Evaluation of genetic components and the effect of non-genetic factors on seminal traits and semen rejection rate in Murrah buffalo bulls reared in tropical climatic condition

Ramajayan. P[1*], S.N. Sivaselvam[1], S.M.K. Karthickeyan[1], R. Venkataramanan[1], A. Gopinathan[1] and S. Poobitha[2]

[*]Corresponding Author: Ramajayan. P, E-mail ID: dr.ramvet1990@gmail.com

[1] Department of Animal Genetics and Breeding, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

[2] Department of Veterinary Pathology, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, India.

Abstract

The present study was designed to determine the effect of genetic and non-genetic factors on semen production and semen discard patterns in Murrah buffalo bulls. Data on 54,268 ejaculates covering a period of 11 years were collected from 77 Murrah bulls maintained in the semen station. The effect of non-genetic factors on seminal parameters was analyzed using least-squares analysis of variance under general linear model. The repeatability estimates were computed by REML using WOMBAT program. Semen rejection rate among the main factors was evaluated using Chi-square test. The overall least-**s**quares means for semen volume, sperm concentration, total sperm per ejaculate, mass activity, initial motility, post-thaw motility, and frozen semen doses per ejaculate were 2.65 ml, 1222.04 million per ml, 3030.10 million, 2.64, 67.45%, 51.73%, and 128.80 doses. Similarly, repeatability estimates were 0.27, 0.25, 0.22, 0.28, 0.34, 0.27 and 0.23, respectively. The non-genetic factors, ejaculate number, period, season, and bull age, had a highly significant (P <0.01) effect on all the seminal traits. The bull-to-bull variation was also found to be significant (P <0.01). The phenotypic correlation coefficient between the traits was highly significant and positive for most traits. The semen rejection rate was high in pre-freezing (2.07%) than post-freezing (17.91%). Better seminal

quality was observed in the first ejaculate, period V, south-west monsoon, and summer seasons. Bulls above eight years of age performed better than younger bulls, with best performance in bulls 12 years and above. The repeatability estimates were low to moderate, indicating a scope for selection based on moderate repeatable traits. This would be useful to the semen stations in tropical regions to frame suitable strategies to improve the quality and quantity of semen production.

Keywords: Semen production; Non-genetic factors; Repeatability, Correlation, Murrah buffalo bulls

Introduction

Buffalo (*Bubalus bubalis*) is an important livestock genetic resource of India in terms of milk and meat production, creating a socio-economic impact among the rural population. The total milk production of India is about 187.75 million tonnes, of which a major share of 49% is contributed by indigenous and non-descript buffaloes (Basic Animal Husbandry and Fisheries Statistics 2019). As per the breeding policy of India, Murrah buffalo is the recommended breed for genetic up-gradation of non-descript buffaloes to enhance their genetic quality and milk production potential. Murrah buffalo is used for grading up as the breed is well recognized for its high-yield milk production and adaptability to survive in different climatic conditions. The modern breeding program utilizes Artificial Insemination (AI) using frozen semen of genetically superior germplasm acquired through pedigree selection and progeny testing to improve the genetic merit of larger non-descriptive populations in shorter intervals. Breeding bulls play a significant role in improving the genetic potential of low-producing non-descript populations as genetic gain associated with production performance comes mainly from sires. Therefore, a larger number of quality frozen semen doses from genetically superior breeding bulls is required to implement the genetic improvement program on a large scale in extensively distributed non-descript buffalo populations through AI under field conditions for successful service and conception. Thus, semen stations are established across the country to produce quality semen from high genetic merit bulls free of genetically transmitted and contagious diseases to ensure better fertility and faster genetic gain. There are about 56 semen stations in the country, of which 49 semen stations are graded by the central monitoring unit of India (Minimum Standard for Production of Bovine Frozen Semen (MSP) 2012). Though genomic selection with SNP markers, pedigree selection, and progeny testing is adopted to select high genetic merit breeding bulls, the semen production process is governed by genetic as well as environmental factors. The micro and macro environment of the bulls, nutrition, managemental practices, and environment during semen collection influences the semen quality (Mukhopadhyay et al. 2010). Therefore, understanding the role of genetic and non-genetic components on semen production would help to assess the environmental causes of variation in seminal traits to improve the production performance of the bulls.

Studies on the effect of genetic and environmental factors on seminal characteristics in an organized conditions are limited in Murrah bulls (Rao et al. 1991; Bhosrekar et al. 1992; Ravimurugan 2001 and Bhakat et al. 2015). However, the reported studies were also performed with minimal data and shorter study duration period without addressing the impact of the factors on the entire production period and also not included other random factors like bull effects. Besides, there is a lack of information on genetic parameters like repeatability and non-genetic factors impact on rejection rate of semen in Murrah buffalo bulls. Since repeatability is considered as upper value for heritability, estimation of repeatability for seminal traits would be useful in assessing the future performance of bulls and culling non-productive animals.

With this background, the present study was performed with a larger set of semen production data to investigate the effect of genetic and non-genetic factors on semen production traits in Murrah buffalo bulls which will be helpful for the semen stations to adopting suitable measures to improve the quality of frozen semen used for AI.

Materials and Methods

Farm and management

Data on seminal parameters were collected from 77 Murrah buffalo bulls maintained in frozen semen production unit, Exotic Cattle Breeding Farm, Thanjavur, Tamil Nadu, India. The semen station was established in 1977 in 513.86 acres, where the mean temperature and relative humidity range from 22° to 39°C and 72 to 90%, respectively. The average annual rainfall is about 914.31 mm, with major contribution from north-east monsoon. The bulls were fed concentrate (18.5% CP and 70% TDN) along with mineral mixture, green and dry fodders. They are housed in individual pens with loafing area and fog system (sprinklers and fans) to control heat stress.

Semen collection and examination

Semen was collected twice a week in the morning by artificial vagina method using a dummy bull. On each collection, two ejaculates are taken with a time interval of 20-30 minutes between the ejaculates. Semen volume was measured directly from the graduated semen collection tube (ml). Sperm concentration ($\times 10^6$/ml) was estimated with the help of bovine photometer. The mass activity was assessed on a scale of 0 (immotile) to 5 (very fast swirling) at 10x magnification using a phase-contrast microscope. Similarly, initial motility (percent) was examined in egg-yolk-tris diluted semen at 40x magnification. After 24 hours of freezing in liquid nitrogen, semen straw thawed at 37ºC for 30 seconds are used to evaluate post-thaw motility. Total sperm per ejaculate was calculated as a product of volume and sperm concentration.

Data structure

54,268 ejaculates data on semen volume (SV), sperm concentration (SC), total sperm per ejaculate (TSE), mass activity (MA), initial motility (IM), post-thaw motility (PTM), and frozen semen doses per ejaculate (FSD) were used to study the effect of non-genetic factors such as ejaculate number, period, season, and age of the bulls. Genetic parameters *i.e.*, repeatability and phenotypic correlations, were studied, however heritability could not be estimated for want of pedigree records.

The seasons were classified into four, winter (December to February), summer (March to May), south-west monsoon (June to August), and north-east monsoon (September to November). The entire collection period of 11 years was grouped into five periods, Period I (2005-2007), II (2008-2009), III (2010-2011), IV (2012-2013), and V (2014-2015). The bulls age was categorized into six groups, A_1 - 18 to 42 months, A_2 - 43 to 67 months, A_3 - 68 to 92 months, A_4 - 93 to 117 months, A_5 - 118 to 142 months, and A_6 - above 142 months.

Statistical analyses

Data were tested for normality and variance homogeneity using mean ± 3 Standard Deviation to remove outliers. The seminal traits were subjected to single trait univariate analysis of variance under general linear model, and bull effect was studied adjusting the data for main factors (Harvey 1996). All interaction effects were explored among the factors, and effects revealing significance were included in the analysis. The difference between the least-squares means for sub-classes under a particular effect was tested by Duncan's Multiple Range Test. As initial motility and post-thaw motility are expressed in percentages, arcsine angular transformation was performed as per Snedecor and Cochran (1989). While representing the means and standard errors, angles were reconverted to percentages to a precision of two decimals.

The statistical model used for analysis:

$$Y_{ijklmno} = \mu + E_i + P_j + S_k + A_l + (A_l \times E_i)_m + (A_l \times S_k)_n + e_{ijklmno}$$

Where,

$Y_{ijklmno}$ = semen production trait of o^{th} individual observation belonging to i^{th} ejaculate, j^{th} period, k^{th} season, l^{th} age, m^{th} age x ejaculate and n^{th} age x season effects

μ = overall mean

E_i = effect of i^{th} ejaculate (i= 1, 2)

P_j = effect of j^{th} period (j = 1 to 5)

S_k = effect of k^{th} season (k = 1 to 4)

A_l = effect of l^{th} age (l = 1 to 6)

$(A_l \times E_i)_m$ = effect of m^{th} age x ejaculate interaction

$(A_l \times S_k)_n$ = effect of n^{th} age x season interaction

$e_{ijklmno}$ = residual random error, NID (0 and σ^2_e)

Repeatability was estimated as an intra-class correlation between records of the same bull (Becker 1975). The variance components were estimated by Restricted Maximum Likelihood (REML) using WOMBAT program (Meyer 2007).

$$Y_{ijk} = \mu + B_i + a_j + e_{ijk}$$

Where,

Y_{ijk} = the k^{th} record of i^{th} bull in the j^{th} non-genetic effect

μ = overall mean

B_i = effect of i^{th} bull (random effect)

a_j = effect of j^{th} non-genetic effect (fixed effect) ‘a’ may be one or more significant non-genetic effects

e_{ijk} = random error NID (0 and σ^2_e)

Repeatability value was obtained as intra-class correlation (r)

$$r = \frac{\sigma^2_b}{\sigma^2_b + \sigma^2_e}$$

The phenotypic correlations were obtained from the estimates of (co) variance components as the ratio of respective covariances to the square root of the products of variances. Sampling errors for correlations were obtained using a two-tailed test of significance (Meyer 2007).

$$r_p = (\sigma_{w(xy)} + \sigma_{s(xy)}) / \sqrt{(\sigma^2_{w(x)} + \sigma^2_{s(x)})\,(\sigma^2_{w(y)} + \sigma^2_{s(y)})}$$

Where,

$\sigma_{w(xy)}$ = residual component of covariance between traits x and y

$\sigma_{s(xy)}$ = sire component of covariance between traits x and y

$\sigma^2_{w(x)}$ = residual component of variance for trait x

$\sigma^2_{w(y)}$ = residual component of variance for trait y

$\sigma^2_{s(x)}$ = sire component of variance for trait x

$\sigma^2_{s(y)}$ = sire component of variance for trait y

The rejection rate of semen was calculated based on the number of ejaculates discarded before and after freezing, which fails to satisfy the minimum standards, *i.e.*, less than 0.5 ml, 500 million/ml, +2 grade, 70% and 50% for semen volume, sperm concentration, mass activity, initial motility and post-thaw motility (MSP 2012). The discard rate among the non-genetic factors was analyzed with Chi-square test using SPSS version 20.

Results

The overall least-**s**quares means for semen volume, sperm concentration, total sperm per ejaculate, mass activity, initial motility, post-thaw motility, and frozen semen doses per ejaculate were 2.65 ml, 1222.04 million per ml, 3030.10 million, 2.64, 67.45%, 51.73%, and 128.80 doses, respectively. The bull effect was found to be a significant source of variation among seminal traits ($P < 0.01$). Among interaction between effects, age x ejaculate and age x season interactions were significant. The effects of non-genetic factors and their analysis of variance were presented in Tables 1 and 2.

Effect of ejaculate numbers

Ejaculate numbers had a highly significant effect (P <0.01) on all the traits. The first ejaculate expressed significantly higher value for semen volume (2.96 ± 0.01 ml), sperm concentration (1260.73 ± 5.43 million/ml), Total sperm per ejaculate (3533.29 ± 20.24 million) and frozen semen doses (146.91 ± 0.91). However, increasing trend was observed between the successive ejaculates for mass activity, initial motility, and post-thaw motility. In general, the first ejaculate had better semen quality and frozen semen doses compared to the second ejaculate.

Effect of period

The period showed a highly significant (P <0.01) influence on all the seminal traits (Table 2). A gradual increase in semen volume (2.38 ± 0.01 to 2.91 ± 0.01 ml) and total sperm per ejaculate (2483.42 ± 28.31 to 3529.43 ± 22.99 million) were observed from period I to V. Sperm concentration also revealed an increasing curve from period I to IV (1168.66 ± 7.60 to 1295.98 ± 6.40 million/ml) and declined in period V (1273.72 ± 6.17 million/ml). Similarly, highest and lowest value for mass activity was observed in periods V and III. A significant variation was noticed among periods for initial motility with the highest value in period V (74.11 ± 0.18%), followed by periods I and IV. Period III recorded the highest post-thaw motility of 52.27 ± 0.01%, succeeded by period I and V without significant difference (P >0.05). The number of frozen semen doses per ejaculate was maximum during period V (161.73 ± 0.99) and least during period I (112.17 ± 1.26).

Effect of season

The effect of season on semen production traits was highly significant (P <0.01), Table 2. The highest value for semen volume, total sperm per ejaculate, and initial motility were observed in south-west monsoon and summer seasons, which did not differ significantly between the seasons. The sperm concentration was higher in summer season (1247.95 ± 12.11 million/ml), followed by south-west monsoon (1231.62 ± 7.32 million/ml) with the lowest value in winter season (1199.19 ± 4.93 million/ml). Among different seasons, winter and south-west monsoon seasons recorded higher value for post-thaw motility (52.04 ± 0.07% and 51.97 ± 0.16%) compared to other seasons. The frozen semen doses per ejaculate were relatively higher in south-west monsoon and summer seasons than north-east monsoon and winter.

Effect of age

All the seminal traits were significantly affected (P <0.01) by bull age. Semen volume showed an increasing trend as the age advances (2.25 ± 0.02 ml to 2.95 ± 0.08 ml). Sperm concentration was highest in age group II (1252.09 ± 4.38 million/ml) and thereafter declined in other age groups, without significant difference (P >0.05). Total sperm per ejaculate (2454.04 ± 28.87 to 3291.76 ± 19.31 million) and initial motility (63.65 ± 0.22 to 69.44 ± 0.15%) increased gradually from age group I to group IV, declined at group V and improved in group VI with highest value. The bulls in the age group VI and V exhibited higher mass activity (2.75 ± 0.07; 2.71 ± 0.01), whereas bulls in the age group III recorded the lowest mass activity (2.55 ± 0.01) along with age group II. The post-thaw motility was highest in age groups VI, III, and IV (52.41 ± 0.50, 52.19 ± 0.07 and 52.02 ± 0.07%, respectively) whereas, the lowest was observed in age group I (51.20 ± 0.11%). As the age progressed, the number of frozen semen doses per ejaculate increased from 104.14 ± 1.39 to 145.87 ± 6.30 doses.

Repeatability

The repeatability estimates ranged from 0.22 to 0.34, which were low to medium. Total sperm per ejaculate was the least repeatable trait, whereas highest repeatability was observed in initial motility. The repeatability of semen production traits is given in Table 3.

Phenotypic correlations

All semen production traits except semen volume and post-thaw motility were highly significant (P <0.01) and positively correlated. Semen volume had a highly significant (P <0.01) positive correlation with total sperm per ejaculate, mass activity, and frozen semen doses per ejaculate but exhibited a very low negative association with sperm concentration

(-0.10), initial motility (-0.02), and post-thaw motility (-0.03). Similarly, post-thaw motility possessed a positive correlation with all traits except total sperm per ejaculate. The phenotypic correlations between seminal attributes are presented in Table 4.

The rejection rate of semen

The rejection rate of semen and its non-genetic effects are presented in Table 5. The overall discard rate of semen was 19.98%, with the highest rejection rate at pre-freezing stage than the post-thaw stage. The first ejaculate had a relatively higher rejection rate (27.08%) than the second ejaculate (11.00%). North-east monsoon expressed the highest rejection rate (21.05%), followed by winter, south-west monsoon, summer seasons, and period V (2014-2015) recorded the least discard rate compared to other periods. There was a gradual decrease in rejection rate as the age of bulls advanced, young bulls had a higher rejection rate (31.08%) compared to adult bulls (7.54%).

Discussion

The mean semen volume observed in the present study is similar to the findings of Kumar et al. (2014) and Bhakat et al. (2015); however, higher (Mukhopadhyay et al. 2010; Tiwar et al. 2011 and Singh et al. 2013) and lower values (Bhakat et al. 2011) were also recorded. The earlier reports on sperm concentration were comparatively lower than the present findings (Bhakat et al. 2011; Tiwar et al. 2011 and Bhakat et al. 2015). Dhami et al. 1998 expressed higher (1264.86 million/ml) sperm concentration and lower post-thaw motility (47.26%) than the present observations. Concerning total sperm per ejaculate, mass activity and initial motility, comparatively higher (Mukhopadhyay et al. 2010; Tiwari et al. 2011 and Kumar et al. 2014) and lower values (Rao and Sreemannarayana 1996 and Bhakat et al. 2015) were observed. The mean initial motility obtained is similar (68.10%) to the report of Khatun et al. (2013). However, Singh et al. (2013) recorded higher initial motility (79.5%) with lower mass activity and post-thaw motility (2.25 and 38.33%, respectively) in Murrah bulls. The findings on frozen semen doses per ejaculate are consistent (129.84 doses) with Bhosrekar et al. (1992). The variation in values might be due to the different climatic regions where the bulls maintained and trained for semen production, the feeding regime and other external factors, and the shorter study period with lesser observations in the previous studies.

The significant effect of ejaculate numbers on seminal traits is consistent with Mishra et al. (1994), as they reported that ejaculate numbers significantly affected semen volume, sperm concentration, post-thaw motility, and frozen semen doses, except initial motility. In contrast, a non-significant difference in semen volume and sperm concentration on ejaculate frequency was also seen in a decreasing trend with an increase in ejaculate numbers (Kumar et al. 1988).

The findings regarding the effect of period are in agree with Ravimurugan (2001), where period was reported as a highly significant source of variation for most of the seminal parameters studied in Murrah bulls. However, a non-significant effect of period on semen volume and mass activity was also observed (Mukhopadhyay et al. 2010). The better performance in period V (2014-2015) might be due to good management practices and feeding regimes followed during this period. In general, the more pronounced variations in the period of semen collection reveals the differences in management, quality of bulls maintained, evaluation methods followed, and quality control measures adopted during various periods. However, the performances of the bulls were found to be better during the periods after 2009. This could be due to the selection of genetically superior bulls from the home track based on pedigree records and progeny testing, better maintenance condition, and culling of non-performance bulls in the early stage of collection.

The present outcome on season suggests that the quality of semen was best during south-west monsoon and summer seasons which is in opine with Rao et al. (1991) and Ravimurugan (2001). It was reported that seminal traits differ significantly between seasons with better initial motility and post-thaw motility in winter and summer seasons and suggested south-west monsoon and rainy season as favourable seasons. This might be due to the existence of semen stations in similar agroclimatic conditions. Contrary to the present study, a non-significant effect of season on semen volume, sperm concentration, total sperm per ejaculate, mass activity, and initial motility was also noticed (Bhosrekar et al. 1992 and Tiwari et al. 2011). Similarly, Mandal et al. (2000) and Bhakat et al. (2015) reported winter as most favourable season for quality semen production followed by rainy season, with least in the summer (hot-dry) season, which differs due to different climatic conditions in the region. The variations in semen characteristics in winter season could be due to the cold climatic condition, which alters the thermoregulation leading to the displacement of testis from the scrotal pouch to the abdomen, causing stress to the bulls (Zafar et al. 1988). The optimal production in summer season would be because of good summer management practices with fogging systems, ventilators, and feeding regimes.

Rao et al. (1991) reported that bulls beyond seven years of age showed an increase in semen volume and total sperm per ejaculate, with highest mean in bulls above 10 years of age. Similarly, Rao and Sreemannarayana (1996) recorded higher value for seminal parameters in bulls 10 to 14 years of age than below 7 years of age. These findings agree with the present work as bulls aged from 7 years to 12 years and above had better performance with increased semen volume and frozen semen doses. This indicates the better sexual maturity of older buffalo bulls compared to younger bulls and their adaptation to the process of semen collections over a time which improves the quality of semen with minimal rejection. However, Ravimurugan (2001) revealed a non-significant effect of age on sperm concentration and higher sperm concentration and post-thaw motility in young bulls, which is contrary to the present study.

When compared with the repeatability estimates of this study, Taraphder et al. (2001) from 110 semen collection reported lower estimates for semen volume (0.27) and similar estimates for initial motility (0.34). A perusal of literature revealed that studies on repeatability estimates of semen production traits are very scanty in Murrah buffalo bulls. The present findings acquired from such a large set of data would be more accurate, which can be used for the prediction of semen production potential in young Murrah bulls. Since repeatability is an upper limit to heritability, it gives an idea about the heritability values for the traits. Selection of bulls based on repeatability would be more beneficial because the bull calves are purchased from different sources, and grouping of progenies per sire is almost impossible to estimate the heritability of the semen production traits. It was observed that the semen production traits such as sperm concentration, total sperm per ejaculate, and frozen semen doses per ejaculate showed low repeatability, indicating that these traits were influenced more by the non-genetic factors rather than heritable causes. The traits with moderate to high repeatability were not affected much by environmental factors and, therefore, helpful in selecting breeding bulls at an early age.

The significant positive correlation between the seminal traits and negative correlation of semen volume with sperm concentration and initial motility observed in the present work is on par with previous reports (Kumar et al. 1993 and Dhami et al. 1995). This negative association reflects the relationship between watery semen and low sperm concentration and motility. However, a non-significant correlation between sperm concentration and other seminal characteristics, except initial motility (Shukla and Misra 2005) and positive correlation between semen volume and sperm concentration (Pant et al. 2003), are inconsistent with the current findings.

Literature on the discard rate of semen is very scanty in Murrah buffalo bulls. The higher discard rate in north-east monsoon and winter might be due to the climatic condition of the seasons, as the temperature is relatively lower compared to other seasons, which affects the quality of semen. In general, higher discard rate in pre-freezing than post-thaw might be due to the stringent standards followed in selecting quality semen ejaculates for frozen dose preparation. Due to strict implementation of Minimum Standards for Production of Bovine Frozen Semen (2012), the quality of frozen semen has improved over the period of time, and further implementation of appropriate managemental practices as per the season and semen collection requirement will even reduce the discard percentage of semen in the semen stations.

In conclusion, the semen production traits studied were significantly influenced by ejaculate, period, season, and age. The first ejaculate recorded better performance than second ejaculate. Period V (2014-2015) had higher values for all the seminal traits than other periods. Interestingly, semen production performance was better during south-west monsoon and summer seasons than other seasons. The bulls above eight years of age produced better quality semen than the younger bulls, and the best performance was seen in bulls above 12 years of age. The repeatability estimates were low to medium, indicating the scope of selection for moderate repeatable traits. The rejection rate of semen was high at pre-freeze stage than the post-thawing shows better freezability. These findings suggest that proper selection of bulls at an early age and maintaining under appropriate uniform management practice might improve the quality of semen and quantity of frozen semen doses to meet the demand in field conditions for AI.

Declarations

Funding

Not applicable

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Availability of data and material

We certify that all raw data and data set pertaining to experiments and experiment results are available

Author's contributions

RP, SNS and SMKK conceived and designed the study. RP and SP acquisition of data and entering of data. RV and RP statistical analysis and interpretation of data. RP, SNS and SP drafting of the manuscript. All authors have critically reviewed and approved the manuscript.

Statement of Animal Rights (Ethics approval)

Not applicable. No experiments were performed on animals.

Acknowledgement

The authors are thankful to the Director, Department of Animal Husbandry, Tamil Nadu, and Exotic Cattle Breeding Farm officials for providing necessary support in data collection for the present study.

References

1. Becker, WA., 1975. Manual of Quantitative Genetics, (Washington State University, Pullman, Washington).
2. Bhakat, M., Mohanty, T.K., Raina, V.S., Gupta, A.K. and Khan, H.M., 2011. Frozen semen production performance of Murrah buffalo bulls. Buffalo Bulletin, 30(2), 157-162.
3. Bhakat, M., Mohanty, T.K., Gupta, A.K., Prasad, S., Chakravarty, A.K. and Khan, H.M., 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. Buffalo Bulletin, 34(1), 100-112.
4. Bhosrekar, M.R., Purohit, J.R., Gokhale, S.B. and Mangurkar, B.R., 1992. Semen characteristics and behavioural patterns of buffalo bulls in relation to age and seasons. Indian Journal of Animal Sciences, 62(3), 251-255.
5. Department of animal husbandry and dairying, Government of India, 2019. Basic Animal Husbandry and Fisheries Statistics. Ministry of Fisheries, Animal Husbandry and Dairying. http://www.dahd.nic.in/Division/statistics/animal-husbandry-statistics-division. Accessed 25 June 2021.
6. Dhami, A.J., Mohan, G. and Sahni, K.L., 1995. Seminal *LDH* and trace elements profile in the static versus motile ejaculates of Friesian and Murrah buffalo bulls. Indian Journal of Animal Sciences, 10, 151-156.
7. Dhami, A.J., Mohan, G. and Sahni, K.L., 1998. Seasonal influence on the quality and freezability of semen of Friesian and Murrah buffalo bulls. Indian Journal of Animal Reproduction, 19(1), 55-58.
8. Harvey, W.R., 1996. Mixed model least-squares and Maximum likelihood computer programme. PC-2 version. Ohio State University, Columbus.

9. Khatun, M., Kaur, S., Kanchan, and Mukhopadhyay, C.S., 2013. Subfertility problems leading to disposal of breeding bulls. Asian-Australasian Journal of Animal Sciences, 26(3), 303-308.
10. Kumar, A.S. and Krupakaran, R.P., 2014. Comparative study on physico-morphological characteristics of semen from Murrah buffaloes and Jersey crossbred cattle. CIBTech Journal of Bio-Protocols, 3(3), 12-14.
11. Kumar, S., Sahni, K.L. and Bishta, G.S., 1993. Correlation studies among various seminal characteristics of motile and static ejaculates of Murrah bulls. Indian Journal of Dairy Science, 46(5), 223-226.
12. Kumar, S., Tripathi, S.S. and Saxena, V.B., 1988. Cytomorphological characters and their correlation studies of successive semen ejaculates of Murrah bulls. Indian Veterinary Journal, 65(9), 842-844.
13. Mandal, D.K., Nagpaul, P.K. and Gupta, A.K., 2000. Seasonal variation in seminal attributes and sexual behavior of Murrah buffalo bulls. Indian Journal of Dairy Science, 53(4), 278-283.
14. Meyer, K., 2007. WOMBAT-A tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). Journal of Zhejiang University- Sciences. 8(11), 815-821.
15. Minimum Standards for Production (MSP) of Bovine Frozen Semen, 2012. Department of Animal Husbandry and Dairying, Government of India. http://dahd.nic.in. Accessed 25 June 2021.
16. Mukhopadhyay, C.S., Gupta, A.K., Yadav, B.R., Khate, K., Raina, V.S., Mohanty, T.K. and Dubey, P.P., 2010. Subfertility in males: An important cause of bull disposal in bovines. Asian-Australasian Journal of Animal Sciences, 23(4), 450-455.
17. Pant, H.C., Sharma, R.K., Patel, S.H., Shukla, H.R., Mittal, A.K., Kasiraj, R., Misra, A.K. and Prabhakar, J.H., 2003. Testicular development and its relationship to semen production in Murrah buffalo bulls. Theriogenology, 60(1), 27-34.
18. Rao, A.V.N., Rao, G.V. and Haranath, G.B., 1991. Effect of collection frequency, age and season on quantitative seminal characteristics in Murrah bulls. Indian Veterinary Journal, 68(11),1084-1085.
19. Rao, A.V.N. and Sreemannarayana, O., 1996. Seminal traits and frozen semen production in relation to age in Murrah bulls. Indian Veterinary Journal,73(5), 526-530.
20. Ravimurugan, T., 2001. Semen production capacity of Murrah buffalo bulls in Tamil Nadu. M.V.Sc. dissertation. Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.
21. Shukla, M.K. and Misra, A.K., 2005. Correlation between seminal characteristics in Murrah bulls. Indian Journal of Animal Sciences, 75(3), 263-266.
22. Singh, A.P., Singh, R., Singh, A.K., Gupta, A.K. and Raina, V.S., 2013. Influence of microclimate modification on sexual behavior and semen characteristics of Murrah buffalo bull during hot humid period in India. Indian Journal of Animal Sciences, 83(4), 431-434.
23. Snedecor, G.W. and Cochran, W.G., 1989. Statistical Methods, (Iowa state University Press, USA).
24. Taraphder, S., Gupta, A.K. and Tomar, S.S., 2001. Repeatability estimates of seminal characteristics of Murrah buffalo bulls. Indian Journal of Animal Research, 35(1), 71-72.

25. Tiwari, R., Mishra, G.K., Shukla, M.K., Singh, R.B., Saxena, S.K. and Siddiqui, M.U., 2011. Seasonal variations in semen production of Murrah buffalo bulls. Indian Journal of Animal Reproduction, 32(2), 5-7.
26. Zafar, A.H., Ahmed, N. and Shah, S.K., 1988. Effect of seasonal variation on semen production of Nili-Ravi buffalo bulls. Buffalo Journal, 4, 61-68.

Table 1 Least-squares means ± standard errors for non-genetic factors affecting semen characteristics of Murrah bulls

Factors	No. of records	Semen volume (ml)	Sperm concentration (million/ml)	Total sperm per ejaculate (million)	Mass activity (0 to 5 scale)	Initial motility (%)	No. of records	Post-thaw motility (%)	Frozen semen doses per ejaculate
Overall	54268	2.65 ± 0.01	1222.04 ± 4.23	3030.10 ± 15.76	2.64 ± 0.01	67.45 ± 0.12	44548	51.73 ± 0.06	128.80 ± 0.70
Ejaculate		**	**	**	**	**		**	**
I	30321	2.96 ± 0.01	1260.73 ± 5.43	3533.29 ± 20.24	2.60 ± 0.01	64.96 ± 0.15	22934	51.04 ± 0.07	146.91 ± 0.91
II	23947	2.34 ± 0.01	1183.35 ± 5.74	2526.92 ± 21.41	2.69 ± 0.01	69.95 ± 0.16	21614	52.41 ± 0.07	110.69 ± 0.93
Period		**	**	**	**	**		**	**
P_1	6468	2.38^{e} ± 0.01	1168.66^{d} ± 7.60	2483.42^{e} ± 28.31	2.80^{c} ± 0.01	71.46^{b} ± 0.22	5527	52.01^{b} ± 0.10	112.17^{d} ± 1.26
P_2	12246	2.61^{d} ± 0.01	1182.47^{c} ± 6.34	2950.02^{d} ± 23.62	1.93^{d} ± 0.01	61.77^{d} ± 0.18	8228	51.56^{c} ± 0.09	123.11^{c} ± 1.14
P_3	11844	2.66^{c} ± 0.01	1189.38^{c} ± 6.57	2978.52^{c} ± 24.48	1.84^{e} ± 0.01	61.66^{e} ± 0.19	8564	52.27^{a} ± 0.01	106.38^{d} ± 1.15
P_4	11961	2.71^{b} ± 0.02	1295.98^{a} ± 6.40	3209.13^{b} ± 23.86	2.99^{b} ± 0.01	68.24^{c} ± 0.18	10906	51.01^{c} ± 0.08	140.61^{b} ± 1.05
P_5	11749	2.91^{a} ± 0.01	1273.72^{b} ± 6.17	3529.43^{a} ± 22.99	3.65^{a} ± 0.01	74.11^{a} ± 0.18	11323	51.77^{b} ± 0.08	161.73^{a} ± 0.99
Season		**	**	**	**	**		**	**
S_1	13413	2.58^{b} ± 0.01	1199.19^{c} ± 4.93	2959.94^{b} ± 18.36	2.62^{c} ± 0.01	67.42^{b} ± 0.14	10864	52.04^{a} ± 0.07	125.07^{c} ± 0.83
S_2	13984	2.66^{a} ± 0.03	1247.95^{a} ± 12.11	3068.60^{a} ± 45.12	2.68^{a} ± 0.02	67.44^{a} ± 0.34	11584	51.39^{b} ± 0.09	130.41^{a} ± 1.99
S_3	13817	2.72^{a} ± 0.02	1231.62^{b} ± 7.32	3099.73^{a} ± 27.29	2.64^{b} ± 0.01	68.00^{a} ± 0.21	11449	51.97^{a} ± 0.16	132.27^{a} ± 1.19
S_4	13054	2.64^{b} ± 0.01	1202.30^{c} ± 4.97	2970.52^{b} ± 18.52	2.62^{c} ± 0.01	66.82^{b} ± 0.14	10651	51.53^{b} ± 0.07	126.43^{b} ± 0.86
Age		**	**	**	**	**		**	**
A_1	5390	2.25^{f} ± 0.02	1199.89^{c} ± 7.75	2454.04^{f} ± 28.87	2.65^{c} ± 0.01	63.65^{e} ± 0.22	3833	51.20^{c} ± 0.11	104.14^{f} ± 1.39
A_2	17019	2.33^{e} ± 0.01	1252.09^{a} ± 4.38	2813.30^{e} ± 16.34	2.57^{d} ± 0.01	65.60^{e} ± 0.12	12646	51.26^{bc} ± 0.06	121.01^{e} ± 0.77
A_3	13020	2.62^{d} ± 0.01	1226.86^{a} ± 5.02	3132.17^{d} ± 18.69	2.55^{d} ± 0.01	68.58^{d} ± 0.14	10613	52.19^{ab} ± 0.07	132.36^{d} ± 0.85
A_4	12021	2.76^{c} ± 0.01	1222.03^{b} ± 5.18	3291.76^{b} ± 19.31	2.68^{b} ± 0.01	69.44^{b} ± 0.15	11061	52.02^{abc} ± 0.07	137.95^{c} ± 1.20
A_5	6553	2.88^{b} ± 0.02	1208.44^{b} ± 7.36	3277.87^{c} ± 27.45	2.71^{a} ± 0.01	68.82^{c} ± 0.21	6144	51.62^{bc} ± 0.10	140.00^{b} ± 0.84
A_6	265	2.95^{a} ± 0.08	1223.82^{b} ± 38.68	3392.85^{a} ± 14.18	2.75^{a} ± 0.07	69.78^{a} ± 1.10	251	52.41^{a} ± 0.50	145.87^{a} ± 6.30

**** -** Highly significant (P <0.01). Least-squares means with at least one common superscript within classes do not differ significantly (P >0.05). P_1 = 2005 to 2007, P_2 = 2008 to 2009, P_3 = 2010 to 2011, P_4 = 2012 to 2013, P_5 = 2014 to 2015; S_1 = winter, S_2 = summer, S_3 = south-west monsoon, S_4 = north-east monsoon; A_1 = 18 to 42 months (m), A_2 = 43 to 67 m, A_3 = 68 to 92 m, A_4 = 93 to 117 m, A_5 = 118 to 142 m, A_6 = >142 m

Table 2 Least-squares analysis of variance for factors affecting semen characteristics in Murrah bulls

Source of variation	df	Semen volume MSS	Sperm concentration MSS	Total sperm per ejaculate MSS	Mass activity MSS	Initial motility MSS	df	Post-thaw motility MSS	Frozen semen doses per ejaculate MSS
Ejaculate	1	5556.89**	11891659.24**	2061754684.15**	13.65**	47817.76**	1	3907.57**	2536621.90**
Period	4	309.41**	25466009.30**	954372293.79**	4611.98**	251660.12**	4	1950.24**	3359388.84**
Season	3	6.42**	6741915.98**	12742250.77**	2.51**	920.64**	3	709.11**	25961.66**
Age	5	434.65**	2846219.29**	529532492.55**	32.16**	28589.13**	5	1034.89**	711088.59**
Bull	76	237.82**	44347790.37**	353861835.40**	71.29**	20764.04**	76	1061.77**	443701.76**
A x E	5	1.65**	866803.45**	14474320.90**	4.29**	9694.90**	5	202.70**	58971.56**
A x S	13	5.84**	1760497.63**	11301034.30**	24.11**	2569.51**	13	860.59**	16273.50**
Error	54236	1.35	265416.20	3687309.7	0.88	212.59	44516	39.44	6287.44

** - Highly significant (P <0.01); df = degrees of freedom; MSS = mean sum of square

Table 3 Repeatability estimates of semen production traits in Murrah bulls

Traits	No. of records	Residual variance ± S.E. ($\sigma^2 e$)	Repeatability ± S.E.
Semen volume	54268	0.73 ± 0.03	0.27 ± 0.03
Sperm concentration	54268	0.75 ± 0.001	0.25 ± 0.001
Total sperm per ejaculate	54268	0.88 ± 0.001	0.22 ± 0.001
Mass activity	54268	0.82 ± 0.03	0.28 ± 0.03
Initial motility	54268	0.66 ± 0.004	0.34 ± 0.04
Post-thaw motility	44548	0.83 ± 0.01	0.27 ± 0.01
Frozen semen doses/ejaculate	44548	0.86 ± 0.02	0.23 ± 0.02

Table 4 Phenotypic correlations between semen production traits in Murrah buffaloes

Traits	Semen volume	Sperm concentration	Total sperm per ejaculate	Mass activity	Initial motility	Post-thaw motility
Sperm concentration	-0.10**	1				
Total sperm per ejaculate	0.71**	0.60**	1			
Mass activity	0.03**	0.42**	0.30**	1		
Initial motility	-0.02**	0.10**	0.02**	0.58**	1	
Post-thaw motility	-0.03**	0.03**	-0.01**	0.44**	0.82**	1
Frozen semen doses/ejaculate	0.44**	0.45**	0.68**	0.56**	0.51**	0.52**

** - Highly significant (P <0.01)

Table 5 Discard percentage of semen ejaculates in Murrah bulls

Factors	Total ejaculates	Ejaculates discarded		Discard percent		
		Before freezing	After freezing	Before freezing	After freezing	Overall
Overall	54268	9720	1126	17.91	2.07	19.98
*Ejaculate						
I	30321	7387	825	24.36	2.72	27.08
II	23947	2333	301	9.74	1.26	11.00
#Period						
P_1	6468	941	135	14.55	2.09	16.64
P_2	12246	4018	286	32.81	2.33	35.14
P_3	11844	3280	247	27.69	2.09	29.78
P_4	11961	1055	262	8.82	2.19	11.01
P_5	11749	426	196	3.63	1.67	5.30
$Season						
S_1	13413	2549	246	19.00	1.83	20.83
S_2	13984	2400	252	17.16	1.80	18.96
S_3	13817	2368	283	17.13	2.04	19.17
S_4	13054	2403	345	18.41	2.64	21.05
@Age						
A_1	5390	1557	118	28.89	2.19	31.08
A_2	17019	4373	411	25.69	2.41	28.10
A_3	13020	2407	259	18.49	1.99	20.48
A_4	12021	960	211	7.99	1.76	9.75
A_5	6553	409	121	6.24	1.85	8.09
A_6	265	14	6	5.28	2.26	7.54

χ^2 Value of non-genetic factors *(df-1)-BF-1945.17**, AF-141.27**; #(df-4)-BF-4972.57**, AF 14.45**; $(df-3)-BF-24.03**, AF-29.73**; (df-5)-BF-2586.92**, AF-18.28**; ** - Highly significant ($P < 0.01$); χ^2 – Chi-square; df = degrees of freedom; BF-Before Freezing; AF-After Freezing