

EXPLORING THE INFLUENCE OF NON-GENETIC FACTORS ON SEMEN PRODUCTION TRAITS IN KANNI ADU BUCKS

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ABSTRACT

The selection of breeding bucks stands as a pivotal decision in both organised and field populations aiming for genetic enhancement. Incorporating bucks with high genetic merit into breeding programme holds the potential to significantly elevate the overall productivity of the stock. This, in turn, serves to augment the economic viability of rural goat keepers who adhere to zero-input management systems. Hence, gaining a comprehensive understanding of the influence exerted by non-genetic factors on semen characteristics among bucks within a particular breed is imperative for effective breeding management.

In this study, a total of 108 ejaculates from four adult Kanni Adu 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, pre-freeze and post-thaw sperm motility (per cent) were 0.623 ± 0.056 , 3841.00 ± 238.00 , 4.73 ± 0.08 , 86.87 ± 0.02 , 85.49 ± 0.01 and 56.15 ± 0.01 respectively.

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

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INTRODUCTION

In the realm of livestock, particularly in small ruminant production, goat farming stands out for its advantages of prolificacy, low investment requirements, and minimal maintenance costs. These attributes have paved the way for significant growth in goat farming among smallholders, transforming it

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into a successful enterprise that strengthens the rural income while also providing affordable nutrition. India, boasts a rich genetic diversity with 39 registered breeds alongside numerous non-descript animals. There exists immense potential for enhancing the productive and reproductive efficiency of the non-descript or native goat population. Undoubtedly, artificial insemination stands as an established and proven reproductive biotechnology tool, facilitating the improvement of the genetic makeup of the native population by introducing elite germplasm. This, in turn, fosters sustainable production practices within the goat farming sector.

The state of Tamil Nadu is bestowed with three registered goat breeds, namely, Salem Black, Kanni Adu and Kodi Adu. Among these indigenous breeds, Kanni Adu, also known as Pullaiadu, is hailed as a remarkable meat breed native to Tamil Nadu, thriving particularly well in the tropical drought conditions prevalent in the region. These goats are characterized by their tall stature, primarily black coat, adorned with white or brown stripes along the sides of the face, as well as white or brown underbellies and inner legs. Notably, Kanni Adu goats exhibit early sexual maturity, high prolificacy (with 67 per cent of births resulting in multiple offspring), and low adult mortality rates, as evidenced by research conducted by Thilagam *et al.* (2006). These distinctive attributes position Kanni Adu as a superior indigenous breed capable of enhancing the genetic quality of non-descript goats in neighboring areas

through selective breeding programs aimed at genetic improvement.

Selecting superior bucks is the crucial step in formulating genetic improvement programs, both within organized farms and at the field level. This process involves evaluating conventional semen production parameters such as volume of semen, concentration of sperm, mass activity and motility of sperm. It is well-known that semen production parameters can be influenced by various factors, including 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). Therefore, understanding the impact of different non-genetic factors on semen characteristics among bucks of the same breed could greatly assist in breeding management practices. Thus, the present investigation was undertaken to examine the effect of non-genetic factors on conventional semen production traits of Kanni Adu bucks. Notably, there have been no previous studies conducted on this particular breed.

MATERIALS AND METHODS

In this study, data were collected from 108 ejaculates obtained from four Kanni Adu bucks over a span of five months, from March to July. These bucks were procured from various farmers' flocks at the age of 10-12 months and were not related to each other. They were housed under standardized conditions at the Frozen Semen Bank,

Department of Animal Genetics and Breeding, Madras Veterinary College, Chennai, Tamil Nadu. The bucks were fed a consistent diet, receiving 300 g of concentrate feed per day per animal, along with *ad libitum* access to green fodder and potable water. Semen collection was performed thrice a week from each buck using the artificial vagina method, with a restrained buck serving as a teaser. The semen samples (first and second ejaculates) were collected in graduated collection tubes and maintained at 37°C for both macroscopic and microscopic evaluations.

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 initial, pre-freeze and post-thaw motility (per cent) estimated using microscope and number of frozen semen doses produced per ejaculate. Other traits such as total initial motile spermatozoa (as a product of volume of ejaculate, concentration of spermatozoa and initial motility of spermatozoa), total pre-freeze motile spermatozoa (as a product of volume of ejaculate, concentration of spermatozoa and pre-freeze motility of spermatozoa) and total post-thaw motile spermatozoa (as a product of volume of ejaculate, concentration of spermatozoa and post-thaw motility of spermatozoa) were calculated.

The semen volume, colour, and consistency of each ejaculate were assessed through visual inspection of the semen within the graduated collection tube. Sperm

concentration was determined using a sperm photometer (Accucell, IMV technologies) that was specifically calibrated for goat semen analysis. The mass activity of the semen was graded on a scale from 0 to 5 based on the observation of sperm wave motion, which was examined from a drop of semen placed on a glass slide under 10x magnification using a phase-contrast microscope maintained at 37°C.

For the production of frozen semen straws intended for artificial insemination, the neat semen was diluted to a concentration of 100 million sperm per dose using a Tris-egg yolk-glycerol diluent. The percentage of progressively motile spermatozoa after dilution, referred to as the initial sperm motility, was subjectively evaluated under 20x and 40x magnification by covering a 10 µl drop of diluted semen with a thin cover slip.

In the analysis of semen production attributes, the data were categorized into random and fixed effects. The buck was regarded as a random effect, implying that its influence varied randomly across the sample. Meanwhile, the order of the ejaculate and season were considered fixed effects, indicating that their impact remained constant throughout the study. The order of the ejaculate had two levels, namely first and second, as ejaculates were collected twice from each buck. Similarly, the season was divided into two levels: summer (March to May) and south-west monsoon (June and July). To ensure the validity of statistical analyses, data measured as percentages were subjected to arcsine

transformation before being analyzed. This transformation helped to stabilize the variance and ensured that the data met the assumptions of the statistical tests applied.

In this study, each attribute was subjected to exploratory analysis to understand its distribution pattern. Outliers were identified and removed using Z-scores, with values beyond ± 4 standard deviations from the mean considered as significant outliers. Given the repeated observations of semen production traits over time for the same buck, which illustrated non-independence in the data, it was imperative to account for this non-independence in the analysis. Therefore, a linear mixed model was employed to address this issue. Specifically, a random intercept statistical model was constructed using the lme4 package (Bates *et al.*, 2015) in R for analyzing semen production traits. In this model, the random effect allows each buck to have a different intercept, thereby accommodating the non-independence in the data, while the effects of other variables remain fixed. After fitting the linear mixed model, significant fixed factors were subjected to pairwise comparisons using Tukey's test to discern any differences between groups. This comprehensive approach enabled the analysis to account for both fixed and random effects, providing a robust evaluation of the factors influencing semen production traits in Kanni Adu bucks. 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 ($i = 1-2$), E_j is effect of j^{th} ejaculate ($j = 1-2$), B_k is effect of k^{th} buck ($k = 1-4$) considered as a random effect and e_{ijkl} is the random error.

RESULTS AND DISCUSSION

In the study, the majority of semen samples exhibited a creamy white to whitish yellow color, although a few samples were yellow, possibly due to the presence of riboflavin in the seminal plasma (Mendoza *et al.*, 1989). Additionally, all samples exhibited a thick consistency, indicative of a higher sperm concentration. The semen volume produced by the bucks ranged from 0.2 ml to 1.2 ml, with a mean of 0.65 ± 0.02 ml. Concentration of sperm varied between 2000 million/ml and 7322 million/ml, with a mean of 3787.31 ± 103.30 million/ml. On average, the mass activity among the bucks was 4.72 ± 0.05 , with values ranging from 3 to 5. Initial sperm motility was recorded at a mean of 87.09 ± 0.004 per cent, while pre-freeze motility averaged 85.14 ± 0.005 per cent. Post-thaw motility among the bucks had an average of 55.85 ± 0.004 per cent.

The overall estimated marginal mean for the volume of ejaculate was 0.623 ± 0.056 ml. Volume of semen produced by Kanni Adu 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), Sirohi bucks (Khadse *et al.*, 2019) and Salem Black (Madhuri *et al.*, 2023), where, the mean volume was 0.840 ± 0.020 , 1.970 ± 0.120 , 0.850 ± 0.070 , 1.060 ± 0.010 and 0.692 ± 0.052 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 Kanni Adu 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. The volume of semen produced in south-west monsoon was 0.732 ± 0.059 ml, while summer season recorded a semen volume of 0.513 ± 0.062 ml. Order of the ejaculate had significant influence on the amount of volume produced, where the first ejaculate had shown higher volume (0.686 ± 0.060 ml) when compared to the second ejaculate (0.559 ± 0.061 ml).

The estimated marginal mean for the sperm concentration was 3841.00 ± 238.00 million/ml. The sperm concentration observed in Kanni Adu goat was more than the Black Bengal (2980.20 ± 0.960 million/ml Sultana *et al.*, 2013), Jamunapari goats (3573.04 ± 1.050 million/ml Kharche *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), and Salem Black (3253.00 ± 167.00 , Madhuri *et al.*, 2023). The differences might probably be due to the genetic group, age, sexual maturity of the bucks, feeding regime and environmental conditions. Season had significant effect on concentration of the sperm. The highest sperm concentration was recorded in summer (4102.00 ± 266.00 million/ml) followed by south west monsoon (3579.00 ± 253.00 million/ml). The order of the ejaculate had also shown to significantly influence the sperm concentration. The first ejaculate had a mean sperm concentration of 4061 ± 253.00 million/ml, while the second ejaculate had a mean sperm concentrate of 3621 ± 261.00 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 4.73 ± 0.08 . The mass activity value observed in this study was comparatively lower than Malabari goats (4.80 ± 0.02 ; Gopinathan *et al.*, 2021) and almost similar to Salem Black bucks (4.70 ± 0.09 ; Madhuri *et al.*, 2023). It 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. Neither season nor the order of ejaculate showed any significant influence on mass activity. Second ejaculate had a slightly higher

mass activity (4.76 ± 0.09) than the first ejaculate (4.69 ± 0.09). Mass activity was more in summer season (4.80 ± 0.10) than the south-west monsoon (4.66 ± 0.09).

The overall estimated marginal means for initial sperm motility and pre-freeze sperm motility were 86.87 ± 0.023 and 85.49 ± 0.012 per cent respectively. The initial sperm motility observed in Beetle (89.80 ± 1.26 per cent; Ahmad *et al.*, 2014), 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 Kanni Adu goats was higher than the Black Bengal (85.40 ± 8.20 per cent; Karunakaran *et al.*, 2015), Jamunapari (78.41 ± 0.67 per cent; Bastola *et al.*, 2018) and Malabari goats (79.60 ± 0.00 per cent; 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 managerial conditions.

Season was not having a significant influence on the initial sperm motility. The estimated means for the initial sperm motility noticed in summer (86.87 ± 0.026 per cent) and south-west monsoon (86.87 ± 0.029 per cent). 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 86.87 ± 0.026 per cent, while the second ejaculate had an estimate marginal mean of 86.87 ± 0.028 . No significant differences

between the season and ejaculates could be due to the genetic merit of the bucks.

Both season and order of the ejaculate did not influence the pre-freeze motility. The motility of pre-freeze semen in the first and second ejaculate had the estimated marginal mean of 85.49 ± 0.012 and 84.78 ± 0.016 per cent. Highest estimated marginal mean was reported in summer (86.19 ± 0.019 per cent), followed by south-west monsoon (84.05 ± 0.016 per cent). Pre-freeze motility was observed to be slightly 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 56.145 ± 0.013 per cent. Significant seasonal variation was observed for post-thaw sperm motility while, the order of the ejaculate had no significant effect. The estimated marginal means for the summer season was 58.321 ± 0.018 per cent, while the south west monsoon was 54.056 ± 0.016 . The first ejaculate had a post-thaw motility 55.847 ± 0.016 per cent, whereas the second ejaculate displayed 56.541 ± 0.017 per cent.

The estimated marginal means were 2109.00 ± 151.00 , 2059.00 ± 141.00 and 1358.00 ± 111.00 million respectively for concentration of motile spermatozoa in initial, pre-freeze and post-thaw semen. Season had no significant effect on the outcome of the initial motile sperm concentration. High concentration of motile sperm (2243.00 ± 173.00 million) was recorded in south-

west monsoon, while summer season had a motile sperm concentration of 1975.00 ± 193.00 million. The order of the ejaculate had significant effect on the concentration of initial motile spermatozoa. The estimated marginal means of concentration of initial motile spermatozoa for the first and second ejaculate were 2489.00 ± 174.00 and 1729.00 ± 185.00 million respectively. This could be due to the higher volume of semen produced and higher concentration of the spermatozoa in the first ejaculate when compared to the second ejaculate. Order of the ejaculate had a significant effect on the pre-freeze motile sperm concentration, whereas, the season was not significant. The first ejaculate had a high motile sperm concentration (2415.00 ± 165.00) in the pre-freeze stage, while the second ejaculate had a motile sperm concentration of 170200 ± 176.00 . In the post-thaw semen, the motile sperm concentration was observed to be significantly influenced by the order of the ejaculate but season had no significant impact. The first ejaculate had displayed a concentration of 1589.00 ± 125.00 million, while the second ejaculate had a motile sperm concentration of 1127.00 ± 132.00 million.

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 24.88 ± 1.17 . The season did not influence the number of frozen semen doses produced. Significant differences were observed in the frozen doses produced

for the order of ejaculate, and first ejaculate had higher doses of semen due to higher volume and concentration.

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 volume and concentration with respect to season and ejaculate is depicted in Fig. 1a and Fig. 1b. On a conclusive note, the season had a greater influence on semen production traits such as volume, concentration and post-thaw motility. The higher ambient temperature and humidity during the monsoon season might have contributed to the observed increase in the ejaculate volume. According to Nuti, (2016), seasonal breeders like goats exhibit higher ejaculate volumes during the breeding season, which typically declines during the non-breeding season and for the concentration of sperm, it is vice versa. In addition, adequate sexual rest could be provided to breeding bucks to obtain better results with respect to sperm motility parameters in both ejaculates.

CONCLUSION

Season was a significant factor affecting volume of semen, concentration of sperm and post-thaw motility. Meanwhile, order of ejaculate demonstrated noteworthy variations across most of the observed traits, except for mass activity, initial motility, and pre-freeze motility. This study highlighted the phenotypic superiority of the Kanni Adu bucks in semen production traits compared to other breeds.

Table 1. Estimated marginal mean (\pm S.E.) for 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	2168.19 \pm 104.01	2110.174 \pm 100.09	1386.54 \pm 67.93	24.88 \pm 1.17
Estimated marginal mean (overall)	2109.00 \pm 151.00	2059.00 \pm 141.00	1358.00 \pm 111.00	24.30 \pm 1.48
Season	NS	NS	NS	NS
Summer	1975.00 \pm 193.00	1953.00 \pm 183.00	1324.00 \pm 136.00	22.70 \pm 2.00
South-west Monsoon	2243.00 \pm 173.00	2165.00 \pm 164.00	1392.00 \pm 124.00	25.80 \pm 1.77
Ejaculate (E,J)	***	***	***	***
EJ1	2489.00 \pm 174.00	2415.00 \pm 165.00	1589.00 \pm 125.00	28.60 \pm 1.78
EJ2	1729.00 \pm 185.00	1702.00 \pm 176.00	1127.00 \pm 132.00	19.90 \pm 1.91

*: 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	0.65 \pm 0.02	3787.31 \pm 103.304	4.72 \pm 0.045	87.09 \pm 0.004	85.14 \pm 0.005	55.85 \pm 0.004
Estimated marginal mean (overall)	0.623 \pm 0.056	3841.00 \pm 238.00	4.73 \pm 0.08	86.87 \pm 0.023	85.49 \pm 0.01	56.15 \pm 0.01
Season	***	*	NS	NS	NS	***
Summer	0.513 ^b \pm 0.062	4102.00 \pm 266.00	4.80 \pm 0.10	86.87 \pm 0.026	86.19 \pm 0.02	58.32 \pm 0.02
South-west Monsoon	0.732 ^a \pm 0.059	3579.00 ^b \pm 253.00	4.76 \pm 0.09	86.87 \pm 0.029	84.05 \pm 0.02	54.06 \pm 0.02
Ejaculate (EJ)	**	*	NS	NS	NS	NS
EJ1	0.686 ^a \pm 0.060	4061.00 \pm 253.00	4.69 \pm 0.09	86.87 \pm 0.026	85.49 \pm 0.01	55.85 \pm 0.02
EJ2	0.559 ^b \pm 0.061	3621.00 \pm 261.00	4.76 \pm 0.09	86.87 \pm 0.028	84.78 \pm 0.02	56.54 \pm 0.02

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

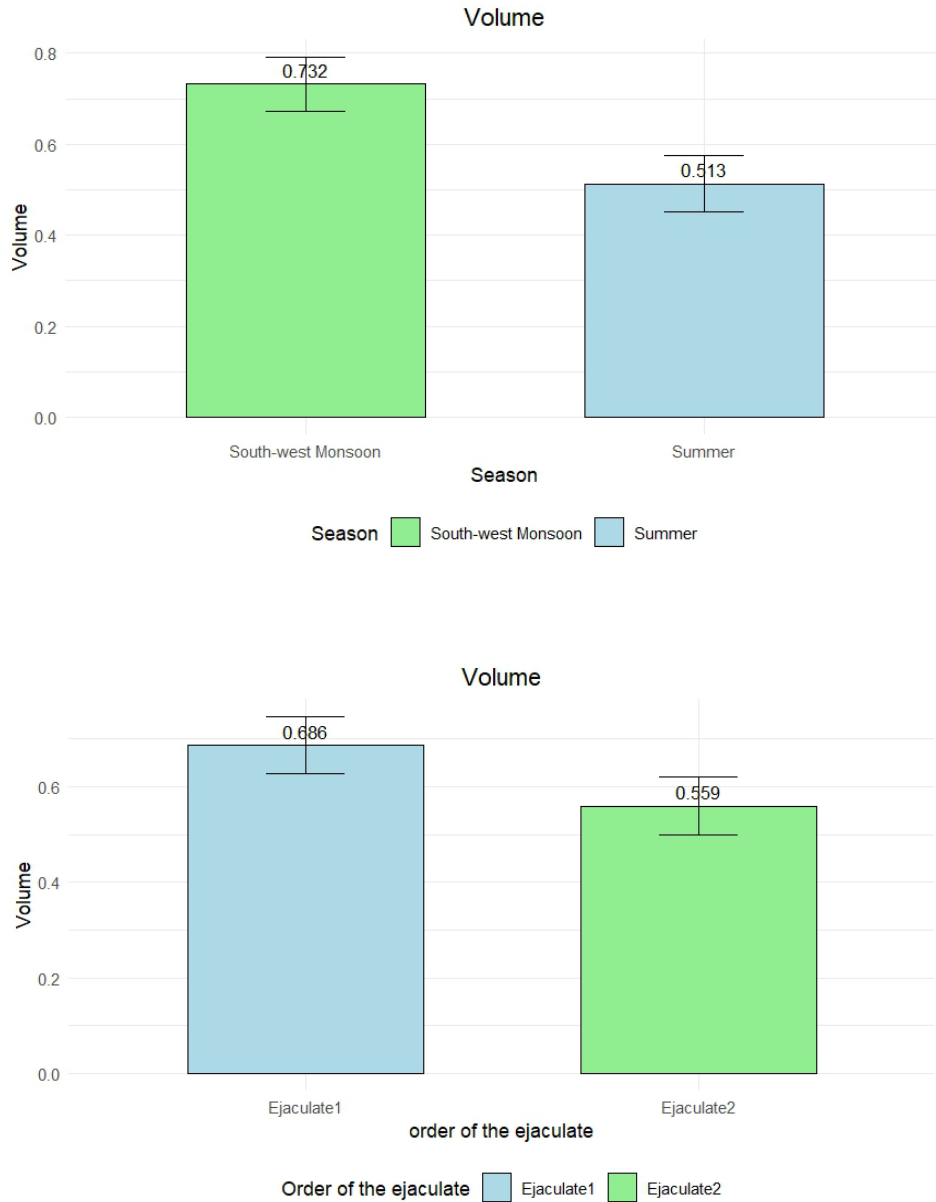


Fig. 1a) Plot of estimated marginal mean \pm S.E. for volume with respect to season and order of ejaculate

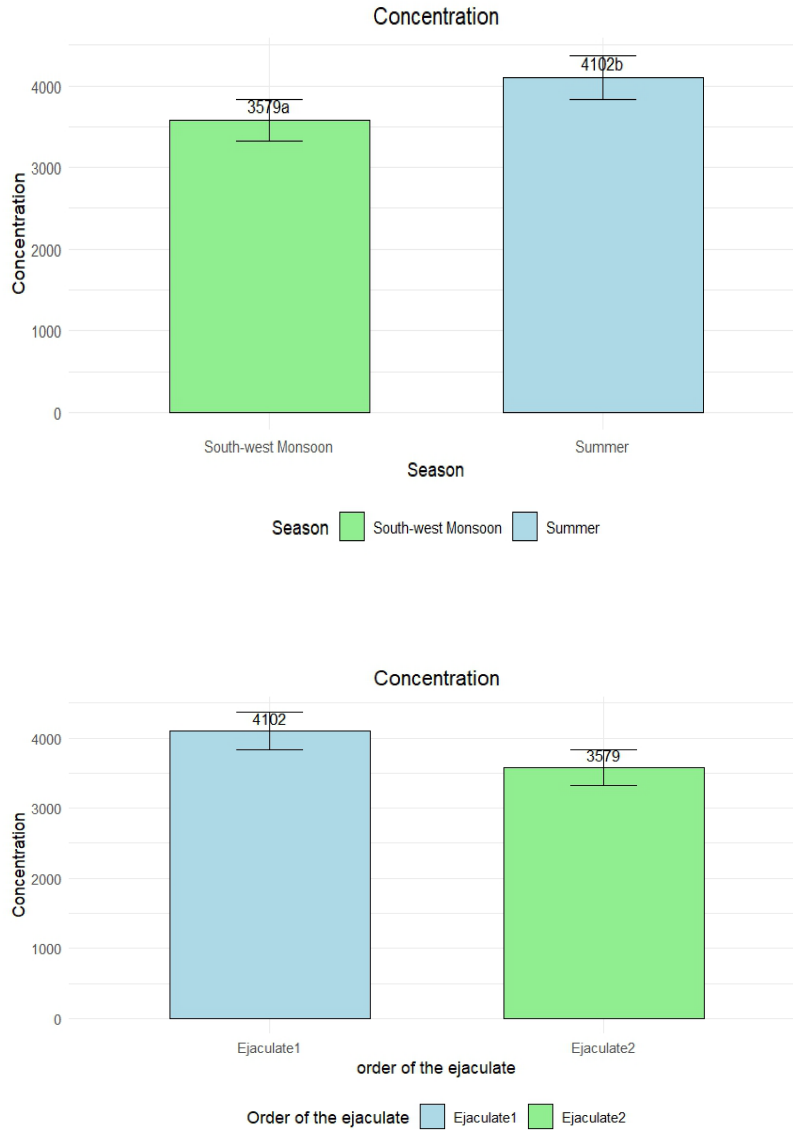


Fig. 1b. Plot of estimated marginal mean \pm S.E. for concentration with respect to season and order of ejaculate

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