Effect of genetic and non-genetic factors on semen production traits in indigenous Bucks (Salem Black, Kanni Adu and Kodi Adu)

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Abstract

Selection of breeding bucks plays a pivotal role in exploiting elite germplasm for genetic improvement and economic sustainability of goat production systems, and therefore understanding the influence of genetic and non-genetic factors on semen characteristics within indigenous breeds is essential for effective breeding management. Hence, the present study was carried out on a total of 389 ejaculates, of which 164 were from Salem Black bucks, 108 and 117 ejaculates from Kanni Adu and Kodi Adu bucks respectively to determine the effect of breed, 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, pre-freeze and post-thaw sperm motility (per cent) were 0.597 ± 0.031 ml, 3944.000 ± 164.000 million per ml, 4.830 ± 0.053, 86.187 ± 0.004 per cent, 84.778 ± 0.004 per cent and 55.946 ± 0.003 per cent respectively. Both season and breed had a greater influence on semen production traits such as volume, concentration, mass activity, pre-freeze and post-thaw motility. Order of the ejaculate significantly impacted the all the parameters under study except the initial motility of sperm. These findings emphasize the prominence of managing non-genetic factors to optimize semen quality. Understanding effects of breed, seasonal variation and order of ejaculate could help in designing effective semen collection protocols and breeding schedules for enhanced reproductive performance.

Keywords: Breed; Conventional semen traits; ejaculate; Season; Semen production traits.

Introduction

Goat farming occupies a vital role in the realm of livestock rearing, largely due to its inherent advantages such as prolificacy, disease resistance and cost -effective management. maintenance. These attributes make goat rearing particularly attractive to small holder farmers, providing them a steady and reliable source of income while also contributing to household nutrition. In India, where the goat population stands tall at 148.88 million as per the 2019 Livestock, goat rearing has seen remarkable growth. With rich genetic diversity of 39 registered goat breeds and huge non-descript goat populations, the country holds the second-largest goat population globally. Furthermore, India also ranks second in chevon production, contributing 1,097.91 thousand metric tons, adding to 13.53% of the nation's total meat output.

This vast genetic repository provides a significant opportunity to enhance the productive and reproductive efficiency of the non-descript or native goat populations. Artificial Insemination (AI), a well-established reproductive biotechnology tool, offers a tremendous potential to improve both productive and reproductive traits by enabling the dissemination of elite germplasm. Through strategic AI programs, genetic progress could be achieved at a faster pace, resulting in a more productive and sustainable production goat farming system.

The state of Tamil Nadu is conferred with three registered goat breeds, namely, Salem Black, Kanni Adu and Kodi Adu. Each of these indigenous breeds possesses unique and valuable traits suited to their native agro-climatic zones. Salem Black goats, known for their tall and lean build, exhibit a completely black coat and are recognized for their hardiness, early sexual maturity, prolificacy and resistance to harsh climatic conditions (Thiruvenkadan and Karunanithi, 2006). Kanni Adu goats are tall animals that thrive in dry, tropical conditions, with characteristic black coats marked by white or brown stripes and noted for their early maturity, high rate of multiple births and low adult mortality (Thilagam *et al*., 2006). Kodi Adu goats, primarily found in Thoothukudi and Ramanathapuram districts, are tall, long, lean and leggy animals with compact bodies and are classified into two colour types, Chem-Porai (white with reddish-brown patches) and Karum-Porai (white with black splashes). Known for their docile temperament, these goats also exhibit early maturity and faster growth rates (Thiruvenkadan *et al*., 2020). The distinctive attributes of these breeds make them valuable genetic resources for selective breeding and grading up programs. Their incorporation into well-planned breeding strategies could significantly improve the genetic potential of non-descript goats in neighbouring regions, fostering sustainable and efficient goat production systems.

The selection of superior breeding bucks is one of the decisive factors in devising genetic improvement programs, whether implemented in organized farms or under field conditions (Eloriaga et al., 2024). This selection process primarily relies on evaluating conventional semen production traits, such as volume of semen, concentration of sperm, mass activity and progressive motility of sperm. It is well-documented that these parameters could be influenced by various non-genetic factors, such as season (Elsheikh and Elhammali, 2015), genetic group (Bastola *et al*., 2018; Islam *et al*., 2019), method of collection (Bopape *et al*., 2015), frequency of collection and plane of nutrition (Arangasamy *et al*., 2018). Understanding the impact of different non-genetic factors on semen characteristics among different indigenous bucks is crucial for optimizing reproductive efficiency and designing effective breeding strategies. Therefore, the present study was undertaken to assess the effect of non-genetic factors on conventional semen production traits in indigenous bucks of Tamil Nadu. Given the dearth of literature on these native breeds, the study aims to generate valuable baseline data and emphasize the need to account for environmental and management-related influences when evaluating the reproductive potential of indigenous goat germplasm.

Materials and methods

Semen samples were collected from 14 bucks (6 from Salem Black, 4 each from Kanni Adu and Kodi Adu) maintained under standard managemental conditions at Frozen Semen Bank, Department of Animal Genetics and Breeding, Madras Veterinary College, Chennai, Tamil Nadu. Bucks were stall-fed with 300 g to 500 g concentrate per head per day, supplemented with *ad libitum* green fodder and potable water. Semen (two ejaculates per buck per day and two days per week) was collected from each buck during summer, south-west monsoon and north-east monsoon seasons using the artificial vagina method, with a restrained buck serving as teaser, following aseptic procedures to avoid contamination. Semen samples were collected in a graduated collection tubes and maintained at 37° C for macroscopic and microscopic evaluations.

A total of 389 ejaculates were used in this study, of which 164 were from Salem Black bucks, 108 and 117 ejaculates from Kanni Adu and Kodi Adu bucks respectively. Data recorded for each ejaculate included buck identification number, date of 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 derived.

Semen volume, colour and consistency of each ejaculate were assessed visually from the graduated collection tube. Sperm concentration was estimated using a photometer standardized for goat semen. Mass activity was graded on a 0 to 5 scale based on wave motion observed under 10x magnification using a phase contrast microscope at 37° C. For production of frozen semen straws required for artificial insemination, neat semen was diluted to a concentration of 100 million sperm/ml using Tris - Egg yolk - Glycerol diluent. The percentage of progressively motile spermatozoa after dilution, i.e., the initial sperm motility was evaluated subjectively under 20x and 40x magnification using a 10 µl drop of diluted semen.

Different factors were hypothesized to influence the semen production traits. These were classified into genetic (buck and breed) and non-genetic effects (season and order of ejaculate). Season, order of ejaculate and breed were considered as fixed factors, while the buck was treated as a random effect to account for individual variability. The factor breed had three levels (Salem Black, Kanni Adu and Kodi Adu), the order of ejaculate had two levels (first and second) and the season had three levels (summer, south-west monsoon and north-east monsoon). Season was classified as winter (December to February), summer (March to April), south-west monsoon (SWM) (June to August) and north-east monsoon (NEM) (September to November).

Percentage-based semen traits were arcsine-transformed prior to analysis. An initial exploratory data analysis was performed to assess distribution patterns and identify outliers. Outliers were defined as observations falling beyond Z ± 4 S.D and were excluded from further analysis. Given that multiple semen samples were collected over time from the same buck, the dataset exhibited non-independence. To address this, a linear mixed model was applied, with buck as a random effect to accommodate within-individual variation. A univariate linear mixed model, specifically, the random intercept statistical model was constructed using the lme4 package (Bates *et al*., 2015) in R. This allowed each buck to have a different intercept in the model, while estimating the fixed effects. The significant fixed factors were further subjected to pair-wise comparison using Tukey's test. Variance components were computed using restricted maximum likelihood (REML) methodology. The statistical model used for estimating the semen production traits was

$$Y_{ijklm} = \mu + S_i + E_j + G_k + B_l + e_{ijklm},$$

where, Y_{ijklm} is the observation of respective semen traits corresponding to i^{th} season, j^{th} ejaculate, k^{th} genetic group, l^{th} buck and µ is the overall mean, S_i is the effect of i^{th} season (i = 1-3), E_j is the effect of j^{th} ejaculate (j = 1-2), G_k is the effect of k^{th} genetic group (k = 1-3), B_l - Effect of l^{th} buck considered as a random effect and e_{ijklm} is the random error.

Results and Discussion

Descriptive statistics of semen production parameters

Descriptive statistics were used to summarize semen production traits of indigenous Salem Black, Kanni Adu and Kodia Adu bucks, followed by mixed model analysis to estimate the effects of genetic and non-genetic factors using estimated marginal means. The semen production traits exhibited considerable variation across breeds, seasons and order of ejaculate, indicating the combined influence of genetic and environmental factors.

The colour of the semen ranged from creamy white to whitish yellow, with few ejaculates exhibiting a yellowish hue, possibly due to the presence of riboflavin in the seminal plasma. Semen consistency was predominantly thick, indicating higher sperm concentration. The overall mean ± S.E for the ejaculate volume, sperm concentration, mass activity, initial motility, total initial motile spermatozoa, pre-freeze motility, pre-freeze motile spermatozoa, frozen semen doses produced, post-thaw motility and total post-thaw motile spermatozoa were 0.614 ± 0.013 ml, 3803.542 ± 66.252 million/ml, 4.722 ± 0.025, 85.370 ± 0.001 per cent, 1904.516 ± 43.600 million, 83.850 ± 0.001 per cent, 1866.109 ± 42.331 million, 22.376 ± 0.504 doses, 55.667 ± 0.001 per cent and 1238.014 ± 28.227 million respectively. A comprehensive summary of the descriptive statistics (mean ± S.E.) for semen production attributes for all the breeds is presented in Table 1.

Among the three breeds, Salem Black bucks produced a higher volume of semen (0.710 ± 0.0181 ml), whereas Kodi Adu bucks exhibited higher sperm concentration (4701.111 ± 136.397 million/ml), mass activity (4.838 ± 0.036), initial (88.159 ± 0.002 per cent), pre-freeze (86.826 ± 0.003 per cent) and post-thaw motility (59.115 ± 0.004 per cent). Further delineation of attributes revealed that Kanni Adu bucks recorded higher total initial (2 168.187 ± 104.006 million), pre-freeze (2110.174 ± 100.091 million) and post-thaw motile spermatozoa (1386.537 ± 67.934 million), as well as total doses produced (24.880 ± 1.168 straws).

Effect of non-genetic and genetic factors on semen production traits

The effect of the factors *viz*. breed, season and order of the ejaculate on the semen production traits are presented in Table 2 along with their overall and factor-wise estimated marginal means. The overall estimated marginal means for volume, concentration of spermatozoa, mass activity, initial motility, pre-freeze motility and post-thaw motility were 0.597 ± 0.031 ml, 3944.000 ± 164.000 million per ml, 4.830 ± 0.053, 86.187 ± 0.004 per cent, 84.778 ± 0.004 per cent and 55.946 ± 0.003 per cent respectively. The overall estimated marginal means for total initial motile spermatozoa, total pre-freeze motile spermatozoa, total post-thaw motile spermatozoa and frozen semen doses produced per ejaculate were 1957.000 ± 106.000 million, 1917.000 ± 103.000 million, 1262.000 ± 72.000 million and 22.800 ± 1.230 doses, respectively.

Volume of Semen

Breed exerted a significant influence on semen volume, indicating inherent genetic differences among the three indigenous breeds. Salem Black bucks produced the highest semen volume (0.710 ± 0.018 ml), followed by Kanni Adu (0.652 ± 0.024 ml) and Kodi Adu bucks (0.443 ± 0.015 ml). The mean semen volume recorded in the present study was lower than those reported for several Indian goat breeds such as Mehsana (Parmar *et al*., 2011), Black Bengal (Sultana *et al*., 2013; Karunakaran *et al*., 2015; Islam *et al*., 2019), Jamunapari (Bastola *et al*., 2018; Islam *et al*., 2019), Beetal (Dhillon *et al*., 2019; Sinha *et al*., 2019), Sirohi (Khadse *et al*., 2019) and Chegu (Sharma and Sood, 2019) bucks which ranged from 0.75 ± 0.12 to 1.97 ± 0.12 ml. but higher than that reported for Assam Hill goats (0.39 ± 0.01 ml, Deori *et al*., 2018). The disparities in semen volume among breeds might plausibly stem from differences in the genetic makeup of the breeds (Bastola *et al*., 2018). Also, variations observed could be ascribed to factors such as the age of the bucks, differences in body weights and scrotal circumference (Souza *et al*., 2011), diverse methods of collection (Bopape *et al*., 2015) and plane of nutrition (Arangasamy *et al*., 2018).

Order of ejaculate had a significant effect on semen volume, with the first ejaculate yielding a higher volume than the second. This observation is consistent with earlier reports and might be attributed to longer sexual rest prior to the first collection, allowing replenishment of seminal reserves, leading to higher volume in the subsequent ejaculates. The reduced semen volume in the second ejaculate is physiologically expected due to the short intervals between successive collections (Furstoss *et al*., 2009). These findings highlighted the importance of considering the time interval between collections and period of sexual rest in optimizing semen collection and quality in goat breeding programs.

Seasonal variation significantly influenced semen volume, with higher values observed during the south-west monsoon compared to the summer season, while differences between south-west and north-east monsoon seasons were non-significant. Goats being seasonal breeders tend to exhibit higher ejaculate volume during the breeding season (Nuti, 2016). Seasonal fluctuations in semen volume might be associated with variations in ambient temperature, photoperiod and endocrine activity, particularly testosterone secretion, which regulates accessory sex gland function. These findings underlined the significant influence of seasonal factors, such as ambient conditions, on modulating the volume of semen in goats.

Table 1. Means ± S.E. of semen production traits in indigenous goat breeds of Tamil Nadu

Trait	Overall	Kanni Adu	Kodi Adu	Salem Black
No. of ejaculates	389	108	117	164
Volume of ejaculate (ml)	0.614 ±0.013	0.652 ±0.024	0.443 ±0.015	0.710 ±0.018
Concentration of spermatozoa (million/ml)	3803.542 ±66. 252	3787.306 ±103.304	4701.111 ±136.397	3173.896 ±69.025
Mass activity	4.722 ±0.025	4.722 ±0.05	4.838 ±0.036	4.640 ±0.044
Initial motility (per cent)	85.370 ±0.001	87.087 ±0.004	88.159 ±0.002	81.987 ±0.003
Pre-freeze motility (per cent)	83.850 ±0.001	85.142 ±0.005	86.826 ±0.003	80.657 ±0.002
Post-thaw motility (per cent)	55.667 ±0.001	55.850 ±0.004	59. 115 ±0.004	53.068 ±0.001
Total initial motile spermatozoa per ejaculate (million)	1904.516 ±43.600	2168.187 ±104.006	1769.128 ±61.462	1827.467 ±61.166
Total pre-freeze motile spermatozoa per ejaculate (million)	1866.109 ±42.331	2110.174 ±100.091	1736.539 ±59.630	1797.820 ±60.319
Total post-thaw motile spermatozoa per ejaculate (million)	1238.014 ±28.227	1386.537 ±67.934	1184.057 ±42.436	1178.699 ±38.288
Total doses produced per ejaculate	22.376 ±0.504	24.880 ±1.168	20.149 ±0.716	22.316 ±0.735

Table 2 Estimated marginal means ± S.E. of semen production traits in indigenous goat breeds of Tamil Nadu

Factors	No. of ejaculates	Volume of ejaculate (ml)	Concentration of spermatozoa (million/ml)	Mass activity (grade 0-5)	Initial motility (per cent)	Pre-freeze motility (per cent)
Overall	389	0.597 ± 0.031	3944.000 ± 164.000	4.830 ± 0.053	86.187 ± 0.004	84.778 ± 0.004
Genetic group		*	*	NS	***	***
Kanni Adu	108	0.644^{ab} ± 0.054	3913.000^{ab} ± 289.000	4.840 ± 0.089	87.538^{a} ± 0.010	85.489^{a} ± 0.011
Kodi Adu	117	0.460^{b} ± 0.054	4622.000^{a} ± 288.000	4.960 ± 0.088	88.829^{a} ± 0.009	87.538^{a} ± 0.011
Salem Black	164	0.688^{a} ± 0.044	3298.000^{b} ± 236.000	4.680 ± 0.071	82.562^{b} ± 0.006	81.018^{b} ± 0.007
Order of the ejaculate		*	*	**	NS	**
First ejaculate	210	0.621^{a} ± 0.032	4063.000^{a} ± 169.000	4.670^{b} ± 0.038	86.187 ± 0.004	84.053^{b} ± 0.005
Second ejaculate	179	0.574^{b} ± 0.033	3826.000^{b} ± 174.000	4.790^{a} ± 0.032	86.187 ± 0.005	85.489^{a} ± 0.005
Season		***	***	**	NS	*
Summer	141	0.502^{b} ± 0.032	4236.00^{a} ± 170.000	4.700^{b} ± 0.057	85.489 ± 0.005	84.778^{ab} ± 0.005
South west monsoon	219	0.666^{a} ± 0.029	3558.00^{b} ± 160.00	4.710^{b} ± 0.050	85.489 ± 0.003	84.053^{b} ± 0.003
North east monsoon	29	0.624^{a} ± 0.048	4039.00^{ab} ± 250.00	5.000^{a} ± 0.104	87.538 ± 0.017	85.489^{a} ± 0.018
* - P<0.05, ** - P<0.01, *** - P<0.001, NS - non-significant.						

(Contd…)

Table 2 Estimated marginal means ± S.E. of semen production traits in indigenous goat breeds of Tamil Nadu (Contd…)

Factors	**Post-thaw motility (per cent)**	**Total initial motile spermatozoa per ejaculate (million)**	**Total pre-freeze motile spermatozoa per ejaculate (million)**	**Total doses produced per ejaculate**	**Total post-thaw motile spermatozoa per ejaculate (million)**
Overall	55.946 ±0.003	1957.000 ± 106.000	1917.000 ± 103.000	22.800 ± 1.230	1262.000 ± 72.000
Genetic group	**	NS	NS	NS	NS
Kanni Adu	55.747^{b} ±0.008	2222.000 ± 183.000	2162.000 ± 178.000	25.400 ± 2.140	1408.000 ± 125.000
Kodi Adu	58.715^{a} ±0.008	1829.000 ± 182.000	1791.000 ± 177.000	20.700 ± 2.130	1200.000 ± 124.000
Salem Black	53.358^{b} ±0.005	1821.000 ± 149.000	1799.000 ± 144.000	22.200 ± 1.740	1177.000 ± 102.000
Order of the ejaculate	**	***	***	***	***
First ejaculate	55.151^{b} ±0.004	2122.000^{a} ± 111.000	2063.000^{a} ± 108.000	24.700^{a} ± 1.290	1352.000^{a} ± 75.200
Second ejaculate	56.740^{a} ±0.004	1793.000^{b} ± 115.000	1771.000^{b} ± 112.000	20.800^{b} ± 1.340	1171.000^{b} ±77.900
Season	*	NS	NS	NS	NS
Summer	57.235^{a} ±0.015	1942.000 ± 112.000	1786.000 ± 108.600	21.100 ± 1.300	1248.000 ± 69.600
South west monsoon	55.350^{ab} ±0.004	1942.000 ± 102.000	1891.000 ± 99.100	22.700 ± 1.190	1206.000 ±75.700
North east monsoon	55.251^{b} ±0.003	2131.000 ± 183.000	2075.000 ± 178.500	24.500 ± 2.120	1331.000 ±121.000
* - P<0.05, ** - P<0.01, *** - P<0.001, NS - non-significant.					

Concentration of sperm

The concentration of spermatozoa in the semen of Kanni Adu, Kodi Adu and Salem Black breeds averaged 3787.31 ± 103.304, 4701.11 ± 136.397 and 3173.90 ± 69.025 million/ml, respectively, with Kodi Adu bucks exhibiting the highest concentration. The values recorded in the present study were higher than those reported for several Indian goat breeds, including Mehasana (3099.10 ± 59.48 million/ml, Parmar *et al*., 2011), Jamunapari (1760.00 ± 0.09 million/ml, Bastola *et al*., 2018), Beetal 2185.50 ± 118.1 million/ml, Dhillon *et al*., 2019) and Sirohi (2621.73 ± 17.60 million/ml, Khadse *et al*., 2019), while being comparable to or higher than those reported for Jamunapari and Assam Hill goats as reported by Kharche *et al*. (2013) and Deori *et al*. (2018) were 3201.00 ± 143.78 and 3573.04 ± 1.05 million/ml, respectively.

The higher sperm concentration observed in Kodi Adu might partly be attributed to their lower semen volume, indicating an inverse relationship between ejaculate volume and sperm concentration. These differences among the breeds could be attributed to underlying genetic disparities among the breeds, as well as variations in age, season, method of estimation, body weights and testicular characteristics mong the bucks.

Order of ejaculate was found to significantly influence sperm concentration, with the first ejaculate exhibiting a higher concentration than the second. This might be due to adequate sexual rest between successive collections, enabling replenishment of sperm reserves. Seasonal variation also had a significant impact on sperm concentration, with higher values recorded during summer. This finding supports earlier reports that seasonal breeders might exhibit increased sperm concentration during the non-breeding season (Nuti, 2016). Also, the negative correlation between the volume of semen and the concentration of sperm could not be neglected as the volume of semen was low in summer season, ultimately resulting in high concentration of sperm. Overall, these findings accentuated the intricate interplay of genetic factors, physiological mechanisms and management practices in influencing the concentration of sperm in goat breeds, highlighting the importance of comprehensive considerations in reproductive management and breeding programs.

Mass activity of semen

The mean mass activity of semen in Kanni Adu, Kodi Adu and Salem Black were 4.72 ± 0.05, 4.84 ± 0.036 and 4.64 ± 0.04, respectively. The mass activity observed in these breeds was higher than the mass activity reported in various other goat breeds of India, like Black Bengal (3.75 ± 0.20, Mishra *et al*., 2010), Mehsana (4.03 ± 0.06, Parmar *et al*., 2011), Jamunapari (3.58 ± 0.14, Kharche *et al*., 2013) and Beetal bucks (3.48 ± 0.08, Bastola *et al*., 2018). These differences might be attributed to the subjective nature of mass activity assessment, which would vary between evaluators and hence, across the studies and breeds.

Breed did not exert a significant effect on mass activity, indicating similar levels across the three breeds. However, order of ejaculate significantly influenced mass activity, with the second ejaculate exhibiting higher wave motion than the first. This discrepancy might be due to the presence of a higher proportion of aged or dead spermatozoa in the first ejaculate following a longer sexual rest period. Seasonal variation significantly affected mass activity, with higher values observed during the north-east monsoon compared to the southwest monsoon and summer seasons. The relatively moderate ambient temperature and favourable climatic conditions during the monsoon period may have contributed to improved sperm wave motion and overall semen quality.

Initial motility of spermatozoa

The mean initial motility of semen in Kanni Adu, Kodi Adu and Salem Black bucks was 87.09 ± 0.004, 88.16 ± 0.002 and 81.99 ± 0.003 per cent, respectively. However, compared to per cent of initial motility in other breeds, such as Beetal (89.80 ± 1.26), Barbari (88.32 ± 1.55) and Sirohi (90.10 ± 1.15) bucks as reported respectively by Ahmad *et al*. (2014), Anand *et al*. (2016) and Anand *et al*. (2016), the initial motility investigated in the breeds under study was lower. A report on Black Bengal bucks by Karunakaran *et al*. (2015) indicated an initial motility of 85.40 ± 8.20 per cent, which was higher than that of Salem Black bucks but lower than Kanni Adu and Kodi Adu breeds, indicating considerable inter-study and inter-breed variation in initial sperm motility

Breed had a significant effect on initial motility, with Kodi Adu exhibiting the highest motility followed by Kanni Adu and Salem Black. However, neither the order of ejaculate nor the season significantly influenced initial motility. This indicates that initial sperm motility in these indigenous breeds is predominantly governed by genetic factors rather than short-term environmental or management-related influences. The observed differences among breeds might also reflect inherent genetic variation and, to some extent, the subjective nature of microscopic motility assessment.

Pre freeze motility of spermatozoa

The mean estimates of pre-freeze motility of sperm observed in the study for Kanni Adu, Kodi Adu and Salem Black bucks were 85.14 ± 0.005, 86.83 ± 0.003 and 80.66 ± 0.002 per cent, respectively. The decrease in pre-freeze motility compared to initial motility suggests that the reduction in sperm motility could be attributed to dilution, equilibration prior to freezing, likely due to gradual temperature reduction and osmotic stress.

Breed, order of ejaculate and season had a significant impact on pre-freeze motility. The differences observed between breeds might be attributed to their genetic merit in withstanding thermal and osmotic stress during equilibration. Higher pre-freeze motility observed in the second ejaculate is likely due to the presence of a greater proportion of aged or dead spermatozoa in the first ejaculate following a longer sexual rest. Seasonal variation was evident, with higher pre-freeze motility observed during the north-east monsoon compared to the south-west monsoon and summer seasons, which may be attributed to more favourable ambient temperatures during equilibration.

Post-thaw motility of spermatozoa

The average post-thaw motility of spermatozoa in Kanni Adu, Kodi Adu and Salem Black breeds was 55.85 ± 0.004, 59.12 ± 0.004 and 53.07 ± 0.001 per cent, respectively. The post-thaw motility observed in the present study was higher than that reported for Beetal bucks (42.30 ± 7.5 per cent; Ahmad *et al*., 2014). Additionally, the post-thaw motility of the Ajmeri breed reported by Bastola *et al*. (2018) was 54.73 ± 2.97 per cent, which was higher than Salem Black bucks but lower than Kanni Adu and Kodi Adu bucks. The post-thaw motility of crossbred Boer bucks (75 per cent) reported by Bastola *et al*. (2018) as 53.27 ± 1.69 per cent was similar to Salem Black bucks, but, lower than Kanni Adu and Kodi Adu breeds. Conversely, the post-thaw motility of crossbred Boer buck (50 per cent) reported in the same study was 55.05 ± 2.59 per cent, similar to Kanni Adu, but higher than Salem Black and lower than Kodi Adu bucks. The post-thaw motility of Boer breed reported by Bastola *et al*. (2018) was 56.93 ± 3.02 per cent, which was lower than Kodi Adu but higher than Kanni Adu and Salem Black bucks. These comparisons highlighted the variability in post-thaw motility across different goat breeds, emphasizing the importance of selection of breed in semen cryopreservation programs.

Breed, order of ejaculate and season significantly influenced post-thaw motility. The superior post-thaw motility observed in Kodi Adu bucks suggests better adaptability of this breed to the cryopreservation process, possibly due to inherent genetic differences in seminal plasma composition and sperm membrane integrity. This finding supports the observations of Longobardi et al. (2020), who reported breed as a major determinant of semen freezability in goats. The second ejaculate exhibited higher post-thaw motility than the first, likely due to the presence of a higher proportion of aged or dead spermatozoa in the first ejaculate following a longer sexual rest. Seasonal variation was evident, with slightly higher post-thaw motility recorded during the summer season; however, this trend should be interpreted cautiously, as a lower number of ejaculates were processed during the north-east monsoon. Furthermore, it is noteworthy that bucks of Kanni Adu and Kodi Adu breeds, which exhibited high post-thaw motility, were predominantly processed during the summer season.

Frozen doses of semen produced

The mean frozen semen doses produced per ejaculate in Kanni Adu, Kodi Adu and Salem Black goats were 24.88 ± 1.168, 20.15 ± 0.716 and 22.32 ± 0.735, respectively. The higher number of doses obtained from Kanni Adu might be attributed to its optimal production of semen volume with sperm concentration. lthough Kodi Adu bucks exhibited higher sperm concentration, their comparatively lower ejaculate volume resulted in fewer doses produced. Among the non-genetic factors included in the study, only the order of ejaculate showed a significant influence on the number of frozen doses produced, with the first ejaculate yielding a higher number of doses than the second. This is consistent with the greater semen volume and sperm output typically associated with the first ejaculate.

Motile spermatozoa per ejaculate

The motile spermatozoa per ejaculate calculated at neat, pre-freeze and post-thaw stages, were not significantly influenced by breed or season. However, the order of ejaculate had a significant effect, with the first ejaculate containing a higher number of motile spermatozoa across all stages. This can be attributed to longer sexual rest before the first ejaculate, which allows accumulation of sperm reserves in the reproductive tract, resulting in higher sperm output compared to the subsequent ejaculate.

Conclusion

Based on the analysis of 389 ejaculates from three indigenous goat breeds of Tamil Nadu using a linear mixed model, semen production and quality traits were significantly influenced by genetic group, ejaculate order and season. These findings highlight the need to account for both genetic and non-genetic factors to optimize semen collection and cryopreservation strategies for effective use in artificial insemination–based genetic improvement programmes.

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