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Genetic and Non-genetic Factors Influencing Semen Production Traits in Dairy Bulls

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ABSTRACT

Background: The choice of superior herd sires is essential for genetic improvement in dairy cattle, with sperm quality serving as a key factor. Bull semen's superior qualities are essential for accelerating genetic improvement through AI. These characteristics are affected by several factors, including the environment, management techniques, physiological condition and hereditary factors. This study involved in evaluating genetic and non-genetic factors influencing seminal attributes.

Methods: Data on 1,85,413 ejaculates from nine genetic groups *viz*. Jersey, Holstein Friesian, crossbred Jersey, crossbred Holstein Friesian, Bargur, Kangayam, Pulikulam, Umblachery and Red Sindhi were collected from four frozen semen stations of Tamil Nadu State. Semen production data *viz*. ejaculate volume (mL), spermatozoa concentration (million per mL), initial motility (percentage), post-thaw motility (percentage), total initial motile spermatozoa and total post-thaw motile spermatozoa were also examined for various genetic and non-genetic factors affecting them using linear mixed model with repeated measures.

Result: The overall estimated marginal means for Volume of ejaculate, Concentration of spermatozoa, Initial motility, Total initial motile spermatozoa per ejaculate, Total doses per ejaculate, Post-thaw motility and Total post-thaw motile spermatozoa per ejaculate were 3.11 ± 0.11 (mL), 963 ± 30.60 (10^6/mL), 79.50 ± 1.29 (per cent), 1847 ± 118 (10^6), 151.00 ± 7.20, 51.00 ± 0.42 (per cent) and 1535 ± 80.20 (10^6) respectively.The effect of all genetic and non-genetic factors (farms, ejaculates, period, seasons, age) were observed to be significantly influencing the seminal traits considered in the study. However, the estimated marginal means of each trait displayed varied ranges of mean against non-genetic factors.

Key words: Cattle, Semen, Genetic factors, Non-genetic factors.

INTRODUCTION

The characteristics of semen can be influenced by a wide range of environmental and management factors. These factors include the age of the bull at the time of semen collection, the order of ejaculates collected on the same day, the month, season and year of collection, the breed of the bull, the collection interval, the semen collector, the level of inbreeding and the ambient temperature. Mathevon *et al*. (1998), Brito *et al*. (2002), Mandal *et al*. (2010), Karoui *et al*. (2011), Bhakat *et al*. (2015) and Sarakul *et al*. (2018) have conducted studies to identify and understand the effects of these factors on semen production and quality parameters. As it is important to assess the genetic and non-genetic factors affecting the semen production traits of bull, this study was undertaken to evaluate the breeding bulls of various genetic groups involved in frozen semen production in Tamil Nadu.

MATERIALS AND METHODS

Data collection was conducted at four frozen semen stations in Tamil Nadu, *viz.* ECBF in Eachekottai (Farm I), DLF in Hosur (Farm II), NJF in Udhagamandalam (Farm III) and DLF in Udhagamandalam (Farm IV). The data encompassed semen production from year 2001 to 2020. Data pertaining to a total of 1,85,413 ejaculates from nine genetic groups were considered after filtering the ejaculates data with missing values and outliers. Semen production data included ejaculate volume (mL), spermatozoa concentration (million per mL), initial motility (percentage) and post-thaw motility (percentage). Additional traits, such as total initial motile spermatozoa (calculated as the product of ejaculate volume, spermatozoa concentration and initial motility) and total post-thaw motile spermatozoa (calculated as the product of ejaculate volume, spermatozoa concentration and post-thaw motility), were also examined.

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Statistical analyses

Linear mixed models were employed to investigate the effects of genetic and non-genetic factors on semen production traits across different genetic groups, including Bos indicus, Bos taurus, crossbred cattle and Bubalus Bubalis. Variance components were estimated using the animal repeatability model and the restricted maximum likelihood (REML) method. The estimated marginal mean, analysis of variance and pairwise comparison tests were conducted using the 'lmerTest,' 'emmeans,' and 'agricolae' packages of the R statistical software.

The data on semen production attributes were categorized into random and fixed effects. Bulls were considered as random effects, while farms, order of ejaculates, period, seasons, age and genetic groups were regarded as fixed effects. Bulls and genetic groups were identified as genetic factors, while the remaining factors were considered non-genetic.

The semen production data were divided into five periods: I (2001-2004), II (2005-2008), III (2009-2012), IV (2013-2016) and V (2017-2020). Each year was further subdivided into four seasons: winter (December to February), summer (March to April), south-west monsoon (June to August) and north-east monsoon (September to November). Semen collection in the four farms was classified into ten age groups: 18 to 30 months, 31 to 42 months, 43 to 54 months, 55 to 66 months, 67 to 78 months, 79 to 90 months, 91 to 102 months, 103 to 114 months, 115 to 126 months and more than 126 months. The data were also categorized into ten genetic groups, which included breeds of Bos taurus, Bos indicus and crossbred cattlesuch as Jersey, Holstein Friesian, crossbred Jersey, crossbred Holstein Friesian, Bargur, Kangayam, Pulikulam, Umblachery and Red Sindhi

Given the presence of repeated measurements, a linear mixed model was employed to analyze the semen production traits of cattle bulls. The statistical model used for analyzing semen production traits was as follows:

$$Y_{ijklmnop} = \mu + F_i + G_j + E_k + S_l + A_m + P_n + B_o + e_{ijklmnop}$$

Where:

$Y_{ijklmnop}$ = Semen production trait.

μ = Overall mean.

F_i = Effect of i^{th} farm.

G_j = Effect of j^{th} genetic group.

E_k = Effect of k^{th} ejaculate.

S_l = Effect of l^{th} season of collection.

A_m = Effect of the m^{th} age (in month) at collection.

P_n = Effect of n^{th} period of production.

B_o = Effect of o^{th} bull.

$e_{ijklmnop}$ = Random error associated with each observation which is assumed to be normally and independently distributed with mean zero and constant variance.

RESULTS AND DISCUSSIONS

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

Table 1 presents the overall estimated marginal means for various semen production traits and the influence of genetic and non-genetic factors.The results for each trait considered in the study are detailed as follows:

Volume of ejaculate (mL)

The overall estimated marginal mean for volume of ejaculate was determined to be 3.11 ± 0.11 mL. Various genetic and non-genetic factors were found to significantly contribute ($P \leq 0.001$) to the variation in volume of ejaculate. Among the genetic groups studied, the Jersey breed exhibited a significantly higher volume compared to other genetic groups. Bos indicus breeds, on the other hand, produced relatively lower volumes of ejaculate compared to Bos taurus breeds and crossbred bulls.

The farm where semen collection took place also had a significant impact on volume, with farm-III recording a significantly higher mean volume, compared to other farms, which could be attributed to factors such as its location at higher altitudes, better management practices and relatively conducive temperature throughout the year. Additionally, farm-III maintains a larger number of exotic breeds (Jersey and Holstein Friesian) and their crossbreds, which typically yield semen with higher volumes. Furthermore, the order of ejaculate influenced volume, with the first ejaculate yielding a significantly higher volume of semen compared to the second and third ejaculates, which can be attributed to the shorter interval between collections. Collecting multiple ejaculates on the same day is done to increase daily sperm production without compromising the functional attributes of the spermatozoa.

Seasonal variations were observed, with the south-west monsoon season showing a significantly higher mean volume of semen compared to other seasons. However, no significant differences were found between the winter and north-east monsoon seasons. The age of the bull also played a role in volume, with a gradual increase in semen volume observed as age advanced up to 90 months, followed by a gradual decrease. The highest mean volume of was observed between 79 and 90 months of age. This finding aligns with previous studies that have reported a direct proportional relationship between the volume of ejaculate and the age of the bull. It is worth noting that the recommended minimum standards for production of bovine frozen semen suggest culling sires after 8 years of age. The volume of ejaculate and the age of the bull are closely linked and support this recommendation.

Concentration of spermatozoa

The estimated marginal mean for concentration of spermatozoa was 963±30.60 million per mL. The genetic group, farm, order of ejaculate, age and period had highly

Table 1: Estimated marginal means±S.E. of semen production potential of cattle.

Factors	No. of ejaculate	Volume of ejaculate (mL)	Concentration of spermatozoa (10^6/mL)	Initial motility (in per cent)	Total initial motile spermatozoa per ejaculate (10^6)	Total doses perejaculate	Post-thaw motility (in per cent)	Total post-thaw per motile spermatozoa ejaculate (10^6)
Overall	1,85,413	3.11±0.11	963±30.60	79.50±1.29	1847±118	151.00±7.20	51.00±0.42	1535±80.20
Genetic group		***	***	***	***	***	***	***
Jersey	48,365	4.75^{e}±0.11	896^{b}±29.50	82.03^{cd}±1.23	2819^{d}±115	211.10^{c}±7.09	52.20^{c}±0.41	2198^{d}±78.80
Holstein Friesian	4,823	3.99^{d}±0.12	1031^{d}±30.80	82.77^{d}±1.27	2638^{c}±121	202.20^{c}±7.57	52.10^{c}±0.45	2075^{d}±85.60
Crossbred Jersey	88,083	3.13^{c}±0.11	979^{c}±29.10	79.31^{c}±1.21	1891^{b}±113	154.30^{b}±6.96	51.10^{b}±0.41	1597^{c}±77.60
Crossbred Holstein Friesian	7,672	2.80^{b}±0.12	1015^{cd}±30.90	82.60^{d}±1.28	1943^{b}±122	155.80^{b}±7.72	49.90^{a}±0.45	1375^{ab}±87.10
Bargur	1,440	2.42^{a}±0.12	981^{c}±32.90	77.13^{b}±1.33	1209^{a}±129	113.20^{a}±8.16	50.40^{ab}±0.50	1332^{ab}±95.30
Kangayam	16,148	2.43^{a}±0.11	1136^{e}±29.90	79.49^{c}±1.24	1839^{b}±116	149.00^{b}±7.21	51.90^{c}±0.44	1497^{b}±85.30
Pulikulam	2,050	2.80^{b}±0.12	903^{b}±33.10	78.76^{c}±1.34	1360^{a}±130	116.80^{a}±8.40	49.70^{a}±0.51	1273^{a}±97.30
Red Sindhi	14,398	2.68^{b}±0.11	1048^{d}±29.80	79.37^{c}±1.23	1853^{b}±116	153.20^{b}±7.16	51.00^{b}±0.45	1364^{ab}±85.70
Umblachery	2,434	2.99^{bc}±0.38	678^{a}±99.10	73.68^{a}±4.58	1069^{a}±350	99.20^{a}±20.04	51.10^{b}±1.08	1103^{a}±219.10
Farm		***	***	***	***	***	***	***
Farm-I	28,925	2.55^{a}±0.12	1173^{c}±31.70	80.99^{b}±1.33	2021^{c}±123	153.00^{b}±7.62	53.50^{d}±0.44	1699^{c}±85.40
Farm-II	79,000	2.94^{b}±0.12	912^{b}±30.90	81.80^{c}±1.30	1721^{b}±119	137.00^{a}±7.33	49.30^{a}±0.43	1208^{a}±82.30
Farm-III	49,198	3.56^{d}±0.12	837^{a}±30.90	73.31^{a}±1.30	1579^{a}±119	160.00^{b}±7.33	50.00^{b}±0.43	1666^{c}±82.30
Farm-IV	28,290	3.39^{c}±0.12	930^{b}±31.20	81.45^{bc}±1.31	2066^{c}±120	153.00^{b}±7.47	51.40^{c}±0.43	1567^{b}±83.50
Order of ejaculate		***	***	***	***	***	***	***
1	94,865	3.51^{b}±0.06	1146^{c}±17.30	77.58^{a}±0.88	2540^{c}±59	201.10^{c}±3.44	49.30^{a}±0.19	2061^{c}±39.30
2	90,523	3.23^{a}±0.06	993^{b}±17.40	79.37^{b}±0.88	2053^{b}±59	160.90^{b}±3.44	51.00^{b}±0.19	1669^{b}±39.30
3	25	2.60^{a}±0.29	750^{a}±77.60	81.57^{b}±2.93	947^{a}±311	89.70^{a}±19.31	52.80^{b}±1.13	875^{a}±213.80
Season		***	*	***	***	***	***	***
Winter	46,552	3.09^{b}±0.11	965^{ab}±30.60	79.74^{b}±1.29	1869^{c}±118	150.00^{ab}±7.21	51.10^{b}±0.42	1550^{b}±80.50
Summer	47,778	3.11^{a}±0.11	962^{ab}±30.60	79.37^{a}±1.29	1840^{ab}±118	151.00^{bc}±7.22	50.90^{a}±0.42	1521^{a}±80.40
South-west monsoon	45,567	3.15^{c}±0.11	967^{b}±30.60	79.31^{a}±1.29	1855^{bc}±118	153.00^{c}±7.21	51.20^{b}±0.42	1566^{b}±80.40
North-east monsoon	45,516	3.09^{b}±0.11	958^{a}±30.60	79.62^{b}±1.29	1824^{a}±118	148.00^{a}±7.20	51.00^{b}±0.41	1503^{a}±80.20
Age of bull (in months)		***	***	***	***	***	***	***
18 to 30	21,515	2.27^{a}±0.11	1010^{f}±30.60	79.01^{a}±1.29	1390^{a}±118	121.00^{a}±7.19	50.60^{a}±0.41	1158^{a}±80.10
31 to 42	38,505	2.56^{b}±0.11	987^{e}±30.50	79.37^{b}±1.28	1512^{b}±117	129.00^{b}±7.18	51.00^{bc}±0.41	1304^{b}±79.90
43 to 54	36,415	2.92^{c}±0.11	979^{e}±30.60	79.68^{c}±1.28	1770^{c}±118	145.00^{c}±7.19	50.90^{ab}±0.41	1497^{c}±80.10
55 to 66	27,623	3.17^{d}±0.11	959^{cd}±30.60	79.31^{b}±1.29	1898^{d}±118	154.00^{d}±7.19	50.80^{ab}±0.42	1617^{d}±80.20
67 to 78	19,399	3.34^{e}±0.11	954^{c}±30.60	79.37^{b}±1.29	2038^{e}±118	162.00^{e}±7.21	51.20^{c}±0.42	1693^{e}±80.50
79 to 90	14,665	3.49^{g}±0.11	936^{ab}±30.70	79.25^{ab}±1.29	2087^{e}±118	166.00^{g}±7.24	51.20^{c}±0.42	1723^{e}±81.00

Table 1: Continue...

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91 to 102	12,551	3.40^{f}±0.11	960^{cd}±30.90	79.62^{bc}±1.29	2068^{e}±119	165.00^{fg}±7.28	51.20^{c}±0.42	1679de±81.70
103 to 114	6,552	3.33^{e}±0.11	952^{bc}±31.10	79.19^{ab}±1.30	1924^{d}±120	159.00^{de}±7.37	51.20^{c}±0.43	1621^{d}±82.80
115 to 126	4303	3.33^{e}±0.11	980de±31.60	80.16^{c}±1.32	2047^{e}±122	164.00^{ef}±7.53	51.70^{cd}±0.44	1671de±84.70
>126	3,885	3.28^{e}±0.12	912^{a}±31.90	80.16^{c}±1.33	1734^{c}±123	142.00^{c}±7.61	50.70^{ab}±0.45	1386^{b}±86.80
Period		***	***	***	***	***	***	***
2001-2004	954	2.79^{a}±0.12	1011^{c}±33.40	79.19^{ab}±1.38	1610^{a}±130	140.00^{a}±8.06	52.50^{d}±0.48	1458^{b}±93.10
2005-2008	10,916	3.15^{c}±0.11	1073^{d}±31.00	78.95^{a}±1.30	2008^{c}±119	163.00^{b}±7.34	51.40^{c}±0.42	1727^{c}±82.00
2009-2012	36,853	3.30^{d}±0.11	1009^{c}±30.50	79.43^{b}±1.28	2136^{d}±117	171.00^{c}±7.17	50.70^{b}±0.42	1745^{c}±79.80
2013-2016	68,211	3.04^{b}±0.11	897^{b}±30.40	80.75^{c}±1.28	1774^{b}±117	139.00^{a}±7.14	50.70^{b}±0.41	1446^{b}±79.30
2017-2020	68,479	3.28^{d}±0.11	825^{a}±30.40	79.19^{ab}±1.28	1706^{a}±117	140.00^{a}±7.13	49.90^{a}±0.41	1299^{a}±79.40

significant ($P \leq 0.001$) influences on concentration. Among the genetic groups, Kangayam exhibited significantly higher concentration, while Umblachery had significantly lower concentration. Farm-I had a significantly higher mean concentration compared to other farms; This can be attributed to the negative correlation between semen volume and the concentration of spermatozoa. As a result, the lower volume of ejaculate observed in farm-I contributed to the higher concentration of spermatozoa. The first ejaculate contained significantly more concentration of spermatozoa compared to the second and third ejaculates. Seasonally, higher concentrations were observed during the south-west monsoon and winter seasons. In certain tropical and semi-tropical regions, a drop in sperm production during the hot season was observed only in taurine and crossbred bulls, while the indicine counterparts remained unaffected.

Several studies have reported a significant effect of the age of the bull on the concentration of spermatozoa. Mature bulls were found to have a higher mean concentration of spermatozoa than younger bulls in accordance with Ahmad *et al*. (2011). However, significant variations in sperm concentration were observed up to 126 months of age, after which the concentration started to decline in this study. It is worth noting that Singh *et al*. (1967) found no significant differences between age groups in Haryana bulls.

Initial motility

The overall estimated marginal mean for initial motility was 79.50±1.29 percent. Genetic group, farm, order of ejaculate, season, age and period significantly influenced initial motility. Holstein Friesianand its crossbred exhibited significantly higher initial motility compared to Bos indicus and crossbred Jersey bulls. Farm-II had significantly higher initial motility than farm-I and farm-III. The location of the farm and the genetic groups present in those farms could be plausible reasons for the higher initial motility observed. Second and third ejaculates showed significantly higher initial motility compared to the first ejaculate.

Winter season and north-east monsoon had significantly higher initial motility compared to summer and south-west monsoon. Several studies have reported that the season has a significant impact on sperm initial motility, which aligns with the findings of the present study. Some studies, including Mukherjee and Bhattacharya (1952), Kodagali (1962), Sinha and Prasad (1966), Tomar *et al*. (1970), Rao and Rao (1975) and Tomar and Gupta (1984), have reported higher initial motility during winter compared to summer, which is consistent with the results of this study. However, there have been conflicting findings, with studies such as Tomar *et al*. (1966), Sinha and Prasad (1966), Mandal *et al*. (2005), Khan *et al*. (2007) and Tiwari *et al*. (2012) reporting no influence of season on initial motility.

The highest and lowest initial motility were observed in the age groups of 115 to 126 months and 18 to 30 months, respectively. Period-IV (2015-2017) recorded significantly higher initial motility compared to other periods.

Total initial motile spermatozoa

The estimated marginal mean for total initial motile spermatozoa was 1847±118 million. Genetic group, farm, order of ejaculate, season, age and period significantly influenced the total initial motile spermatozoa. Jersey exhibited significantly higher total initial motile spermatozoa compared to other genetic groups. Umblachery had lower initial motile spermatozoa. Farm-I and farm-IV showed significantly higher means for total initial motile spermatozoa compared to farm-II and farm-III. These differences can be attributed to the genetic composition of the maintained groups in each respective farm.

The first ejaculate contained significantly higher mean total initial motile spermatozoa compared to the second and third ejaculates. Winter season had the highest total initial motile spermatozoa followed by south-west monsoon, summer and north-east monsoon. Interestingly, while the volume of ejaculate and concentration of spermatozoa were found to be higher during the south-west monsoon season, the total initial motile spermatozoa were observed to be higher during the winter season. This disparity is likely due to the influence of higher initial motility during the winter season.

The highest total initial motile spermatozoa were observed between 79 and 90 months of age. Period-III had significantly better mean total initial motile spermatozoa compared to other periods.

Total doses per ejaculate

The estimated marginal mean for total doses per ejaculate was 151±7.20. According to Salisbury *et al.* (1978), various factors, such as volume, motility rate, spermatozoa concentration per mL of ejaculate, percentage of normal spermatozoa and required concentration for artificial insemination, have a significant impact on the potential number of doses that can be produced from a single ejaculate. Genetic group, farm, order of ejaculate, age and period significantly influenced the total doses per ejaculate. Jersey had the highest mean total doses per ejaculate, while Umblachery had the lowest.

The estimated marginal means revealed higher total doses produced per ejaculate in farm-III (160.00±7.33). The reason might be due to presence of Bos taurus breeds and their crossbred bulls under semen collection. Studies specific to genetic groups can reveal the true effect of farm on total doses produced per ejaculate. More doses were produced from the first ejaculate compared to subsequent ejaculates. As the volume of ejaculate and concentration of spermatozoa were observed to be higher in first ejaculate and during south-west monsoon season, the total doses produced were higher as expected in first ejaculate and during south-west monsoon season. The highest mean total doses per ejaculate were observed between 79 and 90 months of age. Period-III had significantly higher mean total doses per ejaculate compared to other periods. The marginal means showed decreasing trend after 90 months of age, clearly justifying the recommendation of MSP (2012) on replacement of stock.South-west monsoon had the highest mean total doses per ejaculate, followed by summer, winter and north-east monsoon.

Post-thaw motility

The estimated marginal mean for post-thaw motility was 51.00±0.42 per cent. All the genetic and non-genetic factors significantly influenced post-thaw motility. Jersey had the highest mean post-thaw motility, while Pulikulam had the lowest. Farm-I had significantly higher post-thaw motility compared to other farms. Third ejaculate exhibited significantly higher post-thaw motility compared to the first ejaculate. South-west monsoon had the highest post-thaw motility followed by winter, north-east monsoon and summer; while a report by Boujenane and Boussaq (2014) indicated better post-thaw motility during spring.

The highest and lowest post-thaw motility were observed during the age groups of 115 to 126 months and 18 to 30 months, respectively. Similar to the present study, Javed *et al.* (2000), Brito *et al*. (2002), Bhakat *et al*. (2011) and Boujenane and Boussaq (2014) reported a significant effect of age on post-thaw motility. Period-I showed significantly higher post-thaw motility compared to other periods. Although post-thaw motility showed a significant effect, the variation among different levels of the factors was minimal due to subjective assessment. Additionally, this could be attributed to the implementation of better and standardized freezing protocols at frozen semen stations.

Total post-thaw motile spermatozoa

The estimated marginal mean for total post-thaw motile spermatozoa per ejaculate was 1535 ± 80.20 million. Genetic group, farm, order of ejaculate, season, age and period significantly influenced the total post-thaw motile spermatozoa per ejaculate. Jersey had the highest mean, while Umblachery had the lowest. Farm-I had the highest total post-thaw motile spermatozoa per ejaculate, followed by farm-III, farm-IV and farm-II. The first ejaculate possessed significantly higher mean total post-thaw motile spermatozoa compared to the second and third ejaculates.

South-west monsoon had the highest total post-thaw motile spermatozoa, followed by winter, summer and north-east monsoon. Interestingly, unlike the total initial motile spermatozoa, the total post-thaw motile spermatozoa were higher during the south-west monsoon season and lowest during the North-east monsoon season. The highest total post-thaw motile spermatozoa were observed during the age group of 79 to 90 months. Period-III exhibited higher estimates compared to other periods.

However, it is important to note that there is a lack of available literature specifically addressing the total post-thaw motile spermatozoa, which limits our ability to discuss these results in detail. Further research in this area is needed to gain a deeper understanding of this aspect.

CONCLUSION

The results demonstrate that various factors, including genetic group, farm, order of ejaculate, season, age and period, significantly influence semen production traits. The findings highlight the importance of considering these factors in breeding programs to optimize semen quality and enhance genetic improvement in the dairy sector. Understanding the influences of these factors on semen production can aid in the development of strategies to increase milk production, preserve indigenous breeds and provide sustainable economic support to the agricultural community.

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Conflict of interest

On behalf of all authors, the corresponding authors state that there is no conflict of interest.

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