production traits. In this case, the random effect allows each buck to have a different intercept in the model, while the effects of the other variables remain fixed. The significant fixed factors were further subjected to pair-wise comparison

using Tukey's test. The model used in this study was,

$$Y_{ijkl} = \mu + S_i + E_j + B_k + e_{ijkl}$$

Where, Y_{ijkl} is the observation of respective semen traits corresponding to i^{th} season, j^{th} ejaculate, k^{th} buck and μ is the general mean, S_i is effect of i^{th} season ($i = 1-3$), E_j is effect of j^{th} season ($j = 1-2$), B_k is effect of k^{th} buck ($k = 1-6$) considered as a random effect and e_{ijkl} is the random error.

RESULTS AND DISCUSSION

In the study, almost all the samples were creamy white in colour, with few samples being yellow in colour. Yellow colour of the semen might be attributed to the presence of riboflavin in the seminal plasma (Mendoza *et al.*, 1989). All the samples in the study were thick in consistency indicating a higher sperm concentration. Volume produced by the bucks ranged from 0.2 ml to 1.2 ml with a mean of 0.708 ± 0.018 ml. The lowest and highest observed sperm concentration were 2000 million / ml and 6161 million /ml respectively with a mean of 3141.982 ± 66.485 million / ml. The average mass activity among the bucks was 4.707 ± 0.038 with a minimum value of 3 and a maximum of 5. The initial sperm motility had a mean of 82.104 ± 0.002 per cent, whereas, the mean pre-freeze motility was 79.522 ± 0.001 per cent and the post-thaw motility observed among the bucks had an average of 53.587 ± 0.001 per cent.

The overall estimated marginal mean for the volume of ejaculate was 0.692 ± 0.052 ml. Volume of semen produced by Salem Black goats, was lower to the volume

donated by Black Bengal bucks as reported by Sultana *et al.* (2013) and Karunakaran *et al.* (2015), where, the recorded volume was 0.750 ± 0.120 and 0.750 ± 0.250 ml respectively. It was also lower than the volume produced by the Mehsana (Parmar *et al.*, 2011), Jamunapari (Bastola *et al.*, 2018), Chegu (Sharma and Sood, 2019) and Sirohi bucks (Khadse *et al.*, 2019), where, the mean volume was 0.840 ± 0.020 , 1.970 ± 0.120 , 0.850 ± 0.070 and 1.060 ± 0.010 ml respectively. Chegu (Thakur *et al.*, 2005), Pantja (Patni *et al.*, 2016) and Assam Hill (Deori *et al.*, 2018) goats, which were reported to produce a mean volume of 0.470 ± 0.030 , 0.530 ± 0.050 and 0.390 ± 0.010 ml respectively, had a lower mean volume when compared to Salem Black bucks. The differences in semen volume might be attributed to various factors such as genetic group, age of the bucks and individual variations. Season had a significant influence on the semen volume observed in this study ($p < 0.001$). The highest semen volume was produced in south-west monsoon (0.759 ± 0.052 ml), followed by north-east monsoon (0.726 ± 0.061 ml), while the summer season recorded the lowest semen volume among the three seasons (0.590 ± 0.059). Order of the ejaculate had no significant influence on the amount of volume produced, but the second ejaculate had shown higher volume (0.708 ± 0.055 ml) when compared to the first ejaculate (0.675 ± 0.053).

The estimated marginal mean for the sperm concentration was 3253.00 ± 167.00 million/ml. The sperm concentration observed in Salem Black goat was more than the Black Bengal (2980.20 ± 0.960 million/ml; Sultana *et al.*, 2013), Assam Hill goats (3201.00 ± 143.78

million/ml; Deori *et al.*, 2018), Chegu (2238.50 ± 231.00 million/ml; Sharma and Sood, 2019), while, the mean sperm concentration in Jamunapari goats (3573.04 ± 1.050 million/ml; Kharche *et al.*, 2013) was on the higher side. The differences might probably be due to the genetic group, age, sexual maturity of the bucks, feeding regime and environmental conditions. Season had significant ($p < 0.001$) effect on sperm concentration. The highest sperm concentration was recorded in north-east monsoon (3426.00 ± 210.00 million/ml) followed by summer (3404.00 ± 200.00 million/ml) and south-west monsoon (2929.00 ± 170.00 million/ml). High temperature and humidity during the south-west monsoon might be the reason behind the decreased concentration of sperm. The order of the ejaculate was not shown to be significantly influencing the sperm concentration. The first ejaculate had a mean sperm concentration of 3357 ± 174.00 million/ml, while the second ejaculate had a mean sperm concentrate of 3148 ± 180.00 million/ml. The adequate sexual rest given to the bucks would have resulted in a slightly higher concentration of sperm in the first ejaculate.

The overall estimated marginal mean for the mass activity was 4.70 ± 0.09 . The mass activity value observed in this study was comparatively lower than Malabari goats (4.80 ± 0.02 ; Gopinathan *et al.*, 2021). The mass activity in Salem Black was higher when compared to Mehsana (Parmar *et al.*, 2011), Pantja (Patni *et al.*, 2016), Jamunapari (Bastola *et al.*, 2018), Chegu (Sharma and Sood, 2019), which had a mean score of 4.03 ± 0.06 , 3.60 ± 0.22 , 3.48 ± 0.08 and 3.92 ± 0.03 respectively. Since mass activity is a subjective assessment,

chances of variation among the evaluators could also play a major role. Significant influence of season ($p < 0.001$) and the order of ejaculate ($p < 0.05$) were observed on the mass activity. Second ejaculate had higher mass activity (4.77 ± 0.09) than the first ejaculate (4.63 ± 0.09). Mass activity was more in north-east monsoon (4.92 ± 0.12) followed by the south-west monsoon (4.69 ± 0.09) and summer (4.49 ± 0.11) seasons.

The estimated marginal means for initial sperm motility and pre-freeze motility were 81.80 ± 0.005 and 79.43 ± 0.006 per cent respectively. The initial sperm motility observed in Beetle (89.80 ± 1.26 per cent; Ahmad *et al.*, 2014), Black Bengal (85.40 ± 8.20 per cent; Karunakaran *et al.*, 2015), Barbari (88.32 ± 1.55 per cent; Anand *et al.*, 2016) and Sirohi (90.10 ± 1.15 per cent; Anand and Yadav, 2016) were higher than the initial sperm motility observed in this study. But the initial sperm motility in Salem Black goats was higher than the Jamunapari (78.41 ± 0.67 per cent; Bastola *et al.*, 2018) and Malabari goats (79.60 ± 0.00 ; Gopinathan *et al.*, 2021). These variations could be probably attributed to the genetic group, age, individual variations among the bucks, experience of the evaluator, environmental and managemental conditions.

Season was significantly ($p < 0.05$) affecting the sperm motility of neat and pre-freeze semen. The estimated means for the initial sperm motility in north-east monsoon (83.31 ± 0.011 per cent) and south-west monsoon (82.56 ± 0.003 per cent) were not significantly differing from each other, whereas, only north-east monsoon significantly varied from summer (80.23 ± 0.009 per cent) for

initial sperm motility. Order of the ejaculate didn't have a significant effect on the neat sperm motility. The estimated marginal means for initial sperm motility observed in the first ejaculate was 82.56 ± 0.004 per cent, while the second ejaculate had an estimate marginal mean of 81.80 ± 0.005 .

The motility of pre-freeze semen had the estimated marginal mean of 79.43 ± 0.003 per cent. Season had shown a significant influence on the pre-freeze motility, whereas the order of the ejaculate had no significant effect on the pre-freeze motility. Lowest estimated marginal mean was reported in summer (77.79 ± 0.009 per cent), followed by south-west monsoon (79.43 ± 0.004 per cent) and north-east monsoon (81.02 ± 0.009 per cent). The pre-freeze sperm motility in north-east summer significantly differed from summer season. High ambient temperature in summer is attributed to be the reason behind decreased sperm motility, while the favorable environment in north-east monsoon might be the reason for higher sperm motility among the bucks. Pre-freeze motility was observed to be lower than initial sperm motility, which might be due to equilibration and a gradual decrease in temperature to 4°C .

The overall estimated marginal means motility of post-thaw semen was 53.557 ± 0.002 per cent. The post-thaw sperm motility observed in this study was not significantly affected by season and the order of the ejaculate. This might be plausibly due to the phenotypic merit of the bucks, optimal semen collection and processing conditions followed during the equilibration, cooling and freezing procedures.

The estimated marginal means were 1809 ± 166 , 1755 ± 156 and 1174 ± 108 million/ml respectively for concentration of motile spermatozoa in initial, pre-freeze and post-thaw semen. Season had a significant ($p < 0.05$) effect on the outcome of the initial motile sperm concentration with north-east monsoon having a high concentration of motile sperm (2030.00 ± 197.000) followed by south-west monsoon (1818.00 ± 168.00) and summer (1579.00 ± 190.00 million/ml). Estimated marginal means of the initial motile spermatozoa for the first and second ejaculate were 1824 ± 170 and 1794 ± 175 million/ml respectively. The overall estimated marginal means for pre-freeze and post -thaw motile spermatozoa and the effects of non-genetic factors on them are given in Table 1.

The estimated marginal mean of frozen semen doses per ejaculate was 22.20 ± 2.02 . The season as well as the order of the ejaculate did not influence the number of frozen semen doses produced. The highest number of frozen semen doses were produced in the north-east monsoon (24.50 ± 2.40) followed by the south-west monsoon (22.10 ± 2.04) and summer (19.90 ± 2.31 straws). Although there was no overall significance observed for the doses produced among seasons, significant difference was observed between north-east monsoon and summer. The descriptive mean, overall marginal mean and marginal mean estimated for the fixed factors are presented in Tables 1 and 2. The estimated marginal mean for different semen production traits is depicted in Fig. 1a, 1b, 2a, 2b, 3a, 3b, 4a and 4b. On a conclusive note, the season had a greater influence on most of the semen production traits. The hot and humid

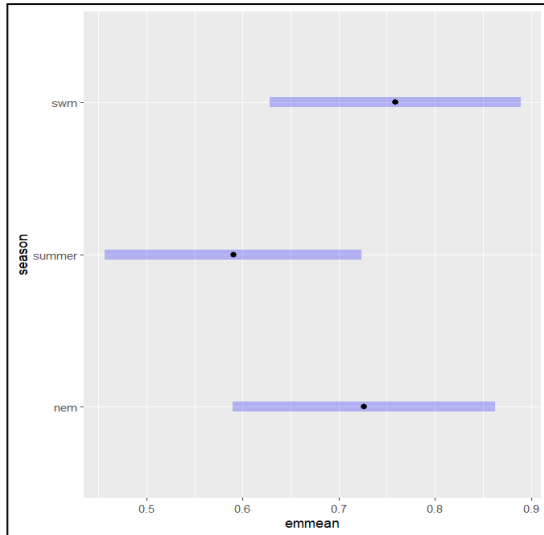


Fig. 1a) Plot of estimated marginal mean (black point) $\pm$ S.E. (blue band) for volume with respect to season

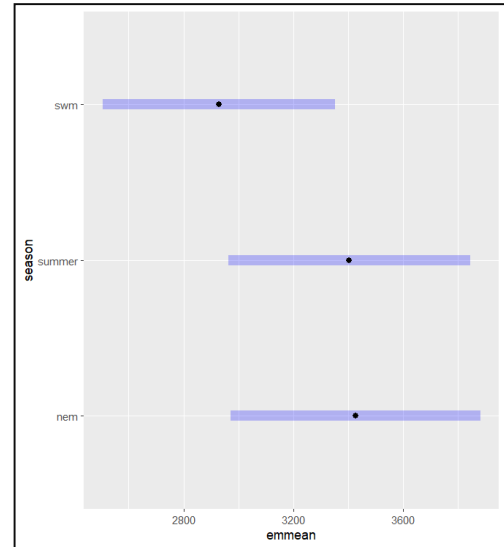


Fig. 1b) Plot of estimated marginal mean (black point) $\pm$ S.E. (blue band) for concentration with respect to season

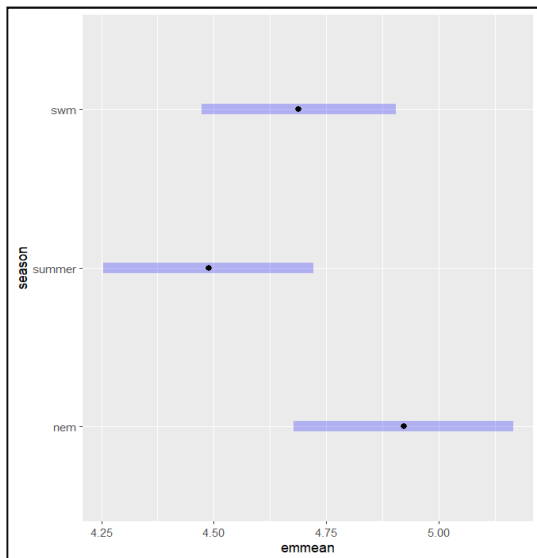


Fig. 2a) Plot of estimated marginal means (black point) $\pm$ S.E. (blue band) for mass activity with respect to season

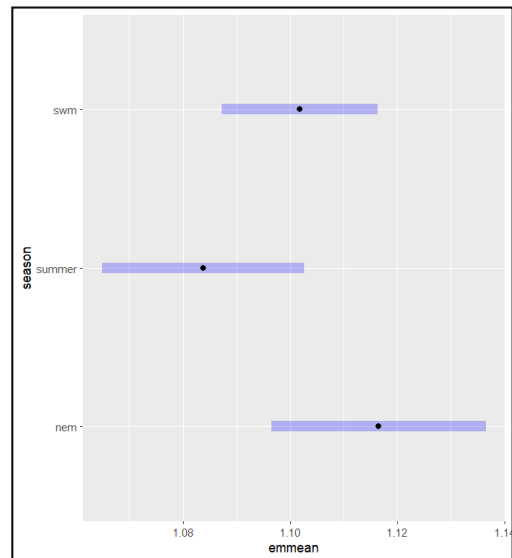


Fig. 2b) Plot of estimated marginal means (black point) $\pm$ S.E. (blue band) for mass activity with respect to ejaculate

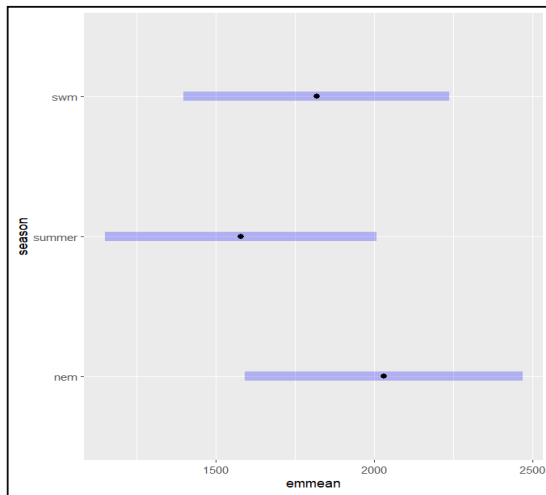


Fig. 3a) Plot of estimated marginal mean (black point) $\pm$ S.E. (blue line) for initial motility with respect to season

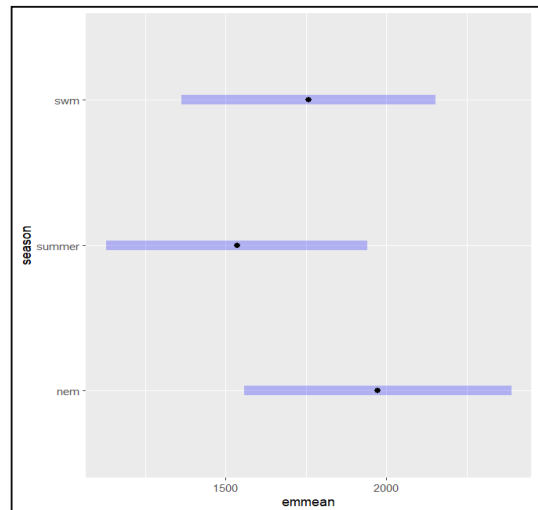


Fig. 3b) Plot of estimated marginal mean (black point) $\pm$ S.E. (blue line) for pre-freeze motility with respect to season

Table 1. Semen production traits in Salem Black bucks

Effects	Initial motile spermatozoa (million/ml)	Pre-freeze motile spermatozoa (million/ml)	Post-thaw motile spermatozoa (million/ml)	Doses
Descriptive mean	1801.58 $\pm$ 56.58	1745.52 $\pm$ 54.50	1172.45 $\pm$ 36.29	21.99 $\pm$ 0.68
Estimated marginal mean (overall)	1809.00 $\pm$ 166.00	1755.00 $\pm$ 156.00	1174.00 $\pm$ 108.00	22.20 $\pm$ 2.02
Season	*	*	NS	NS
North-east Monsoon	2030.00 ^a $\pm$ 197.00	1974.00 ^a $\pm$ 188.00	1285.00 $\pm$ 128.00	24.50 ^a $\pm$ 2.40
Summer	1579.00 ^b $\pm$ 190.00	1535.00 ^b $\pm$ 180.00	1053.00 $\pm$ 123.00	19.90 ^b $\pm$ 2.31
South-west Monsoon	1818.00 ^a $\pm$ 168.00	1758.00 ^a $\pm$ 158.00	1183.00 $\pm$ 109.00	22.10 ^a $\pm$ 2.04
EJ	NS	NS	NS	NS
EJ2	1824.00 $\pm$ 170.00	1763.00 $\pm$ 161.00	1177.00 $\pm$ 111.00	22.30 $\pm$ 2.08
EJ1	1794.00 $\pm$ 175.00	1748.00 $\pm$ 166.00	1170.00 $\pm$ 114	22.00 $\pm$ 2.13

*: Significant ($p < 0.05$), NS – Not Significant,

Table 2. Estimated marginal mean ($\pm$ S.E.) for semen production traits in Salem Black bucks

Effects	Volume (ml)	Concentration (million/ml)	Mass activity	Initial motility (per cent)	Pre-freeze motility (per cent)	Post-thaw motility (per cent)
Descriptive mean	0.71 $\pm$ 0.02	3141.98 $\pm$ 66.48	4.71 $\pm$ 0.04	82.09 $\pm$ 0.002	79.53 $\pm$ 0.001	53.58 $\pm$ 0.001
Estimated marginal mean (overall)	0.69 $\pm$ 0.05	3253.00 $\pm$ 167.00	4.70 $\pm$ 0.09	81.80 $\pm$ 0.002	79.43 $\pm$ 0.003	53.58 $\pm$ 0.001
Season	***	***	**	*	*	NS
North-east	0.73 ^a $\pm$ 0.06	3426.00 ^a $\pm$	4.92 ^a $\pm$	83.31 ^a $\pm$ 0.01	81.02 ^a $\pm$	52.96 $\pm$ 0.01
Monsoon		210.00	0.12		0.01	
Summer	0.59 ^b $\pm$ 0.06	3404.00 ^a $\pm$	4.49 ^b $\pm$	80.23 ^b $\pm$ 0.01	77.79 ^b $\pm$	53.96 $\pm$ 0.01
		200.00	0.11		0.01	
South-west	0.76 ^a $\pm$ 0.05	2929.00 ^b $\pm$	4.69 ^b $\pm$	82.56 ^a $\pm$ 0.01	79.43 ^a $\pm$	53.66 $\pm$ 0.01
Monsoon		170.00	0.09		0.01	
EJ	NS	NS	*	NS	NS	NS
EJ2	0.68 $\pm$ 0.05	3148.00 $\pm$	4.77 ^a $\pm$	82.56 $\pm$ 0.01	79.43 $\pm$ 0.01	53.56 $\pm$ 0.01
		180.00	0.09			
EJ1	0.71 $\pm$ 0.05	3357.00 $\pm$	4.63 ^b $\pm$	81.80 $\pm$ 0.01	79.43 $\pm$ 0.01	53.46 $\pm$ 0.01
		174.00	0.09			

***: Significant ($p<0.0001$), **: Significant ($p<0.001$), *: Significant ($p<0.05$), NS: Non-Significant

temperature might be the reason for decreased values for sperm motility traits in summer and among the seasons, the south-west monsoon was shown to have comparatively better results for the sperm motility parameters. In addition, adequate sexual rest could be provided to breeding bucks to obtain better results with respect to sperm motility parameters in both ejaculates. Overall, Salem Black bucks used in this study were phenotypically superior with respect to the semen production traits.

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